# A morphometric and electrophoretic analysis of *Labeo* capensis and *Labeo* umbratus from two localities in southern Africa

NG van Vuuren, PFS Mulder, IT Ferreira\* and FH van der Bank

Department of Zoology, Rand Afrikaans University, PO Box 524, Johannesburg 2000, South Africa.

#### **Abstract**

The external morphology of Labeo capensis and L. umbratus from the Barrage (Vaal River) and Hardap Dam are compared. Although the characters overlap in some cases, the mean and modal values are sufficient to differentiate between the species. Electrophoresis was carried out on two blood and five muscle proteins. The results proved useful as an additional means of identification. The electrophoretograms revealed important biochemical population genetic information for the species. With this information it is possible to commence with a genetic data bank for L. capensis and L. umbratus.

#### Introduction

The need to conserve fish genetic resources has been recognised by fishery scientists and aquaculturists for some time, especially in relation to overfishing of natural stocks, the effects of large-scale alterations to river systems and domestication of species through aquaculture (FAO, 1981). Natural genetic resources are of vital importance because the ability to adapt to certain environmental changes is often determined by the degree of genetic variability within a population (FAO, 1981). An essential prerequisite to any broad programme of genetic resource conservation is the correct identification of the taxon, since an erroneous identification would lead to an incorrect impression of a specific population's biochemical genetic data (as expressed by their allele frequencies) and inferences on the ability of that population to adapt to subsequent natural or man-made selection pressures.

Apart from the difficulty to distinguish between closely related taxa by using only traditional morphological characteristics, the problem becomes even more complex in situations where hybrids are involved (Allendorf and Utter, 1979). However, this problem has previously been successfully resolved with the aid of gel electrophoresis (Allendorf and Utter, 1979; Ferguson, 1980; Stratil et al., 1983). It is interesting to note that the possibility of hybridisation between L. capensis and L. umbratus has been mentioned in the Orange Free State Nature Conservation Report of 1972/73 as well as by Gaigher and Bloemhof (1975).

This paper presents the results of an investigation into the morphological and electrophoretic differences between *L. capensis* and *L. umbratus*. An objective of the investigation is to initiate a genetic data bank (as expressed by their biochemical genetic variation) for each of these two economically important species. The alarming dearth of population genetic information on South African freshwater fish species was the initial motive for this investigation. It was, however, also thought necessary to compare the traditionally considered external morphological characteristics with the biochemical genetic data to evaluate the reliability of these two approaches when distinguishing between closely-related species.

## \*To whom all correspondence should be addressed. Received 22 February 1989; accepted in revised form 30 January 1990

#### Materials and methods

#### Sample collection

Fifty specimens of both *Labeo* species were collected from two populations: the Barrage in the Vaal River (South Africa) and Hardap Dam in the Fish River (Namibia). The total body length of the fish analysed varied between 350 mm and 420 mm. It was necessary to include Hardap Dam populations in this study in view of the report by Gaigher and Bloemhof (1975) of hybridisation species in this impoundment. A 0,6 ml sample of blood was taken from the caudal artery of each fish and mixed with 0,4 ml of 0,9% saline. After coagulation the serum was removed and stored on ice. In addition 40 g of skeletal muscle was removed, bottled and placed in a coolbag with ice to minimise the loss in enzyme activity. The samples were stored at -20°C in the laboratory for subsequent electrophoretic analysis.

#### Morphometric and meristic analysis

Counts were taken of the number of scales on the lateral line and around the caudal peduncle, the rays and spines in the dorsal and anal fins and the total gill-rakers on the anterior gill arch. The number of vertebrae was counted according to the technique described by Van der Bank and Ferreira (1987). The following morphometric measurements were determined and the relative proportions calculated: standard length/head length (SL/HL), standard length/body depth (SL/BD), head length/head width (HL/HW) and head length/eye diameter (HL/ED).

#### Electrophoretic analysis

#### Polyacrylamide gel electrophoresis

The extraction procedures, electrophoresis and staining methods employed were as described by Avtalion and Wojdani (1971). Serum transferrin and serum esterase phenotypes were determined by polyacrylamide gel electrophoresis of sera, using a continuous triscitrate buffer system and 6% gels.

#### Starch gel electrophoresis

Tissue extracts for electrophoresis were dissected from the frozen fish. The extracts were manually homogenised in a plastic vial

with 0,25 m $\ell$  distilled water added for every gram of tissue. The homogenate was centrifuged at 3 000 rpm for 15 min and prepared according to the electrophoretic techniques described by May et al. (1979). The gels consisted of 13% hydrolysed potato starch (Sigma, No. S-4501) and one of the following buffer systems.

RW) Gel (pH 8,5):	Tris Citric acid	0,03M 0,005M
Tray (pH 8,1):	Lithium hydroxide Boric acid	0,06M 0,3M
(as described by R	idoway et al. 1970)	

TC) Gel (pl	H 6,9)	1:15 dilution of tray solution	
Tray (1	H 6,9)	Tris	0,15M
•		Citric acid	0,05M
(as descri	bed by Wh	nitt, 1970)	
MF) Gel (pl	H 8,7)	1:4 dilution of tray solution	
Tray (1	oH 8,7):	Tris	0,18M
•	· • •	Boric acid	0,1M

Na EDTA 0,0 (as described by Markert and Faulhaber, 1965)

0,004M

(as	described	by	Ridgway	et al.,	1970)		

LATERAL LINE SO	CALE COUNTS OF <i>L</i> . IN	TABLE 1 CAPENSIS AND L. SOUTHERN AFRIC		TWO LOCAL	
Species	Labeo	Labeo capensis		Labeo umbratus  Barrage Hardap	
Locality	Barrage	Hardap	Barrage	Hardap	
Range (mode)	43-50(46)	38-48(45)	58-71(65)	49-60(55)	
Sample size	50	33	50	30	
Lateral line*					
- 38		3,04	_	_	
43	2	6,06		_	
	16	9,09	_	_	
44			-		
45	32	48,48	-	•	
46	34	18,18	-	-	
47	12	9,09	-	-	
48	2	6,06	-	-	
49	-	-	•	3,33	
50	2	-	-	3,33	
51	-	-	-	3,33	
52		-	-	6,67	
53		_	_	6,67	
	-	<u>-</u>	_	16,67	
54 55	-	-	<del>-</del>	26,67	
55	•	•	-	6,67	
56	-	•	•		
57	•	-	-	10,00	
58	-	-	4	6,67	
59	•	-	-	6,67	
60	-	=	•	3,33	
61	-	•	4	3,33	
62	-	_	12	-	
63	_	•	12	-	
64	_		16	-	
-	•	-	22	_	
65	-	-		-	
66	-	-	16	-	
67	-	•	8	-	
68	-	-	4	-	
71	-	-	2	<u>.</u> -	

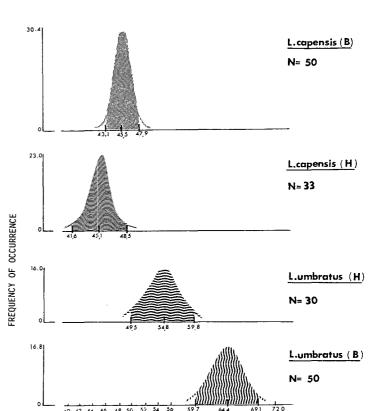


Figure 1 Comparison of the total number of scales on the lateral line of L. capensis and L. umbratus. Fish were collected at the Barrage (B) and Hardap Dam (H)

TOTAL NUMBER OF SCALES ON LATERAL LINE

TABLE 2
CAUDAL PEDUNCLE SCALE COUNTS OF L. CAPENSIS AND L. UMBRATUS FROM TWO LOCA-
LITIES IN SOLITHERN AFRICA

Species	Labeo	capensis	Lab	eo umbratus
Locality	Barrage	Hardap Barrage		Hardap
Range (mode)	21-25(22)	20-25(22)	26-33(28)	28-36(32)
Sample size	50	33	50 30	
Caudal peduncle* count				
20	-	3,03	-	-
21	10	27,27	-	-
22	54	30,30	-	-
23	26	27,27	•	•
24	8	9,09	-	-
25	2	3,04	-	-
26	-	•	6	-
27	•	-	26	-
28	-	-	32	3,33
29	•	-	20	6,67
30	-	-	10	10,00
31	-	-	4	13,33
32	-	-	•	26,67
33	-	-	2	20,00
34	-	•	-	10,00
35	•	<del>-</del>	-	6,67
36	•	•	-	3,33

ual counts are given as a percentage of the sample size.

TABLE 3
TOTAL GILL RAKER COUNT ON THE ANTERIOR ARCH OF *L. CAPENSIS* AND *L. UMBRATUS*,
FROM TWO LOCALITIES IN SOUTHERN AFRICA

Species		Labeo capensis	Labe	Labeo umbratus		
Locality	Barrage	Hardap	Barrage	Hardap		
Range (mode)	40-57(50)	42-53(48)	39-55(49)	43-53(47)		
Sample size	50	33	50	30		
Gill raker* count						
39	-	-	2	-		
40	2	•	2	-		
41	2	-	2	-		
42	-	3,03	-	-		
43	-	3,03	-	3,33		
44	6	6,06	•	3,33		
45	6	6,06	2	6,67		
46	6	6,06	6	16,67		
47	8	12,12	8	30		
48	8	27,27	11	16,67		
49	10	15,16	21	•		
50	16	9,09	12	10		
51	12	6,06	10	6,67		
52	8	3,03	8	3,33		
53	6	3,03	8	3,33		
54	4	-	6	-		
55	2	•	2	-		
56	2	-	-	-		
57	2	-	-	-		

After 3 to 5 h of electrophoresis, specific enzymes were stained for (Table 8), using chemical solutions described by Shaw and Prasad (1970) and Harris and Hopkinson (1976). Locus and allelic nomenclature was applied as described by Allendorf and Utter (1979), where the products of multiple loci, coding for functionally

similar proteins, were designated by their mobilities relative to a

Statistical procedures

common allele.

#### Morphometric analysis

The mean and standard deviation was calculated for morphometric data. Testing of the data was done using the chi-square test. All calculations were done with the SPSS computer package on a Sperry 1100 main frame computer.

#### Electrophoretic analysis

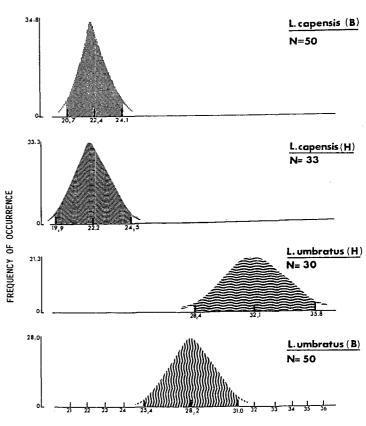
Statistical procedures were conducted as described by Grant et al. (1983), and Grant and Leslie (1983). Allelic frequencies were

estimated from genotypic frequencies by gene counting, since all protein variants observed in this study were interpreted to reflect products coded by co-dominant, Mendelian alleles. Departures from Hardy-Weinberg were detected using the likelihood-ratio test for goodness of fit (Sokal and Rohlf, 1973).

#### Results and discussion

Of all the African Labeo species, L. umbratus most closely resembles L. capensis (Reid, 1985). According to this author the anal fin tip of L. umbratus does not reach the caudal fin base and (in preserved material at least) a faint caudal peduncle spot is usually evident. Reid (1985) also shows that there are differences in proportions (HL/SL; HL/ED; dorsal fin base length/SL) and modal meristics (scales) in L. capensis.

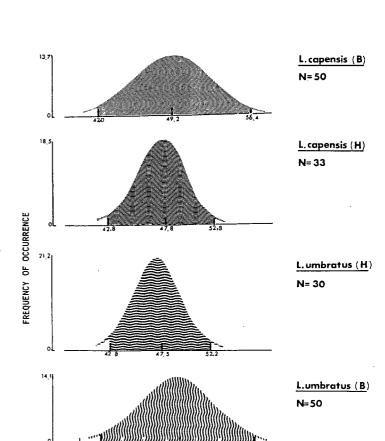
The close resemblance between the two species as pointed out by Reid (1985) is shown in Tables 1 to 6 and Figs. 1 to 7. Only two characters of *L. capensis* do not overlap with that of *L. umbratus*, namely the number of scales around the caudal peduncle and the number of dorsal fin spines. It is of interest to note that the



TOTAL NUMBER OF SCALES AROUND PEDUNCLE

Figure 2

Comparison of the total number of scales around the caudal peduncle of L. capensis and L. umbratus. Fish were collected at the Barrage (B) and Hardap Dam (H)



TOTAL NUMBER OF GILL RAKERS

Figure 3

Comparison of the total number of gill rakers on the first anterior gill arch of L. capensis and L. umbratus. Fish were collected at the Barrage (B) and Hardap Dam (H)

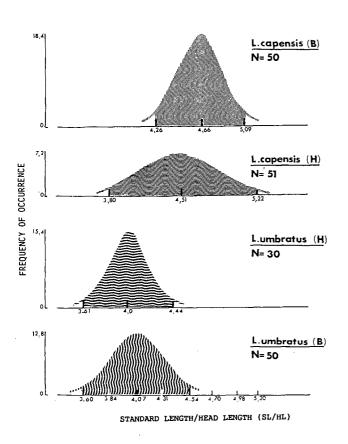


Figure 4

Comparison of the morphological proportion SL/HL of L. capensis and L. umbratus. Fish were collected at the Barrage (B) and Hardap Dam (H)

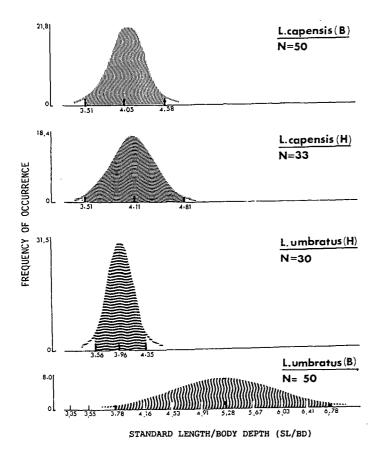
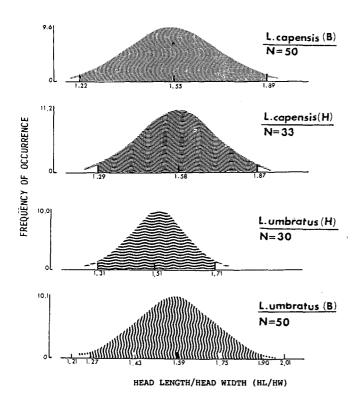


Figure 5
Comparison of the morphological proportion SL/BD of L. capensis and L. umbratus. Fish were collected at the Barrage (B) and Hardap Dam (H)



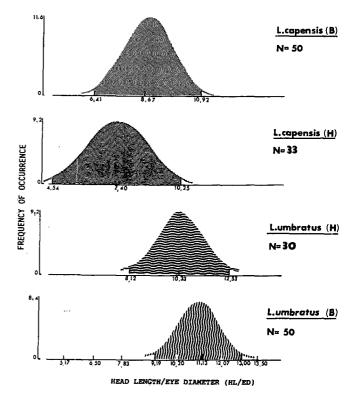


Figure 6
Comparison of the morphological proportion HL/HW of L. capensis and L. umbratus. Fish were collected at the Barrage (B) and Hardap Dam (H)

Figure 7
Comparison of the morphological proportion HL/ED of L. capensis and L. umbratus. Fish were collected at the Barrage (B) and Hardap Dam (H)

TABLE 4 THE TOTAL NUMBER OF SPINES AND RAYS ON THE DORSAL FIN OF <i>L. CAPENSIS A UMBRATUS</i> FROM TWO LOCALITIES IN SOUTHERN AFRICA					
Species	Labeo	capensis	Lab	eo umbratus	
Locality	Barrage	Hardap	Barrage	Hardap	
Range (mode)	iii 10-12(11)	iii 10-11(10)	iv 9-10(9)	iv 9-10(9)	
Sample size	50	33	50	30	
Dorsal spines* and rays					
iii	100	100	_		
iv	-	-	100	100	
9	-	-	64,0	86,67	
10	12	90,91	36,0	13,33	
11	72	9,09	•	-	
12	16	-	•	•	

number of scales around the caudal peduncle of *L. capensis* from the Barrage (21 to 25) differs only by one from that of *L. umbratus* from the same locality (26 to 33). As these values are not fixed a change in the number of scales may be induced by different environmental conditions and hence it is clear that this character may be insufficient to distinguish between hybrids and the pure species.

An example of the above-mentioned problem is reflected by the lateral line scale counts of the *L. umbratus* populations from the Barrage and Hardap Dam, respectively. The *L. umbratus* population from Hardap Dam has a much lower scale count in general than the Barrage population. It is thus clear that there will be no difficulty in distinguishing between *L. umbratus* and *L. capensis* 

from the Barrage. However, this is not the case with the two populations from Hardap Dam where hybridisation occurs between the two populations. Difficulties with identification of hybrids emphasise the need for an alternative method of identification. The morphometric characters (Table 7 and Figs. 1 to 7) indicate that the head of *L. umbratus* is longer and wider than that of *L. capensis* and that *L. umbratus* has a smaller eye than *L. capensis*.

These results thus support the findings of Du Plessis (1963), Jubb (1967), Le Roux and Steyn (1968) and Reid (1985) that the two species can be identified on external morphology. Gaigher and Bloemhof (1975) could not easily identify the hybrids in Hardap Dam. The following quotation from their publication is an example of the problems they had to deal with: "The L. umbratus with

TABLE 5 THE TOTAL NUMBER OF SPINES AND RAYS ON THE ANAL FIN OF L. CAPENSIS AND L. UMBRATUS, FROM TWO LOCALITIES IN SOUTHERN AFRICA

Species	Labeo	capensis	Labe	Labeo umbratus	
Locality	Barrage	Hardap	Barrage	Hardap	
Range (mode)	iii-5	iii-5	iii-5	iii-5	
Sample size	50	33	50	30	
Anal spines* and rays					
iii 5	100 100	100 100	100 100	100 100	

<sup>\*</sup>Individual counts are given as a percentage of the sample size.

TABLE 6
THE TOTAL NUMBER OF VERTEBRAL COMBINATIONS OF *L. CAPENSIS* AND *L. UMBRATUS*FROM TWO LOCALITIES IN SOUTHERN AFRICA

Species	Labeo co	apensis	Labeo umbratus		
Locality	Barrage	Hardap	Barrage	Hardap	
Range (mode)	39-40(39)	39-41(40)	39(39)	39-40(39)	
Sample size	50	33	50	30	
Vertebral count*					
(20 + 19)	30	-	30	30	
(20 + 20)	10	20	-	20	
(21 + 18)	40	10	70	40	
(21 + 19)	20	60	-	10	
(21 + 20)	-	10	•	-	

<sup>\*</sup>Individual counts are given as a percentage of the sample size.

TABLE 7
THE MEANS AND STANDARD DEVIATIONS OF THE MORPHOMETRIC PROPORTIONS FOR L.
CAPENSIS AND L. UMBRATUS FROM TWO LOCALITIES IN SOUTHERN AFRICA

Species	Labeo	capensis	o umbratus		
Locality	Barrage	Hardap	Barrage	Hardap	
Sample size	50	33	50 30		
Proportion					
SL/HL	$4,656 \pm 0,219$	$4,507 \pm 0,352$	$4,073 \pm 0,235$	$4,022 \pm 0,208$	
SL/BD	$4,047 \pm 0,072$	$4,159 \pm 0,323$	$5,280 \pm 0,748$	$3,955 \pm 0,196$	
HL/HW	$1,554 \pm 0,167$	$1,579 \pm 0,145$	$1,588 \pm 0,160$	$1,508 \pm 0,103$	
HL/ED	$8,665 \pm 1,126$	$7,397 \pm 1,428$	$11,095 \pm 0,952$	$10,325 \pm 1,101$	

SL: Standard length HL: Head length HW: Head width

BD: Body depth ED: Eye diameter

TABLE 8
A SUMMARY OF THE PROTEINS STAINED FOR *L. CAPENSIS* AND *L. UMBRATUS*. IN EACH CASE THE BUFFER SYSTEM THAT PROVIDED THE BEST RESOLUTION AND THE NUMBER OF LOCI CODING EACH PROTEIN ARE GIVEN

Protein	Locus abbreviation	EC No.	Buffer	Number of loci
Glyceraldehyde phosphate	GAP	12.1.12	RW	2
Glycerol-3-phosphate	GPD	1.1.1.8	RW	2
Isocitrate dehydrogenase	IDH	1.1.1.42	MF	2
Lactate dehydrogenase	LDH	1.1.1.27	RW	2
Serum esterases	EST	3.1.1.1	Tris citrate	1
Serum transferrins	TF	-	Tris citrate	1

46 lateral line scales was possibly a hybrid and not a true *L. umbratus*." In view of difficulties stated the two species were analysed electrophoretically to determine whether a better means of identification could be provided. The genetic information, as expressed by the allelic frequencies, can also be used to initiate a genetic data bank for SA freshwater fish species.

#### Electrophoretic analysis

Table 8 lists the enzymes studied with locus abbreviation, the number of loci found and the buffer system that showed the best resolution. A summary of the results is provided in Table 9 and further details are described below under separate headings for each enzyme.

#### Glyceraldehyde phosphate dehydrogenase (GAP)

Two loci, with heterotetrameric bands, were observed. The least anodal zone, GAP<sup>2</sup>1, was monomorphic for both *L. capensis* and *L. umbratus* and showed the same migration rate for both species. GAP-2 was polymorphic for three alleles that consisted of single banded homozygotes (AA) and triple banded heterozygotes. Similar polymorphisms, in which only one of the homozygotes was found along with the heterozygotes, have been reported for *Onchorhynchus garbusa* (May et al., 1975). No variation was observed in the banding patterns and hence GAP is not suitable as a genetic marker to distinguish between *L. capensis* and *L. umbratus*.

#### Glycerol-3-phosphate dehydrogenase (GPD)

The products of two loci were found where the least anodal zone, GPD-1, was monomorphic for both L. capensis and L. umbratus. GPD-2 consisted of slow migrating, single banded homozygotes and triple banded heterozygotes, as would be expected for a dimeric enzyme. As in the case of GAP, no difference was observed in the banding patterns of the two species and thus GPD cannot be used as a genetic marker to distinguish between them either.

#### Isocitrate dehydrogenase (IDH)

Two zones of activity appeared on gels stained for this enzyme. The least anodal zone, IDH-1, was polymorphic for both L. capensis and L. umbratus, consisting of a single banded homozygote and double banded heterozygotes. This unexpected monomeric composition for IDH, a dimeric enzyme (Kirpichnikov, 1981), was also observed by Grant and Leslie (1983) for Lophius upsicephalus. The migration rate of the homozygotic phenotype was distinctly

TABLE 9 ALLELIC FREQUENCIES OF ELECTROPHORETIC VARIANTS OF L. CAPENSIS AND L. UM-BRATUS FROM THE BARRAGE (B) AND HARDAP DAM (H). ALLELES ARE DESIGNATED BY THEIR MOBILITIES RELATIVE TO THE COMMON ALLELE

Locus	Allele	L. capensis (B)	L. capensis (H)	L. umbratus (B)	L. umbratus(H)
TF	84	0,060	_	0.040	
••	93	0,480	-	0,040	-
	100	0,160	0,636	0,440	- 0.000
	109	0,080	0,061	0,250	0,983
	113	-	0,030	-	•
	116	•	0,273	-	
	122	0,220	-	0,260	0,017
	N	50		-	-
	IN	<b>5</b> 0	33	50	30
EST	100	-	0,076	0,860	0,967
	103	0,280	0,045	0,140	<u>.</u>
	108	0,570	0,879	-	0,033
	113	0,150	-	-	-
	N	50	33	50	30
LDH-1	100	1,0	1,0	1.0	1.0
	N ·	50	33	1,0	1,0
	14	50	<b>J</b> J	50	30
LDH-2	100	1,0	1,0	1,0	1,0
	N	50	33	50	30
IDH-1	77	0,050	0,121	0.000	0.017
	100	0,950	0,879	0,900	0,917
	N	50	33	0,100	0,083
	11	30	33	50	30
IDH-2	84	-	-	1,0	0,933
	100	1,0	1,0	-	0,067
	N	50	33	50	30
GAP-1	100	1,0	1,0		1.0
	N	50	33	1,0	1,0
	N	50	· 99	50	30
GAP-2	100	0,150	0,045	0,190	0,050
	127	0,850	0,955	0,810	0,950
	N	50	33	50	30
GPD-1	100	1.0	1.0		
01 D-1	100	1,0	1,0	1,0	1,0
	N	50	33	50	30
GPD-2	100	0,170	0,091	0,180	0,100
	120	0,830	0,909	0,820	0,900
	N	50	33	50	33
				50	23

faster for L. capensis than for L. umbratus.

IDH-2 was monomorphic for both *L. capensis* populations as well as for *L. umbratus* from the Barrage. *L. umbratus* from Hardap Dam showed a few double banded heterozygotes in addition to the single banded homozygote. The homozygotes of *L. capensis* populations were again faster migrating phenotypes than the homozygotes of populations of *L. umbratus*. It appears that IDH can be used with success as a genetic marker to distinguish between *L. capensis* and *L. umbratus*. The poor resolution obtained for this locus is probably due to the fact that muscle and not liver was analysed (Grant and Leslie, 1983). Further studies should use liver and other tissues as a source for this enzyme.

#### Lactate dehydrogenase (LDH)

The common phenotype was five-banded and was interpreted to result from the products of two monomorphic loci with heterote-trameric bands that formed between LDH-1 and LDH-2. LDH-1 and LDH-2 are probably the A and B loci mentioned by Markert and Faulhaber (1965). Further research on heart tissue is needed before an opinion can be given on whether either LDH-1 or LDH-2 is LDH-A, and *vice versa*, as LDH-B is most prominently active in heart tissue and LDH-A in skeletal muscle (Johnson and Utter, 1976; McAndrew and Majumdar, 1983; Philipp *et al.*, 1983; Basio and Taniguchi, 1984; Grant, 1985).

#### Serum esterases

A total of four bands (A,B,C and D) were observed at a single locus in the four populations. The double-banded heterozygotes that were observed in all four populations are distinct proof of the monomeric structure of this enzyme in *L. capensis* and *L. umbratus*. However, it was necessary to interpret esterase electrophoretograms of the presence of satellite (ghost) bands (Kirpichnikov, 1981). Satellite bands, which are of no genetic significance, were prominent in the *L. umbratus* population from the Barrage and both populations from Hardap Dam. It is interesting to note that *L. capensis* from the Barrage did not show any occurrence of the D-band (100) and the *L. umbratus* population no indication of the B-band (108). The presence of the abovementioned alleles in low frequencies in the *L. capensis* (D) and *L. umbratus* (B) populations from Hardap Dam could be due to hybrids that had been wrongly identified.

#### Serum transferrins

One zone of activity was observed for the four populations of *Labeo* with variants for seven different bands (A,B,C,D,E,F and G). Fishes of both species from the Barrage showed a total of five bands. The high degree of polymorphism obtained is characteristic for this protein (Manwell and Baker, 1970; Kirpichnikov, 1981). The individuals from Hardap Dam did not show the same degree of polymorphism as the Barrage populations and no satellite bands were observed. The heterozygotes from Hardap Dam also showed the typical banded variant for a monomer. The most common band (A) of these four populations was designated the 100-band.

#### Hardy-Weinberg proportions

The gene products of 10 protein coding loci were investigated in L. capensis and L. umbratus. Six of these loci (TC, EST, IDH-2, GAP-2 and GPD-2) exhibited variable phenotypes. These were all found to be amenable to Hardy-Weinberg proportions expected with random mating. One significant departure from the Hardy-

TABLE 10
AVERAGE POPULATION HETEROZYGOSITY OF
L. CAPENSIS AND L. UMBRATUS FROM THE
BARRAGE (B) AND HARDAP DAM (H)

Species	Average popula- tion heterozygosity	Standard deviation
L. capensis (B)	0,1892	0,8113
L. capensis (H)	0,1200	0,0528
L. umbratus (B)	0,1698	0,0698
L. umbratus (H)	0,0649	0,0219

Weinberg proportions was detected (P>0.05) at the transferrin locus of *L. capensis* from the Barrage, observing a shortage of heterozygotes. This may be explained by the high degree of polymorphism that was observed at this locus. It may be possible that the sample was too small to contain all of the expected phenotypes. If this is the case, this population (as the other three) is a panmictic one.

### Average population heterozygosity

The average population heterozygosity (Table 10) of *L. capensis* (0,1892) and *L. umbratus* (0,1698) from the Barrage is higher than those of *L. capensis* (0,1200) and *L. umbratus* (0,0649) from Hardap Dam. These values are, however, close to those of other natural populations of fish.

Fujio and Kato (1979) found an average population heterozygosity of 0,067 for *Engraulis japonicus* and Grant (1985) found an average population heterozygosity for *Engraulis japonicus* that varied between 0,110 and 0,128.

The difference between the average population heterozygosity of the two populations from the Barrage and the two from Hardap Dam is a very complex issue, as a combination of various factors may be responsible. Hardap Dam is geographically situated in the upper reaches of the Fish River. Thus, with the construction of the dam wall, any new genetic material from the main body of the river was restricted to the lower reaches, resulting in isolated nonrandom populations. The erection of the dam wall has also led to the destruction of the natural habitat of L. capensis and L. umbratus, both of which are normally adapted to a riverine habitat (Jubb, 1967; Reid, 1985). The Barrage populations on the other hand probably originated from a much larger, existing population (more random) and also occur in a much more natural habitat. It appears thus that these manmade alterations have influenced the genetic variability of the Hardap Dam populations. However, a more intensive genetic investigation will be needed to determine other effects of these unnatural pressures on SA freshwater fish stocks. The inability to come to a comprehensive conclusion on the population genetic structure of SA freshwater fish stocks, emphasises the dearth of information and the need to analyse our natural fish stocks genetically.

#### **Conclusions**

This study supports previous studies (Du Plessis, 1963; Jubb, 1967; Reid, 1985) which showed that the two species can be clearly separated on morphometric and meristic characters alone. It is, however, obvious that the data may be insufficient when hybri-

disation occurs between closely-related species. Electrophoresis proved to be useful in solving this problem. An important fact that arose from these results is the lack of population genetic data on our natural fish stocks. Hopefully this will promote an awareness of the need for establishing genetic data bases for fishes.

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