93/64 Stock structure and species identification of school and gummy sharks in Australasian waters

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2. To determine whether there is more than one species of school shark world-wide.
3. To determine the genetic stock structures of school and gummy sharks in Australasian waters.

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Genetic analysis of school shark collections from Australia, New Zealand, South Africa, Argentina and the UK, using both allozyme and mitochondrial DNA techniques, confirmed that this is a single, widely distributed species, *Galeorhinus galeus*.

There was no evidence for more than a single stock of school shark in the south-east Australian waters of Tasmania, Victoria and South Australia. No collections from Western Australia could be obtained, so the genetic status of fish in that area remains unknown. There were some genetic differences in New Zealand fish, which thus appeared to constitute a distinct stock from the south-east Australian stock. However, these differences were small, and not incompatible with some very limited exchange between the two areas. More fish need to be sampled to confirm (or refute) this apparent stock separation.

Genetic (allozyme and mitochondrial DNA) and morphometric analysis confirmed the existence of four species of gummy sharks in the Australasian region: the non-spotted *Mustelus species A* (a single specimen examined from the North West Shelf), and the three, spotted, species *M. species B* (specimens examined from Western Australia: the North West Shelf south to Perth), *M. antarcticus* (specimens examined from Bunbury in Western Australia around the southern Australian coast and as far north east as Townsville), and *M. lenticulatus* (from New Zealand).

Three genetic stocks of the common gummy shark, *Mustelus antarcticus*, were identified. One ranged along the southern coast of Australia from Bunbury in the west to Eden in the east, a second was located off New South Wales, in the region of Newcastle to Clarence River, and a third located off Townsville, Queensland. More collections need to be examined from the east coast to determine whether the stocks in this region form part of a cline or whether they are discrete entities.

Levels of allozyme heterozygosity were quite high in *M. antarcticus* ($H = 0.101$) but much lower in the other four shark species examined (ranging from 0.025 to 0.000). It is suggested that the higher variability of *M. antarcticus* relates to its larger population size than at least the other three *Mustelus* species examined, although if H is directly related to population size, then perhaps the school shark would also be expected to show more variation than the very low level (0.008) observed.

Levels of mitochondrial DNA variation showed similar trends, from *M. antarcticus* with moderately high sequence divergence of 0.25% and haplotype diversity of 0.483 to *M. lenticulatus* which showed zero mtDNA variation.

KEYWORDS:

Genes, allozymes, heterozygosity, mitochondrial DNA, morphometrics, taxonomy, sharks, *Galeorhinus, Mustelus,*

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2. BACKGROUND

The southern shark fishery is currently worth $20 million to fishermen in Victoria, Tasmania and South Australia, with annual landings exceeding 5000 tonnes live weight. The stocks of both school (*Galeorhinus galeus*) and gummy (*Mustelus antarcticus*) shark, the two target species, are considered by scientists and many fishermen to be over-exploited (Walker 1988). These sharks are members of the family of hound sharks, Triakidae.

About 20% of the catch of the southern Western Australian shark fishery comprises gummy shark; other sharks taken in large amounts include the bronze whaler (*Carcharhinus obscurus*) and whiskery shark (*Furgaleus macki*). There are concerns about declining catch rates in this fishery which is currently worth about $7 million.

Management of the southern shark fishery in particular, and shark fisheries in general, is hindered by uncertainties in stock assessments; key uncertainties relate to the spatial structure of the populations and mixing rates of fish between different regions. Fundamental to these spatial problems are the unknown stock structures of both school and gummy sharks. It has been generally assumed that both species are comprised of single stocks in southern Australian waters, but only limited work has addressed this issue with respect to gummy sharks, and there has been none at all with respect to school sharks.

The limited work on gummy shark stock structure was carried out by MacDonald (1988). He examined patterns of variation at a single weakly polymorphic locus, and could find no evidence for more than a single stock in southern Australian waters. However, this was very much a provisional assessment, as the power of the study was low. Recently, it has become apparent that there are gummy shark populations in tropical waters off western and eastern Australia which are currently not easily separable from *M. antarcticus* using classical taxonomic techniques. Based on vertebral counts, as many as four forms may occur in Australian waters but whether these constitute distinct species or separate populations of the southern form is unknown. Furthermore, the New Zealand gummy shark, *M. lenticulatus*, may not truly be specifically distinct from *M. antarcticus*, and it is possible that a single species occurs in both regions with intermixing. There is therefore a need to investigate these important taxonomic and stock structure issues for gummy sharks, and this reports seeks to address these issues using both genetic and morphological approaches.

The lack of work on school shark stock structure has been highlighted by the recent recapture in southern Australia of school sharks tagged in New Zealand. These recaptures have renewed interest in determining whether stocks in these two countries are shared or separate. This is examined by the joint application of allozyme electrophoresis and mitochondrial DNA analysis. School sharks were also sampled from different areas of the world and examined genetically to see if they represent more than one species.

3. NEED

The species problems surrounding the gummy shark need to be resolved. Such basic information is essential for effective management of the fishery. It is anticipated that resolution of these taxonomic issues will be achieved through the joint use of morphometrics, allozyme electrophoresis, and mitochondrial DNA analysis.

It is also necessary to gather further information on the stock structure of southern gummy sharks. This issue will be re-examined using allozyme electrophoresis together with the more powerful technique of mitochondrial DNA analysis.

There are similar uncertainties concerning the stock structure of school shark. These have been highlighted recently with the capture of New Zealand tagged fish in Australian waters suggesting the possibility that the two fisheries share a single stock. This issue will also be examined using allozyme electrophoresis and mitochondrial DNA analysis.

4. OBJECTIVES

1. To determine how many species of gummy sharks exist in Australasian waters and to determine their distributions.

2. To determine whether there is more than one species of school shark world-wide.

3. To determine the genetic stock structures of school and gummy sharks in Australasian waters.

5. METHODS

5.1 Specimen acquisition

5.1.1. Frozen/fresh specimens

Most fish (all school and most gummy sharks) were tissue sampled soon after capture, with samples of liver and muscle being taken and frozen immediately. Depending on logistics and availability of equipment, some collections were snap frozen in liquid nitrogen, others were maintained at -20°C. A minority of gummy sharks were sent frozen, intact, to the CSIRO laboratory. In the laboratory these sharks were thawed, labelled, sexed, and samples of muscle and liver were dissected. The fish were refrozen at -20°C for later measurement, possible radiographing for vertebral counts, or for fixing and adding to the CSIRO fish collection. All tissue samples in the laboratory were maintained at -70°C.

Collection details are given in Tables 5.1a (school sharks) and 5.1b (gummy sharks), and approximate map locations shown in Figs 5.1-5.3.

5.1.2. Stored museum specimens

Specimens of fixed (preserved) gummy sharks from the CSIRO fish collection were used to provide additional specimens for x-ray (vertebral count) analysis. Details of these specimens are given in Table 5.2.

5.2. Genetic examination

Both nuclear DNA, as represented by allozymes, and mitochondrial DNA, were examined.

5.2.1. Allozyme electrophoresis

Allozyme variation was examined using Helena Titan III cellulose acetate plates with a tris-glycine (0.02 M tris, 0.192 M glycine; see Hebert and Beaton (1989) for further details) or a 75 mM tris-citrate (pH 7.0) buffer system (see Richardson *et al.* (1986) for further details). Small pieces of liver or muscle were placed in 1.5 ml micro centrifuge tubes, homogenised manually with a few drops of distilled water, and spun in a micro centrifuge at 10 000 g for 5 min. The supernatant was used for electrophoresis. Table 5.3. lists the enzymes and buffers used. Tris-glycine gels were run at 200V at room temperature, typically for 30 min. Tris-citrate gels were run at 100v at 4°C, typically for 60 min. Staining procedures follow those of Hebert and Beaton (1989) and Richardson *et al.* (1986). Phenylalanine leucine was used for the peptidase stain. Coomassie Blue was used for the general protein stain.

School shark alleles were identified by the anodal electrophoretic mobility of their product relative to that of the most common allele (= 100) in the Tasmanian collections of school sharks, rounded to the nearest 5%. Gummy shark alleles were identified by the anodal electrophoretic mobility of their product relative to that of the most common allele (= 100) in the Tasmanian collections of gummy sharks, rounded to the nearest 5%. Locus notation follows that of Shaklee *et al.* (1990). When an enzyme was encoded by two loci, the more anodally migrating enzyme was suffixed as 1.

For the school shark, 20 enzymes and 29 loci could be resolved (Table 5.3), although tissue degradation in some collections meant that not all collections could be examined for all loci.

For the gummy shark, 21 enzymes and 28 loci could be resolved (Table 5.3), although again not all collections could be examined for all loci.

Notes on some of the enzymes:

*ACP** This enzyme was scorable in gummy sharks but not in school sharks. It is presented in the gummy shark frequency table as a di-allelic polymorphism. In good samples, four alleles could be detected but the mobility differences between the products of the two faster alleles, and between the two slower alleles, were small and could not be clearly distinguished in many fish. Here the two faster alleles have been pooled (as allele -100), as have the two slower alleles (as allele -200).

*CK-A** Like carcharhinid sharks (Lavery and Shaklee 1989), *CK-A** in *M. antarcticus* showed three-banded heterozygotes – teleosts show two-banded heterozygotes (Ferris and Whitt 1978; Elliott and Ward 1992) for this dimeric enzyme. The *CK-A** polymorphism could be scored on Coomassie blue protein stained gels and is not either of the general proteins *PROT-1** or *PROT-2**.

*ESTD-1&2*ESTD-1** was detectable with methylumbelliferyl acetate, the normal substrate for ESTD, but gave two-banded heterozygotes typical of a monomer. It is probably not a "true" esterase-D, which is a dimeric enzyme. The *ESTD-2** locus did give three-banded heterozygotes, typical of a dimer, and is a "true" esterase-D.

*PEP-1** Individual bands in heterozygotes for the aminopeptidase *PEP-1** were unable to be resolved, but were consistent with it being a hexamer. The usual dipeptide used as the substrate was phenylalanyl-leucine, but the dipeptide leucyl-tyrosine gave identical banding patterns. Lavery and Shaklee (1989) found cytosolic leu-tyr amino peptidase in carcharinid sharks to be hexameric.

5.2.2. Mitochondrial DNA procedures

Total DNA was extracted from approximately 100 mg of white muscle tissue using a modified CTAB (hexadecyltrimethylammoniumbromide) protocol described by Grewe *et al.* (1993). Animals from both species were screened for 10 restriction enzymes (*Apa*I, *Ava*I, *Bcl*I, *EcoR*I, *Hind*III, *Nco*I, *Pst*I, *Pvu*II, *Sac*II and *Xba*I).

Restriction fragments were separated in horizontal 1.0% agarose gels submerged in a tris-borate-EDTA (TBE) buffer system (Sambrook *et al.* 1989). DNA was transferred to a nylon membrane filter (Hybond N+, Amersham Ltd.) by southern transfer (Sambrook *et al.* 1989). The nylon membrane filters were probed with blue eye trevalla (*Hyperoglyphe antarctica*, Teleostei: Stromateoidei) mtDNA (50 ng used per ten 20 cm x 20 cm blots) purified by caesium chloride ultra centrifugation. The trevalla probe was labelled with [^{32}P]dCTP (Bresatec Pty Ltd.) by a GIGAprime DNA labelling kit (Bresatec Pty Ltd.). The membrane filters were then exposed to Kodak X-ray film for 12-48 h, routinely at -20°C without intensifying screens.

Restriction profiles for each enzyme were given letter designations in order of discovery, with the first pattern being designated "A", the second "B", and so on. Haplotypes of each fish were then identified by the combination of letters representing the restriction profiles for each restriction enzyme used. Restriction fragments were sized with the assistance of the program DNAGEL (Kieser 1984; modified by P. Grewe in QuickBasic) run on an IBM PC computer.

5.2.3. Statistical analysis

Generally the 0.95 criterion was used to define loci as polymorphic: the most common allele under this definition having to have a frequency of 0.95 or less. In comparing our data with other data (Chapter 9), the 0.99 definition was used.

Polymorphic loci were tested for goodness-of-fit to Hardy-Weinberg expectations using two alleles and single degrees of freedom, as rare alleles were pooled to reduce the number of genotype classes with small expectations.

Allele frequency homogeneity between specified collections was tested using the randomised Monte Carlo chi-square procedure of Roff and Bentzen (1989), which obviates the need to pool rare alleles. For each test, 1000 randomisations of the data were carried out, each giving a randomised chi-square value. The number of times each of the randomised replicates was greater than or equal to the observed value, divided by 1000, provides an estimate of the probability of obtaining the observed value by chance.

Nei's (1973) gene diversity (G_{ST}) statistics were used to quantify the extent of differentiation among collections. G_{ST} is equal to $(H_T-H_S)/H_T$, where H_T (total genetic diversity) is the average of the Hardy-Weinberg expected heterozygosity across all collections and H_S (mean genetic diversity per area) is the average Hardy-Weinberg expected heterozygosity within collections. Across all loci, G_{ST} was estimated from the mean of the H_T and H_S values. The G_{ST} value represents the proportion of genetic diversity that can be attributed to differences between collections. A bootstrapping procedure (Elliott and Ward 1992) was used to estimate the magnitude of G_{ST} that could be attributed to sampling error alone. This quantity is termed *G_{ST}-null* and a mean value of *G_{ST}-null* was estimated for each locus from 1000 replications. The number of times each of the 1000 estimates of *G_{ST}-null* was equal to or greater than the observed G_{ST} was determined, and this divided by 1000 gave the probability of obtaining the observed G_{ST} by chance.

Genetic distances between collections were assessed using Nei's (1978) unbiased genetic distance measure. The resulting genetic distance matrix was converted to a dendrogram of collection relationships by cluster analysis using the UPGMA (unweighted pair-group method with averaging) algorithm. This method assumes a constant rate of evolution. Nei's genetic distance takes a range of 0 (total similarity) to infinity (total dissimilarity), and his unbiased estimates take collection size into account. In situations where pairwise distance estimates between collections were infinity (no shared alleles), Nei's (1978) genetic identity was used. This takes a tractable range of 1 (total similarity) to 0 (total dissimilarity). In a few instances, Rogers (1972) distance was used, as it takes the tractable range of 0 (total similarity) to 1 (total dissimilarity). Calculations used the computer program BIOSYS-1 (Swofford and Selander 1989).

Mitochondrial DNA haplotype (nucleon) and nucleotide diversity, and sequence divergence between specified collections was computed using the REAP package (McElroy *et al.* 1992) and the formulations of Nei and Tajima (1981) and Nei (1987).

In all analyses involving multiple tests, such as testing for the same effect in each of several loci, the predetermined experimentwise significance level, α, of 0.05 was adjusted using Bonferroni procedures. Generally, the α level was divided by the number of tests to derive a new α level, and *P* values had to be less than this corrected α value to be deemed significant. Sometimes the the sequential Bonferroni procedure of Hochberg (1988) was used.

5.3. Morphometric examination and vertebral counts of gummy sharks

5.3.1. Methods

A total of 102 measurements (Table 5.4) were taken following Compagno (1984b), using vernier callipers or a standard fish measuring board (for total length and snout to vent length). All measurements were taken to the nearest mm, and only fresh (frozen) specimens were examined. Initially, extensive measurements were taken from four fish from each of three areas (off Townsville, Queensland; off the east coast of Tasmania; off Newcastle, New South Wales), and three fish from one area (in Shark Bay, Western Australia). All measurements were standardised by expressing as a percentage of total length.

A subset of 16 measurements (Table 5.5 and Figure 5.4) was selected that showed the least overlap (in measurements expressed as a percentage of total length) between these initial areas. A total of 84 fish from 11 sites (Table 5.6) were analysed for these measurements.

Some of these fish were radiographed, as well as fixed sharks in the CSIRO fish collection (Table 5.7). Monospondylic, diplospondylic and precaudal vertebrae were counted from X ray films (except for the New Zealand fish and *Mustelus sp. A*, where only precaudal vertebrae were counted). The transition from monospondylic to diplospondylic vertebrae was indicated by a marked shortening of the centra. The precaudal vertebrae were delineated from the anterior caudal vertebrae by inserting a pin perpendicularly at the upper origin of the caudal fin and counting vertebrae only to this pin.

5.3.2 Statistical analysis

For statistical analysis, fish were pooled into five groups based on their genetic similarity (from the allozyme and mitochondrial DNA analysis) as follows:

Group 1. *Mustelus sp. B.* (Western Australia)
Group 2. *M. antarcticus*, Esperance to Eden
Group 3. *M. antarcticus*, Newcastle and Clarence River
Group 4. *M. antarcticus*, Townsville
Group 5. *M. lenticulatus* (New Zealand)

Only a single specimen of *Mustelus sp. A* was available for morphometric examination, and it could not be included in the statistical analyses.

Males were compared with females within groups using t-tests. There were no overall significant differences so sexes were pooled for subsequent analyses. Differences between groups were examined using analysis of variance and discriminant analysis with the statistical package SYSTAT. In the discriminant analysis, prior probabilities were specified to adjust for differences in collection sizes. Fixed sharks were not compared as the number of animals was low in some areas, and pooling morphometric measurements of fresh and fixed animals could have confounded any conclusions.

Precaudal vertebral counts were compared between the four *Mustelus* species and within the *M. antarcticus* collections by analyses of variance. Less attention was paid to the monospondylic and diplospondylic vertebral counts as these counts were not done for all populations.

Table 5.1a. School sharks. Collection details. n=number of individuals, na=not available.

Locality	Abbrev	n	Latitude	Longitude	Depth (m)	Date collected	Collector
North Atlantic	NATL	16	51.5°N	4.5°W	na	Aug 95	Jim Ellis: University College, Swansea
Argentina	ARG	17	38-40°S	58-62°W	na	Dec 94	Gustavo Chiaramonte: Argentine Natural Science Museum
South Africa	SAFR	23	33.5-36°S	20-26°E	42-160	Nov 93	Michelle van der Merwe: South African Museum
South Australia	SA	143	33-35°S	134-135°E	30-132	Jun 94	Russel Hudson: VFRI
Tasmania (west)	WTAS	60	41-43°S	143-145°E	na	May 95	Grant West: CSIRO
Tasmania (east)	ETAS	90	41°30'-43°40'S	147°3'-148°4'E	na	Dec 93-March 94	Brian Baily: "Katrina B" John Stevens: CSIRO John Pitsilidis: "Sou Wester"
Tasmania (shelf)	STAS	35	43-44°S	146-149°E	na	June 92-Aug 94	Japanese Long-liners: Observer program
Victoria	VIC	80	40-41°S	144-146°E	na	Feb 95	Natalie Bridge: VFRI
NZ (East coast Sth Isl)	ENZ	55	43-45°S	171-173.5°E	44-105	May-Jun 93	Malcolm Francis: Fisheries Research Centre NZ
NZ (West coast Sth Isl)	WNZ	11	42.5-43.5°S	169.5-170.5°E	45-91	Apr 95	Malcolm Francis: Fisheries Research Centre NZ
NZ (Bottom Sth Isl)	WNZ	18	46-48°S	166.5-170°E	69-300	Feb-Mar 93	Malcolm Francis: Fisheries Research Centre NZ

Table 5.1b. Gummy sharks. Sampling details. n(1)=number of individuals sampled, n(2)=number of whole fish collected, na=not available.

Locality	Abbrev	n(1)	n(2)	Lat.°S	Long.°E	Depth (m)	Date collected	Collector
North West Shelf	NWS	7	7	18.5-20°	114.5-118.5°	27-150	Aug 95	Gorden Yearsley: Southern Surveyor
Shark Bay	SBAY	3	3	27°	112°	303	Feb 91	Colin Simpfendorfer, Phil Unsworth: W.A. Fisheries
Kalbarri	KALB	2	2	27-28°	113°	161-163	Mar 94	Colin Simpfendorfer, Phil Unsworth: W.A. Fisheries
Perth	PERTH	3	3	32°4'	115° 10.25	207	Nov 91	Colin Simpfendorfer, Phil Unsworth: W.A. Fisheries
Bunbury	BUN	23	0	33°23'	115°10'	91	Mar 94	Colin Simpfendorfer, Phil Unsworth: W.A. Fisheries
Augusta	AUG	21	0	34°40'	115°	18-58	Mar 94	Colin Simpfendorfer, Phil Unsworth: W.A. Fisheries
Doubtful Isls	DISL	16	0	34°30'	120°	36-84	Mar 95	Colin Simpfendorfer, Phil Unsworth: W.A. Fisheries
Esperance	ESP	16	16	34°20'	123°	71-75	Oct 95	Colin Simpfendorfer. Phil Unsworth: W.A. Fisheries
Isralite Bay	IBAY	33	0	34°	124°	18-91	Mar 95	Colin Simpfendorfer, Phil Unsworth: W.A. Fisheries
South Aust	SA	123	0	34°-35°	134-135°	30-110	Jun 94	Russel Hudson: VFRI
Tas (south)	STAS	33	0	43°03'	147°03'	na	Dec 93	John Pitsilidis: "Sou' Wester"
Tas (west)	WTAS	15	0	42-43°	145°	na	May 95	Grant West: CSIRO
Tas (east)	ETAS	85	26	41°3'-43°	148°-148°40'	na	Dec 93	Brian Baily: "Katrina B" John Stevens: CSIRO
Victoria	VIC	100	0	39°-40°	145°	50-86	Oct 94	Treena Bath: VFRI
NSW (Eden)	EDEN	14	14	36°40'-37°20'	150°10'	37-135	Feb-Mar94	NSW Fisheries / CSIRO Southern Surveyor
NSW (Newcastle)	NEWC	22	22	33°	152°	115-124	Feb-Mar94	Ken Graham: NSW Fisheries
NSW (Clarence R)	CLR	45	45	29-30°	153°3'-153°45'	27-73	Aug-Dec 95	Ken Graham: NSW Fisheries
Queensland (Townsville)	TOWN	4	4	18°	147°	205	Dec 93	Gorden Yearsley: Southern Surveyor
New Zealand	NZ	110	16	41°	174°	na	Nov 94	Malcolm Francis: F.R.C., NZ

Table 5.2. Gummy sharks. Additional (fixed) specimens used for X-ray examination. CSIRO fish collection number given. Abbrev. as in Table 5.1b

Specimen	Abbrev.	Locality
CA3038	NWS	Western Australia (North West Shelf) sp.A
CA3367	NWS	Western Australia (North West Shelf) sp.A
CA3037	NWS	Western Australia (North West Shelf) sp.A
CA3319	NWS	Western Australia (North West Shelf) sp.A
CA3368	NWS	Western Australia (North West Shelf) sp.A
CA3369	NWS	Western Australia (North West Shelf) sp.B
H1370.01	NWS	Western Australia (North West Shelf) sp.B
CA3317	NWS	Western Australia (North West Shelf) sp.B
CA3370	NWS	Western Australia (North West Shelf) sp.B
H2356.02	SBAY	Western Australia (Shark Bay)
H3599.03	SBAY	Western Australia (Shark Bay)
H3599.02	SBAY	Western Australia (Shark Bay)
H3599.01	SBAY	Western Australia (Shark Bay)
H822.14	SBAY	Western Australia (Shark Bay)
H2501.01	ETAS	Tasmania (Norfolk bay)
H2501.03	ETAS	Tasmania (Norfolk bay)
H2501.02	ETAS	Tasmania (Norfolk bay)
H1330.02	ETAS	Tasmania
C4693	ETAS	Tasmania
H1330.02	ETAS	Tasmania
H2690.05	CLR	New South Wales (North)
H2690.04	CLR	New South Wales (North)
H2690.01	CLR	New South Wales (North)
H2690.02	CLR	New South Wales (North)
H2690.03	CLR	New South Wales (North)
H2488.03	CLR	New South Wales (Brunswick/Tweed)
H2488.01	CLR	New South Wales (Brunswick/Tweed)
H2488.04	CLR	New South Wales (Brunswick/Tweed)
H2488.02	CLR	New South Wales (Brunswick/Tweed)
H1362.02	TOWN	Queensland (North)
H2468.01	TOWN	Queensland (North)
H632.02	TOWN	Queensland (North)
H1367.01	TOWN	Queensland (North)
H2469.02	TOWN	Queensland (North)
H1362.01	TOWN	Queensland (North)
H2471.01	TOWN	Queensland (North)
H1367.01	TOWN	Queensland (North)
H2469.01	TOWN	Queensland (North)
H2471.02	TOWN	Queensland (Hinchinbrook Island)
H460.02	TOWN	Queensland (Hinchinbrook Island)

Table 5.3. School and gummy sharks. Loci assayed with buffer type (TC = Tris citrate, TG = Tris glycine), tissue used (m = white muscle, l = liver, preferred tissue first), and species (g=gummy sharks, s=school sharks). Assumed quaternary structure (from heterozygote banding patterns) given for polymorphic enzymes.+ see text for details

Enzyme or protein name	Locus	EC No.	Buffer	Tissue	Species	Structure
Acid phosphatase	*ACP**	3.1.3.2	TG	l	g	dimer
Adenosine deaminase	*ADA-1**	3.5.4.4	TC	l	g, s	
	*ADA-2**	3.5.4.4	TC	l	s	monomer
Adenylate kinase	*AK**	2.7.4.3	TC	m	g, s	monomer
Aldehyde oxidase	*AO**	1.2.3.1	TC	l	g, s	
Aspartate aminotransferase	*AAT-1**	2.6.1.1	TC	m, l	g, s	
	*AAT-2**	2.6.1.1	TC	m, l	g, s	dimer
Creatine kinase	*CK-A**	2.7.3.2	TG	m	g, s	dimer+
Diaphorase	*DIA**	1.6.*.*	TG	l	g, s	
Esterase-D	*ESTD-1**	3.1.1.1	TG	l	s	monomer+
	*ESTD-2**	3.1.1.1	TG	l	g, s	dimer+
Fumarase	*FH**	4.2.1.2	TG	l, m	g, s	tetramer
Glucose-6-phosphate dehydrogenase	*G6PDH**	1.1.1.49	TC	l, m	g, s	dimer
Hexokinase	*HK**	2.7.1.1	TG	l	g, s	
Isocitrate dehydrogenase	*IDHP-1**	1.1.1.42	TC	m, l	g, s	
	*IDHP-2**	1.1.1.42	TC	l, m	g, s	
Lactate dehydrogenase	*LDH-1**	1.1.1.27	TG	m	g, s	
	*LDH-2**	1.1.1.27	TG	m	g, s	tetramer
Malate dehydrogenase	*MDH-1**	1.1.1.37	TC	m	g, s	
	*MDH-2**	1.1.1.37	TC	m	g, s	
Malic enzyme	*MEP-1**	1.1.1.40	TG	m	g, s	tetramer (?)
	*MEP-2**	1.1.1.40	TG	m	g, s	
Mannose-6-phosphate isomerase	*MPI**	5.3.1.8	TG	l	g, s	monomer
Octanol dehydrogenase	*ODH**	1.1.1.73	TG	l	g, s	
Peptidase	*PEP-1**	3.4.11/13	TG	l, m	g, s	hexamer+
	*PEP-2**				s	
General protein stain	*PROT-1**	-	TG	m	g, s	
	*PROT-2**	-	TG	m	g	
Superoxide dismutase	*sSOD**	1.15.1.1	TG	l	g, s	
Triose-phosphate isomerase	*TPI-1**	5.3.1.1	TC	m	g, s	
	*TPI-2**	5.3.1.1	TC	l	g, s	

Table 5.4. Gummy sharks. Morphometric measurements used. Suffix 'h' or 'p' in the Abbreviation column indicates 'Horizontal measurement' or 'Point to point measurement' respectively.

Abbrev.	Measurement	Abbrev.	Measurement
TOT	Total length	CSW	Subterminal caudal width
FOR	Fork length	CTR	Terminal caudal margin
PRC	Precaudal length	CTL	Terminal caudal lobe
PD2 h	Pre-second dorsal length	D1L	First dorsal length
PD2 p	Pre-second dorsal length	D1A	First dorsal anterior margin
PD1 h	Pre-first dorsal length	D1B	First dorsal base
PD1 p	Pre-first dorsal length	D1H	First dorsal height
HDL h	Head length	D1I	First dorsal inner margin
HDL p	Head length	DIP	First dorsal posterior margin
PG1 h	Prebranchial length	D2L	Second dorsal length
PG1 p	Prebranchial length	D2A	Second dorsal anterior margin
PSP h	Prespiracular length	D2B	Second dorsal base
PSP p	Prespiracular length	D2H	Second dorsal height
POB h	Preorbital length	D2I	Second dorsal inner margin
POB p	Preorbital length	D2P	Second dorsal posterior margin
PP1 h	Prepectoral length	P2L	Pelvic length
PP1 p	Prepectoral length	P2A	Pelvic anterior margin
PP2 h	Prepelvic length	P2B	Pelvic base
PP2 p	Prepelvic length	P2H	Pelvic height
SVL	Snout-vent length	P2I	Pelvic inner margin length
PAL h	Preanal length	P2P	Pelvic posterior margin length
PAL p	Preanal length	ANL	Anal length
IDS	Interdorsal space	ANA	Anal anterior margin
DCS	Dorsal-caudal space	ANB	Anal base
PPS	Pectoral-pelvic space	ANH	Anal height
PAS	Pelvic-anal space	ANI	Anal inner margin
ACS	Anal-caudal space	ANP	Anal posterior margin
PCA	Pelvic-caudal space	HDH	Head height
VCL	Vent-caudal length	TRH	Trunk height
PRN	Prenarial length	ABH	Abdomen height
POR	Preoral length	TAH	Tail height
EYL	Eye length	CPH	Caudal peduncle height
EYH	Eye height	DPO	First dorsal midpoint-pelvic origin
ING	Intergill length	MOL	Mouth Length
GS1	First gill slit height	MOW	Mouth width
GS2	Second gill slit height	ULA	Upper labial furrow length
GS3	Third gill slit height	LLA	Lower labial furrow length
GS4	Fourth gill slit height	NOW	Nostril width
GS5	Fifth gill slit height	INW	Internarial space
P1A	Pectoral anterior margin	ANF	Anterior nasal flap length
P1B	Pectoral base	CLO	Clasper outer length
P1I	Pectoral inner margin	CLI	clasper inner length
P1P	Pectoral posterior margin	CLB	clasper base width
SOD	Subocular pocket depth	INO	Interorbital space
CDM	Dorsal-caudal margin	SPL	Spiracle length
CPV	Preventral caudal margin	ESL	Eye spiracle space
CPU	Upper postventral caudal margin	HDW	Head width
CPL	Lower postventral caudal margin	ABW	Abdomen width
CFW	Caudal fork width	TAW	Tail width
CFL	Caudal fork length	CPW	Caudal peduncle width
CST	Subterminal caudal margin	GIR	Girth

Table 5.5. Gummy sharks. The subset of morphometric measurements used for routine analysis. See also Fig. 5.4.

Abbreviation	Measurement	
TOT	Total length	Measured from tip of snout to end of tail whilst resting on right side with dorsal lobe of caudal fin placed in line with the longitudinal axis of the body
PG1	Prebranchial length	Point to point measurement taken from tip of snout to top of first gill arch
PSP	Prespiracular length	Point to point measurement from tip of snout to anterior most point of spiracular whilst lying on ventral surface
SVL	Snout-vent length	Point to point measurement to anterior most point of vent whilst resting on right side
ACS	Anal-caudal space	Point to point measurement whilst resting on right side including the flap of skin at the insertion
EYL	Eye length	Visible part of orbital measured with small vernier calipers
POR	Preoral length	Measured with small vernier calipers excluding the jaw
D1B	First dorsal base	Measured from where origin of fin can be felt under surface of dorsal line to include webbing at posterior of fin whilst resting on right side
D1P	First dorsal posterior margin	Measured with shark resting on right side and fin flattened on table
D2A	Second dorsal anterior margin	Measured from where origin of fin can be felt under surface of dorsal line to upper posterior most point of fin
D2B	Second dorsal base	As for first dorsal base
ABH	Abdomen height	Taken directly behind first dorsal fin with shark resting on right side
TAH	Tail height	Taken directly behind pelvic fins with shark resting on right side
CPH	Caudal peduncle height	Taken where origin of upper caudal fin is felt under dorsal line
HDW	Head width	Measured with shark resting on ventral surface across the origin of the pectoral fins
ABW	Abdomen width	Measured with shark resting on ventral surface directly behind first dorsal fin
TAW	Tail width	Measured with shark resting on ventral surface across the anterior origin of the pelvic fins

Table 5.6. Gummy sharks. List of fish analysed for the subset of 16 morphometric measurements.
(M)m = mature male, M(sub) = submature male, M(i) = immature male, F = female, TL = total length (mm)

Locality		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NWS	ID#	GS726	GS727	GS728	GS729	GS730	GS731									
	Sex	M(m)	M(m)	M(m)	M(i)	M(i)	M(i)									
	TL	948	650	644	288	450	480									
KALB	ID#	GS201A	GS202A													
	Sex	F	F													
	TL	810	660													
SBAY	ID#	GS133a/ H3599.01	GS134a/ H3599.02	GS135a/ H3599.03												
	Sex	M(m)	M(m)	M(m)												
	TL	590	701	698												
PERTH	ID#	GS716	GS717	GS718												
	Sex	F	F	F												
	TL	755	804	739												
ESP	ID#	GS732	GS733	GS734	GS735	GS736	GS737	GS738	GS739	GS740	GS741	GS742	GS743	GS744	GS745	GS746
	Sex	M(m)	F	M(m)	M(m)	M(m)	M(m)	F	F	m(sub)	F	F	M(m)	F	F	F
	TL	1003	1156	1110	1208	1200	1160	1007.5	1015	1025	1115	1272	1230	1290	1340	1400
ETAS	ID#	GS122	GS698	GS699	GS700	GS701	GS188	GS189	GS190	GS191	GS107	GS108				
	Sex	M(m)	M(sub)	M(i)	M(i)	M(m)	F	M(m)	M(m)	F	?	?				
	TL	1012	1050	1005	970	1025	931	1016	985	984	713	875				
EDEN	ID#	GS160	GS161	GS162	GS163	GS164	GS165									
	Sex	M(i)	F	F	F	F	F									
	TL	842	535	607	397	571	57?									
NEWC	ID#	GS167	GS168	GS169	GS170	GS301A	GS303A	GS304A								
	Sex	F	M(m)	M(m)	M(m)	M(i)	M(m)	M(m)								
	TL	636	723	751	699	325	785	820								
CLR	ID#	GS711	GS712	GS713	GS714	GS715	gS756	GS757	GS772	GS773	GS776	GS777	GS786	GS787		
	Sex	M(i)	F	F	F	M(i)	M(m)	M(m)	M(m)	M(m)	M(m)	M(m)	F	F		
	TL	482.7	488.2	487.8	467.6	475.9	802	672	668	725	726	833	973	832		
TOWN	ID#	GS101	GS102	GS103	GS104											
	Sex	F	F	F	F											
	TL	1007	1027	1136	1013											
NZ	ID#	GS703	GS704	GS705	GS706	GS707	GS708	GS709	GS710	GS719	GS720	GS721	GS722	GS723	GS724	
	Sex	M(m)	M(i)	M(i)	M(m)	M(m)	M(m)	M(m)	M(m)	M(m)	M(m)	F	F	F	M(i)	
	TL	866	577	535	885	822	905	850	870	785	836	815	676	710	652	

Table 5.7. Gummy sharks. List of radiographed fish. C, CA and H prefixed fish belong to the CSIRO Fish Collection.

NWS SpA	NWS SpB	SBAY	PERTH	ETAS	EDEN	NEWC	CLR	TOWN	NZ
GS725	GS731	H2356.02	GS716	H2501.01	GS165	GS186	H2690.05	H1362.02	GS706
CA3038	GS727	H3599.03	GS717	GS114	GS172	GS185	H2690.04	H2468.01	GS703
CA3367	GS726	H3599.02	GS718	GS108	GS163	GS177	H2690.01	H632.02	GS707
CA3037	GS729	H3599.01		GS116	GS162	GS181	GS714	GS102	GS709
CA3319	GS728	H822.14		GS109	GS164	GS167	H2488.03	GS103	GS710
CA3368	GS730			GS110	GS171	GS184	H2488.01	H2471.02	GS704
	CA3369			H2501.03	GS161	GS170	H2690.03	H2469.02	GS708
	H1370.01			GS120		GS180	H2690.02	H1362.01	GS705
	CA3317			GS118		GS183	GS711	H460.02	
	CA3370			GS112		GS182	GS713	H1367.01	
				GS113		GS187	H2488.04	H2469.01	
				GS107		GS168	GS712	H2471.01	
				H1330.02		GS179	H2488.02	H1367.01	
				GS117			GS715		
				GS115					
				GS119					
				H2501.02					
				C4693					
				H1330.02					

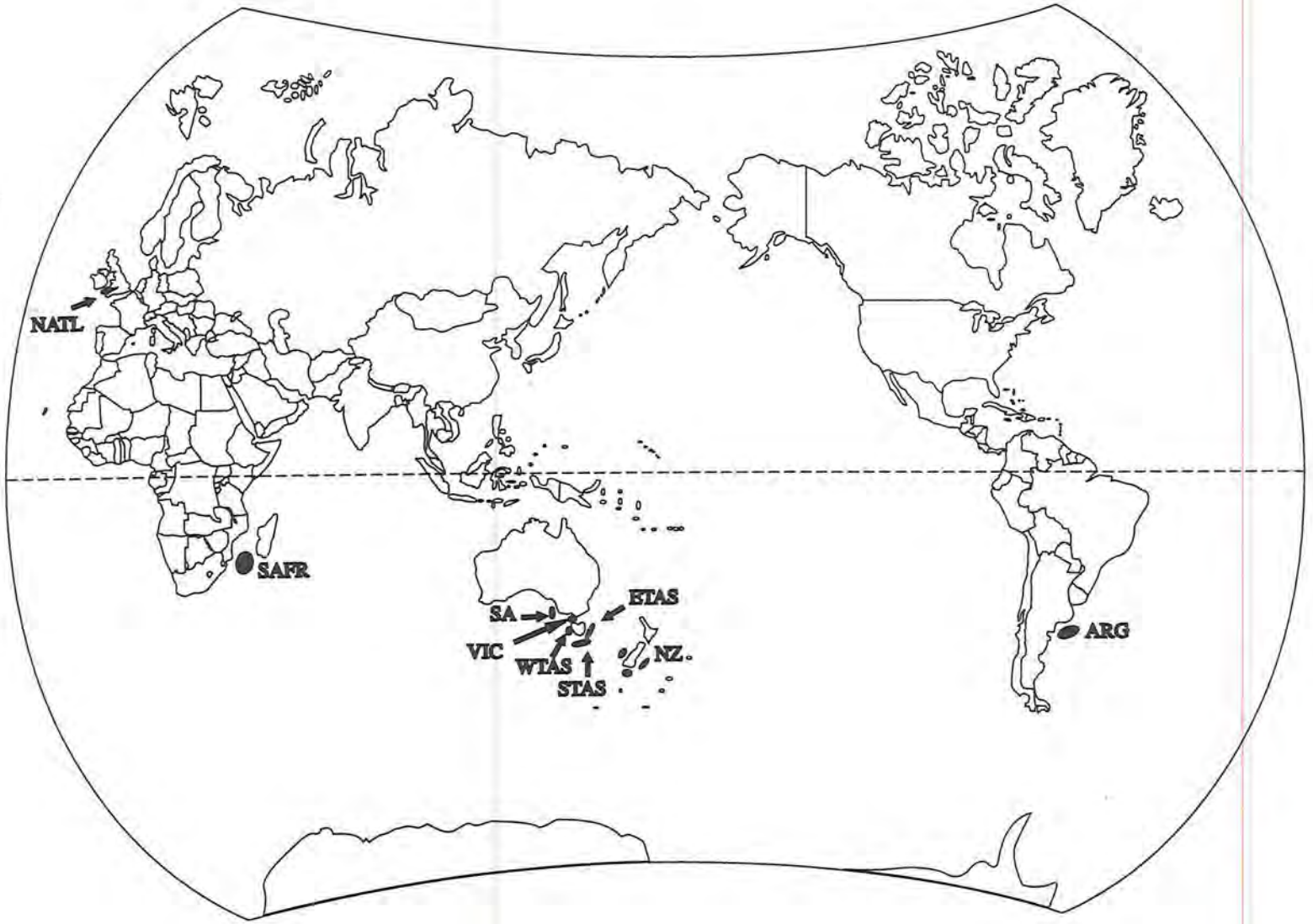

Fig. 5.1. School shark localities: global. See Table 5.1a for location details

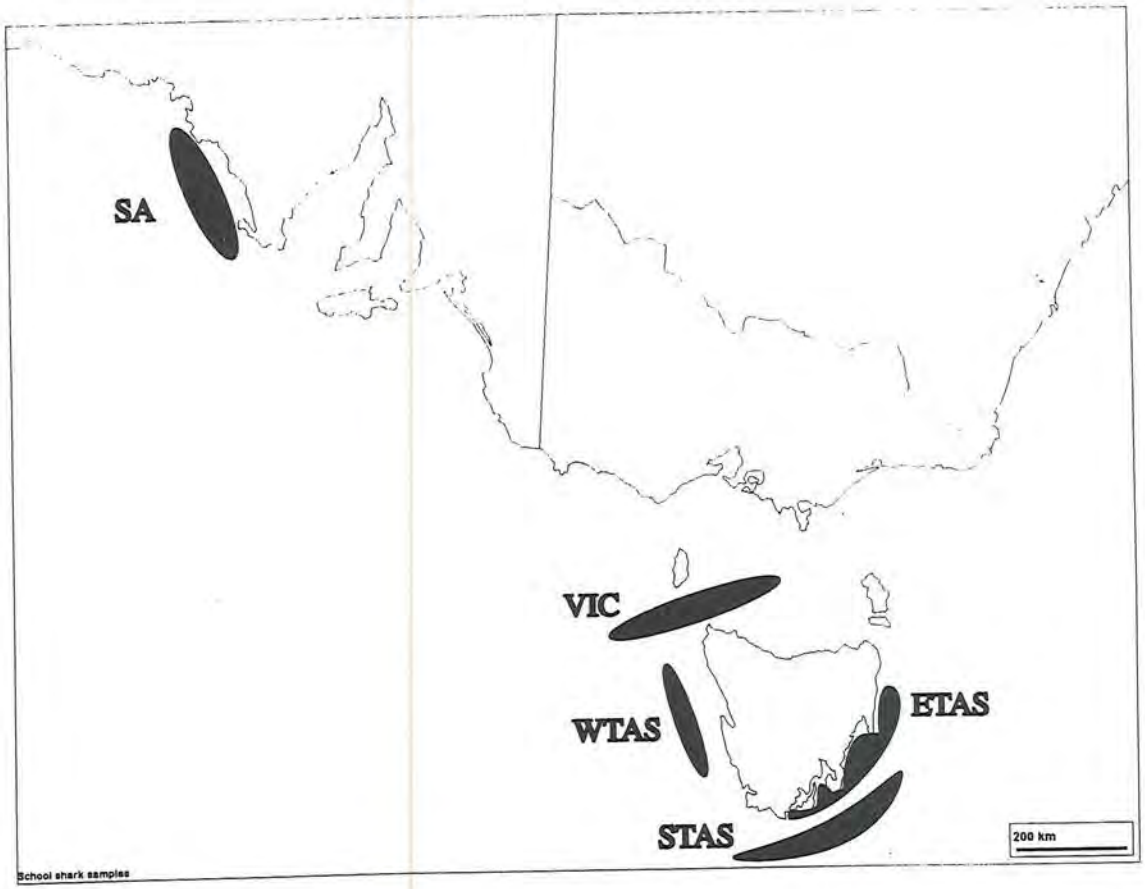

Fig. 5.2. School shark localities: Australia. See Table 5.1a for location details

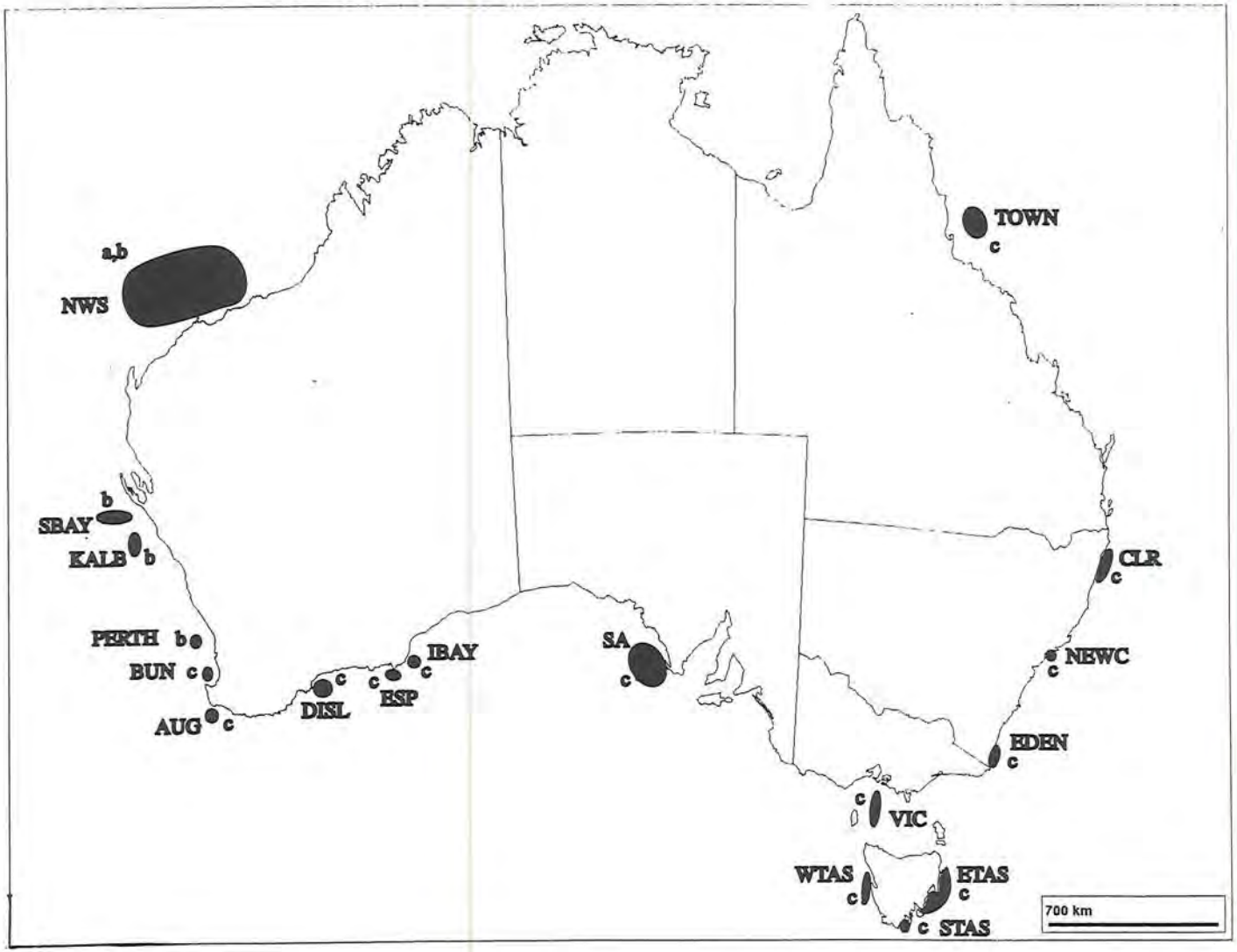

Fig. 5.3. Gummy shark localities: Australia
a = *M. sp.A*; b = *M. sp.B*; c = *M. antarcticus*

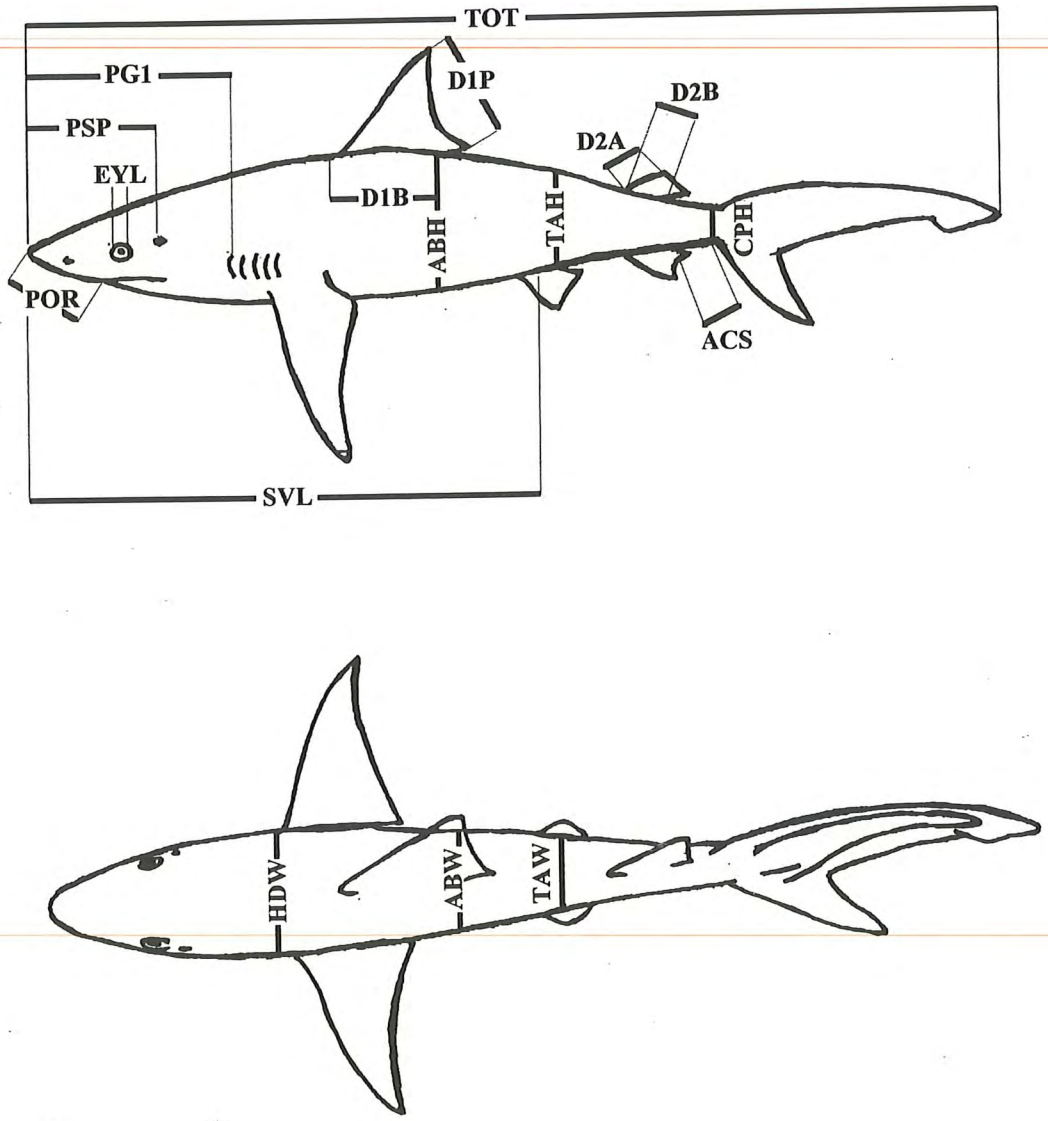

Fig. 5.4. Gummy sharks. The subset of 16 measurements (plus total length) that were used in morphometric analyses. See Table 5.5 for explanation of abbreviations.

6. RESULTS: TAXONOMY AND STOCK STRUCTURE OF SCHOOL SHARKS

6.1. Introduction

Much early work on Australian school sharks referred to the species as *Galeorhinus galeus* (Linnaeus), but until recently subsequent workers almost uniformly referred to it as *G. australis* (Macleay) (e.g. Olsen, 1954, 1984), one of a number of closely-related *Galeorhinus* species each with distinct allopatric distributions in different parts of the world. A closer study of this genus by Compagno (e.g. 1970, 1984a,b) suggested that the four nominal species *G. australis* (southern Australia and New Zealand), *G. zygopterus* (western America and Canada), *G. chilensis* (Chile and Peru) and *G. vitaminicus* (Uruguay and Brazil) should be regarded as synonyms of the European *G. galeus*. The FAO Species Catalogue now recognises a single species of *Galeorhinus* , *G. galeus* (Compagno 1984a).

Three of these five nominal species were sampled for our genetic study: *G. galeus* (England), *G. vitaminicus* (Argentina) and *G. australis* (Australia and New Zealand). A collection from South Africa was also studied. In total, eleven collections (Table 5.1a) were examined for both allozyme and mtDNA variation.

6.2 Allozyme data

Allozyme allele frequencies are given in Table 6.1. Only the New Zealand collection from the east coast of the north island (ENZ) was examined. A cursory look at this table will convey three impressions: firstly, that there is little allozyme variation, secondly, that all collections are extremely similar to one another, and, thirdly, that there are significant gaps in the data set, with some loci not being screened in some collections. The reason for the latter is that several allozymes, especially the liver-specific allozymes, only worked acceptably in well-preserved frozen samples. Unfortunately by the time we received some samples, some allozymes had degraded and therefore could not be scored.

Levels of allozyme variation are low, with only two loci polymorphic. The variation in eacg collection is summarised in Table 6.2. Only one collection (WTAS) could be examined for all 29 loci; it had an average Hardy-Weinberg expected heterozygosity per locus of 0.021.

Fifteen loci were scored in all collections, but few of these loci showed variation. Average heterozygosity per locus for these 15 loci ranged from 0.000 (six collections) to 0.003 (two collections) and 0.004 (one collection).

Only two loci showed sufficient variation that they could be used for population structure analysis - *ESTD-1** and *PEP-2** - but because of enzymatic degradation, in most collections these two loci could not be reliably scored. In fact, only one Australian collection, WTAS, was scored for these loci, and so the allozyme data could not be used for examining within-Australia stock structure. The New Zealand school sharks could not be examined for *ESTD-1**, but for *PEP-2** there was some evidence of a

small but just significant difference in allele frequencies from the WTAS collection (P = 0.04).

The South African collection was able to be examined for both *ESTD-1** and *PEP-2**. The South African collection was not differentiated from WTAS for *ESTD-1** (P = 0.29), but was for *PEP-2** (P<0.001).

Seven populations (NATL, SAFR, WTAS, ETAS, STAS, VIC, ENZ) were examined for 25 loci (all loci except *ESTD-1**, *MPI**, *PEP-2**, *PROT-1**) and genetic similarities among populations determined using Nei's (1978) unbiased genetic distance. These were all very small, ranging from 0.000 to 0.027. The conclusion that school sharks from sites as distant as the North Atlantic (U.K.), South Africa, Australia, and New Zealand do in fact represent a single globally-distributed species is inescapable.

6.3. Mitochondrial DNA data

Only one (*Hind*III) of the 10 restriction enzymes used showed variation among individuals (Table 6.3a). Fragment sizes are given in the Appendix. The mean size of the mitochondrial DNA molecule was estimated at 16,476bp (SD=1,151).

*Hin*d III was used to examine mtDNA variation among a total of 473 school sharks, including 342 from south-east Australia (Table 6.3b). For these analyses, the two smaller New Zealand collections (from the west coast of the south island and the south of the south island were combined into a single collection, WNZ).

There was very significant heterogeneity with respect to mtDNA haplotypes among the nine collections, ranging from the North Atlantic to South Africa and to Australia and New Zealand (P<0.001, Table 6.4). Inspection of the data showed that this was largely due to differences between the two Atlantic Ocean collections (the North Atlantic (U.K.) and the South Atlantic (Argentina)), and the other collections. Haplotype C, which was fixed in the Atlantic collections, was present at much lower frequencies (0.05 to 0.52) in all other collections, and haplotype A, common in all non-Atlantic collections (frequencies 0.38 to 0.64) was absent from the Atlantic collections. The South African collection also appeared different from other collections in having the highest frequency of haplotype A and the lowest frequency of haplotype C.

On the other hand, the five collections from south-east Australia (SA, WTAS, ETAS, STAS, VIC) showed no significant differentiation (P = 0.284, Table 6.4), so that the null hypothesis that these sharks came from a single genetic stock cannot be rejected.

Likewise, the two New Zealand collections, ENZ and WNZ, were not significantly different from each other (P = 0.407, Table 6.4).

Pooling the five Australian collections, a justifiable procedure as there was no significant heterogeneity among them, and comparing this pooled collection with the pooled New Zealand collection, similarly justifiable, gave a result bordering on statistical significance (P = 0.052, Table 6.4). The B haplotype was more common in the Australian population (0.175) than the New Zealand population (0.079), and the C

haplotype was less common in the Australian population (0.370) than in the New Zealand population (0.487). This is therefore evidence of some restriction on gene flow between the two populations.

Finally, there was a very significant difference between the pooled Australian collection and the South African collection ($P = 0.001$, Table 6.4). As mentioned above, this is because the A and C haplotypes are respectively more and less common in the South African collection than in the Australian collections.

A matrix of Nei's unbiased identities and distances based on haplotype frequency differences among pairs of collections was estimated (Table 6.5), and a dendrogram derived from it using the UPGMA algorithm (Fig. 6.1). Relationships thus shown support the statements above, with the Australian and New Zealand collections forming one cluster, separated from the South African collection and finally from the clustered Argentina/North Atlantic collections. The striking difference in haplotype frequencies between the Argentina and North Atlantic collections and all other collections is evident. In fact, the Atlantic collections are more different from the South African collections than they are from the Australian/New Zealand collections; an unexpected observation and one that needs to be confirmed (or refuted) with data from other variable genetic markers. The two NZ collections fall within the Australian cluster, rather than outside it, indicating that the difference between the pooled Australian collection and the pooled New Zealand collection, while bordering on statistical significance, is small.

As with the allozyme data, the striking similarities of mtDNA sequences (nine out ten restriction enzymes giving identical restriction profiles in fish from the North Atlantic to New Zealand) support the contention that this is a single, globally-distributed, species.

6.4. Conclusions

There was little allozyme variation in the school sharks, and gaps in the dataset caused by enzymatic degradation meant that the allozyme data were of no use for examining stock structure within Australian waters. There was, however, evidence of significant differentiation of a South African collection from a West Tasmanian collection (*PEP-2**, $P<0.001$), and weak evidence of differentiation of a New Zealand collection from a West Tasmanian collection (*PEP-2**, $P = 0.040$).

The mitochondrial DNA data were more useful, as all collections could be analysed using the restriction enzyme *Hind*III and there were no gaps in the dataset. *Hind*III produced six different haplotypes. However, there was no evidence for stock structuring among the Australian collections. The South African collection was significantly differentiated from the pooled Australian collection ($P = 0.001$). There was also weak evidence that the New Zealand fish were differentiated from the Australian fish, although the extent of differentiation only bordered on statistical significance ($P = 0.052$).

The observation that the two polymorphic genetic markers examined in both Australian and New Zealand collections (*PEP-2** and mtDNA *Hind*III) showed frequency differences bordering on significance ($P = 0.040$ and 0.052 respectively) suggests that

there is some restriction on gene flow between these two areas, and that it would be wrong to consider the two areas as simply constituting different parts of a single panmictic population. However, migration between these two areas of small numbers of sharks cannot be discounted. The analysis of additional samples from these two areas, ideally with genetic markers that are more variable than allozymes (e.g. microsatellites), is required to confirm (or refute) the probable existence of two discrete stocks.

Finally, the high degree of both allozyme and mitochondrial DNA similarity of all populations suggests that, as Compagno (1984a,b) suggested, school sharks do constitute a single globally distributed species, *Galeorhinus galeus*. The only caveat on this conclusion is that no specimens from the north-east Pacific or south-east Pacific could be examined.

Table 6.1. School sharks. Collection allele frequencies. *n* = number of fish, *n.s.* = not scored

Locus	Allele	NATL	ARG	SAFR	SA	WTAS	ETAS	STAS	VIC	ENZ
*ADA-1**	100	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*n.s.*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*ADA-2**	110	-	-	0.03	-	0.01	-	-	-	-
	100	1.00	-	0.97	-	0.98	1.00	1.00	1.00	1.00
	90	-	-	-	-	0.01	-	-	-	-
	n	*16*	*n.s.*	*18*	*n.s.*	*58*	*39*	*20*	*20*	*20*
*AK**	100	-	-	-	0.02	0.01	-	-	-	-
	80	1.00	1.00	1.00	0.98	0.99	1.00	1.00	1.00	1.00
	n	*16*	*17*	*8*	*20*	*58*	*39*	*20*	*20*	*20*
*AO**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*AAT-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*AAT-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*CK-A**	100	1.00	-	1.00	1.00	1.00	0.99	1.00	1.00	1.00
	40	-	-	-	-	-	0.01	-	-	-
	n	*16*	*n.s.*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*DIA**	100	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*n.s.*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*ESTD-1**	110	-	-	0.18	-	0.27	-	-	-	-
	100	1.00	-	0.82	-	0.73	-	-	-	-
	n	*1*	*n.s.*	*17*	*n.s.*	*41*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
*ESTD-2**	150	-	-	-	-	-	-	-	0.02	-
	100	1.00	-	1.00	1.00	1.00	1.00	1.00	0.98	1.00
	n	*16*	*n.s.*	*18*	*20*	*42*	*39*	*20*	*32*	*20*
*FH**	100	1.00	1.00	1.00	0.98	0.99	1.00	1.00	1.00	1.00
	60	-	-	-	0.02	0.01	-	-	-	-
	n	*6*	*17*	*18*	*20*	*58*	*39*	*20*	*20*	*20*
*G6PDH**	115	-	-	-	0.02	0.01	0.03	-	0.02	0.03
	100	0.97	-	1.00	0.98	0.99	0.95	1.00	0.98	0.98
	90	0.03	-	-	-	-	0.02	-	-	-
	n	*16*	*n.s.*	*18*	*20*	*58*	*20*	*20*	*32*	*20*
*HK**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*IDHP-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*IDHP-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*

Table.6.1 cont.

Locus	Allele	NATL	ARG	SAFR	SA	WTAS	ETAS	STAS	VIC	ENZ
*LDH-1**	120	-	-	-	-	-	-	-	-	0.02
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*LDH-2**	-100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*MDH-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*MDH-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*MEP-1**	100	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*n.s.*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*MEP-2**	100	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*n.s.*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*MPI**	110	-	-	-	-	-	-	-	-	-
	100	0.97	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00
	90	0.03	-	-	-	-	-	-	-	-
	n	*16*	*17*	*n.s.*	*8*	*21*	*39*	*20*	*20*	*20*
*ODH**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*9*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*PEP-1**	100	1.00	1.00	1.00	1.00	0.97	1.00	1.00	0.98	1.00
	80	-	-	-	-	0.03	-	-	-	-
	30	-	-	-	-	-	-	-	0.02	-
	n	*16*	*17*	*18*	*20*	*58*	*39*	*20*	*32*	*20*
*PEP-2**	100	-	-	0.68	-	0.98	-	-	-	0.90
	70	-	-	0.32	-	0.02	-	-	-	0.10
	n	*n.s.*	*n.s.*	*17*	*n.s.*	*58*	*n.s.*	*n.s.*	*n.s.*	*20*
*PROT-1**	100	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*n.s.*	*20*	*21*	*39*	*20*	*20*	*20*
*sSOD**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*17*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*TPI-1**	100	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*n.s.*	*18*	*20*	*21*	*39*	*20*	*20*	*20*
*TPI-2**	100	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*16*	*n.s.*	*18*	*20*	*21*	*39*	*20*	*20*	*20*

Table 6.2. School sharks. Summary of allozyme variation. H = average heterozygosity per locus, % P = percent loci polymorphic (0.95 criterion).

	NATL	ARG	SAFR	SA	WTAS	ETAS	STAS	VIC	ENZ
no. loci scored	28	17	27	26	29	27	27	27	28
mean sample size	15.67	17.00	17.56	19.54	29.77	38.29	20.73	21.33	20.00
H	0.004	0	0.029	0.004	0.021	0.004	0	0.004	0.009
% P (0.95)	0	0	7.41	0	3.44	3.70	0	0	3.57
15 loci common to all collections:									
mean sample size	15.33	17.00	17.33	20.00	28.40	39.00	20.00	20.80	20.00
H	0	0	0	0	0.004	0	0	0.003	0.003
% P (0.95)	0	0	0	0	0	0	0	0	0

Table 6.3. School shark. Mitochondrial DNA haplotype frequencies (a) Composite haplotypes from the restriction enzymes (in order) *Apa*I, *Ava*I, *Bcl*I, *Eco*RI, *Hind*III, *Nco*I, *Pst*I, *Pvu*II, *Sac*II and *Xba*I, (b) single haplotypes from the polymorphic restriction enzyme *Hind*III.

Haplotype	NATL	ARG	SAFR	SA	WTAS	ETAS	STAS	VIC	ENZ	WNZ
(a) Composite haplotype from 10 restriction enzymes										
AAAAAAAAAA	-	-	0.60	0.43	-	0.52	0.33	0.60	0.50	-
AAAABAAAAA	-	-	0.13	0.29	-	0.19	0.20	0.07	0.13	-
AAAACAAAAA	1.00	1.00	0.07	0.29	-	0.24	0.47	0.33	0.38	-
AAAADAAAAA	-	-	0.13	-	-	0.05	-	-	-	-
AAAAFAAAAA	-	-	0.07	-	-	-	-	-	-	-
n	*16*	*17*	*15*	*14*	*0*	*21*	*15*	*15*	*16*	*0*
(b) Single haplotype from the polymorphic restriction enzyme HindIII										
A	-	-	0.64	0.39	0.45	0.39	0.43	0.38	0.38	0.41
B	-	-	0.18	0.19	0.16	0.26	0.11	0.11	0.11	0.03
C	1.00	1.00	0.05	0.39	0.33	0.33	0.43	0.40	0.47	0.52
D	-	-	0.09	0.03	0.05	0.02	0.03	0.11	0.04	-
F	-	-	0.05	-	-	-	-	-	-	0.03
n	*16*	*17*	*22*	*97*	*55*	*82*	*35*	*73*	*47*	*29*

Table 6.4. School shark. Statistical tests for heterogeneity in the mtDNA haplotype data

Comparison	Number of groups	$\chi 2$	*P*
All populations	9	99.85	<0.001
Australia (SA, WTAS, ETAS, STAS, VIC)	5	18.41	0.284
New Zealand (ENZ, WNZ)	2	4.161	0.407
Australia (pooled) vs. New Zealand (pooled)	2	11.284	0.052
Australia (pooled) vs. South Africa	2	22.56	0.001

Table 6.5. School shark. Mitochondrial DNA genetic distance measures. Above diagonal: Nei's (1978) unbiased genetic identity. Below diagonal: Nei's (1978) unbiased genetic distance.

	NATL	ARG	SAFR	SA	WTAS	ETAS	STAS	VIC	ENZ	WNZ
NATL	-	1.000	0.069	0.671	0.570	0.577	0.703	0.703	0.767	0.788
ARG	0.000	-	0.069	0.671	0.570	0.577	0.703	0.703	0.767	0.788
SAFR	2.678	2.678	-	0.789	0.877	0.828	0.776	0.770	0.714	0.678
SA	0.399	0.399	0.237	-	1.000	0.998	1.000	0.993	0.997	0.968
WTAS	0.561	0.561	0.131	0.000	-	0.998	1.000	0.993	0.981	0.953
ETAS	0.550	0.550	0.189	0.002	0.006	-	0.973	0.957	0.954	0.909
STAS	0.352	0.352	0.253	0.000	0.000	0.028	-	1.000	1.000	1.000
VIC	0.352	0.352	0.262	0.007	0.007	0.044	0.000	-	1.000	0.984
ENZ	0.265	0.265	0.337	0.003	0.019	0.047	0.000	0.000	-	1.000
WNZ	0.239	0.239	0.388	0.032	0.048	0.095	0.000	0.016	0.000	-

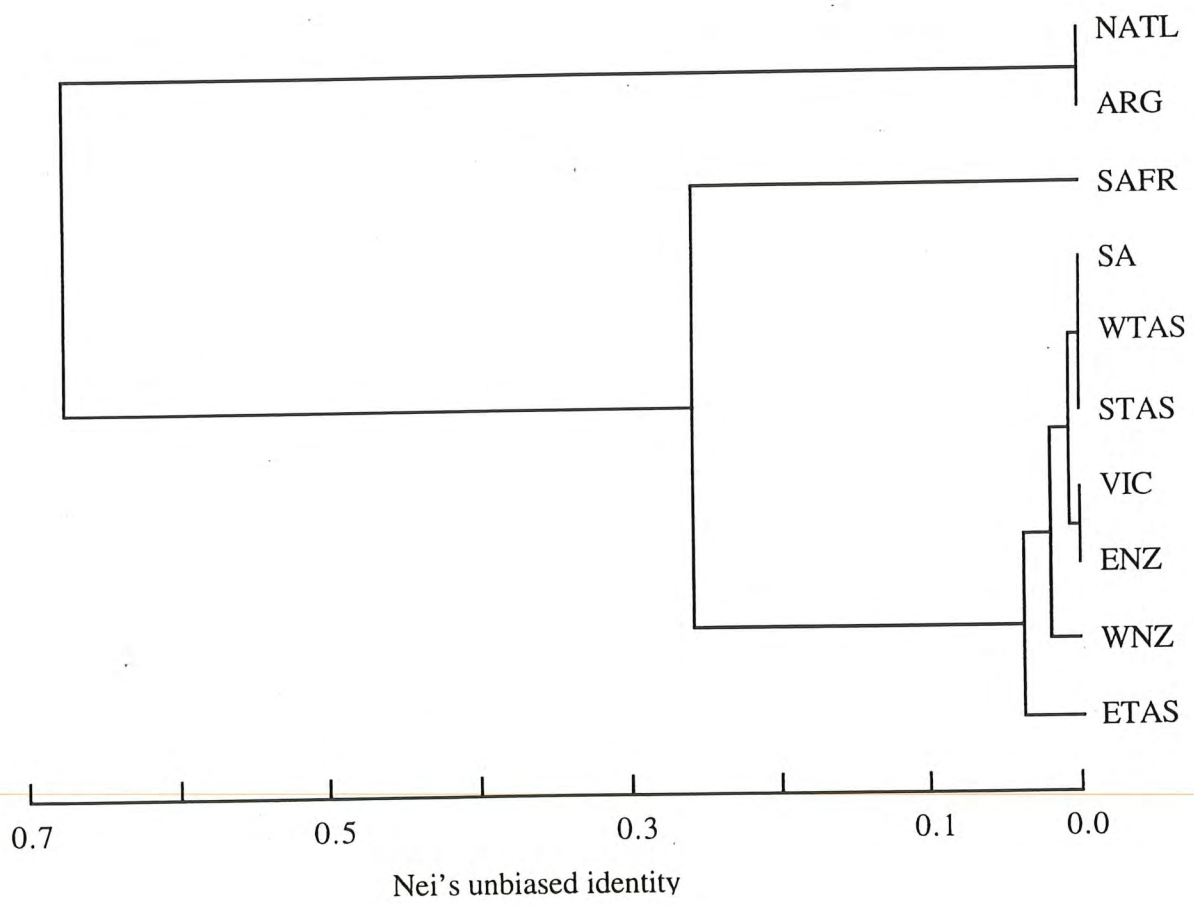

Figure 6.1. School sharks. Mitochondrial DNA genetic relationship among samples using Nei's (1978) unbiased genetic identity and UPGMA clustering.

7. RESULTS: STOCK STRUCTURE OF SOUTHERN AUSTRALIAN GUMMY SHARKS

Eleven collections of gummy sharks, *M. antarcticus*, were obtained from southern Australian localities from Bunbury in the west to Eden in the east (Table 5.1b). Four additional small collections of gummy sharks were obtained from the west coast of Australia (from Perth in the south to the north-west shelf) and three additional collections were obtained from the east coast (from Newcastle to Townsville). The west coast collections proved not to be *M. antarcticus* but *Mustelus* sp. B (see Chapter 8).

In this chapter, only the twelve collections from Bunbury to Newcastle are considered. These were all *M. antarcticus*. The Clarence River and Townsville collections are considered in Chapter 8, as initially it was considered that they may not be *M. antarcticus.*

7.1. Allozyme data

Allele frequency data for 28 loci in the twelve collections are given in Table 7.1.

Two collections (DISLE and IBAY) were in poor condition on arrival at Hobart and could not be typed for ten loci. Three other collections could not be typed for *HK** (BUN, AUG and SA), two collections (BUN and SA) could not be typed for *MPI**, and AUG could not be typed for *ODH**. Thus only seven collections could be typed for all 28 loci. For these seven collections and 28 loci, average heterozygosities ranged from 0.076-0.094 and proportion of polymorphic loci from 21.43%-25.00% (Table 7.2).

Seventeen loci were scored in all 12 collections. Average heterozygosities here ranged from 0.095-0.137 and proportion of polymorphic loci from 29.41%-35.29% (Table 7.2).

Six loci were polymorphic (*ACP**, *CK-A**, *ESTD-2**, *G6PDH**, *MPI**, *PEP-1**), showing enough variation that they could be used for genetic stock delineation purposes. With the exception of *MPI**, which could not be scored in the BUN and SA collections, each of these loci was scored in each collection. In addition, *LDH-2** showed variation in nine of the 12 collections.

MacDonald (1988) screened 32 loci in *M. antarcticus* and found only one locus (*LDH-2**) to show variation (compared with ten of the 28 loci in our study) and a mean heterozygosity per locus, *H*, of 0.006 ± 0.002 (compared with our mean value over the seven collections scored for all 28 loci of 0.084). The one enzyme, *LDH-2**, found by MacDonald to be variable enough (frequency of the most common allele < 0.95) to carry out a study of the genetic stock structure, exhibited similar amounts of variation in our collections. However, we found six additional loci to be polymorphic. Four of these loci (*ACP**, *ESTD-2**, *CK-A** and *PEP-1**) were not screened in MacDonald's study, accounting for some of the difference in mean heterozygosity between the two studies. But two loci, *G6PDH** and *MPI**, which were reported as invariant in his study, were highly polymorphic in our collections. The most likely explanation for this discrepancy

is the use of differing electrophoretic techniques with different types of gels and buffer systems.

Following chi-square tests for inter-collection allozyme heterogeneity at polymorphic loci (Table 7.3), two loci showed significant heterogeneity, *CK-A** and *LDH-2**. The *CK-A** result ($P = 0.019$) became non-significant following Bonferroni corrections of significance levels to account for the seven tests that were carried out. The *LDH-2** result ($P<0.001$) remained highly significant even after Bonferroni correction. Inspection of the allele frequency data for this locus (Table 7.1) showed that this could be largely attributed to the NEWC collection. In this collection, the frequency of *LDH-2**-60 was 0.273, much higher than the pooled frequency of 0.038 in the remaining 11 collections. Eliminating this collection from the analyses also eliminated the significant heterogeneity ($\chi^2=16.979$, $P = 0.509$).

Results following the G_{ST} analysis were similar, in that the only locus showing significant spatial differentiation was *LDH-2**. For this locus, about 6 per cent of the observed differentiation could be attributed to spatial differences (the figure of 6 per cent being the observed G_{ST} minus $G_{ST.null}$)

MacDonald (1988) found evidence of significant differentiation of *LDH-2** allele frequencies between sexes, with males having a significantly higher frequency of the rarer allele (number of fish examined = 525, $P = 0.02$). Our data failed to confirm this observation (number of fish examined (excluding the *LDH-2** distinct NEWC collection) = 314, $P = 0.377$).

7.2. Mitochondrial DNA data

The mtDNA fragment patterns produced by 10 restriction enzymes were examined in representative individuals from nine of the 12 collections, from Bunbury to Newcastle (Table 7.4a), totalling 113 fish. There was no significant differentiation among localities ($P = 0.191$).

Five of the 10 restriction enzymes used showed variation among individuals and were examined in the great majority of *M. antarcticus* individuals. A sixth enzyme, *Nco* I, distinguished *M. sp. B* from *M. antarcticus* (see Chapter 8) and was also examined in most *M. antarcticus* individuals. Fragment sizes are given in the Appendix. The mean size of the mitochondrial DNA molecule was estimated at 15,943 bp (SD = 1,389).

Two common mtDNA variants were seen among the individuals examined using the six restriction enzymes (Table 7.4b), and these differed in the mtDNA fragments produced by the *Apa* I enzyme. Haplotype C for *Apa* I has gained a restriction site from haplotype B. A third *Apa* I haplotype, E, was rare. This haplotype had lost a restriction site with respect to haplotype B. Four other composite haplotypes were seen, following digestion with four further enzymes, but all were rare.

All twelve collections (a total of 464 fish) were scored for mtDNA variability using the six restriction enzymes (Table 7.4b), but no significant inter-collection differentiation

was observed (Table 7.3; $P = 0.139$ following chi-square analysis and $P = 0.533$ following G_{ST} analysis).

7.3. Conclusions

Twelve collections of gummy sharks, from Bunbury in the south-west to Newcastle in the mid-east, were examined for allozyme and mtDNA variation. Only one of the seven polymorphic allozyme loci, *LDH-2**, showed significant inter-collection differentiation. This was due to different allele frequencies in the Newcastle collection. There was no significant mtDNA differentiation.

This significant *LDH-2** heterogeneity forces us to reject the null hypothesis of a single genetic stock of gummy sharks from Bunbury to Newcastle. We conclude instead that there are at least two stocks of gummy sharks in this region, one stretching from Bunbury to Eden, and the other found in the vicinity of Newcastle.

Table 7.1. Gummy sharks. Allele frequencies in collections of *M. antarcticus*. *n* = number of fish. *ns.* = not scored.

Locus	Allele	BUN	AUG	DISLE	ESP	IBAY	SA	STAS	WTAS	ETAS	VIC	EDEN	NEWC
*AAT-1**	115	-	-	-	-	-	-	0.03	0.04	-	-	-	-
	100	1.00	1.00	1.00	1.00	1.00	0.99	0.97	0.96	1.00	1.00	1.00	1.00
	90	-	-	-	-	-	0.01	-	-	-	-	-	-
	n	*22*	*7*	*9*	*15*	*20*	*95*	*32*	*14*	*66*	*99*	*11*	*22*
*AAT-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*7*	*9*	*15*	*20*	*95*	*32*	*14*	*66*	*99*	*11*	*22*
*ADA-1**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*13*	*1*	*n.s.*	*15*	*n.s.*	*47*	*26*	*11*	*13*	*23*	*11*	*21*
*AK**	100	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	1.00
	75	-	-	-	-	-	0.01	-	-	0.01	-	-	-
	n	*22*	*15*	*9*	*15*	*20*	*94*	*20*	*3*	*65*	*99*	*11*	*22*
*ACP**	-100	0.67	0.74	0.57	0.63	0.67	0.57	0.55	0.53	0.56	0.58	0.73	0.62
	-200	0.33	0.26	0.43	0.38	0.33	0.43	0.45	0.47	0.44	0.42	0.27	0.38
	n	*24*	*21*	*14*	*16*	*32*	*76*	*31*	*15*	*65*	*95*	*13*	*21*
*AO**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*13*	*1*	*n.s.*	*15*	*n.s.*	*47*	*25*	*15*	*13*	*23*	*11*	*22*
*CK-A**	90	0.08	0.24	0.23	0.16	0.11	0.11	0.25	0.14	0.10	0.13	0.32	0.16
	-100	0.92	0.76	0.77	0.84	0.89	0.89	0.75	0.86	0.90	0.88	0.68	0.84
	n	*24*	*21*	*15*	*16*	*32*	*95*	*32*	*11*	*66*	*100*	*11*	*22*
*ESTD-2**	150	0.21	0.28	0.28	0.25	0.17	0.21	0.15	0.20	0.24	0.24	0.25	0.34
	100	0.79	0.72	0.72	0.75	0.83	0.79	0.85	0.80	0.76	0.76	0.75	0.66
	n	*7*	*9*	*16*	*2*	*33*	*7*	*31*	*15*	*75*	*97*	*14*	*16*
*FH**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*5*	*2*	*n.s.*	*1*	*n.s.*	*70*	*28*	*15*	*46*	*47*	*11*	*8*
*G6PDH**	140	-	-	-	-	-	-	-	-	-	0.01	-	-
	120	0.33	0.36	0.31	0.38	0.35	0.42	0.27	0.43	0.39	0.37	0.40	0.50
	100	0.67	0.57	0.69	0.62	0.59	0.58	0.73	0.57	0.61	0.61	0.60	0.46
	80	-	0.07	-	-	0.06	-	-	-	-	0.02	-	0.04
	n	*18*	*7*	*16*	*13*	*33*	*55*	*26*	*15*	*53*	*94*	*10*	*12*
*DIA**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*9*	*10*	*n.s.*	*15*	*n.s.*	*30*	*20*	*14*	*6*	*23*	*11*	*18*
*HK**	100	-	-	-	1.00	-	-	1.00	1.00	1.00	1.00	1.00	1.00
	n	*n.s.*	*n.s.*	*n.s.*	*16*	*n.s.*	*n.s.*	*20*	*4*	*7*	*23*	*11*	*4*
*IDHP-1**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*11*	*n.s.*	*1*	*n.s.*	*30*	*31*	*14*	*18*	*23*	*11*	*22*
*IDHP-2**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*11*	*n.s.*	*15*	*n.s.*	*30*	*31*	*14*	*18*	*23*	*11*	*22*
*LDH-1**	120	-	-	-	-	0.02	-	-	-	-	0.01	-	-
	100	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*25*	*21*	*16*	*16*	*33*	*94*	*31*	*14*	*70*	*100*	*11*	*22*

Table 7.1 cont.

Locus	Allele	BUN	AUG	DISLE	ESP	IBAY	SA	STAS	WTAS	ETAS	VIC	EDEN	NEWC
*LDH-2**	-60	-	-	0.06	-	0.05	0.04	0.06	0.07	0.03	0.04	0.14	0.27
	-100	1.00	1.00	0.94	1.00	0.95	0.95	0.94	0.93	0.97	0.96	0.86	0.73
	-120	-	-	-	-	-	-	-	-	-	0.01	-	-
	n	*25*	*21*	*16*	*16*	*33*	*95*	*31*	*14*	*70*	*100*	*11*	*22*
*ME-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*12*	*9*	*15*	*13*	*30*	*25*	*14*	*7*	*23*	*11*	*18*
*ME-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*12*	*9*	*15*	*13*	*30*	*20*	*14*	*7*	*23*	*11*	*18*
*MDH-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*12*	*9*	*16*	*13*	*30*	*20*	*14*	*7*	*23*	*11*	*18*
*MDH-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*12*	*9*	*16*	*13*	*30*	*20*	*14*	*7*	*23*	*11*	*18*
*MPI**	120	-	-	-	-	-	-	-	0.03	0.01	0.01	-	-
	110	-	0.14	0.31	0.50	0.23	-	0.21	0.17	0.20	0.28	0.19	0.15
	100	-	0.86	0.66	0.50	0.68	-	0.73	0.77	0.75	0.65	0.73	0.83
	90	-	-	-	-	0.05	-	0.03	0.03	0.03	0.04	0.04	0.03
	80	-	-	0.03	-	0.05	-	0.03	-	0.01	0.02	0.04	-
	n	*n.s.*	*7*	*16*	*1*	*33*	*n.s.*	*31*	*15*	*61*	*97*	*13*	*20*
*ODH**	100	1.00	-	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	85	-	-	-	-	-	-	-	-	-	-	-	-
	n	*1*	*n.s.*	*n.s.*	*15*	*n.s.*	*30*	*20*	*14*	*7*	*23*	*11*	*13*
*PEP-1**	120	0.09	0.20	-	0.13	0.03	0.07	0.08	-	0.09	0.08	0.09	0.12
	100	0.86	0.78	0.91	0.84	0.95	0.89	0.85	1.00	0.87	0.89	0.91	0.88
	75	0.05	0.03	0.09	0.03	0.02	0.04	0.06	-	0.04	0.04	-	-
	n	*22*	*20*	*16*	*16*	*33*	*95*	*31*	*15*	*70*	*96*	*11*	*21*
*PROT-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		24	*21*	*16*	*16*	*33*	*95*	*24*	*14*	*70*	*100*	*11*	*18*
*PROT-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*24*	*21*	*16*	*16*	*33*	*95*	*26*	*14*	*70*	*100*	*11*	*18*
*sSOD**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*11*	*16*	*16*	*33*	*69*	*31*	*14*	*36*	*23*	*11*	*22*
*TPI-1**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*11*	*n.s.*	*15*	*n.s.*	*52*	*26*	*15*	*7*	*47*	*11*	*22*
*TPI-2**	100	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*22*	*11*	*n.s.*	*15*	*n.s.*	*52*	*26*	*15*	*7*	*25*	*11*	*22*

Table 7.2. Gummy sharks. Summary of allozyme variation. n = mean sample size per locus, H = average heterozygosity per locus, % P = percent loci polymorphic (0.95 criterion).

	BUN	AUG	DISLE	ESP	IBAY	SA	STAS	WTAS	ETAS	VIC	EDEN	NEWC
loci scored	26	26	18	28	18	27	28	28	28	28	28	28
n	16.89	11.67	13.11	13.36	26.28	58.07	26.68	13.36	38.32	59.68	11.21	18.79
H	0.097	0.122	0.134	0.084	0.120	0.105	0.085	0.076	0.079	0.083	0.090	0.094
% P (0.95)	18.52	22.22	38.89	21.43	38.89	22.22	25.00	21.43	21.43	21.43	25.00	25.00
17 loci common to all collections												
n	19.24	14.71	12.94	14.71	25.88	69.41	27.24	13.41	51.18	76.12	11.24	19.53
H	0.095	0.120	0.115	0.109	0.099	0.108	0.115	0.103	0.107	0.108	0.123	0.137
% P (0.95)	29.41	29.41	35.29	29.41	35.29	35.29	35.29	29.41	29.41	29.41	35.29	35.29

Table 7.3. Gummy sharks (BUN-NEWC). Analyses of genetic differentiation at polymorphic loci.
H_S = average Hardy-Weinberg expected heterozygosity (genetic diversity for mtDNA) per collection.
G_{ST} = proportion of genetic variation attributable to inter-collection differentiation.
Twelve collections scored for each locus except *MPI** where only ten collections were scored.

	Number of			Chi-square analysis		Genetic diversity analysis		
Loci	alleles	fish	H_S	$\chi 2$	P	G_{ST}	$G_{ST.null}\pm SD$	P
*ACP**	2	423	0.463	10.428	0.504	0.019	0.020±0.010	0.448
*CK-A**	2	445	0.269	23.315	0.019	0.036	0.020±0.010	0.056
*ESTD-2**	2	322	0.355	7.492	0.768	0.014	0.047±0.028	0.942
*G6PDH**	4	352	0.480	32.381	0.454	0.017	0.027±0.013	0.761
*LDH-2**	3	453	0.109	62.920	<0.001	0.089	0.020±0.009	<0.001
*MPI**	5	294	0.412	24.167	0.846	0.047	0.064±0.042	0.657
*PEP-1**	3	450	0.200	27.192	0.198	0.031	0.020±0.008	0.075
mtDNA	7	464	0.465	84.936	0.139	0.034	0.038±0.016	0.533

Table 7.4. Gummy sharks, Bunbury to Newcastle. Mitochondrial DNA haplotype frequencies .
n = number of fish, *n.s.* = not scored

(a) Composite haplotypes from the 10 restriction enzymes (in order) *Apa*I, *Ava*I, *Bcl*I, *Eco*RI, *Hind*III, *Nco*I, *Pst*I, *Pvu*II, *Sac*II and *Xba*I,

(b) Composite haplotypes from the 6 restriction enzymes (in order) *Apa*I, *Ava*I, *Bcl*I, *Eco*RI, *Nco*I and *Xba*I which detect polymorphic cut sites (note: *Nco*I is invariant here but is distinctive in gummy shark species B).

Haplotype	BUN	AUG	DISLE	ESP	IBAY	SA	STAS	WTAS	ETAS	VIC	EDEN	NEWC
(a) Composite haplotypes from 10 restriction enzymes												
BBBBEABBAB	0.64	1.00	-	0.64	-	0.77	0.67	0.53	-	0.75	-	0.64
BBBBEABBAD	-	-	-	-	-	-	-	-	-	-	-	0.07
CBBBEABBAB	0.29	-	-	0.36	-	0.23	0.22	0.40	-	0.25	0.67	0.29
CBBDEABBAB	-	-	-	-	-	-	-	0.07	-	-	0.33	-
ECBBEABBAB	-	-	-	-	-	-	0.11	-	-	-	-	-
BBDBEABBAB	0.07	-	-	-	-	-	-	-	-	-	-	-
n	14	2	n.s	14	n.s	13	18	15	n.s	20	3	14
(b) Composite haplotypes from 6 restriction enzymes												
BBBBAB	0.52	0.81	0.63	0.69	0.67	0.66	0.77	0.53	0.55	0.62	0.69	0.67
BBBBAD	-	-	-	-	-	-	-	-	0.01	0.01	-	0.05
CBBBAB	0.43	0.14	0.31	0.31	0.33	0.32	0.16	0.40	0.40	0.36	0.23	0.29
CBBDAB	-	-	-	-	-	-	-	0.07	-	-	0.08	-
CCBBAB	-	-	-	-	-	0.01	-	-	-	-	-	-
ECBBAB	-	0.05	-	-	-	-	0.06	-	0.01	-	-	-
BBDBAB	0.04	-	0.06	-	-	0.01	-	-	0.02	0.01	-	-
n	23	21	16	16	33	90	31	15	85	100	13	21

8. RESULTS: TAXONOMY OF AUSTRALASIAN GUMMY SHARKS

8.1. Introduction

In Last and Stevens (1994), three species of Australian gummy sharks are identified. These are the common southern gummy shark (*Mustelus antarcticus* Günther), the grey gummy shark (*Mustelus sp. A*), and the white-spotted gummy shark (*Mustelus sp. B*). In addition, there is the rig, *M. lenticulatus* Phillipps, in New Zealand waters. *Mustelus sp. B* has been identified from tropical Australia, from both Western Australia (Dampier) and Queensland (Cairns to Bowen), but is said to be 'almost indistinguishable' (Last and Stevens, 1994) from *M. antarcticus. Mustelus sp. A* has also been identified from tropical Australia, from off north-western Australia (Dampier to Darwin), and possibly also off Queensland (Townsville). This is a non-spotted species, and Last and Stevens suggest that it may be more widespread than current records imply.

The New Zealand species *M. lenticulatus* is morphologically very similar to *M. antarcticus*, and is primarily distinguishable from it by a precaudal vertebral count of 87-95 (Heemstra 1973; Francis and Mace 1980) as opposed to 79-86 of *M. antarcticus* (Last and Stevens 1994). *Mustelus sp. B* has a count of 76-80 (Western Australia) and 88-95 (Queensland), and *M. sp. A* a count of 90-92 (Last and Stevens, 1994).

Last and Stevens (1994) are uncertain whether the Western Australian and Queensland populations of *M. sp. B* are truly just variants of *M. antarcticus*, or whether they represent one or two separate species. It is, for example, known that vertebral counts of sharks can show considerable differences throughout the range of a single species (Springer and Garrick 1964), and thus by themselves may not always be reliable indicators of species status. Last and Stevens conclude that more taxonomic work is required on members of this genus in Australasian waters.

In order to further clarify these taxonomic relationships, we carried out a genetic comparison of these species, using both allozyme and mitochondrial DNA analysis.

The power of genetic techniques to discriminate species is well known (Ward and Grewe 1994; Davis 1994). Allozymes are especially useful for comparing closely related genera, resolving taxonomic problems at the species level, and identifying species that are difficult to distinguish morphologically (Richardson *et al.* 1986; Ward 1990). Lavery and Shaklee (1991) used allozyme electrophoresis to differentiate between two very similar northern Australian blacktip sharks, *Carcharhinus limbatus* and *C. tilstoni.* Two of 48 loci, *GPI** and *PEP**, exhibited dramatic (almost fixed) differences in allele frequencies with significant differences at four other polymorphic loci: the two forms (species) showed a Nei's genetic distance of 0.045. Two sibling species of the genus *Squatina* in South America showed fixed differences at two of 14 allozyme loci (*EST-2** and *EST-4**) and a large genetic distance of 0.34 (Solé-Cava *et al.* 1983). Phylogenetic relationships of carcharhinid and thresher sharks have also been examined by allozyme analysis (Naylor 1989; Lavery 1992; Eitner 1995).

Increasingly, mitochondrial DNA analysis is also being used in studies of population structure and species identification (Ward and Grewe 1994). Examples of mtDNA-based identification include tunas (Bartlett and Davidson 1991; Chow and Inoue 1993;

Ward *et al.* 1995), snappers (Chow *et al.* 1993) and wrasse (Hare *et al.* 1994). Martin (1993) presents preliminary data for some carcharhinid sharks, suggesting that levels of within-species mtDNA sequence diversity are "remarkably low", and Dunn and Morrissey (1995) have used mtDNA sequencing for comparing the five orders of elasmobranchs.

For our study, eighteen Australian collections of gummy sharks were obtained from the North-West Shelf southwards to Perth, and then along the southern coast of Australia and northwards to Townsville in north Queensland (Table 5.1b and Fig. 5.3). A large collection of *M. lenticulatus* was examined from New Zealand.

Unfortunately, it proved extremely difficult to collect the tropical Australian gummies, and collection sizes from these regions were very small (1 to 6 per collection). A single specimen of *Mustelus sp. A* was collected from the North West Shelf. A total of 14 specimens of *Mustelus sp. B* were collected from Western Australia, from the North West Shelf south to Perth. Twelve collections of *M. antarcticus*, from Bunbury to Newcastle, were obtained. The classification of two collections of gummy sharks from Australia's east coast, from Clarence River to Townsville, was initially unclear.

8.2. Allozyme data

Allele frequency data for 28 loci are presented in Table 8.1. Note that not all collections could be examined for all loci. Three collections in particular were poorly preserved on arrival in Hobart: PERTH (*M. sp. B*), DISLE (*M. antarcticus*), and IBAY (*M. antarcticus*).

Only 11 collections could be examined for all 28 loci: NWS (*M. sp. B*), ESP, STAS, WTAS, ETAS, VIC, EDEN, NEWC, CLR, TOWN (*M. antarcticus*), NZ (*M. lenticulatus*). The two *PROT** loci were not scored from *M. sp. A*, but the remaining 26 loci were scored. Thus 12 collections were scored for 26 loci, and 11 collections scored for 28 loci. The average heterozygosities and proportion of polymorphic loci of these collections were estimated (Table 8.2).

The CLR and TOWN collections appeared to be *M. antarcticus* (see later in this section), so four species of gummy sharks are represented here. *M. sp. A* was the least variable, the single specimen being homozygous at all 26 loci thereby giving heterozygosity and polymorphism levels of zero. *M. sp. B* and *M. lenticulatus* showed a few polymorphic loci out of the 28 examined, having percentage polymorphism levels (*P*) of 14.29 and 7.14 respectively, and average heterozygosities per locus (*H*) of 0.046 and 0.019 respectively. Note that Smith (1986) identified three out of 38 loci as polymorphic in *M. lenticulatus*: *G6PDH**, *GPI** and *SOD**. While allele frequencies were not given by Smith, each was specified to have the most common allele at a frequency greater than 0.80, and variation was therefore limited. In our collection, *G6PDH** and *SOD** were invariant, and *GPI** was not resolved satisfactorily. Two additional loci, *AAT-2** and *MEP-1**, reported as monomorphic by Smith, showed limited variation in our study. Both studies thus found low levels of genetic diversity in *M. lenticulatus*; Smith reporting *P* and *H* estimates of 0.079 and 0.001 $\pm$ 0.001 respectively, compared with our values of 0.071 and 0.019 $\pm$ 0.011 respectively. *M. antarcticus* was considerably more variable, with percentage polymorphism per

collection ranging from 21.43 to 32.14, and average heterozygosities per locus ranging from 0.076 to 0.129.

M. sp. A, although only represented by a single specimen, was well differentiated from all other collections. Six out of the 26 loci examined were diagnostic (*ADA-1**, allele 105; *CK-A**, allele 400; *MPI**, allele 130; *ODH**, allele 85, *PEP-1**, allele 125; *sSOD**, allele 95).

None of the other species showed absolutely diagnostic loci, although the collections of *M. sp. B* from Western Australia differed from the collections of *M. antarcticus* in having a very high frequency of allele *ESTD-2**150 (*M. antarcticus* has *ESTD-2**100 as its common allele), and alleles *LDH-2**-60 and *PEP-1**120 fixed (these alleles are uncommon in at least southern populations of *M. antarcticus*). *M. lenticulatus*, like *M. sp. B.*, was fixed for *LDH-2**-60 (an allele uncommon in *M. antarcticus*), monomorphic for several loci that are highly polymorphic in *M. antarcticus* (*ACP**, *CK-A**, *ESTD-2**, *G6PDH**, *PEP-1**), and nearly monomorphic for *MPI**.

There are thus allozyme differences between *M. sp. A.*, *M. sp. B* from Western Australia, *M. antarcticus*, and *M. lenticulatus*, consistent with these four taxa representing four distinct species. However, *M.sp. B*, *M. antarcticus*, and *M. lenticulatus* are genetically very similar to one another, with *M. sp. A* being the most divergent species.

Within the group provisionally identified as *M. antarcticus*, from BUN to TOWN, seven polymorphic loci (*ACP**, *CK-A**, *ESTD-2**, *G6PDH**, *LDH-2**, *MPI** and *PEP-1**) were identified, and with the exception of *MPI** (not resolved in BUN nor SA), all these polymorphic loci were examined in all collections. Section 7.3 concluded that data from the collections from BUN to EDEN were consistent with these collections coming from a single genetic stock, and that the *LDH-2** allele frequencies showed that NEWC represented a different stock. In Chapter 7, the CLR and TOWN collections were not considered, as their taxonomic status was uncertain.

Gene frequencies for the seven polymorphic loci for the combined BUN-EDEN collections were calculated, and compared to the NEWC, CLR, and TOWN frequencies (Table 8.3). After making Bonferroni corrections for the seven multiple tests (thereby reducing the 0.05 significance level to 0.05/7 = 0.007), two loci showed very significant heterogeneity (*LDH-2** and *PEP-1**, both with $P<0.001$), with a third locus (*CK-A**) bordering on significance ($P = 0.009$).

Pairwise collection comparisons of these three loci were carried out in order to locate the source of the heterogeneity (Table 8.4). Six pairwise comparisons were carried out for each locus, so in order to allow for Bonferroni correction for multiple tests, for each locus results were declared significant only if their probability values were equal to or less than 0.05/6 = 0.008.

For *CK-A**, only one comparison, the pooled Bunbury to Eden collection versus Clarence River, was significant ($P = 0.007$).

For *LDH-2**, the pooled Bunbury to Eden collection was significantly different from the remaining three collections, which showed no significant differentiation among themselves.

For *PEP-1**, three pairwise comparisons were significant: BUN-EDEN/CLR, BUN-EDEN/TOWN, and NEWC/TOWN.

CLR and TOWN were not significantly different for any of these three loci, and neither were CLR and NEWC. Since the TOWN collection was only four fish, it was ignored for the remaining analyses, and the CLR and NEWC collections combined. The combined BUN-EDEN collection was compared with the combined NEWC and CLR collection, with the following results: *CK-A**, $P = 0.014$; *LDH-2**, $P<0.001$; *PEP-1**, $P<0.001$. All three loci showed significant differentiation between these two pooled groups, even after Bonferroni correction for three tests (with α being reduced to $0.05/3 = 0.017$).

The allozyme data therefore suggest a genetic break between Eden and Newcastle, with gummy sharks from Eden south comprising one group, and gummy sharks from Newcastle and the Clarence River a second group. Only four fish from the Townsville region were collected - the allozyme data were equivocal on whether these formed part of the Newcastle/Clarence River group or constituted a group by themselves. It is impossible to tell from the present data whether there are indeed genetically discrete groups of *M. antarcticus* on the east coast or whether the groups identified form parts of a gradual cline for *LDH-2** (*LDH-2**-60 allele frequencies ranging from about 0.05 in southern parts of the range through 0.14 at Eden, 0.27 at Newcastle, 0.46 at Clarence River, to 0.75 at Townsville) and, to a lesser extent, for *PEP-1** (*PEP-1**100 ranging from about 0.90 in southern parts of the range as far north as Newcastle, 0.72 at Clarence River, to 0.25 at Townville). Additional sampling is required to solve this question.

Genetic distances (Nei 1978) between the 12 collections scored for 26 loci were estimated (Table 8.5), and a UPGMA dendrogram drawn (Fig 8.1). This multi-locus assessment of collection relationships confirmed the very distinct nature of *M. sp. A. M. lenticulatus* also separated from the major *Mustelus* grouping. Samples from ESP to EDEN showed minimal differentiation. NEWC and CLR formed a distinct cluster that was, however, closely related to the BUN-EDEN cluster. In the UPGMA dendrogram, the TOWN collection clustered with *M. sp. B*. However, this cluster is not supported strongly by the data. Inspection of Table 8.5. shows that the TOWN collection is more closely related to the CLR collection ($D = 0.004$) than to *M. sp. B*. ($D = 0.012$), although it is more closely related to *M. sp. B* than to any *M. antarcticus* collection other than CLR. Thus this multi-locus allozyme approach also failed to resolve the exact genetic nature of the TOWN collection. Subsequent mitochondrial DNA analysis showed it to be *M. antarcticus* rather than *M. sp. B* (see following section).

8.3. Mitochondrial DNA data

Mitochondrial DNA haplotype frequencies are presented in Table 8.6. Fragment sizes are given in the Appendix.

All specimens of *Mustelus sp. A, M. sp. B* and *M. lenticulatus* were examined with all 10 restriction enzymes, as were all the CLR and TOWN specimens of what is provisionally

identified as *M. antarcticus*. Limited numbers of other *M. antarcticus* were also examined for the 10 enzymes.

The single specimen of *M. sp. A* showed the most divergence. It had unique fragment patterns for six of the ten enzymes: *Eco*RI (haplotype E), *Hind*III (haplotype I), *Nco*I (haplotype D), *Pst*I (haplotype D), *Pvu*II (haplotype D) and *Xba*I (haplotype C).

One restriction enzyme, *Nco*I, gave a unique fragment pattern (haplotype C) for *M. sp. B*. *Xba* I gave three haplotypes, B (common in *M. antarcticus*), D (found in a very few M. antarcticus from ETAS, VIC and NEWC, and E (rare, but so far unique to *M. sp. B*).

Two of the 10 restriction enzymes gave nearly unique fragment patterns for *M. lenticulatus*. These were *Apa*I (haplotype E) and *Ava*I (haplotype C). All 94 specimens of *M. lenticulatus* had the same 10 enzyme composite haplotype, but this haplotype was also seen in two of the 162 *M. antarcticus* examined for the 10 enzymes.

What does the mtDNA data tell us about those collections provisionally identified as *M. antarcticus*, from BUN to TOWN? Section 7.2 shows that there was no differentiation among the 464 fish examined from BUN to NEWC for six restriction enzymes, so these collections were pooled into a single group and compared with the CLR collection and with the TOWN collection. These three collections showed a highly significant difference in composite haplotype frequencies ($P<0.001$). The three collections were compared pairwise to locate the source of the differentiation, with the following results: BUN-NEWC v. CLR, $P = 0.045$; BUN-NEWC v. TOWN, $P<0.001$; CLR-TOWN, $P = 0.004$. Therefore, after taking into account Bonferroni corrections for the three multiple tests (thereby reducing the significance level α from 0.05 to $0.05/3 = 0.017$), the source of the deviation is seen to be the TOWN collection.

The TOWN collection was a very small collection, of just four fish, but nevertheless two of these fish had haplotypes not seen in any other *M. antarcticus*. However, these two haplotypes were closely related to the two common *M. antarcticus* haplotypes. Haplotype BBCBAB was related to the most common haplotype BBBBAB by the loss of a single cut site for *Bcl* I, and haplotype CBBCAB was related to the second most common haplotype CBBBAB by the gain of a cut site for *Eco* RI.

Mitochondrial DNA relationships among collections were further assessed using Nei's genetic distance methods. In fact, Nei's genetic distance measures *per se* cannot be used for the mtDNA data set, as several collections (e.g. *M. sp. A*) share no alleles at all with any other collection, giving Nei genetic distance estimates of infinity and posing problems for clustering algorithms. However, Nei's identity parameters can be used (identity takes a range of 1 to 0, whereas corresponding distances are 0 to infinity), and so Nei's (1978) unbiased genetic identity measures were estimated for all pairwise comparisons using the 10 restriction enzyme dataset (Table 8.7). In addition, Rogers (1972) distances were estimated, as these take the tractable range of 0 to 1 (Table 8.7). Rogers distance is a maximal 1 when two collections are monomorphic for different alleles: if one collection has more than one allele, then even if the alleles in this collection are different from the allele(s) in another collection, Rogers distance is less than 1. This explains why, for example, the NWS collections of *M. sp. A* (with one allele) and *M. sp. B* (with three alleles), despite having no alleles in common, have a Rogers distance of 0.866 rather than 1.0 (see Table 8.7), whereas *M. sp. A* (one allele) and the KALB collection of *M. sp. B* (one allele), again with no alleles in common, have

a distance of 1.0. The Nei identity and the Rogers distance matrices were clustered using the UPGMA algorithm to produce dendrograms Figs 8.2. and 8.3. respectively.

These two dendrograms were very similar in general outline. There were four clusters: the single sample of *M. sp. A.*, the four collections of *M. sp. B.*, the eleven collections of *M. antarcticus*, and the single collection of *M. lenticulatus*. Note that the somewhat anomalous position of the EDEN collection, somewhat distant from the other *M. antarcticus* collections, is a sampling artefact caused by the three fish from EDEN sampled for the 10 restriction enzymes having relatively uncommon haplotypes. In the dataset based on only six restriction enzymes (but larger sample sizes), the EDEN fish are very similar to other *M. antarcticus* (see Table 8.6). The TOWN collection clusters with the *M. antarcticus* collections in both dendrograms, not with the *M. sp. B* collections as it did (although rather weakly) in the allozyme dendrogram. In fact, not one of the three *M. sp. B* composite haplotypes was found in TOWN, and the *Nco* I 'C' haplotype, found in all *M. sp. B* fish, was not present in any of the TOWN fish. This strongly suggests that the TOWN collection comprises *M. antarcticus* not *M. sp. B.*

To summarise: the mtDNA data supports the species distinction of *Mustelus sp. A*, *M. sp. B* (but note that all collections came from Western Australia - no Queensland samples were obtained), *M. antarcticus* (with the four fish from TOWN being from a separate genetic stock from remaining *M. antarcticus*), and *M. lenticulatus.*

Thus, considering both the allozyme and mitochondrial DNA data, three genetic stocks of *M. antarcticus* can be recognised: Bunbury to Eden, Newcastle and Clarence River, and Townsville. Whether these stocks are discrete or form part of a genetic cline along the east coast of Australia is unclear and further collections and analyses are required to answer this question.

8.4. Morphometric data

Five groups of gummy sharks were chosen for morphometric examination:

Group 1. *Mustelus sp. B.* (Western Australia)

Group 2. *M. antarcticus*, Esperance to Eden

Group 3. *M. antarcticus*, Newcastle and Clarence River

Group 4. *M. antarcticus*, Townsville

Group 5. *M. lenticulatus* (New Zealand)

The three groups of *M. antarcticus* were those groups that earlier allozyme and mtDNA analysis had shown to be genetically distinguishable.

Seventeen characters (total length plus 16 other characters, see Table 5.5) were measured in each fresh (i.e. frozen then thawed) shark. The 16 characters were then expressed as percentages of total length for each individual, and means and standard deviations estimated for each character in each sex in each group (Table 8.8).

The first stage in analysing these data was to see if there were any sex differences in these measurements. The largest group, group 2 (*M. antarcticus* from Esperance to Eden), was examined first. t-Tests comparing the means between the sexes for each character (expressed as a percentage of total length) were carried out (Table 8.8). After Bonferroni correction of α levels for 16 tests, only one character showed significant differences between the sexes This was SVL, snout-vent length ($P = 0.0028$, $\alpha = 0.05/16 = 0.0031$). The second largest group, group 3 (*M. antarcticus* from Newcastle and Clarence River), did not show any significant differences after Bonferroni correction for any character, and SVL was far from significant ($P = 0.301$). The final *M. antarcticus* group, group 4, comprised females only. Group 1 (*M. sp. B*) showed significant differences for two characters after Bonferroni corrections (ABH, abdomen height, $P = 0.0001$, $\alpha = 0.0031$; HDW, head width, $P = 0.0006$, $\alpha = 0.0031$). Group 5 fish (*M. lenticulatus*) showed no significant differences for any trait after Bonferroni corrections.

The general conclusion drawn from these analyses was that there was no good evidence of sex differences for any of these traits. The few possible sex differences that were observed varied from one group to another, and probably reflected chance differences in small collections. It was therefore decided that it would be reasonable to pool sexes for subsequent analyses.

Means (and standard deviations) for each character for the pooled sexes in each group are given in Table 8.8 and displayed graphically in Fig 8.4.

The next stage was to look at the three groups of *M. antarcticus* to determine whether there were any differences among them for any of the 16 morphological traits (expressed as a percentage of total length). The initial stage of this analysis took the form of an analysis of variance for each trait (Table 8.9). Bartlett *P* values (a measure of variance similarity) were all non-significant after making corrections for multiple tests, justifying the ANOVA approach. The nine characters (PG1, PSP, SVL, EYL, POR, D1P, ABH, CPH, TAW; see Table 8.9) with ANOVA *P* values of 0.003 or less all show significant differences among groups after making Bonferroni corrections (sequential or standard) for multiple tests.

Three of these nine characters were then chosen for discriminant analysis. Only three characters were chosen because one of the groups (group 4) only comprised four fish, and it is recommended that the number of characters for discriminant analysis be less than the minimum collection number. The three characters chosen were POR (preoral length), D1P (first dorsal posterior margin) and CPH (caudal peduncle height). These all had low probabilities of homogeneity (<0.001, 0.001, 0.003, respectively, see Table 8.9) and all measured different aspects of fish shape. Wilks' lambda (likelihood ratio criterion) for the multivariate hypothesis is 0.424 ($F = 8.927$, d.f. = 6,100, $P<0.001$)

Discriminant analysis allows the tabulation of actual group membership against that predicted from a multivariate analysis of the three groups (independent variables) and the three characters (dependent variables). Table 8.10 shows that 28 (90.3%) of the group 2 fish correctly reallocate to group 2 and that 17 (85%) of the group 3 fish correctly reallocate to group 3. However, of the four group 4 fish, only 1 (25%) reallocates to group 4, with one specimen relocating to group 2 and two specimens to group 3.

These results were then plotted in discriminant space (Fig. 8.5). This shows the group 3 fish to cluster in the top part of the figure and the group 2 fish to cluster in the lower part; the first canonical factor thus largely separating these two groups. There is some overlap accounting for the misclassified fish in Table 8.10. Only one group 4 fish (that with the lowest factor 2 score) separates from the group 2 and group 3 fish. The loadings of the three characters onto factor 1 were all roughly equal (if different in sign), suggesting that all characters contribute to the discrimination (POR=0.761, D1P=0.567, CPH=-0.472).

The conclusion from these analyses is that there are significant morphological differences between the southern gummy sharks and those from New South Wales. This supports the genetic data pointing to differences between these two groups of fish. The position of the Queensland fish is ambiguous, possibly reflecting the small collection size, but generally falls within the range of the New South Wales and southern gummies.

The next stage in the analysis of the morphometric data was to determine whether there were differences between the three species of gummy shark. This analysis was carried out three times, twice (analyses A and B) using the three characters used above (i.e. POR, D1P, and CPH), and once using nine characters (analysis C). Analysis A kept the three groups of *M. antarcticus* separate from one another, and analyses B and C pooled these three groups.

In analysis A, Wilks' lambda (likelihood ratio criterion) for the multivariate hypothesis was 0.193 (F = 14.288, d.f. = 12,198, $P<0.001$). Correctly predicted group membership (Table 8.11) of groups 1, 2 and 3 was high (78.6 to 87.1%). Group 4 in this analysis was subsumed by groups 2 and 3. Group 5 was 61.5% correctly reallocated, but 30.8% of specimens were incorrectly assigned to group 2. These results were then plotted in discriminant space (Fig. 8.6). This shows the group 1 fish to cluster in the top part of the figure, group 2 in the lower part, and group 3 in the centre. These three groups separate largely according to factor 1 (with roughly similar loadings for the three characters: POR=0.584, D1P=0.493, CPH=-0.329). Group 5 fish separate more according to factor 2, which is weighted towards character CPH (POR=0.263, D1P=0.597, CPH=0.886).

In analysis B, Wilks' lambda (likelihood ratio criterion) for the multivariate hypothesis was 0.381 (F = 15.910, d.f. = 6, 154, $P<0.001$). Correctly predicted membership (Table 8.12) of groups 1 and 2+3+4 was high, 71.4% and 96.4% respectively, but group 5 fish were correctly allocated only 53.8% of time, being otherwise allocated to group 2+3+4. These results were then plotted in discriminant space (Fig. 8.7). This shows the group 1 fish to cluster in the top part of the figure, with group 2+3+4 in the lower part. Thus these two groups separate largely according to factor 1 (with roughly similar loadings for the three characters: POR=0.600, D1P=0.709, CPH=-0.400). Group 5 partly overlap with group 2+3+4 fish, but otherwise as in Fig 8.6 separate according to factor 2 (again weighted towards character CPH) (POR=0.145, D1P=0.533, CPH=0.866).

The conclusion from these two analyses, A and B, is that *M. sp. B* (group 1; W in Figs. 8.6. and 8.7) is morphologically distinguishable from *M. antarcticus* (groups 2, 3, and 4; N, S, and Q in Fig. 8.6. or C in Fig. 8.7) with only very little overlap. About half the

specimens of *M. lenticulatus* (group 5; Z in Figs. 8.6 and 8.7) fall into their own character space, while the other half overlap with *M. antarcticus*.

Analysis C considered more than just the three characters considered in the preceding analyses. This analysis compared the group 1 fish (*M. sp. B*) with group 2+3+4 fish (*M. antarcticus*) and with group 5 fish (*M. lenticulatus*). Firstly, the results of ANOVAs on the 16 characters (Table 8.13) were considered. Six of these characters showed low probabilities of homogeneity of variance (Bartlett *P* values less than the Bonferroni adjusted α value of 0.05/16 = 0.003). Of the ten characters that did show homogeneous variances, four (PSP, POR, D1P, D2A) showed significant differences in distribution between the species after making Bonferroni corrections to the α level. Nine characters showing variances that did not deviate significantly from homogeneity (POR, D1P, D2A, D2B, TAH, HDW, ABW, TAW and ACS) were used in subsequent discriminant analyses, PSP being dropped as it was not scored in some fish.

Wilks' lambda (likelihood ratio criterion) for the multivariate hypothesis using was 0.321 (F = 5.959, d.f. = 18,140, $P<0.001$). Correctly predicted group membership (Table 8.14) of groups 2+3+4 and 5 was very high (96.4 to 100%), but group 1 was very poorly resolved (only 38.5% correctly assigned, little better than chance expectations). However, the results plotted in discriminant space (Fig. 8.8.) showed that the group 1 fish (*M. sp. B*) did form a loose cluster in the upper right quarter of the figure, suggesting that this species is indeed morphologically distinguishable. Groups 2+3+4 (*M. antarcticus*) and 5 (*M. lenticulatus*) separate largely according to factor 1, while group 1 separates more according to factor 2. Factor loadings (see below) emphasise the importance of two dorsal fin characters (D1P and D2A) to factor 1, and a variety of characters (especially ACS, POR and D1P) to factor 2.

Factor	ACS	POR	D1P	D2A	D2B	TAH	HDW	ABW	TAW
1	-0.265	0.308	0.547	0.431	0.273	0.221	-0.209	-0.111	0.093
2	0.468	0.656	0.563	0.195	-0.124	-0.293	0.356	0.216	-0.304

8.5. X-ray counts

The distributions of monospondylic, diplospondylic and precaudal vertebrae are given in Table 8.15a. Analyses of variances show that means of these characters vary significantly ($P<0.001$) among the nine (monospondylic, diplospondylic) or ten (precaudal vertebrae) collections. Fig. 8.9 gives histograms of the distributions of the precaudal vertebral counts.

The two collections of *M. antarcticus* from east Tasmania and from Eden did not show any significant differences in distributions of monospondylic, diplospondylic and precaudal vertebrae. These two collections form part of group 2 of the morphometric analysis above, and form part of the southern gummy shark genetic stock. The collections from Eden northwards show a gradual increase in mean precaudal vertebral number (ETAS, 82.69; EDEN, 82.00; NEWC, 85.69; CLR, 87.93; TOWN, 88.31). This heterogeneity is statistically significant ($P<0.001$). There was also evidence of a similar, although less striking, increase in both monospondylic and diplospondylic vertebral numbers (both $P<0.001$).

The Townsville sample showed an especially wide range of precaudal vertebral counts. Of the 13 specimens, the lowest had a count was 79, followed by one with a count of 80, up to two specimens with counts of 94 (Fig. 8.9). It is conceiveable that the two specimens with the lowest counts were a distinct species of gummy shark, possibly *M. sp. B*. The two specimens both counted and examined genetically were genetically classified as *M. antarcticus*: they had counts of 87 and 89. Neither of the specimens with low counts was examined genetically, as both were fixed specimens from the museum collection. We assume that all the Townsville specimens were *M. antarcticus*, although the broad spread of precaudal vertebral counts suggests that further investigation of the taxonomy of gummy sharks from this area is warranted: *M. sp. B* may co-occur here.

Mean precaudal vertebral counts for each of the four species, pooling collections within species, are given in Table 8.15b. Distributions are given in Figure 8.10. Mean precaudal vertebral count is lowest in *M. sp. B* (77.39±2.79), followed by *M. antarcticus* (85.61±4.03) and *M. sp. A* (90.50±1.05), with *M. lenticulatus* having the highest counts (94.88±1.46). These differences are highly significant ($P<0.001$).

8.6. Conclusions.

Notes on each species are followed by some general comments.

- *Mustelus species A* Last and Stevens

Examination of this non-spotted species, albeit of a single specimen, confirmed that it was genetically (for both allozymes and mitochondrial DNA) quite distinct from the other, spotted, *Mustelus* species examined. This was the only fresh specimen of this species obtained during the course of the study, and morphological measurements from this single fish could not be used in the multivariate comparison of species. However, it and some stored specimens were examined for vertebral counts: they had a mean precaudal vertebral count of 90.5, higher than *M. sp. B* (77.39) or *M. antarcticus* (85.6) but lower than *M. lenticulatus* (94.9). All specimens examined came from the North West Shelf; we were unable to confirm its possible existence off Townsville, Queensland (Last and Stevens 1994). We are confident that this is a valid species.

- *Mustelus species B* Last and Stevens

The white-spotted gummy shark, *M. sp. B*, was genetically quite similar to the similarly spotted common gummy shark, *M. antarcticus*, although there were large allele frequency differences at several allozyme loci and unique mtDNA fragment patterns for two of the ten restriction enzymes tested. These genetic differences, although limited, serve to confirm its species status. Morphologically, multivariate analysis showed *M. sp. B* to be generally distinct from *M. antarcticus* and *M. lenticulatus*, although some specimens did fall into the *M. antarcticus* space. Precaudal vertebral counts had a mean of 77.4, a little less than the southern collections (east Tasmania and Eden) of *M. antarcticus*, 82.5, and substantially less than the more northern collections (Newcastle and Townsville), 87.3. Three small collections, from the North West Shelf, Shark Bay, and Perth, were confirmed as *M. sp. B*. These findings thus confirm the previously

unconfirmed (Last and Stevens 1994) existence of the species in Shark Bay, and extend its range much further south - its previously sole confirmed locality was off Dampier (Last and Stevens 1994).

- *Mustelus antarcticus* Günther

Collections of gummy sharks from Bunbury around the south coast to Clarence River (NSW) were confirmed genetically (by both allozyme and mtDNA analysis) as *M. antarcticus*. A more northerly collection, from Townsville, showed allozyme similarities to both *M. antarcticus* and to *M. sp. B*. Mitochondrial DNA analysis of these four fish showed two of them to have common *M. antarcticus* haplotypes while the other two had closely related (although otherwise unique) haplotypes: none had *M. sp. B* haplotypes. The genetic data overall supported the identification of the Townsville collection as a discrete genetic stock of *M. antarcticus* rather than *M. sp. B*. This conclusion was also supported by the multivariate analysis of morphological characters, which showed the Townsville collection to fall within the range of other *M. antarcticus* collections and away from the *M. sp. B* collections. Finally, precaudal vertebrae counts of the Townsville collection were very similar (mean of 88.3) to the Clarence River collection of *M. antarcticus* (87.9) and considerably higher than the mean for *M. sp. B* (77.4).

Last and Stevens (1994) write that this temperate species "possibly also ventures into southern Queensland". Our data indicate that it is found at least as far north as Townsville, mid-Queensland. It is possible that the Queensland records ascribed by Last and Stevens to *M. sp. B* are really those of *M. antarcticus*: a conclusion supported by their *M. sp. B* from Queensland having precaudal vertebral counts of 88-95 (similar to those recorded here for Townsville *M. antarcticus*), considerably higher than their *M. sp. B* counts from Western Australia at 76-80 (which were similar to our *M. sp. B* counts). It may be that *M. sp. B* is restricted to the west coast of Australia. However, two fish from the Townsville area had low precaudal vertebral counts (79 and 80): conceivably these were *M. sp. B* : they could not be examined genetically.

Finally, the increase in precaudal vertebral counts from temperate to tropical regions (means: east Tasmania, 82.7; Eden, 82.0; Newcastle, 85.7; Clarence River, 87.9; Townsville, 88.3) is interesting and somewhat unexpected. In a review of factors controlling meristic variation, Lindsey (1988) writes that "the number of vertebrae tends to be higher in fish from more polar or cooler waters than in their relatives from tropical or warm water. The phenomenon, termed Jordan's rule, hold both in Northern and Southern Hemispheres....It occurs in many different fish groups and at taxonomic levels....the operational factor is evidently temperature". This also holds within species, fish hatched at warmer temperatures generally have reduced vertebral counts. Hulme (1995), for example, estimated that for North Sea herring (*Clupea harengus*), a 1°C rise in temperature led to a fall of 0.11 in vertebral count. However, Lindsey (1988) also says that there are also many cases where higher temperature produces more parts, and it appears that the gummy shark falls into this category.

- *Mustelus lenticulatus* Phillipps

The New Zealand gummy, *M. lenticulatus*, was genetically distinct from the Australian gummies at several allozyme loci, and two of the ten restriction enzymes used gave nearly unique mtDNA restriction fragment patterns. These differences support the

identification of this taxon as a distinct species. Morphometrically, multivariate analysis further confirmed its distinctiveness from *M. antarcticus* (and from *M. sp. B*). It also had a higher precaudal vertebral count (94.9) than any of the other *Mustelus* species examined, the next highest being *M. sp. A* with a mean of 90.5.

- Comments on the relationships between *M. antarcticus* and *M. lenticulatus*

Previously *Mustelus antarcticus* and *M. lenticulatus* have been separated on the basis of a difference in precaudal vertebral counts (Heemstra 1973) but as vertebral numbers can vary throughout the range of shark species (Springer and Garrick 1964; Compagno 1984b), further taxonomic work was thought necessary. The existence of nearly diagnostic mtDNA haplotype differences and a nearly fixed allelic difference at one allozyme locus (*LDH-2**) with significant differences at eight other loci supports the current taxonomy, although the small allozyme genetic distance (0.089) and low mtDNA sequence divergences (0.5-0.1%) show that the two species are very closely related. The allozyme genetic distance (0.089) is low, although not as low as the value of 0.045 between the morphologically similar sharks *Carcharhinus limbatus* and *C. tilstoni* (Lavery and Shaklee 1991). However, the two *Mustelus* species are unusually closely related, for about 90% of congeneric fish species have genetic identities less than 0.8 (Thorpe 1983). The mtDNA analysis yielded similar results: mtDNA sequence divergence of 0.5-0.1% is very small, with most congeneric marine fish species having divergences in the range 3.7 - 13.0 % (Billington and Hebert 1991).

Under neutral theory, H is expected to be positively related to effective population size, N_e (Kimura and Crow 1964), although there are strong grounds for believing that equilibrium for H is rarely, if ever, attained (Nei and Graur 1984; Crow 1986). The reduced H estimates in *M. lenticulatus* ($H = 0.019$) might mirror a smaller N_e than that of *M. antarcticus* (mean $H = 0.096$) or it may be that *M. lenticulatus* populations were originally derived from *M. antarcticus*, with a consequent population size bottleneck leading to the loss of variation.

If *M. lenticulatus* populations were derived from *M. antarcticus*, *Apa* I digestion of mtDNA shows a restriction site loss in *M. lenticulatus*, and *Ava* I a site gain. In fact, the *M. lenticulatus* mtDNA haplotype was found, albeit rarely (a frequency of around 0.03), in Tasmanian and Western Australian *M. antarcticus*. These Australian sharks with *M. lenticulatus* haplotypes have allozyme alleles associated with *M. antarcticus* and so are not immigrant sharks from New Zealand. Assuming that this haplotype arose on only one occasion, then either this haplotype represents the mtDNA lineage which later founded the New Zealand *M. lenticulatus*, or these sharks represent the offspring of one or more New Zealand *M. lenticulatus* females which emigrated to Australian waters some generations ago and whose mtDNA has subsequently introgressed into the Australian *Mustelus* population. The flow of mtDNA across a species boundary has been reported on several occasions (e.g. Ferris *et al.* 1983; Tegelström 1987; Billington *et al.* 1988). Although no tagged New Zealand *Mustelus* have been caught in Australian waters or vice versa (J.D. Stevens personal communication), females of both *M. lenticulatus* and *M. antarcticus* are known to move substantial distances (Francis 1988; Walker 1983). However, gummy sharks are, unlike school sharks, relatively benthic, and are unlikely to move across oceans but rather to remain in coastal regions. It is thus unlikely that gummy sharks do interchange between Australia and New Zealand.

Whatever the explanation for the differences in variability between the two species, the genetic data (and precaudal vertebral counts) are consistent with their being two species, albeit closely related. While the *M. antarcticus* - *M. lenticulatus* genetic distances are small, *LDH-2** and mtDNA variants were nearly diagnostic. No such loci were found in comparisons of conspecific populations of teleosts across the Tasman Sea which separates New Zealand from Australia. In these studies, of blue grenadier/hoki (*Macruronus novaezelandiae*) (Milton and Shaklee 1987; Smith *et al.* 1996), morwong/tarakihi (*Nemadactylus macropterus*) (Elliott and Ward 1994; Grewe *et al.* 1994), orange roughy (*Hoplostethus atlanticus*) (Elliott and Ward 1992; Smith *et al.* 1996), and three species of oreos (*Pseudocyttus maculatus*; *Allocyttus niger* and *A. verrucosus*) (Ward *et al.* 1996), no fixed or nearly fixed trans-Tasman allozyme or mtDNA differences were observed.

Table 8.1. Gummy sharks. Population allele frequencies. *n* = number of fish, *n.s.* = not scored

Locus	Allele	*M. sp. A.*	*Mustelus sp. B*				*Mustelus antarcticus*													*M. len.*
		NWS	NWS	SBAY	KALB	PERTH	BUN	AUG	DISLE	ESP	IBAY	SA	STAS	WTAS	ETAS	VIC	EDEN	NEWC	CLR	TOWN	NZ
*AAT-1**	115	-	0.08	-	0.25	-	-	-	-	-	-	-	0.03	0.04	-	-	-	-	-	-	-
	100	1.00	0.92	1.00	0.75	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.97	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	90	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-
	n	*1*	*6*	*3*	*2*	*3*	*22*	*7*	*9*	*15*	*20*	*95*	*32*	*14*	*66*	*99*	*11*	*22*	*45*	*4*	*95*
*AAT-2**	130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06
	115	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.06
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.88
	n	*1*	*6*	*3*	*2*	*3*	*22*	*7*	*9*	*15*	*20*	*95*	*32*	*14*	*66*	*99*	*11*	*22*	*45*	*4*	*96*
*ADA-1**	105	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	1.00	1.00	1.00	-	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*n.s.*	*13*	*1*	*n.s.*	*15*	*n.s.*	*47*	*26*	*11*	*13*	*23*	*11*	*21*	*45*	*4*	*23*
*AK**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	1.00	1.00	0.88	0.99
	75	-	-	-	-	-	-	-	-	-	-	0.01	-	-	0.01	-	-	-	-	0.13	0.01
	n	*1*	*6*	*3*	*2*	*3*	*22*	*15*	*9*	*15*	*20*	*94*	*20*	*3*	*65*	*99*	*11*	*22*	*45*	*4*	*47*
*ACP**	-100	1.00	0.50	0.50	0.50	1.00	0.67	0.74	0.57	0.63	0.67	0.57	0.55	0.53	0.56	0.58	0.73	0.62	0.67	0.63	1.00
	-200	-	0.50	0.50	0.50	-	0.33	0.26	0.43	0.38	0.33	0.43	0.45	0.47	0.44	0.42	0.27	0.38	0.33	0.38	-
	n	*1*	*6*	*3*	*2*	*2*	*24*	*21*	*14*	*16*	*32*	*76*	*31*	*15*	*65*	*95*	*13*	*21*	*45*	*4*	*45*
*AO**	100	1.00	1.00	1.00	1.00	-	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*n.s.*	*13*	*1*	*n.s.*	*15*	*n.s.*	*47*	*25*	*15*	*13*	*23*	*11*	*22*	*45*	*4*	*23*
*CK-A**	400	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	90	-	-	-	-	-	0.08	0.24	0.23	0.16	0.11	0.11	0.25	0.14	0.10	0.13	0.32	0.16	0.26	0.38	-
	-100	-	1.00	1.00	1.00	1.00	0.92	0.76	0.77	0.84	0.89	0.89	0.75	0.86	0.90	0.88	0.68	0.84	0.74	0.63	1.00
	n	*1*	*6*	*3*	*2*	*3*	*24*	*21*	*15*	*16*	*32*	*95*	*32*	*11*	*66*	*100*	*11*	*22*	*45*	*4*	*69*
*ESTD-2**	150	1.00	0.92	-	1.00	-	0.21	0.28	0.28	0.25	0.17	0.21	0.15	0.20	0.24	0.24	0.25	0.34	0.30	0.50	1.00
	100	-	0.08	-	-	-	0.79	0.72	0.72	0.75	0.83	0.79	0.85	0.80	0.76	0.76	0.75	0.66	0.57	0.50	-
	n	*1*	*6*	*n.s.*	*1*	*n.s.*	*7*	*9*	*16*	*2*	*33*	*7*	*31*	*15*	*75*	*97*	*14*	*16*	*45*	*3*	*45*
*FH**	100	1.00	1.00	-	1.00	-	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	1.00
	85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.17	-
	n	*1*	*6*	*n.s.*	*2*	*n.s.*	*5*	*2*	*n.s.*	*1*	*n.s.*	*70*	*28*	*15*	*46*	*47*	*11*	*8*	*4*	*3*	*23*
*G6PDH**	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	0.01	-	-
	120	1.00	0.42	-	-	-	0.33	0.36	0.31	0.38	0.35	0.42	0.27	0.43	0.39	0.37	0.40	0.50	0.38	0.50	1.00
	100	-	0.58	-	-	-	0.67	0.57	0.69	0.62	0.59	0.58	0.73	0.57	0.61	0.61	0.60	0.46	0.59	0.50	-
	80	-	-	-	-	-	-	0.07	-	-	0.06	-	-	-	-	0.02	-	0.04	0.02	-	-
	n	*1*	*6*	*n.s.*	*n.s.*	*n.s.*	*18*	*7*	*16*	*13*	*33*	*55*	*26*	*15*	*53*	*94*	*10*	*12*	*41*	*4*	*45*
*DIA**	100	1.00	1.00	1.00	1.00	-	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*n.s.*	*9*	*10*	*n.s.*	*15*	*n.s.*	*30*	*20*	*14*	*6*	*23*	*11*	*18*	*45*	*4*	*23*

*HK**	100	1.00	1.00	1.00	1.00	-	-	-	-	1.00	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*16*	*n.s.*	*n.s.*	*20*	*4*	*7*	*23*	*11*	*4*	*45*	*4*	*23*
*IDHP-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*3*	*22*	*11*	*n.s.*	*1*	*n.s.*	*30*	*31*	*14*	*18*	*23*	*11*	*22*	*45*	*4*	*69*
*IDHP-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00
	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-
	n	*1*	*6*	*3*	*2*	*3*	*22*	*11*	*n.s.*	*15*	*n.s.*	*30*	*31*	*14*	*18*	*23*	*11*	*22*	*45*	*4*	*69*
*LDH-1**	120	-	-	-	-	-	-	-	-	-	0.02	-	-	-	-	0.01	-	-	0.01	-	-
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00
	n	*1*	*6*	*3*	*2*	*3*	*25*	*21*	*16*	*16*	*33*	*94*	*31*	*14*	*70*	*100*	*11*	*22*	*45*	*4*	*47*
*LDH-2**	-60	1.00	1.00	1.00	1.00	1.00	-	-	0.06	-	0.05	0.04	0.06	0.07	0.03	0.04	0.14	0.27	0.46	0.75	1.00
	-100	-	-	-	-	-	1.00	1.00	0.94	1.00	0.95	0.95	0.94	0.93	0.97	0.96	0.86	0.73	0.54	0.25	-
	-120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-
	n	*1*	*6*	*3*	*2*	*3*	*25*	*21*	*16*	*16*	*33*	*95*	*31*	*14*	*70*	*100*	*11*	*22*	*45*	*4*	*47*
*ME-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84
	85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.16
	n	*1*	*6*	*3*	*2*	*3*	*22*	*12*	*9*	*15*	*13*	*30*	*25*	*14*	*7*	*23*	*11*	*18*	*45*	*4*	*94*
*ME-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*3*	*22*	*12*	*9*	*15*	*13*	*30*	*20*	*14*	*7*	*23*	*11*	*18*	*45*	*4*	*92*
*MDH-1**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*3*	*1*	*12*	*9*	*16*	*13*	*30*	*20*	*14*	*7*	*23*	*11*	*18*	*45*	*4*	*15*
*MDH-2**	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*3*	*1*	*12*	*9*	*16*	*13*	*30*	*20*	*14*	*7*	*23*	*11*	*18*	*45*	*4*	*15*
*MPI**	130	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	120	-	-	-	-	-	-	-	-	-	-	-	-	0.03	0.01	0.01	-	-	-	-	-
	110	-	-	-	-	-	-	0.14	0.31	0.50	0.23	-	0.21	0.17	0.20	0.28	0.19	0.15	0.23	0.38	-
	100	-	1.00	-	-	-	-	0.86	0.66	0.50	0.68	-	0.73	0.77	0.75	0.65	0.73	0.83	0.67	0.63	0.99
	90	-	-	-	-	-	-	-	-	-	0.05	-	0.03	0.03	0.03	0.04	0.04	0.03	0.08	-	-
	80	-	-	-	-	-	-	-	0.03	-	0.05	-	0.03	-	0.01	0.02	0.04	-	0.03	-	0.01
	n	*1*	*6*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*7*	*16*	*1*	*33*	*n.s.*	*31*	*15*	*61*	*97*	*13*	*20*	*39*	*4*	*45*
*ODH**	100	-	1.00	1.00	1.00	-	1.00	-	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	85	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	n	*1*	*6*	*3*	*2*	*n.s.*	*1*	*n.s.*	*n.s.*	*15*	*n.s.*	*30*	*20*	*14*	*7*	*23*	*11*	*13*	*45*	*3*	*23*
*PEP-1**	125	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	120	-	1.00	1.00	1.00	1.00	0.09	0.20	-	0.13	0.03	0.07	0.08	-	0.09	0.08	0.09	0.12	0.27	0.75	-
	100	-	-	-	-	-	0.86	0.78	0.91	0.84	0.95	0.89	0.85	1.00	0.87	0.89	0.91	0.88	0.72	0.25	1.00
	75	-	-	-	-	-	0.05	0.03	0.09	0.03	0.02	0.04	0.06	-	0.04	0.04	-	-	0.01	-	-
	n	*1*	*6*	*3*	*2*	*3*	*22*	*20*	*16*	*16*	*33*	*95*	*31*	*15*	*70*	*96*	*11*	*21*	*45*	*4*	*45*

*PROT-1**	100	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		n.s.	*6*	*3*	*2*	*3*	*24*	*21*	*16*	*16*	*33*	*95*	*24*	*14*	*70*	*100*	*11*	*18*	*45*	*4*	*47*
*PROT-2**	100	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*n.s.*	*6*	*3*	*2*	*3*	*24*	*21*	*16*	*16*	*33*	*95*	*26*	*14*	*70*	*100*	*11*	*18*	*41*	*4*	*47*
*sSOD**	100	-	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	95	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	n	*1*	*6*	*3*	*2*	*n.s.*	*22*	*11*	*16*	*16*	*33*	*69*	*31*	*14*	*36*	*23*	*11*	*22*	*45*	*4*	*45*
*TPI-1**	100	1.00	1.00	1.00	1.00	-	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*n.s.*	*22*	*11*	*n.s.*	*15*	*n.s.*	*52*	*26*	*15*	*7*	*47*	*11*	*22*	*45*	*4*	*22*
*TPI-2**	100	1.00	1.00	1.00	1.00	-	1.00	1.00	-	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	n	*1*	*6*	*3*	*2*	*n.s.*	*22*	*11*	*n.s.*	*15*	*n.s.*	*52*	*26*	*15*	*7*	*25*	*11*	*22*	*45*	*4*	*22*

Table 8.2. Gummy sharks. Summary of allozyme variation. All samples except *M. sp. A* examined for 28 loci; *M.sp.A* for 26 loci. n = mean sample size per locus, H = average heterozygosity per locus, % P = percent loci polymorphic (0.95 criterion).

	M.sp. A	*M.sp. B*	*M. antarcticus*									*M.len.*
	NWS	NWS	ESP	STAS	WTAS	ETAS	VIC	EDEN	NEWC	CLR	TOWN	NZ
n	1	6	13.36	26.68	13.36	38.32	59.68	11.21	18.79	43.04	3.89	46.46
H	0.000	0.046	0.084	0.085	0.076	0.079	0.083	0.090	0.094	0.120	0.129	0.019
% P (0.95)	0	14.29	21.43	25.00	21.43	21.43	21.43	25.00	25.00	25.00	32.14	7.14

Table 8.3. Gummy sharks. Allele frequencies for polymorphic loci in the combined Bunbury to Eden sample, and the Newcastle, Clarence River and Townsville samples.

Locus	Allele	BUN-EDEN	NEWC	CLR	TOWN	$\chi 2$	*P*
*ACP**	-100	0.600	0.619	0.667	0.625		
	-200	0.400	0.381	0.333	0.375		
	n	*402*	*21*	*45*	*4*	1.569	0.656
*CK-A**	90	0.139	0.159	0.256	0.375		
	-100	0.861	0.841	0.744	0.625		
	n	*423*	*22*	*45*	*4*	11.563	0.009
*EST-D2**	150	0.222	0.344	0.346	0.500		
	100	0.778	0.656	0.654	0.500		
	n	*306*	*16*	*39*	*3*	9.865	0.025
*G6PDH**	140	0.001	-	0.012	-		
	120	0.369	0.500	0.378	0.500		
	100	0.618	0.458	0.585	0.500		
	80	0.012	0.042	0.024	0		
	n	*340*	*12*	*41*	*4*	8.354	0.359
*LDH-2**	-60	0.038	0.273	0.456	0.750		
	-100	0.961	0.727	0.544	0.250		
	-120	0.001	-	-	-		
	n	*431*	*22*	*45*	*4*	231.742	<0.001
*MPI**	120	0.005	-	-	-		
	110	0.239	0.150	0.231	0.375		
	100	0.701	0.825	0.667	0.625		
	90	0.035	0.025	0.077	-		
	80	0.020	-	0.026	-		
	n	*274*	*20*	*39*	*4*	8.497	0.615
*PEP-1**	120	0.076	0.119	0.267	0.750		
	100	0.886	0.881	0.722	0.250		
	75	0.038	0	0.011	0		
	n	*425*	*21*	*45*	*4*	72.541	<0.001

Table 8.4. Gummy sharks. Pairwise comparisons of allele frequencies for *CK-A**, *LDH-2**, and *PEP-1** for four samples (Bunbury to Eden combined, Newcastle, Clarence River, and Townsville). Probability figures given above diagonal and chi-square values below diagonal.

	Locus	BUN-EDEN	NEWC	CLR	TOWN
BUN-EDEN	*CK-A**		0.834	0.007	0.083
	*LDH-2**	-	<0.001	<0.001	0.008
	*PEP-1**		0.250	<0.001	<0.001
NEWC	*CK-A**	0.133		0.276	0.325
	*LDH-2**	48.777	-	0.055	0.013
	*PEP-1**	2.507		0.082	0.001
CLR	*CK-A**	8.566	1.583		0.691
	*LDH-2**	197.61	4.132	-	0.143
	*PEP-1**	35.231	4.241		0.092
TOWN	*CK-A**	3.615	2.032	0.538	
	*LDH-2**	93.771	6.813	2.552	-
	*PEP-1**	47.390	15.590	8.092	

Table 8.5. Gummy sharks. Allozyme genetic distance measures. Above diagonal: Nei's (1978) unbiased genetic identity. Below diagonal: Nei's (1978) unbiased genetic distance.

	M.sp A NWS	*M.sp. B* NWS	*M. antarcticus* ESP	STAS	WTAS	ETAS	VIC	EDEN	NEWC	CLR	TOWN	*M.len.* NZ
M. sp. A	-	0.742	0.699	0.686	0.692	0.692	0.695	0.710	0.721	0.737	0.760	0.766
M. sp. B	0.299	-	0.903	0.903	0.903	0.909	0.906	0.912	0.933	0.951	0.988	0.937
ESP	0.359	0.102	-	1.000	1.000	1.000	1.000	1.000	0.996	0.991	0.968	0.906
STAS	0.376	0.102	0.000	-	1.000	0.999	0.999	1.000	0.995	0.989	0.964	0.899
WTAS	0.369	0.102	0.000	0.000	-	1.000	1.000	1.000	0.999	0.989	0.960	0.916
ETAS	0.368	0.095	0.000	0.001	0.000	-	1.000	0.999	0.998	0.989	0.963	0.912
VIC	0.364	0.098	0.000	0.001	0.000	0.000	-	0.999	0.997	0.990	0.964	0.911
EDEN	0.342	0.092	0.000	0.000	0.000	0.001	0.001	-	1.000	0.996	0.973	0.924
NEWC	0.328	0.070	0.004	0.005	0.001	0.002	0.003	0.000	-	0.998	0.980	0.944
CLR	0.305	0.050	0.009	0.011	0.011	0.011	0.010	0.005	0.002	-	0.996	0.946
TOWN	0.275	0.012	0.033	0.037	0.041	0.038	0.037	0.027	0.020	0.004	-	0.947
M.len.	0.266	0.065	0.099	0.106	0.088	0.092	0.093	0.079	0.058	0.055	0.055	-

Table 8.6. Gummy sharks - all species (*Mustelus sp. A., Mustelus sp. B., M. antarcticus, M. lenticulatus)*. Mitochondrial DNA haplotype frequencies (a) Composite haplotypes from the 10 restriction enzymes (in order) *Apa* I, *Ava* I, *Bcl* I, *Eco* RI, *Hind* III, *Nco* I, *Pst* I, *Pvu* II, *Sac* II and *Xba* I, (b) Composite haplotypes from the 6 restriction enzymes (in order) *Apa* I, *Ava* I, *Bcl* I, *Eco* RI, *Nco* I and *Xba* I which detect polymorphic cut sites.

	M. sp. A.	*Mustelus sp. B*				*Mustelus antarcticus*												*M.?*	*M. len.*
Haplotype	NWS	NWS	SBAY	KALB	PERTH	BUN	AUG	DISLE	ESP	IBAY	SA	STAS	WTAS	ETAS	VIC	EDEN	NEWC	CLR	TOWN	NZ
(a) Composite haplotype from 10 restriction enzymes																				
EBCEIDDDAC	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BBBBEABBAB	-	-	-	-	-	0.64	1.00	-	0.64	-	0.77	0.67	0.53	-	0.75	-	0.64	0.38	0.25	-
BBBBEABBAD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-
CBBBEABBAB	-	-	-	-	-	0.29	-	-	0.36	-	0.23	0.22	0.40	-	0.25	0.67	0.29	0.62	0.25	-
CBBDEABBAB	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	0.33	-	-	-	-
CBBCEABBAB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-
BBCBEABBAB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-
ECBBEABBAB	-	-	-	-	-	-	-	-	-	-	-	0.11	-	-	-	-	-	-	-	1.00
BBDBEABBAB	-	-	-	-	-	0.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BBCBECBBAB	-	0.67	0.67	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BBCBECBBAD	-	0.17	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BBCBECBBAE	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
n	*1*	*6*	*3*	*2*	*3*	*14*	*2*	*0*	*14*	*0*	*13*	*18*	*15*	*0*	*20*	*3*	*14*	*45*	*4*	*94*
(b) Composite haplotype from 6 restriction enzymes																				
EBCEDC	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BBBBAB	-	-	-	-	-	0.52	0.81	0.63	0.69	0.67	0.66	0.77	0.53	0.55	0.62	0.69	0.67	0.38	0.25	-
BBBBAD	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.01	-	0.05	-	-	-
CBBBAB	-	-	-	-	-	0.43	0.14	0.31	0.31	0.33	0.32	0.16	0.40	0.40	0.36	0.23	0.29	0.62	0.25	-
CBBDAB	-	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	0.08	-	-	-	-
CBBCAB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-
BBCBAB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.25	-
CCBBAB	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-
ECBBAB	-	-	-	-	-	-	0.05	-	-	-	-	0.06	-	0.01	-	-	-	-	-	1.00
BBDBAB	-	-	-	-	-	0.04	-	0.06	-	-	0.01	-	-	0.02	0.01	-	-	-	-	-
BBCBCB	-	0.67	0.67	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BBCBCD	-	0.17	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BBCBCE	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
n	*1*	*6*	*3*	*2*	*3*	*23*	*21*	*16*	*16*	*33*	*90*	*31*	*15*	*85*	*100*	*13*	*21*	*45*	*4*	*94*

Table 8.7. Gummy sharks. Mitochondrial DNA genetic distance measures based on the 10 restriction enzyme haplotypes.

Above diagonal: Nei's (1978) unbiased genetic identity.
Below diagonal: Rogers (1972) genetic distance

Species	Sample	*M. sp.A* NWS	*M.sp.B* NWS	SBAY	KALB	PERTH	BUN	AUG	ESP	*M. antarcticus* SA	STAS	WTAS	VIC	EDEN	NEWC	CLR	TOWN	*M. len.* NZ
M. sp. A	NWS	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
M. sp. B	NWS	0.866	-	1.000	0.989	0.989	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	SBAY	0.882	0.167	-	0.976	0.976	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	KALB	1.000	0.289	0.333	-	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	PERTH	1.000	0.289	0.333	0.000	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
M. ant.	BUN	0.866	0.707	0.726	0.866	0.866	-	0.926	1.000	1.000	1.000	1.000	1.000	0.402	1.000	0.837	0.885	0.000
	AUG	1.000	0.866	0.882	1.000	1.000	0.327	-	0.888	0.969	0.950	0.813	0.956	0.000	0.926	0.522	0.661	0.000
	ESP	0.878	0.721	0.740	0.878	0.878	0.071	0.357	-	1.000	1.000	1.000	1.000	0.482	1.000	0.887	0.914	0.000
	SA	0.907	0.757	0.775	0.907	0.907	0.110	0.231	0.126	-	1.000	0.965	1.000	0.284	1.000	0.755	0.833	0.000
	STAS	0.868	0.709	0.729	0.868	0.868	0.105	0.294	0.125	0.107	-	0.966	1.000	0.309	1.000	0.768	0.838	0.158
	WTAS	0.851	0.689	0.709	0.851	0.851	0.132	0.437	0.096	0.211	0.182	-	0.972	0.645	1.000	0.948	0.942	0.000
	VIC	0.901	0.750	0.768	0.901	0.901	0.094	0.250	0.107	0.019	0.100	0.192	-	0.311	1.000	0.772	0.843	0.000
	EDEN	0.882	0.726	0.745	0.882	0.882	0.581	0.882	0.557	0.668	0.619	0.462	0.651	-	0.402	0.838	0.645	0.000
	NEWC	0.866	0.707	0.726	0.866	0.866	0.071	0.327	0.071	0.110	0.105	0.132	0.094	0.581	-	0.837	0.885	0.000
	CLR	0.875	0.718	0.737	0.875	0.875	0.307	0.622	0.265	0.391	0.358	0.198	0.372	0.358	0.307	-	0.913	0.000
	TOWN	0.791	0.612	0.635	0.791	0.791	0.378	0.612	0.381	0.444	0.395	0.341	0.433	0.486	0.378	0.374	-	0.000
M. len.	NZ	1.000	0.866	0.882	1.000	1.000	0.866	1.000	0.878	0.907	0.801	0.851	0.901	0.882	0.866	0.875	0.791	-

Table 8.8. Gummy sharks. Means (expressed as a percentage of total length) and standard deviations of the 16 characters in each of the five groups, together with *P* values comparing males and females for the three largest groups

Character		Total			Males			Females		
	n	mean	s.d	n	mean	s.d	n	mean	s.d	*P*
Group 1										
PG1	12	17.18%	0.901%	9	17.41%	0.857%	3	16.49%	0.762%	0.1317
PSP	14	11.65%	0.757%	9	11.79%	0.804%	5	11.39%	0.666%	0.3645
SVL	14	48.15%	3.603%	9	46.92%	3.516%	5	50.38%	2.800%	0.0840
ACS	14	7.57%	0.546%	9	7.73%	0.436%	5	7.29%	0.656%	0.1505
EYL	14	3.40%	0.527%	9	3.46%	0.583%	5	3.29%	0.450%	0.5880
POR	14	6.67%	0.491%	9	6.86%	0.435%	5	6.33%	0.422%	0.0463
D1B	14	13.38%	1.690%	9	13.10%	1.799%	5	13.87%	1.527%	0.4355
DIP	14	11.43%	0.906%	9	11.04%	0.841%	5	12.11%	0.581%	0.0274
D2A	14	11.42%	0.585%	9	11.26%	0.598%	5	11.69%	0.504%	0.2062
D2B	14	10.23%	0.592%	9	10.27%	0.493%	5	10.15%	0.802%	0.7313
ABH	14	10.25%	1.402%	9	9.39%	0.517%	5	11.80%	1.096%	0.0001
TAH	14	6.43%	0.790%	9	6.05%	0.525%	5	7.12%	0.739%	0.0077
CPH	14	2.35%	0.370%	9	2.42%	0.322%	5	2.23%	0.456%	0.3801
HDW	14	10.83%	0.788%	9	10.38%	0.420%	5	11.64%	0.619%	0.0006
ABW	14	9.35%	1.143%	9	8.77%	0.629%	5	10.40%	1.154%	0.0047
TAW	13	7.14%	0.626%	8	6.92%	0.576%	5	7.50%	0.583%	0.1419
Group 2										
PG1	27	16.32%	0.779%	12	16.12%	0.514%	13	16.56%	0.978%	0.1829
PSP	27	10.39%	0.709%	13	10.20%	0.383%	12	10.58%	0.972%	0.2039
SVL	32	47.15%	1.514%	15	46.38%	1.315%	15	47.98%	1.359%	0.0028
ACS	32	7.22%	0.572%	15	7.22%	0.613%	15	7.21%	0.590%	0.9543
EYL	32	2.78%	0.434%	15	2.63%	0.186%	15	2.90%	0.577%	0.0976
POR	32	5.71%	0.450%	15	5.55%	0.200%	15	5.85%	0.597%	0.0762
D1B	32	12.16%	1.033%	15	12.12%	0.725%	15	12.40%	1.190%	0.4367
DIP	31	9.68%	0.748%	15	9.34%	0.566%	15	9.99%	0.799%	0.0159
D2A	32	10.62%	0.958%	15	10.23%	1.071%	15	11.04%	0.723%	0.0205
D2B	32	10.20%	0.585%	15	10.28%	0.614%	15	10.15%	0.597%	0.5470
ABH	32	10.07%	0.681%	15	9.98%	0.423%	15	10.22%	0.870%	0.3410
TAH	32	6.82%	0.760%	15	6.78%	0.341%	15	6.83%	1.061%	0.8545
CPH	32	2.68%	0.204%	15	2.69%	0.139%	15	2.66%	0.245%	0.6035
HDW	32	10.81%	0.723%	15	10.60%	0.672%	15	10.99%	0.785%	0.1559
ABW	32	9.31%	1.009%	15	9.01%	0.529%	15	9.65%	1.308%	0.0892
TAW	32	7.59%	0.645%	15	7.65%	0.546%	15	7.55%	0.766%	0.6894
Group 3										
PG1	13	18.42%	1.371%	9	18.89%	1.306%	4	16.94%	0.922%	0.0616
PSP	16	11.15%	0.677%	12	11.14%	0.644%	4	11.58%	0.878%	0.9470
SVL	20	46.00%	1.050%	15	45.86%	1.167%	5	46.41%	0.402%	0.3012
ACS	20	7.74%	0.750%	15	7.75%	0.704%	5	8.09%	0.965%	0.9225
EYL	20	3.19%	0.453%	15	3.17%	0.451%	5	3.46%	0.502%	0.6471
POR	20	6.42%	0.485%	15	6.43%	0.505%	5	6.58%	0.474%	0.8759
D1B	20	11.60%	0.921%	15	11.59%	1.047%	5	11.43%	0.440%	0.9579
DIP	20	10.48%	0.730%	15	10.27%	0.690%	5	11.01%	0.440%	0.0200
D2A	20	10.76%	1.031%	15	10.63%	1.066%	5	11.06%	0.913%	0.3595
D2B	20	10.05%	0.802%	15	9.94%	0.855%	5	10.42%	0.540%	0.2744
ABH	19	9.24%	0.634%	14	9.06%	0.442%	5	9.99%	0.853%	0.0734

Table 8.8. cont.

Character	n	Total mean	s.d	n	Males mean	s.d	n	Females mean	s.d	*P*
Group 3										
TAH	20	6.24%	0.572%	15	6.14%	0.551%	5	6.41%	0.591%	0.1936
CPH	20	2.53%	0.145%	15	2.52%	0.138%	5	2.60%	0.177%	0.5355
HDW	20	10.62%	0.638%	15	10.63%	0.625%	5	10.35%	0.749%	0.8611
ABW	20	9.16%	0.833%	15	9.08%	0.849%	5	9.20%	0.825%	0.4780
TAW	20	6.90%	0.724%	15	6.87%	0.690%	5	6.75%	0.901%	0.7783
Group 4										
PG1	4	15.65%	0.773%	0	-	-	4	15.65%	0.773%	-
PSP	4	10.11%	0.313%	0	-	-	4	10.11%	0.313%	-
SVL	4	48.14%	0.779%	0	-	-	4	48.14%	0.779%	-
ACS	4	7.29%	0.425%	0	-	-	4	7.29%	0.425%	-
EYL	4	2.49%	0.106%	0	-	-	4	2.49%	0.106%	-
POR	4	5.71%	0.337%	0	-	-	4	5.71%	0.337%	-
D1B	4	12.07%	1.140%	0	-	-	4	12.07%	1.140%	-
DIP	4	10.44%	0.533%	0	-	-	4	10.44%	0.533%	-
D2A	4	11.14%	0.314%	0	-	-	4	11.14%	0.314%	-
D2B	4	9.84%	0.311%	0	-	-	4	9.84%	0.311%	-
ABH	4	9.65%	0.399%	0	-	-	4	9.65%	0.399%	-
TAH	4	6.63%	0.147%	0	-	-	4	6.63%	0.147%	-
CPH	4	2.42%	0.157%	0	-	-	4	2.42%	0.157%	-
HDW	4	10.93%	0.253%	0	-	-	4	10.93%	0.253%	-
ABW	4	9.25%	1.729%	0	-	-	4	9.25%	1.729%	-
TAW	4	7.50%	0.294%	0	-	-	4	7.50%	0.294%	-
Group 5										
PG1	14	16.74%	0.454%	11	16.26%	0.374%	3	16.97%	0.177%	0.0095
PSP	14	11.41%	0.368%	11	10.85%	0.292%	3	11.42%	0.223%	0.0107
SVL	14	46.38%	0.984%	11	46.15%	1.120%	3	46.26%	0.100%	0.8774
ACS	14	6.28%	0.629%	11	6.90%	0.655%	3	6.38%	0.263%	0.2152
EYL	14	2.77%	1.258%	10	2.54%	0.128%	3	2.63%	0.096%	0.3024
POR	14	6.63%	0.239%	11	6.27%	0.195%	3	6.55%	0.243%	0.0701
D1B	13	12.44%	0.551%	10	12.94%	0.593%	3	13.30%	0.236%	0.3391
DIP	13	12.21%	1.267%	10	10.96%	1.089%	3	12.82%	0.463%	0.0180
D2A	14	12.15%	0.608%	11	11.87%	0.683%	3	11.84%	0.219%	0.9430
D2B	14	10.47%	0.577%	11	10.64%	0.653%	3	10.70%	0.130%	0.8752
ABH	14	10.83%	0.347%	11	10.14%	0.371%	3	10.29%	0.215%	0.5284
TAH	14	6.85%	0.320%	11	7.05%	0.294%	3	7.35%	0.291%	0.1604
CPH	14	3.06%	0.133%	11	2.84%	0.101%	3	3.05%	0.081%	0.0066
HDW	14	10.44%	0.527%	11	10.06%	0.436%	3	10.76%	0.430%	0.0354
ABW	14	9.27%	0.551%	11	8.75%	0.524%	3	9.04%	0.577%	0.4490
TAW	14	7.40%	0.297%	11	7.55%	0.274%	3	7.68%	0.344%	0.5274

Table 8.9. Gummy sharks. Analysis of variance for each of 16 morphometric traits in the three groups 2, 3 and 4 of *M. antarcticus*.

Character	df (1)	df (2)	*F*	*P*	Bartlett *P*
PG1	2	41	23.168	0.000	0.058
PSP	2	44	7.555	0.002	0.327
SVL	2	53	6.699	0.003	0.140
ACS	2	53	4.206	0.020	0.302
EYL	2	53	7.836	0.001	0.069
POR	2	53	15.349	<0.001	0.751
D1B	2	53	1.993	0.146	0.821
D1P	2	52	7.984	0.001	0.783
D2A	2	53	0.566	0.571	0.138
D2B	2	53	0.714	0.494	0.113
ABH	2	52	9.760	<0.001	0.565
TAH	2	53	4.603	0.014	0.022
CPH	2	53	6.614	0.003	0.285
HDW	2	53	0.648	0.527	0.174
ABW	2	53	0.143	0.867	0.168
TAW	2	53	6.809	0.002	0.266

df (1) = number of degrees of freedom between groups,
df (2) = number of degrees of freedom within groups,
F = F ration (MS between / MS within)
P = probability of no difference in the trait.
Bartlett *P* is the probability of homogeneity of group variances.

Table 8.10. Gummy sharks. Actual group membership against predicted for the 3 groups 2, 3 and 4 for 3 variables (POR, D1P, and CPH).

	Predicted			
Group observed	2	3	4	Total
2	28	3	0	31
3	3	17	0	20
4	1	2	1	4
Total	32	22	1	55

Table 8.11. Gummy sharks. Actual group membership against predicted for the 5 groups 1, 2, 3, 4, and 5 for 3 variables (POR, D1P, and CPH).

	Predicted					
Group observed	1	2	3	4	5	Total
1	11	0	2	0	1	14
2	1	27	3	0	0	31
3	1	3	16	0	0	20
4	0	1	3	0	0	4
5	0	4	1	0	8	13
Total	13	35	25	0	9	82

Table 8.12. Gummy sharks. Actual group membership against predicted for the 3 groups 1, 2+3+4, and 5 for 3 variables (POR, D1P, and CPH).

	Predicted			
Group observed	1	2+3+4	5	Total
1	10	2	2	14
2+3+4	2	53	0	55
5	0	6	7	13
Total	12	61	9	82

Table 8.13. Gummy sharks. Analysis of variance for each of 16 traits in the three species *M. sp. B*, *M. antarcticus* and *M. lenticulatus* (groups 1, 2+3+4, and 5 respectively).

Character	df (1)	df (2)	*F*	*P*	Bartlett *P*
PG1	2	67	1.387	0.257	<0.001
PSP	2	72	11.374	<0.001	0.017
SVL	2	81	4.023	0.022	<0.001
ACS	2	81	6.310	0.003	0.666
EYL	2	81	3.142	0.049	<0.001
POR	2	81	11.831	<0.001	0.005
D1B	2	80	12.054	<0.001	0.001
D1P	2	79	20.647	<0.001	0.116
D2A	2	81	12.114	<0.001	0.037
D2B	2	81	3.935	0.023	0.792
ABH	2	80	2.712	0.073	<0.001
TAH	2	81	4.062	0.021	0.006
CPH	2	81	19.146	<0.001	<0.001
HDW	2	81	4.117	0.020	0.369
ABW	2	81	1.376	0.258	0.037
TAW	2	80	1.529	0.223	0.004

df (1) = number of degrees of freedom between groups,
df (2) = number of degrees of freedom within groups,
F = F ration (MS between / MS within)
P = probability of no difference for the trait.
Bartlett *P* is the probability of homogeneity of group variances.

Table 8.14. Gummy sharks. Actual group membership against predicted for the 3 groups 1, 2+3+4, and 5 for 9 variables (POR, D1P, D2A, D2B, TAH, HDW, ABW, TAW and ACS).

	Predicted			
Group observed	1	2+3+4	5	Total
1	5	5	3	13
2+3+4	1	53	1	55
5	0	0	13	13
Total	6	58	17	81

Table 8.15. Gummy shark. Vertebral counts. MS=monospondylic, DS=diplospondylic, PCV=precaudal vertebrae

a. By species and by sample

		M. sp.A NWS	*M.sp.B* NWS	SBAY	PERTH	*M. ant.* ETAS	EDEN	NEWC	CLR	TOWN	*M.len.* NZ
MS	n	1	6	4	3	16	6	13	14	10	0
	range	-	33 - 34	33 - 34	34 - 35	35 - 37	35 - 38	33 - 38	35 - 39	35 - 39	-
	X±SD	35	33.33±0.52	33.50±0.58	34.33±0.58	35.81±0.66	36.50±1.22	36.08±1.38	37.50±1.09	37.30±1.34	-
DS	n	1	6	4	3	16	6	13	14	10	0
	range	-	43 - 49	35 - 45	42 - 44	42 - 51	37 - 50	44 - 53	48 - 54	42 - 57	-
	X±SD	54	44.50±2.35	41.75±4.72	43.00±1.00	46.88±2.73	43.83±4.88	49.62±2.81	50.43±1.70	49.80±4.18	-
PCV	n	6	10	5	3	16	6	13	14	13	8
	range	89 - 92	76 - 82	69 - 79	77 - 78	78 - 86	75 - 87	80 - 89	85 - 91	79 - 94	93 - 97
	X±SD	90.50±1.05	78.20±2.25	75.80±4.09	77.33±0.58	82.69±2.63	82.00±4.65	85.69±2.75	87.93±1.54	88.31±4.77	94.88±1.46

b. By species (samples within species pooled)

		M. sp. A	*M.sp. B*	*M.antarcticus*	*M.lenticulatus*
MS	n	1	13	59	0
	range	-	33 - 35	33 - 39	-
	X±SD	35	33.62±0.65	36.59±1.30	-
DS	n	1	13	59	0
	range	-	35 - 49	37 - 57	-
	X±SD	54	43.31±3.09	48.51±3.66	-
PCV	n	6	18	62	8
	range	89 - 92	69 - 82	75 - 94	93 - 97
	X±SD	90.50±1.05	77.39±2.79	85.61±4.03	94.88±1.46

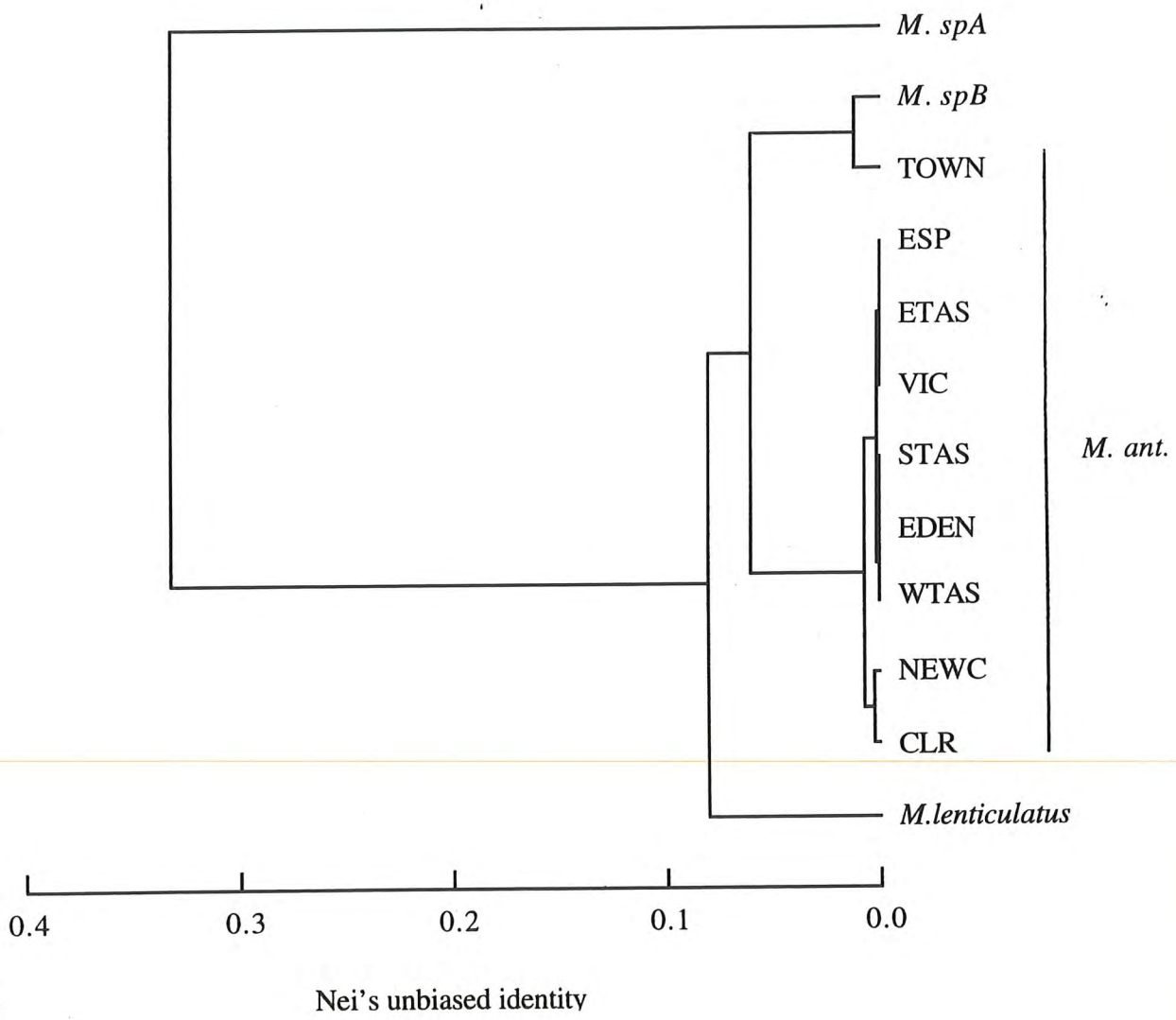

Figure 8.1. Gummy sharks. Allozyme genetic relationship (26 loci) among samples using Nei's (1978) unbiased genetic identity and UPGMA clustering.

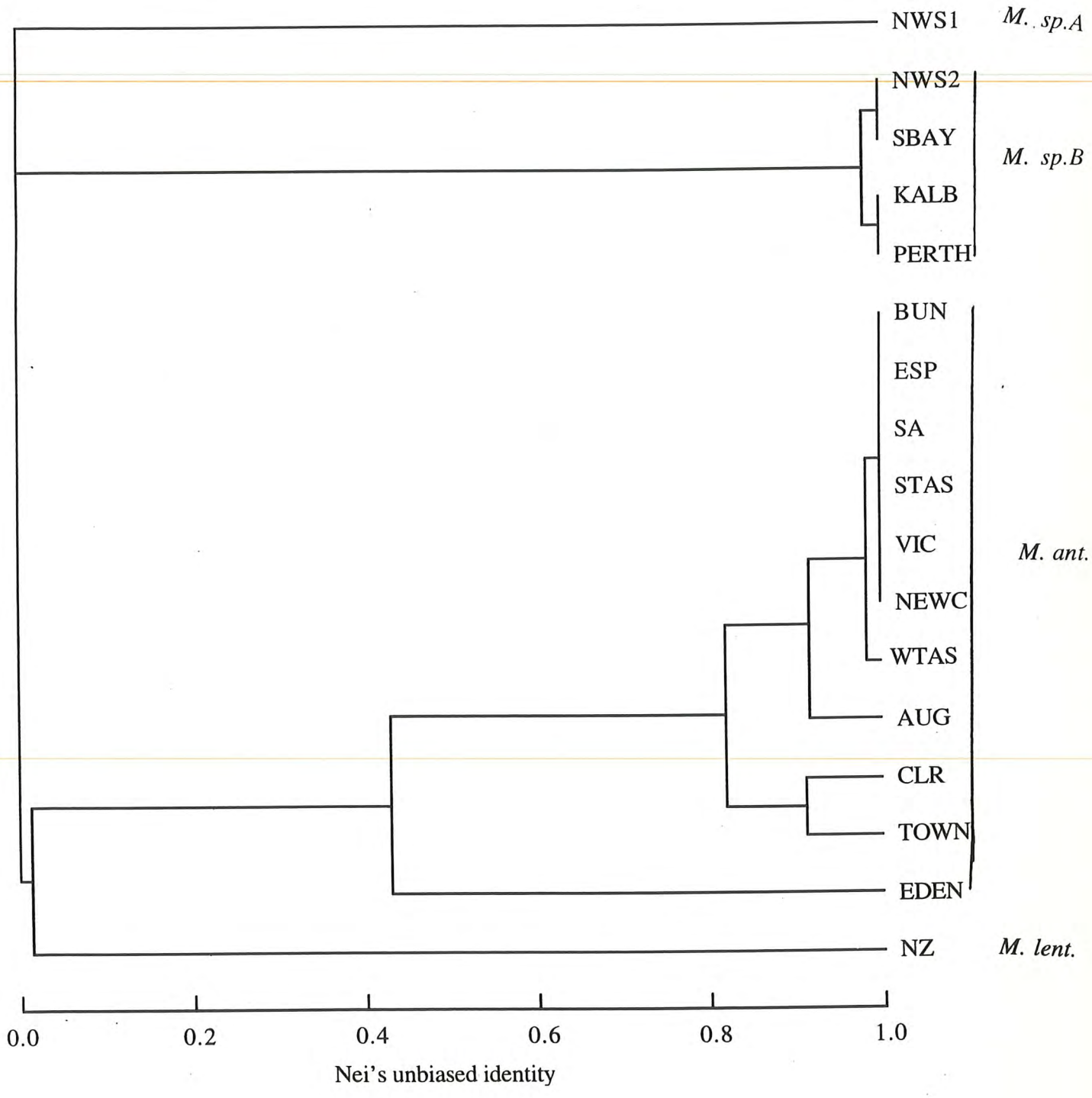

Figure 8.2. Gummy sharks. Mitochondrial DNA genetic relationship among samples using Nei's (1978) unbiased genetic identity and UPGMA clustering.

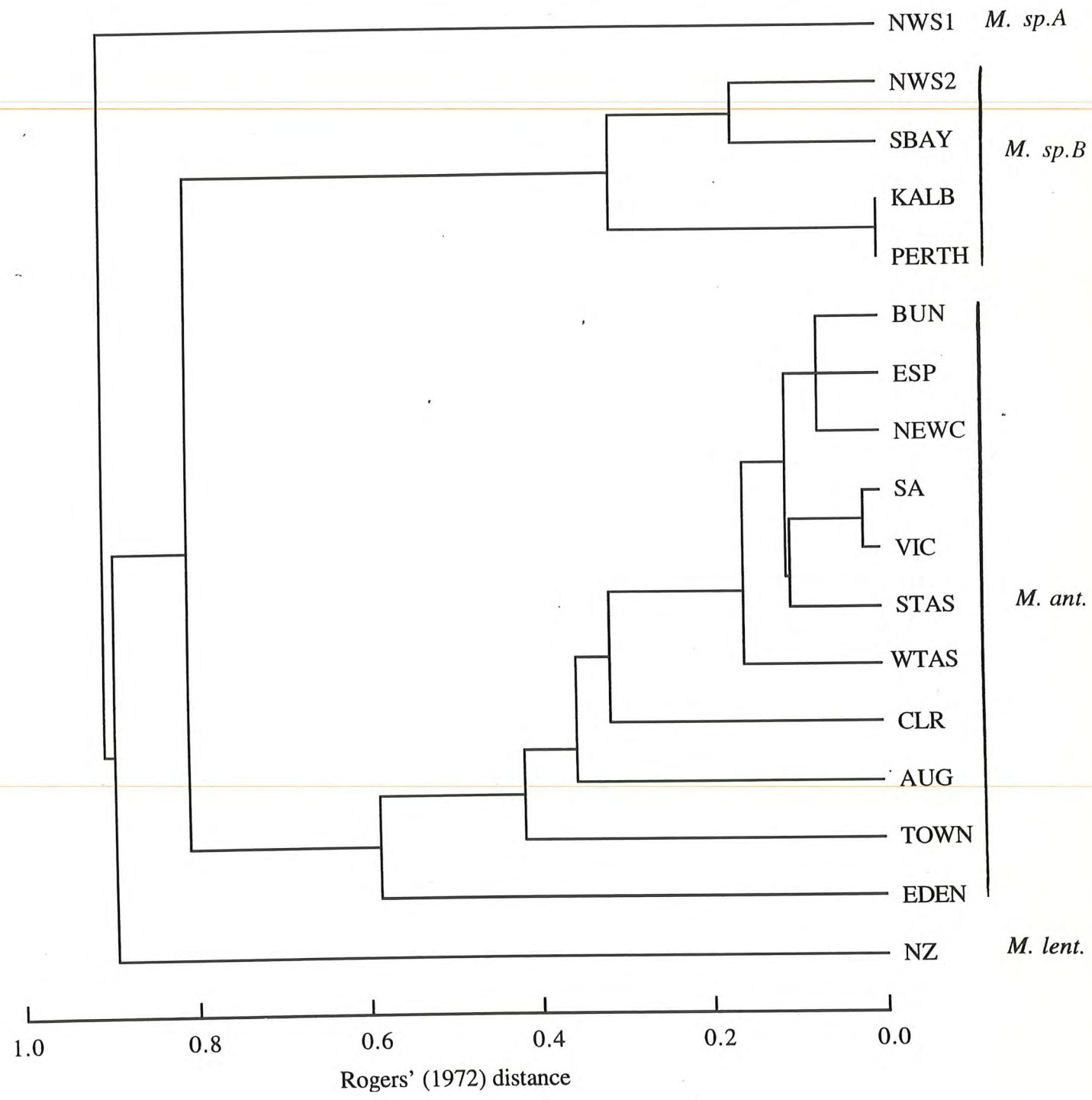

Figure 8.3. Gummy sharks. Mitochondrial DNA genetic relationship among samples using Rogers' (1972) distance and UPGMA clustering

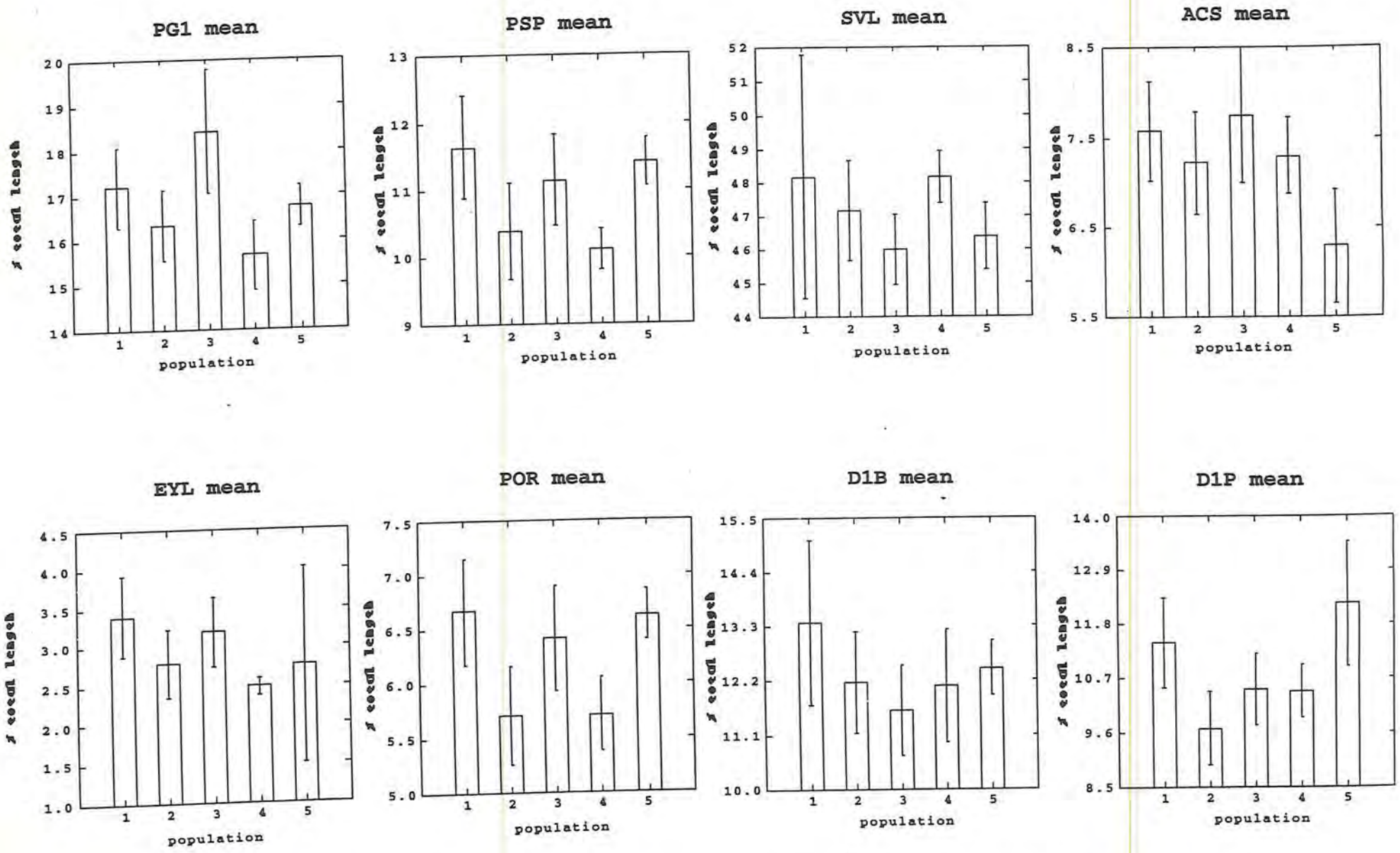

Fig. 8.4. Means and standard deviations of the 16 characters expressed as a percentage of total length for each of the five groups of gummy sharks.

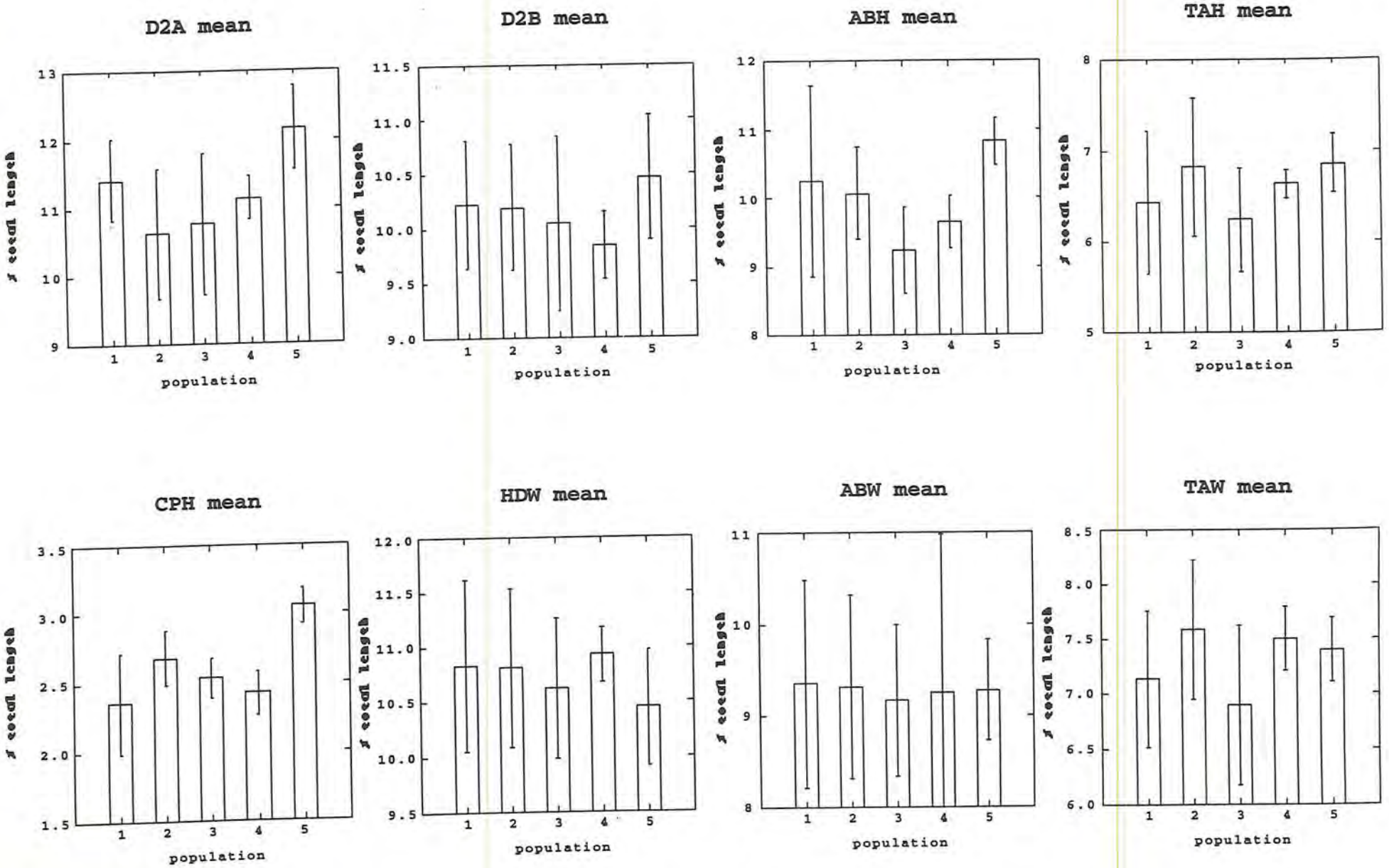

Fig. 8.4. continued.

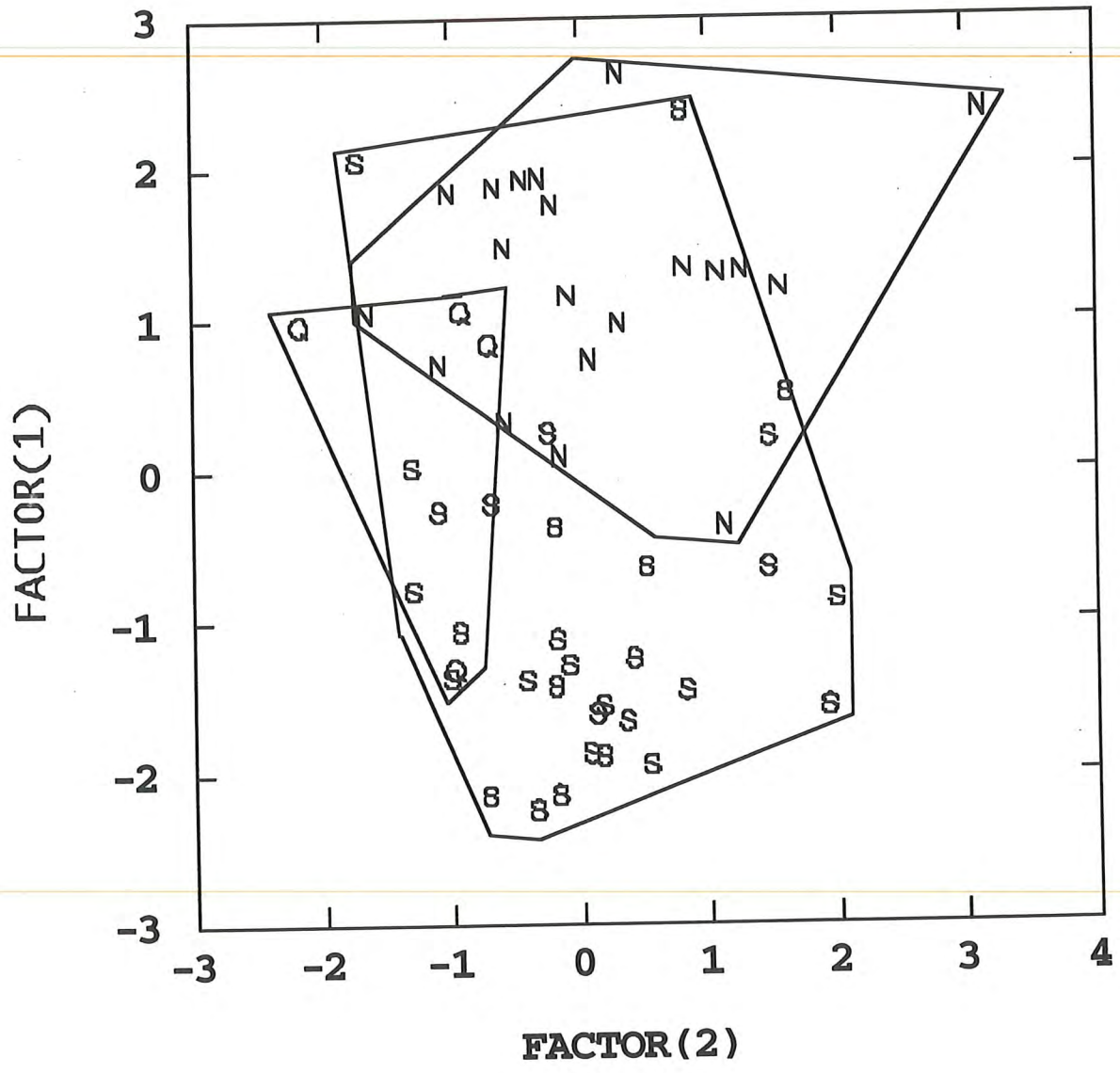

Fig. 8.5. Canonical variate plot. Three characters (POR, D1P, CPH). *M. antarcticus*. Groups 2 (=S, for south), 3 (=N, for north) and 4 (=Q, for Queensland).

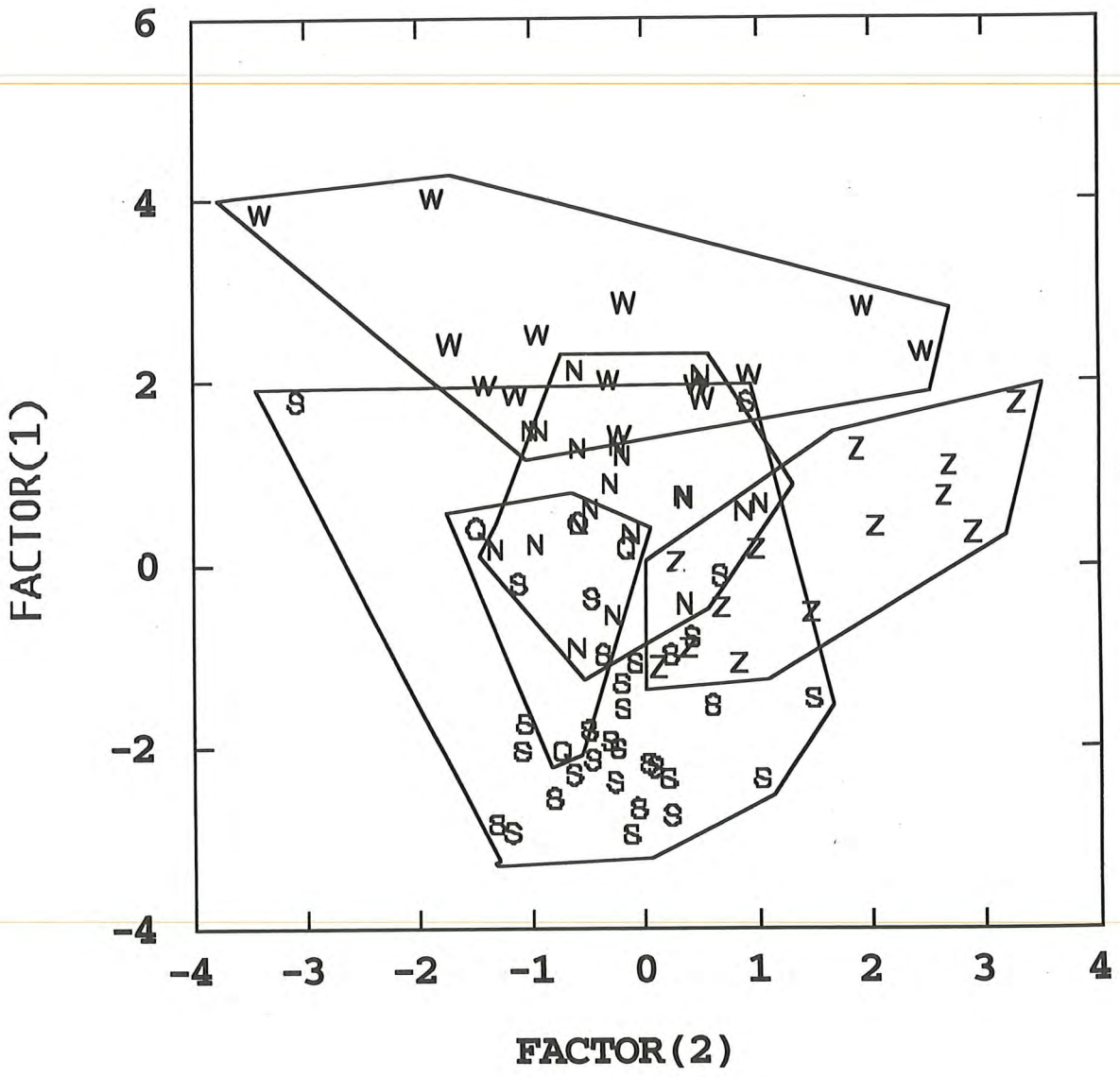

Fig. 8.6. Canonical variate plot. Three characters (POR, D1P, CPH). (Groups 1 (=W, for west, *M. sp. B*), 2 (=S, for south), 3 (=N, for north), 4 (=Q, for Queensland), and 5 (=Z, for New Zealand, *M. lenticulatus*)

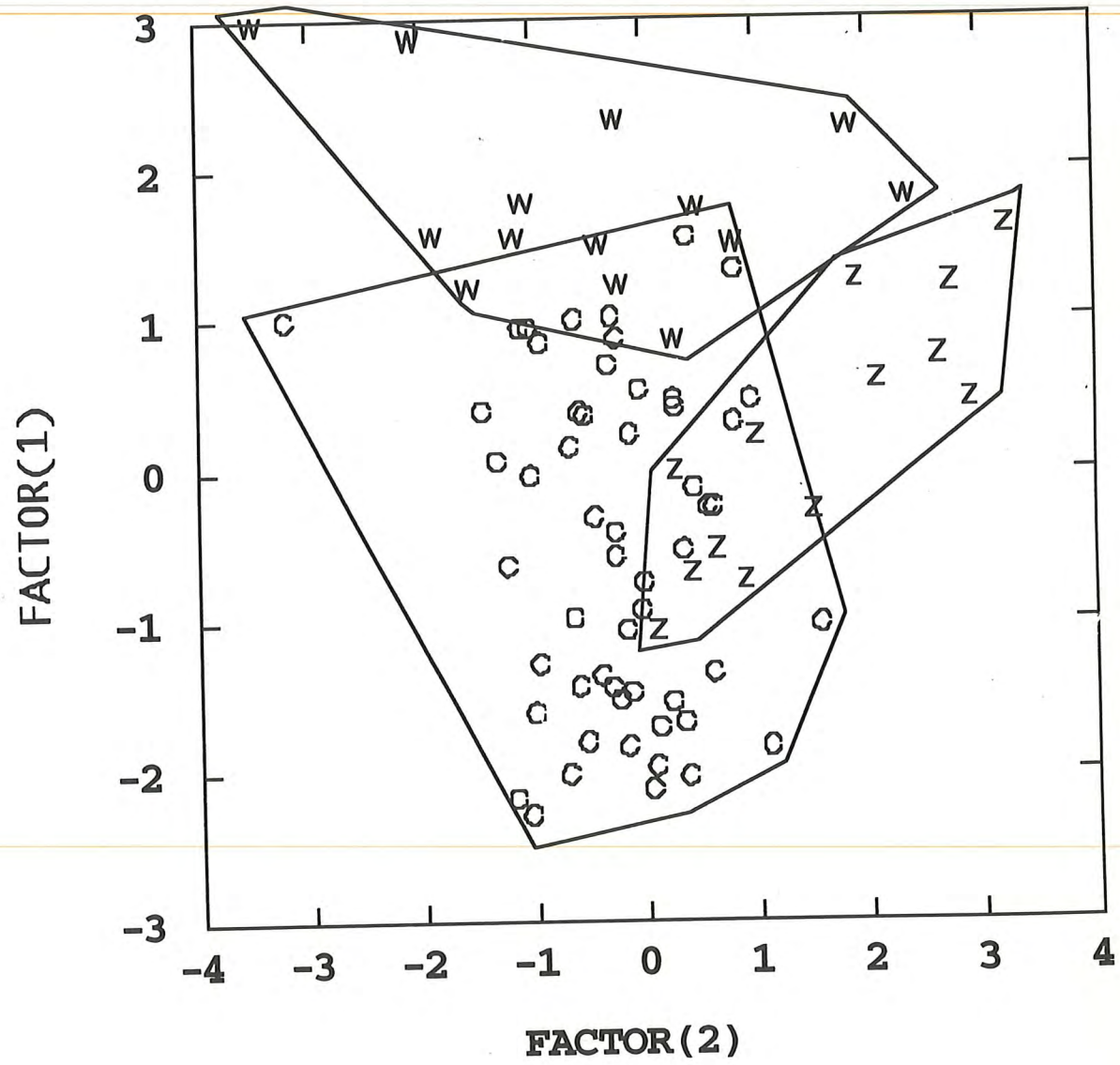

Fig 8.7. Canonical variate plot. Three characters (POR, D1P, CPH). Groups 1 (=W, for west, *M. sp. B*), 2+3+4 (=C, for combined *M. antarcticus*), and 5 (=Z, for New Zealand, *M. lenticulatus*)

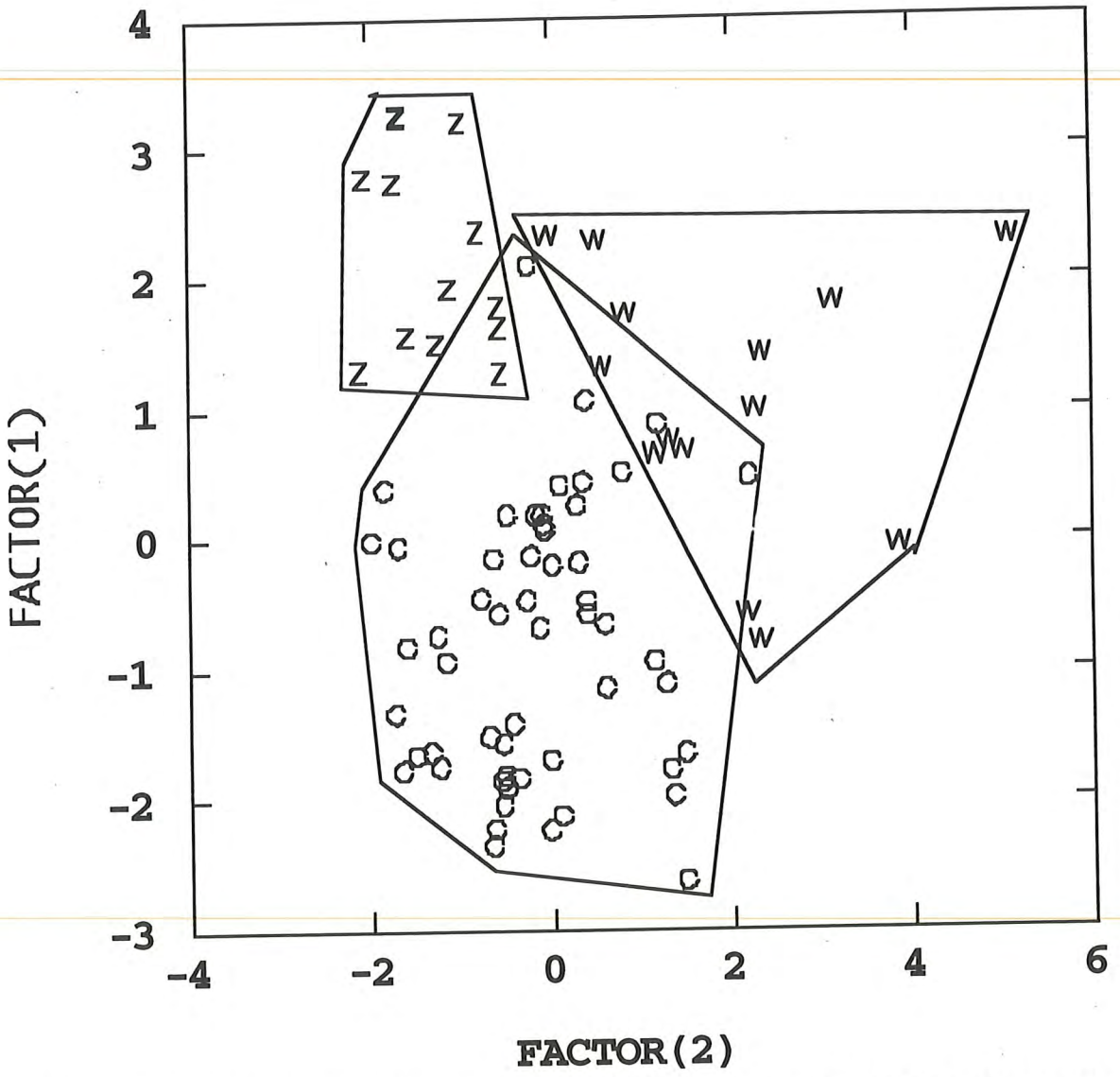

Fig. 8.8. Canonical variate plot. Nine characters (POR, D1P, D2A, D2B, TAH, HDW, ABW, TAW and ACS). Groups 1 (=W, for west, *M. sp. B*), 2+3+4 (=C, for combined, *M. antarcticus*), and 5 (=Z, for New Zealand, *M. lenticulatus*)

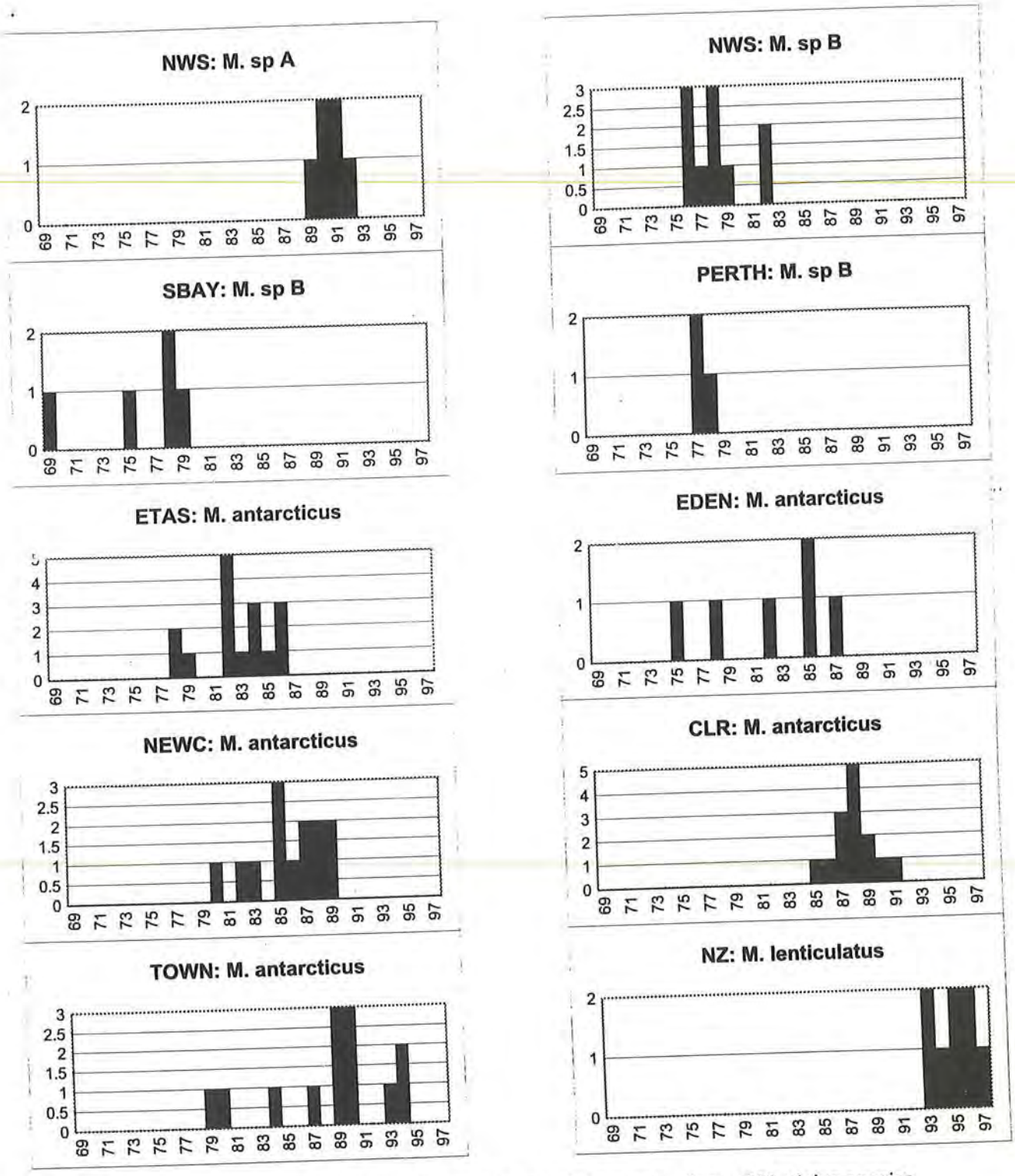

Fig. 8.9. Distributions of precaudal vertebral number in individual collections of Mustelus species

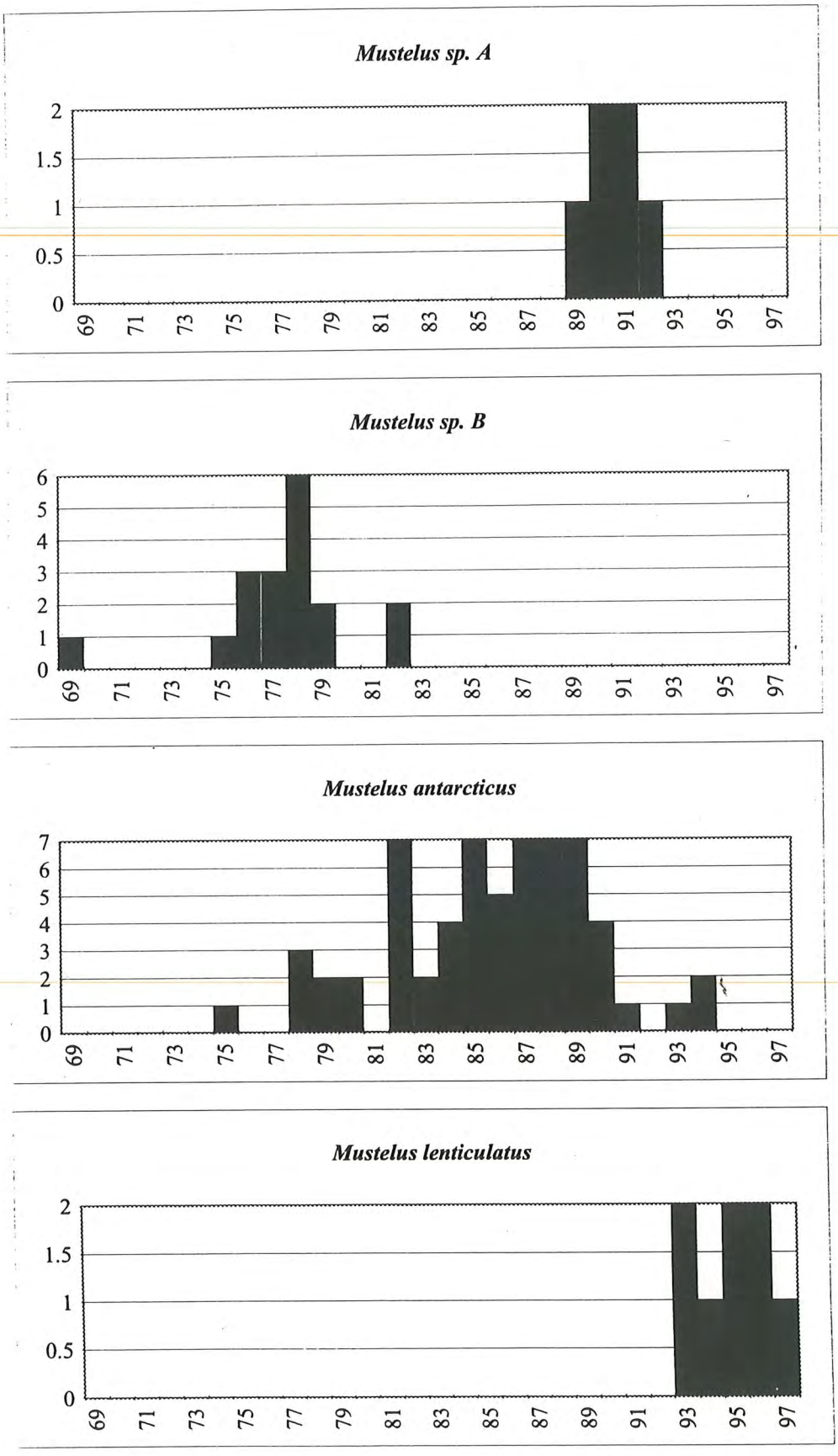

Fig. 8.10. Distributions of precaudal vertebral number in different Mustelus species (multiple collections within species pooled)

9. RESULTS: GENERAL DISCUSSION

9.1 Taxonomy

The taxonomic issues tackled in the course of this project were generally resolved satisfactorily, either by genetic analysis alone (as in the case of the school shark) or by a combination of genetic and morphological approaches (gummy sharks).

Collections of school sharks taken from England, Argentina, South Africa, Australia, and New Zealand were all very similar genetically (although some spatial differences were noted), supporting the current classification of this taxon as a single globally-distributed species, *Galeorhinus galeus* (Compagno 1984a,b).

Genetic and morphometric analysis confirmed the existence of four species of gummy sharks in the Australasian region: the non-spotted *Mustelus species A* (a single specimen examined from the North West Shelf), and three spotted, species; *M. species B* (from Western Australia, the North West Shelf south to Perth), *M. antarcticus* (from Bunbury in Western Australia around the southern Australian coast and as far north as Townsville), and *M. lenticulatus* (from New Zealand). Descriptions of these species may be found in Last and Stevens (1994). While there was no genetic evidence for species other than *M. antarcticus* in the Townsville region, only four specimens from that area could be examined genetically, and the possibility remains that, given the wide vertebral count range there(79-94, $n = 13$), other species such as *M. sp. B* co-occur in that region.

9.2. Levels of genetic variation

Levels of allozyme variation in these five triakid species are summarised in Table 9.1a. Average heterozygosity per locus is a better indicator of levels of genetic variation than percentage loci polymorphic, as it is less sample-size dependent. Heterozygosity was high in *M. antarcticus* (0.101) but much lower in the four other species (0.025, 0.019, 0.008, 0.000). Heterozygosity levels we recorded for *M. antarcticus* and *M. lenticulatus* were higher than those of past studies of these two species (see Table 9.1b); possible explanations for this have been presented earlier (sections 7.1 and 8.2 respectively).

Sharks generally have been found to have low allozyme variability. Table 9 gives data for 16 shark species (17 if the two *Squatina argentina* morphs are attributed to different species), and the overall grand mean heterozygosity figures are 0.031 (16 species) and 0.039 (17 species). These mean values are little more than half those typically found in fish, with Smith and Fujio (1982) and Ward *et al.* (1994) estimating values of 0.055 and 0.064 for 106 and 57 species of marine teleosts respectively.

Smith and Fujio (1982) hypothesised that, in marine teleosts, there is high genetic variability in habitat specialists and low variability in habitat generalists. Thus Smith (1986) attributed the relative lack of variation in sharks to their being habitat generalists. The high variation observed in *Squatina argentina*, (angel shark), (although from only 14 loci see Table 9.1 and Solé-Cava *et al.* 1983), was attributed by Smith to its morphological specialisation and consequent classification as a habitat

specialist. However, we find levels of allozyme variation in *M. antarcticus* to be high, not only for sharks but indeed for fish generally. The various *Mustelus* species would appear to be ecologically and morphologically very similar, and it does not seem appropriate to use Smith and Fujio's hypothesis to classify *M. antarcticus* as a habitat specialist and the other species of *Mustelus* as habitat generalists.

It is arguable that levels of allozyme variation reflect not ecological adaptation but demographic variation in neutral traits: if so, then the generally reduced *H* estimates in sharks compared with teleosts might reflect their position as top predators. Under neutral theory, *H* is expected to be positively related to effective population size, N_e (Kimura and Crow 1964), although there are strong grounds for believing that equilibrium for *H* is rarely, if ever, attained (Nei and Graur 1984; Crow 1986). Be that as it may, sharks are likely, on the whole, to have a relatively small N_e and by neutral theory are thus expected to have low *H*. *Mustelus antarcticus* may be an exception to this general pattern, since it forms the basis of an important fishery, and may well have higher N_e values and thus higher *H* values than most sharks. The reduced *H* estimates in the other *Mustelus* might reflect smaller N_e values, although on this basis it might be expected that the school shark, *Galeorhinus galeus*, which also forms significant fisheries in Australia and other parts of the world, might be expected to have a higher *H* than the very low value (0.008) observed.

The generally low degree of genetic variation in sharks appears to be found not only in allozymes but also in mtDNA. While mtDNA data are less abundant than allozyme data, Martin (1993) presents preliminary data for some carcharhinid sharks, suggesting that levels of within-species mtDNA sequence diversity are "remarkably low". This is supported by Heist *et al.*'s (1995) finding of low diversity in *Carcharhinus plumbeus* (sequence divergence of 0.036%, haplotype diversity of 0.161). Our data are not quite so clear-cut, with mtDNA diversities ranging from zero (in *M. lenticulatus*) to the moderately high sequence divergence of 0.25% and haplotype diversity of 0.483 in *M. antarcticus*. Interestingly, with the exception of the Atlantic populations, the school sharks showed appreciable levels of mtDNA variation, contrasting with the low levels of allozyme variability observed.

9.3. Stock structures

Collections of school sharks from south-east Australian waters (South Australia, Victoria, Tasmania) appeared to constitute a single genetic stock, with some differences from New Zealand fish which appeared to constitute a second stock. The differences between these two stocks were, however, small, and these data therefore did not rule out the possibility of some very limited genetic exchange between fish from these two areas. South African fish showed more pronounced differences, indicating a very low likelihood of genetic exchange between South African and Australian stocks. Unfortunately, no school sharks could be collected from Western Australia, so the genetic status of fish from that area remains unknown.

How do these results accord with tagging results? Early tagging studies indicated 'a complete mixing of sharks from all areas of south eastern Australia' and that there was 'no evidence from tagging of mixing of western Australia and New Zealand stocks...with the southeastern Australian stock' (Olsen 1984). More recent data indicates that the situation is not as clearcut as this. For example, Coutin *et al.* (1992) mentions the recapture of Bass Strait tagged school sharks from Western Australia

(although John Stevens, pers. com. indicates that these are likely to have been from near the border with South Australia rather than more western regions), and, more importantly, describes four school sharks tagged off New Zealand that were recaptured off south-east Australia, two from Tasmania's west coast and two from South Australia. The number of trans-Tasman migrants has now risen to 19 (Neil Bagley and John Stevens, pers. com.), with 17 moving from New Zealand to southern Australia (two being recovered from close to the Western Australia - South Australia border) and two moving from Tasmania to New Zealand. Tagging data continue to suggest a thorough mixing of south-east Australian school sharks, which is reflected in the genetic homogeneity of sharks from this region. The genetic data, indicating small but statistically significant genetic differences between Australian and New Zealand fish, suggest that the interchange of sharks between these two regions is too limited to lead to panmixia (possibly the tagged trans-Tasman sharks do not breed in Australian waters), and that pending the results of further analyses these stocks should continue to be regarded as effectively separate for management purposes.

Three genetic stocks of the common gummy shark, *Mustelus antarcticus*, were identified. One ranged along the southern coast of Australia from Bunbury in the west to Eden in the east, a second was located off New South Wales, in the region of Newcastle to Clarence River, and a third located off Townsville, Queensland. Whether these populations are genetically discrete and reproductively isolated stocks, or form parts of a continuous south to north genetic cline, cannot be resolved from the present data and resolving this issue requires much more extensive sampling off the eastern Australian coast. Certainly there is genetic differentiation in this region, and management as a single stock would be inappropriate.

Lenanton *et al.* (1990) describe some possible differences in the reproductive biology of females collected off Albany and Esperance from those collected off south-eastern Australia. However, our analysis failed to identify any genetic differences between Western Australian and south-eastern Australian gummies, and we cannot reject the null hypothesis of a single southern gummy shark stock. This conclusion accords with the earlier genetic analysis of MacDonald (1988), but is more definitive. We examined several polymorphic allozyme and mitochondrial DNA markers while MacDonald's conclusion rested on the examination of a single, weakly polymorphic, allozyme locus (*LDH-2**, which was among the loci we examined).

There is work in progress analysing tagged gummy shark movements, but little has yet been published. There are records of tagged gummy sharks moving from Bass Strait to South Australia, with one tagged female mving from Bass Strait to waters off Western Australia (Walker 1983). Preliminary analysis of more extensive recent data (J. Stevens, pers. com.) indicates the likelihood of extensive mixture of gummy sharks from Tasmania, Victoria and South Australia. The relative lack of fishing effort off eastern Australia makes it hard to assess the degree of gummy shark movement in this area. The general indications are that gummy sharks are somewhat less mobile than school sharks, a conclusion supported by the presence of a distinct species of gummy shark in New Zealand waters while the school shark there is the same as the Australian species. There are no records of the movements of tagged gummy sharks between the two countries.

The presence of (a minimum of) three distinct stocks of southern gummies along Australia's east coast flags a pronounced restriction of gene flow between these stocks, although the cause of this restriction is unknown. It may reflect a patchy

distribution of sharks in this region; little is known of the distribution of gummy sharks in these more tropical waters. One factor that might be relevant here is that gummy (and school) sharks 'pup', that is, give birth to free-swimming independent juveniles. There is no pelagic larval stage as there is in many teleosts, the drift of which in ocean currents is thought to promote gene flow and retard spatial differentiation. Gene flow in these shark species must be mediated by migrations alone rather than egg/larval drift together with migration, and sharks may thus in general be more likely to show stock differences than teleosts of similar migratory powers. It may be that gummy sharks in these more northern warmer regions are less migratory than in cooler southern waters, and therefore more liable to show genetic differentiation and stock differences. There are no tagging data from northern waters that would allow the independent assessment of this hypothesis.

Table 9.1. Heterozygosity per locus (H) and proportion of loci polymorphic (P) (frequency of common allele < 0.99) in sharks from allozyme data.

(a) Data summary from the current project

species	number of samples	number of fish	mean number of loci	locus range	H mean	H range	P mean	P range
G. galeus	9	59-218	26.2	17-29	0.008	0-0.029	0.020	0-0.074
M. antarcticus	14	134-503	26.4	18-28	0.101	0.076-0.134	0.270	0.185-0.444
M. sp. A	1	1	26	-	0.000	-	0.000	-
M. sp. B	4	6-14	23.5	16-28	0.025	0-0.046	0.064	0-0.143
M. lenticulatus	1	22-96	28	-	0.019	-	0.071	-

(b) Data summary from past studies

species	number of fish	number of loci	H	P	reference
Mustelus antarcticus	60	32	0.006	0.031	b
M. lenticulatus	92	38	0.001	0.053	c
Carcharhinus limbatus	8-20	48	0.023	0.083	d
C. tilstoni	967	48	0.037	0.192	e
C. sorrah	50-655	48	0.035	0.170	e
C. plumbeus	100-395	27	0.005	0.037	f
Squatina argentina Morph. 1	6-36	14	0.130	0.286	a
S. argentina Morph. 2	6-47	14	0.164	0.367	a
Prionace glauca	20	27	0.037	0.111	c
Squalus acanthias	20	30	0.017	0.033	c
Galeorhinus galeus	20	32	0.003	0.063	c
Deania calcea	6	28	0.012	0.036	c
Centroscymnus crepidater	20	29	0.003	0.069	c
Etmopterus baxteri	19	29	0.015	0.138	c

a, Solé-Cava *et al.* 1983; b, MacDonald 1988; c, Smith 1986; d, Lavery and Shaklee 1991; e, Lavery and Shaklee 1989; f, Heist *et al.* 1995.

10. BENEFITS

1. Management of the school shark fishery will benefit from increased confidence that south-east Australian school sharks do comprise a single stock. Genetic data indicates that this stock shows some small differences from New Zealand fish, which therefore appear to constitute a separate stock. While tagging data shows some migration between these stocks, this does not appear to be sufficient to render the stocks panmictic: they are likely to be best managed as separate stocks. The data will assist the setting of quotas or exploitation rates at levels commensurate with ecologically sustainable levels.

2. Uncertainties concerning the taxonomic identification of gummy sharks in Australasian waters have been resolved. Four species have been identified, with the common gummy shark ranging from at least Bunbury in Western Australia around the southern Australian coast to as far up the east coast as Townsville.

3. Three stocks of common gummy shark were identified: one ranged along the southern coast of Australia from Bunbury in the west to Eden in the east, a second was located off New South Wales, in the region of Newcastle to Clarence River, and a third located off Townsville, Queensland. The major fishing ground for gummy sharks is in south-east Australian waters, and management of this fishery will benefit from increased confidence that these fish do comprise a single stock. The data will assist the setting of quotas or exploitation rates at levels commensurate with ecologically sustainable levels.

11. INTELLECTUAL PROPERTY AND VALUABLE INFORMATION

No commercial intellectual property arose from this work.

12. FURTHER DEVELOPMENT

This study did not fully resolve all the issues concerning southern shark stock structure. There appeared to be small but statistically significant differences between SE Australian and New Zealand school sharks, but this needs to be confirmed (or refuted) with additional data. Likewise, whether along the eastern coast of Australia there is a south-north genetic cline in *M. antarcticus* or whether there are discrete reproductively isolated stocks in unclear. Our knowledge of the genetic basis of southern shark stock structure could be improved in several ways. The most obvious is to increase the number of fish examined, both by analysing additional fish from areas already sampled, and by analysing fish from new areas. The analysis of additional fish will increase statistical power. These fish should be analysed for both allozyme and mitochondrial DNA variation. Some collections in the present project had degraded for some allozyme markers, and techniques for preserving samples in better condition for allozyme analysis are required. Samples need to be stored in liquid nitrogen as soon as possible following capture of the fish, and then transferred to an ultra-cold freezer on arrival at the laboratory. Existing allozyme methodologies are perfectly acceptable, but thought should be given to detecting additional mtDNA variants. This goal would be most readily attained using PCR techniques to amplify known sequences of mtDNA, and then using 4-base cutters to digest these fragments.

These genetic approaches should be supplemented with an examination of DNA microsatellite loci. These loci constitute a new class of recently discovered genetic markers. They comprise short one to four nucleotide repeats that are generally less than 300bp in total, but can be much larger (Tautz 1989). Mutation rates are high, estimated at around 0.2% to 0.05% (Huang *et al.* 1992; Kwiatkowski *et al.* 1992), and heterozygosity high. This high mutation rate and high variability suggests that these markers may be more powerful than allozyme and mtDNA markers for stock delineation studies, although at present few comparative studies are available. Their promise was supported by one of the first studies of microsatellite variability in fish, which showed highly significant differences in allele frequencies at several microsatellite loci in samples of Atlantic cod off eastern Canada, whereas allozymes and mtDNA reveal little differentiation among local populations (Wright and Bentzen 1994). The problem with microsatellite loci is that for each new group of fish examined, new markers have to be developed in a process that might take several months before the markers can be applied in a stock structure analysis. Nonetheless, the apparently increased power of the technique means that this investment is likely to be worthwhile. It would be especially valuable for a more in-depth examination of the relationships between Australian and New Zealand school sharks, and resolve the east coast gummy shark relationships. These topics formed minor parts of the present study, but warrant further investigation.

13. STAFF

Bob Ward	CSOF7	Principal Investigator - Project supervisor and genetics management
John Stevens	CSOF7	Advice on shark biology and assistance with sample collections
Peter Last	CSOF7	Taxonomy management and advice
Peter Grewe	CSOF5	Development and implementation of mitochondrial DNA procedures
Michael Gardner	CSOF3	Laboratory assistant: allozyme, mitochondrial DNA, and morphological analysis

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16. FINAL COST

Total funds used are in accordance with the table below

	Funded by FRDC / FRRF 50/50
Salaries	$175,517
Travel	$300
Operating	$30,760
Capital	$0
Total	$206,577

The Final Statement of Receipts and Expenditure was forwarded to FRDC on 16 April 1996.

16. APPENDIX: MITOCHONDRIAL DNA FRAGMENT SIZES

Fragment sizes of shark shark mtDNA following digestion with ten different restriction enzymes. For each restriction enzyme, haplotypes are given designations from A onwards. sch=school shark, gs=gummy shark, sp A=gummy shark species A, sp B=gummy shark species B.

ApaI

A sch	B gs	C gs	E gs
16,100	-	-	-
-	-	-	8,800
-	8,272		8,272
-	7,462	7,462	-
-	-	5,680	-
-	-	2,306	-
-	1,200	1,200	-
16,100	16,934	16,648	17,072

AvaI

A sch	B gs	C gs
7,930	-	-
5,542	5,542	5,542
-	-	5,093
-	3,689	3,689
-	2,376	-
-	1,370	-
1,267	1,267	1,267
14,739	14,244	15,591

BclI

A sch	B gs	C gs	D gs
5,880	-	-	-
-	-	4,620	-
-	4,080	4,080	4,080
4,360	-	-	-
-	3,273	-	-
2,300	-	-	-
-	2,260	2,260	2,260
2,090	-	-	-
-	1,980	1,980	1,980
-	-	-	1,850
-	-	-	1,620
1,412	1,412	1,412	1,412
1,075	1,075	1,075	1,075
17,117	14,080	15,427	14,277

EcoRI

A sch	B gs	C gs	D gs	E gs
-	-	-	17,100	-
16,500	-	-	-	-
-	11,750	-	-	-
-	-	-	-	10,800
-	-	8,660	-	-
-	-	-	-	4,420
-	3,530	3,530	-	-
-	-	3,010	-	-
16,500	15,280	15,200	17,100	15,220

HindIII

A sch	B sch	C sch	D sch	E gs	F sch	H sch	I spA
-	-	-	-	-	-	9,010	9,010
-	-	7,780	-	-	-	-	-
5,730	-	-	-	-	-	-	-
-	5,150	-	-	-	-	-	-
-	-	-	-	4,763	4,763	-	-
-	-	-	4,440	-	-	-	-
-	-	-	-	3,680	-	-	-
-	-	-	-	-	3,400	-	-
3,080	3,080	3,080	3,080	-	-	-	-
2,950	2,950	2,950	2,950	-	2,950	2,950	2,950
2,320	2,320	2,320	2,320	2,320	-	2,320	-
-	-	-	-	-	2,140	-	-
1,910	1,910	-	1,910	-	-	1,910	-
-	-	-	-	-	-	-	1,600
-	-	-	-	1,190	1,190	-	1,190
1,100	1,100	1,100	1,100	-	-	1,100	-
-	-	-	-	980	-	-	980
-	-	-	-	836	836	-	836
540	540	540	540	-	-	540	-
17,630	17,050	17,770	16,340	13,769	15,279	17,830	16,566

NcoI

A sch & gs	C spB	D spA
14,250	14,250	14,250
-	2,880	-
-	-	2,630
600	-	-
14,850	17,130	16,880

PstI

A sch	B gs	D spA
11,300	-	11,300
-	8,880	-
-	-	5,120
-	4,927	-
3,080	3,080	-
1,600	1,600	-
1,010	-	-
925	-	-
17,915	18,487	16,420

PvuII

A sch	B gs	D spA
-	-	12,870
-	12,000	-
9,630	-	-
3,900	3,900	-
-	-	3,600
2,790	-	-
-	1,010	-
-	600	-
16,320	17,510	16,470

SacII

A sch	B gs
12,870	12,870
	2,010
1,790	1,790
14,660	16,670

XbaI A sch	B gs	C spA	D spB (gs)	E spB (gs)
-	-	-	-	16,880
-	-	-	11,975	-
9,600	9,600	-	-	-
-	-	9,420	-	-
3,960	-	3,960	-	-
2,580	2,580	-	2,580	-
-	2,375	-	-	-
-	-	1,700	-	-
-	1,543	-	1,543	-
-		1,410	-	-
-	1,228	-	1,228	-
900	-	-	-	-
17,040	17,326	16,490	17,326	16,880