

**POPULATION GENETIC STUDIES OF HORSE MACKEREL
TRACHURUS TRECAE AND *TRACHURUS TRACHURUS CAPENSIS*
OFF ANGOLA**

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Genetic variability of the Kunene horse mackerel *Trachurus trecae* and the Cape horse mackerel *Trachurus trachurus capensis* was examined using starch gel electrophoresis of enzymatic proteins on individuals collected along the Angolan coast. Six polymorphic enzyme loci were found in both species. The *idh-2** locus displayed differences between the two species, and several alleles at different loci exhibited different allele frequencies between the two species, indicating that they are genetically different. Significant differences found in the distribution of allele frequencies between Kunene horse mackerel from the Benguela region of Angola indicated that this species consist of more than one randomly mating population in Angolan waters.

Key words: Angolan coast, horse mackerel, isozymes, population studies, species identification

There are two species of horse mackerel in Angolan waters, the Kunene horse mackerel *Trachurus trecae*, found between 26°N (Cape Bojador) and 17°S (Kunene River), and the Cape horse mackerel *Trachurus trachurus capensis*, which extends from 15°S (off southern Angola) along the southern African seaboard to about 30°E (Wysokiński 1986). Two main morphological features distinguish the two species. In *T. t. capensis*, the dorsal accessory lateral line is longer and the lateral line has larger scutes than in *T. trecae*. These characteristics are, however, poorly developed in young fish, making distinction between the two species difficult. They have been considered to be subspecies of a single species, *Trachurus trachurus* (e.g. Wysokiński 1986).

Attempts have been made to differentiate horse mackerel stocks in the South-East Atlantic by means of morphological characteristics (Fischer *et al.* 1981). However, such features are not genetically controlled and variations may be caused by ecological factors (Meisfjord and Sundt 1996). Few investigations have studied the genetic population structure of either the Cape or Kunene horse mackerel in the South-East Atlantic. Geographic variations in the Cape horse mackerel were studied using electrophoretic methods (Komarov 1971, Zenkin and Komarov 1981) and DNA techniques (Naish 1990). However, the stock structure of the Kunene horse mackerel has not been studied off Angola using genetic methods. Wysokiński (1986) and Crawford (1989) believed that the species is composed of a randomly mating population.

The present study investigates the amount and distribution of allozyme variation within the Cape and Kunene horse mackerel. It also looks for population genetic structure of the Kunene horse mackerel and

describes interspecific allozyme variations between the two species. Allozyme variability was determined using horizontal starch gel electrophoresis (Chakraborty and Leimar 1987), a simple and cost-effective means of analysing genetic variation in a large number of individuals (Grant 1987).

Two hypotheses were tested: (1) the Kunene and Cape horse mackerel along the Angolan coast are the same species; (2) the Kunene horse mackerel population along the Angolan coast consists of one stock.

MATERIAL AND METHODS

Sampling

A total 1 037 Kunene horse mackerel and 192 Cape horse mackerel was collected from 11 and 2 localities respectively in Angolan waters (Fig. 1, Table I) during a survey by the Norwegian research vessel *Dr Fridtjof Nansen* from 25 August to 22 September 1995. The survey area was divided into three regions: Cabinda, Luanda and Benguela (Fig. 1). Liver and white muscle tissues were removed from each fish and frozen on board in microwells at about -20°C; the samples were later stored at -80°C in the laboratory. The sex and developmental stages were also determined and total length was recorded for each fish.

Starch gel electrophoresis

The principles and techniques used in this study for tissue preparation and electrophoresis analysis followed

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Table I: Cruise and sampling information of *T. trecae* and *T. t. capensis* (Stations 11 and 13 only) collected during the survey, and the number of fish sampled per tissue type

Region	Sample number	Ship's station number	Geographical location	Date	Depth (m)	Muscle	Liver	Mean length (m)
Cabinda	1	614	06°00'S, 11°59'E	27.08.95	88–92	96	–	29.6
	2	621	06°50'S, 12°00'E	28.08.95	41–57	96	96	36.7
	3	623	07°00'S, 12°06'E	28.08.95	103–107	96	96	24.4
	4	646	08°20'S, 13°16'E	01.09.95	31	96	96	24.3
	5	668	09°10'S, 12°44'E	03.09.95	219–271	77	77	38.8
Luanda	6	691	10°05'S, 13°14'E	06.09.95	40	96	96	33.2
	7	722	11°28'S, 13°34'E	10.09.95	72–49	96	96	30.7
	8	740	12°12'S, 13°31'E	12.09.95	84–70	96	96	29.0
	9	758	13°09'S, 12°45'E	15.09.95	99	96	96	30.5
Benguela	10	767	14°45'S, 12°16'E	16.09.95	100–121	96	96	23.5
	11	771	15°10'S, 12°16'E	17.09.95	110–122	96	96	20.2
	12	774	15°31'S, 11°59'E	17.09.95	38–72	96	96	24.2
	13	784	16°40'S, 11°34'E	19.09.95	91	96	96	16.4
<i>T. trecae</i>					192	192		
<i>T. t. capensis</i>					1 229	1 133		

those described by Utter *et al.* (1987) and Murphy *et al.* (1996). Two buffer systems were used: continuous amine-citrate (morpholine), adjusted to pH 6.1 using N-(3-aminopropyl) morpholine; and discontinuous histidine-citrate (adjusted to pH 7 using NaOH). All

tissue/buffer combinations were initially screened for 10 enzyme systems (Table II) on one sample (each of 96 individuals) of each species to identify the combinations that gave the best results for routine analysis. The gene product of 16 presumptive loci were viewed

Table II: Enzyme (Enzyme Commission number), locus abbreviations and buffer systems used, and the intensity of tissue expression in muscle and liver tissues of *T. trecae* and *T. t. capensis*

Enzyme	Locus	Histidine buffer		Amine-citrate buffer	
		Muscle	Liver	Muscle	Liver
Aspartase aminotransferase (2.6.1.1)	<i>aat-1</i>	–++	–	++	
	<i>aat-2</i>	++	–+		
Adenylate kinase (2.7.4.3.)	<i>ak-1</i>	++	+	++	+
	<i>ak-2</i>		+	+	
Creatine kinase (2.7.3.2)	<i>ck-1</i>	++	+	++	+
	<i>ck-2</i>	–+		+	
Esterase (3.1.1.1)	<i>est</i>	–	+	–	–
Glycerophosphate-dehydrogenase (1.1.1.8)	<i>gpd-1*</i>	+	–	++	–
	<i>gpd-2*</i>	–	+	–	++
Isocitrate dehydrogenase (NADPH+) (1.1.1.42)	<i>idh-1*</i>	++	–	++	–
	<i>idh-2*</i>	–	+	–	++
L-Lactate dehydrogenase (1.1.1.27)	<i>ldh</i>	+	+	+	+
Malate dehydrogenase (1.1.1.37)	<i>mdh</i>	+	+	+	+
Glucose phosphate isomerase (5.3.1.9)	<i>gpi-1</i>	+	–	+	–
	<i>gpi-2*</i>	++	+	++	+
Phosphoglucomutase (5.4.2.2)	<i>pgm-1*</i>	+	+	++	++
	<i>pgm-2</i>	–	+	–	+

– No activity
 + Weak activity
 ++ = Strong activity
 * Polymorphic

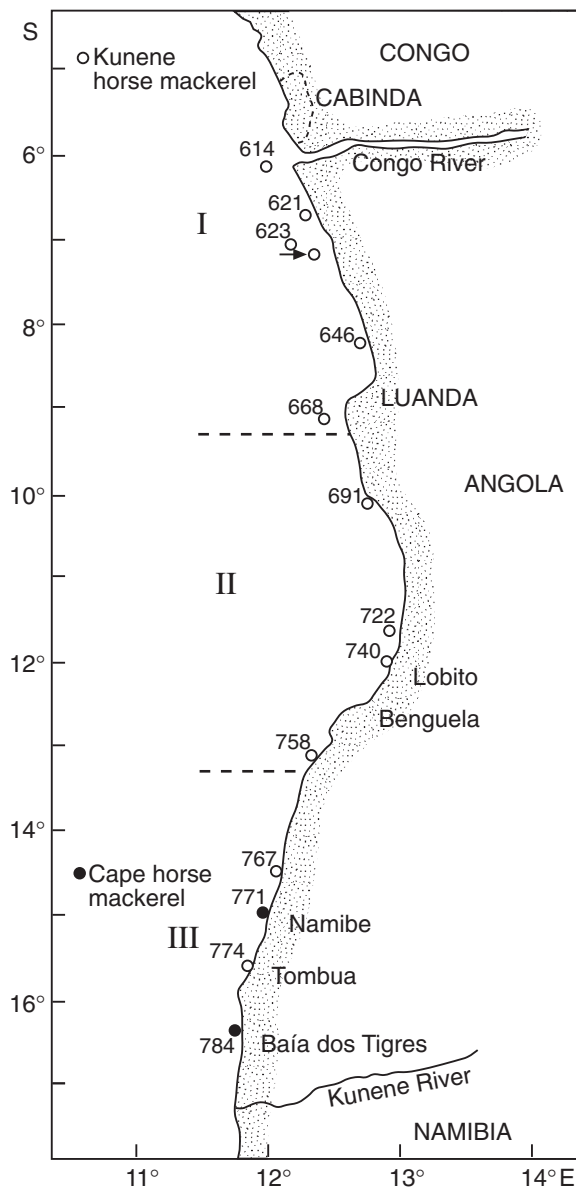


Fig. 1: Sampling locations of horse mackerel collected for electrophoresis studies off the Angolan coast. The three regions are: I Cabina-Luanda; II Luanda-Benguela; III Benguela-Kunene

using histochemical stains and the procedures described by Harris and Hopkinson (1976) and Murphy *et al.* (1996). Enzyme nomenclature followed Shaklee *et al.* (1990). When several forms of the same enzyme were observed, the loci were identified with an Arabic

number, beginning with one for the locus closest to the cathode. Alleles at a locus were designated by the electrophoretic mobilities of the products they encode relative to the most common allele product designated "100". A minus sign was assigned to any allele product exhibiting cathodal mobility. To compare the isoenzyme patterns between the two species and to discern possible mixture in the samples collected in overlapping areas, samples of both species were run on the same gel.

Data analysis

The observed genotypic proportion at polymorphic loci were tested for consistency with Hardy-Weinberg expectations, using the χ^2 goodness-of-fit test (Zar 1984). Rare genotypes were pooled, following Swofford and Selander (1981). Standard genetic distance was calculated for all pairs of samples, according to Nei (1972), and used to measure genetic divergence between the two species. Heterogeneity in allele frequencies between sample sites and between the three areas (Fig. 1) was calculated on grouped data of Kunene horse mackerel. Statistical analyses of genetic data were performed using the BIOSYS-1 computer programme (Swofford and Selander 1981). Homogeneity of genotype distributions was also tested by Monte Carlo simulation, as suggested by Roff and Bentzen (1989) and programmed by Zaykin and Pudovkin (1993). For multiple tests of the same hypothesis, the significance level was adjusted by sequential Bonferroni corrections (Holm 1979, Weir 1990).

The effect of length (reflecting age) on the distribution of genotypes and allele frequencies were tested. For each sample, the specimens were separated into small and large length-classes. Samples with separate modes (possibly reflecting age-classes) were chosen and the distribution of allele frequencies was compared as described above. Likewise, possible sex-dependent effects on the distribution of genotypic and allele frequencies were tested.

RESULTS

Genetic variability within samples

In all, 16 putative loci, encoding 10 enzymes, were analysed (Table II), of which six (*gpd-1**, *gpd-2**, *idh-1**, *idh-2**, *gpi-2** and *pgm-1**) were polymorphic at the 95% level in at least one sample. Histidine gave good resolution for *idh* and *gpi*, but the amine-citrate buffer system gave acceptable resolution and activity

Table III: Allele frequencies at six polymorphic loci for samples of *T. trecae* and *T. t. capensis* (Stations 11 and 13 only) collected in the different regions of the Angolan coast

Locus (Allele)	Allele frequency per station number and region												
	Cabinda				Luanda				Benguela				
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>idh-2</i>													
65		–	–	–	–	–	–	–	–	–	0.043	–	0.047
75		0.005	–	–	0.006	–	0.005	–	–	–	–	0.005	–
95		–	–	–	–	–	–	–	–	–	0.750	–	0.807
100		0.995	0.984	1.000	0.994	1.000	0.984	1.000	0.995	1.000	–	0.995	–
119		–	–	–	–	–	–	–	–	–	0.207	–	0.146
120		–	0.016	–	–	–	0.010	–	0.005	–	–	–	–
<i>n</i>		96	96	96	77	96	96	96	96	85	96	96	96
<i>gpi-2</i>													
80	0.016	0.021	0.010	0.021	–	–	–	0.005	0.005	0.017	0.011	0.016	0.010
100	0.818	0.828	0.792	0.797	0.812	0.830	0.837	0.849	0.811	0.791	0.188	0.818	0.182
120	0.167	0.151	0.198	0.182	0.188	0.170	0.163	0.146	0.184	0.192	0.785	0.016	0.797
125	–	–	–	–	–	–	–	–	–	–	–	0.005	–
140	–	–	–	–	–	–	–	–	–	–	0.005	–	0.026
<i>n</i>	96	96	96	96	77	94	92	96	96	85	96	96	96
<i>pgm-1</i>													
–65	–	–	–	0.005	0.013	–	–	–	0.005	–	0.005	–	0.005
–80	0.016	0.083	0.026	0.036	0.052	0.078	0.016	0.047	0.042	0.094	0.989	0.068	0.969
–100	0.984	0.917	0.969	0.958	0.935	0.917	0.979	0.953	0.953	0.894	0.005	0.932	0.026
–120	–	–	0.005	–	–	0.005	0.005	–	–	0.012	–	–	–
<i>n</i>	–	96	96	96	77	96	96	96	96	85	96	95	96
<i>gpd-1</i>													
–80	0.010	0.005	–	0.021	0.019	0.010	0.016	0.010	0.021	0.035	0.005	0.052	0.005
–100	0.990	0.990	1.000	0.974	0.981	0.990	0.984	0.990	0.979	0.965	0.995	0.948	0.995
–130	–	0.005	–	0.005	–	–	–	–	–	–	–	–	–
<i>n</i>	96	96	96	96	77	96	96	96	96	85	96	96	96
<i>gpd-2</i>													
–75	–	–	0.011	0.010	–	–	–	–	–	–	–	–	0.016
80	–	–	–	–	–	–	0.005	–	–	–	–	–	–
85	–	–	–	–	0.006	–	0.021	0.010	0.011	–	–	–	–
100	–	0.917	0.973	0.974	0.974	0.943	0.922	0.958	0.963	0.959	0.911	0.984	0.901
130	–	0.083	0.016	0.016	0.019	0.057	0.052	0.031	0.026	0.041	0.089	0.016	0.083
<i>n</i>	–	96	96	96	77	96	96	96	96	85	96	96	96
<i>idh-1</i>													
–75	–	–	–	–	–	–	–	–	–	–	0.823	–	0.818
–100	0.969	0.990	0.990	0.969	0.968	0.979	0.969	0.953	0.964	0.965	0.177	0.984	0.182
–120	0.031	0.010	0.031	0.032	0.021	0.031	0.047	0.036	0.035	0.035	–	0.016	–
<i>n</i>	96	96	96	96	77	96	96	96	96	85	96	95	96

n = Number of specimens analysed

for most loci, and was therefore used for routine analyses. The polymorphic systems are briefly described below. The relative allele frequencies and the relative mobilities of the allelic variants are presented in Table III.

Glycerophosphate-dehydrogenase — This dimeric enzyme appeared to be encoded by two polymorphic loci (*gpd-1** and *gpd-2**). “Satellite bands” were observed, especially in the liver tissue. The cathodal *gpd-1** locus (one common and two rare alleles) was expressed in the muscle. The *gpd-1*130* allele was

very rare in the Kunene and absent from the Cape horse mackerel. The anodal *gpd-2* locus was expressed in the liver. Five alleles were found in the Kunene horse mackerel, three of which were expressed in the Cape horse mackerel.

Isocitrate dehydrogenase — This dimeric enzyme exhibited two groups, each assumed to be controlled by alleles at one locus in both species (Fig. 2). *Idh-1** was cathodal and was expressed in the muscle. The frequency of occurrence of the *idh-1*100* allele was between 0.95 and 1.0 in the Kunene horse mackerel,

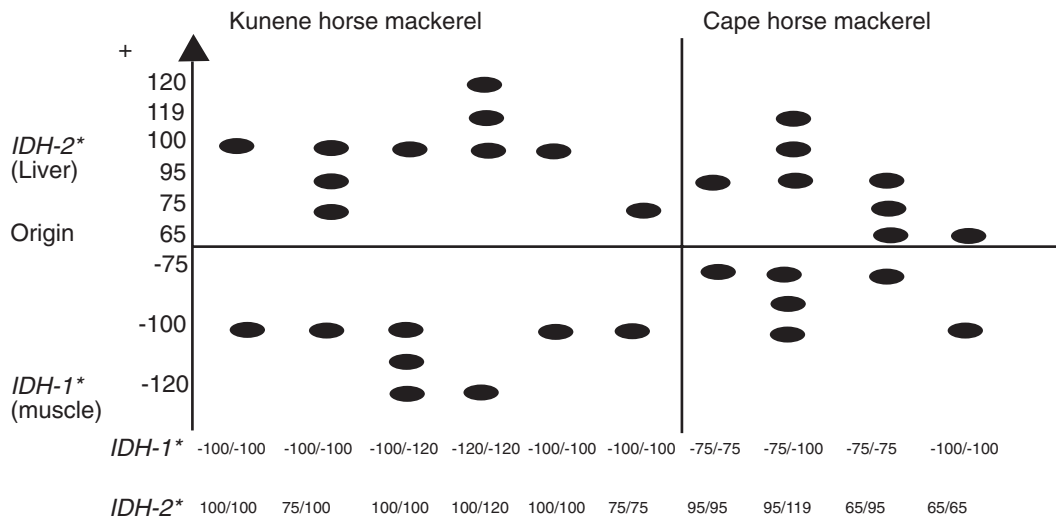


Fig. 2: Diagrammatic representation of variation in IDH patterns of Kunene and Cape horse mackerel in the liver and muscle

but only between 0.177 and 0.182 in the Cape horse mackerel. The common allele in the latter (*idh-1**) was not observed in the former species. *Idh-2** is anodal, expressed in liver tissue, and had fixed differences between the two species. Three unique alleles were observed in each species.

Glucose-6-phosphate isomerase — Two loci, encoding *gpi-1** and *gpi-2** (Fig. 3), were expressed in the muscle, whereas blurred patterns were observed in the liver. The phenotypic pattern observed at *gpi-2** followed the variation expected for this dimeric enzyme. *Gpi-1** banding patterns were unscorable and were omitted from further analysis. Consistent intermediate bands detected between the *gpi-1** and *gpi-2** banding patterns were interpreted as interlocus heterodimers. The highly polymorphic *gpi-2** locus was scored using muscle tissue. The two species shared three out of five alleles expressed by the *gpi-2** locus.

Phosphoglucosmutase — Strong activity for *pgm-1** was detectable in both muscle and liver tissues. *Pgm-1** is cathodal and polymorphic, with a banding pattern typical of a monomeric enzyme. Three alleles were shared by the two species, whereas one additional allele was observed at low frequency in the Kunene horse mackerel. Products of the *pgm-2* locus were observed in liver tissue only. This locus displayed diffuse, unscorable components and was therefore omitted from further analysis.

Other enzymes — The products observed in most of the samples stained for aspartate aminotransferase (*aat*), adenilate kinase (*ak*), creatine kinase (*ck*), esterase (*est*), lactate dehydrogenase (*ldh*) and malate dehydrogenase (*mdh*) either showed monomorphic patterns or were not clear enough to be interpreted with confidence.

The observed genotypic distributions did not deviate significantly ($p > 0.05$) from expected Hardy-Weinberg proportions in any sample when the Bonferroni correction of the significance level was applied. The most deviating distributions (three out of 58 tests for the Kunene and one out of 12 for the Cape horse mackerel) all showed a deficit of observed heterozygotes, albeit not significant.

The χ^2 test revealed no significant genetic differences between the subsamples grouped by size or sex. No subsamples displayed any significant differences between observed and expected Hardy-Weinberg distribution.

Interspecies variation

The result obtained from analysis of the six polymorphic loci indicates that the Kunene and the Cape horse mackerel are distinguished by well-defined gene pools. The two species did not share any alleles at *idh-2** locus and the allele frequencies (Table III) were markedly different at three other loci (*idh-1**,

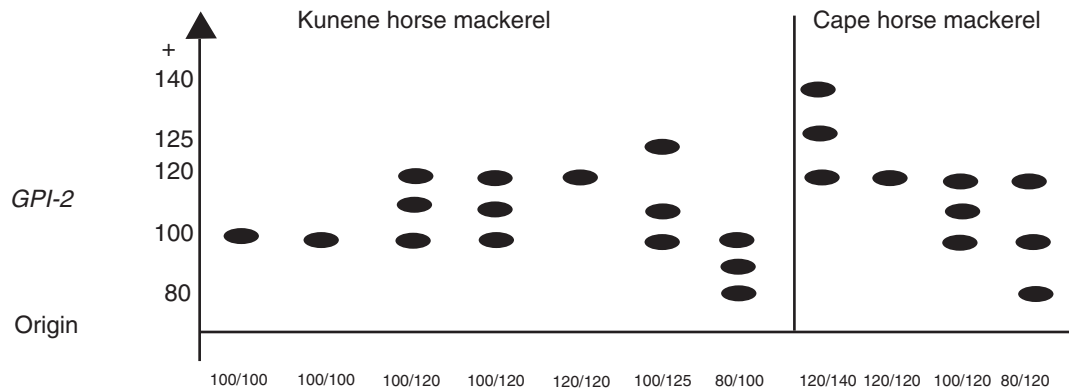


Fig. 3: Diagrammatic representation of variation in *gpi-2** patterns of Kunene and Cape horse mackerel. The *gpi-1** locus displayed diffuse patterns, which could not be interpreted

*gpd-2** and *pgm-1**). Nei's (1972) genetic distance (Table IV) between the two species yielded values ranging from 0.731 to 0.791, which is within the range between congeneric species (Thorpe 1982).

Heterogeneity among samples

The χ^2 test from all loci obtained for the Cape horse mackerel revealed no significant differences ($p > 0.05$) between the two samples. It is therefore unlikely that the samples were drawn from different gene pools.

There was no significant heterogeneity in allele distribution among samples at any locus when a Monte Carlo test was applied with sequential Bonferroni corrections of significance level. However, the distribution of *gpd-2** alleles was close to significant. When combining all loci, there was significant heterogeneity ($p > 0.05$). Samples within each of the geographical regions were tested separately. There were no significant

differences in sample variation within each region for any locus or for all loci combined. Therefore, all samples were pooled for each region and the tests were performed between regions. The Benguela region of Angola differed significantly ($p > 0.05$) from both the Cabinda and Luanda regions in frequency distribution of *gpd-1** alleles and for all loci combined. In addition, the difference between the Benguela and Luanda regions was close to significant for *pgm-1** alleles. No significant differences ($p > 0.05$) were found in any loci or in all loci combined between the Cabinda and Luanda regions.

DISCUSSION

For populations or species to be genetically differentiated, they must be isolated from each other, either geographically or reproductively (Nei 1975). Off the

Table IV: Nei's (1972) genetic distance for all pairs of *T. trecae* and *T. t. capensis* (Stations 11 and 13 only) samples collected along the Angolan coast

Sample number	2	3	4	5	6	7	8	9	10	11	12	13
3	0.001											
4	0.001	0										
5	0	0	0									
6	0	0	0	0								
7	0	0	0	0	0							
8	0	0	0	0	0	0						
9	0	0	0	0	0	0	0					
10	0	0.001	0	0	0	0.001	0	0				
11	0.755	0.760	0.765	0.751	0.750	0.791	0.772	0.762	0.732	0.760		
12	0.001	0	0	0	0	0.001	0	0	0	0		
13	0.754	0.757	0.763	0.749	0.748	0.788	0.771	0.760	0.731	0.759	0	-

Gulf of Guinea, *T. trecae* are found closer inshore than *T. t. capensis* (Troadeç and Garcia 1980), but both species co-occur in Namibian waters (Anon. 1995). Given the present results, the species must therefore have developed efficient means of reproductive isolation. Shaklee *et al.* (1982) argued that the demonstration of qualitative genetic differences between sympatric populations is a sufficient (although not a necessary) criterion to recognize distinct species.

The characteristics of three enzyme loci (*idh-1**, *idh-2** and *pgm-1**) seem promising as diagnostic keys to differentiate between the two species. The *idh-2** locus, with unique alleles in both species, appears to be the most useful for species identification. However, samples from the whole geographical range of the Cape horse mackerel are required to draw more firm conclusions.

The present study indicates that Kunene horse mackerel may be structured into two genetic groups in Angolan waters, one in northern tropical waters (the Cabinda and Luanda populations) and the other in the southern (from about 14°S) cooler Benguela upwelling system (the Benguela population). The characteristically low temperature and high salinity in the south of Angola (see Pereira 1978) may act as a barrier to gene flow between the populations. However, the estimates of genetic distance (Table IV) indicate that the two populations are closely related. According to Shaklee *et al.* (1982), maximizing the number of gene loci screened provides the most efficient means for obtaining accurate estimates of genetic distances between groups and for testing the conspecific status of two or more groups. Nei (1987) showed that the interlocus variance is much larger than the intralocus variance, unless the number of loci is very small. It is therefore important to analyse a large number of loci rather than many individuals per locus in order to reduce the variance of the estimate of genetic distance. Ward and Grewe (1995) point out that a variety of tissues should be examined in order to expand the allozyme information for taxonomic or identification purpose. In future studies, analysing more tissue types may reveal more polymorphic loci for the two species of horse mackerel off western southern Africa.

ACKNOWLEDGEMENTS

This work was supported by a scholarship given to MLS by the Norwegian Agency for Development and Cooperation (NORAD), and she thanks the Fisheries Ministry of Angola and Instituto de Investigação Pesqueira, Luanda, for the opportunity to

study in Norway. The *Dr Fridtjof Nansen* project provided support during the collection of data. The analyses were carried out at the Department of Fisheries and Marine Biology (DFMB), University of Bergen, Norway. We thank Profs T. Jørgensen and J. Kolding, and Ms B. Øglænd of DFMB for their assistance, and Dr G. Bianchi of the Institute of Marine Research, Bergen, who suggested the theme of this work and for her comments on the manuscript. We especially thank Dr R. W. Leslie of Marine & Coastal Management, Cape Town, for his invaluable help in restructuring and focusing the paper towards acceptance for publication.

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