# Allozyme variation in a Johnston's topminnow, *Aplocheilichthys johnstoni*, population from the Zambezi River system

**MKJ Steenkamp*, GD Engelbrecht and PFS Mulder**
*Department of Physiology, University of the North, Private Bag X1106, Sovenga 0727, South Africa*

## Abstract

Twenty five specimens of *Aplocheilichthys johnstoni* (Günther, 1893) were collected from the Cuando River in the Zambezi River system. Protein electrophoresis was used to analyse the genetic structure of this population. Seven of the 20 loci studied, (15% using the 95% criterion) revealed polymorphism. The heterozygosity value obtained ($H_O$ = 0.050) compare favourably to those recorded for other fish species. Observed allele frequencies deviated from expected Hardy-Weinberg proportions at the **EST-3**, **GPD-1** and **GPI-2** protein coding loci. The results from the genetic analysis of *A. johnstoni* are discussed in relation to its role in mosquito larval control.

## Introduction

The Aplocheilichthyinae (African lampeyes) comprise approximately nine genera of which two genera, *Aplocheilichthys* and *Hypsopanchax* are found in Southern Africa. The genus, *Aplocheilichthys*, is characterised by their bright, glossy white or blue eyes and therefore they are sometimes referred to as "lampeyes" (Skelton, 1993). Johnston's topminnow, *A. johnstoni*, is recognised as an aquarium species as well as for mosquito larval control. They are small (<50 mm total length) and keep to shallow, densely vegetated habitats. They primarily utilise the upper 10cm of the water where they feed on insect larvae, daphnia and other small invertebrates. Their anterodorsally located mouths enable them to feed on neustonic organisms and they are therefore extremely vulnerable to the spraying of insecticides (especially those aimed at the killing of mosquito larvae) and other pollutants (Kleynhans, 1986). Johnston's topminnows are serial spawners and eggs are laid on vegetation. The eggs are not drought resistant and excessive water extraction poses a threat to the survival of the species (Kleynhans, 1986).

The distribution of Johnston's topminnow in Southern Africa ranges from the Cunene, Okavango, Zambezi, Pungwe and Busi Rivers. Isolated populations of *A. johnstoni* are also known from the Marico, Notwane, Crocodile and Levuvhu Rivers (Limpopo River system). Further north, the species is found in the Zambian-Zaire and Kasai-Zaire River systems, the catchment areas of Lake Malawi and Lake Rukwa and east-coast rivers of Tanzania (Bell-Cross, 1972; Kleynhans, 1986). It is the presence of these isolated populations of *A. johnstoni* that is of particular interest to conservationists. The biological and commercial potential of this species served as motivation for this study.

## Materials and methods

Twenty five specimens of *A. johnstoni* were collected from the Cuando River, Upper Zambezi River system (18°07'38"S, 23°22'51"E). Reference specimens were donated to the J.L.B. Smith Institute for Ichthyology, Grahamstown (RUSI 61856). They were captured using electro-narcosis and whole fish were frozen in liquid nitrogen (-196°C) and stored at -40°C until electrophoresis. Each specimen was homogenised in 1ml distilled water prior to electrophoresis. The samples were prepared as described in Engelbrecht and Mulder (1999) and analysed by means of horizontal starch gel-electrophoresis. The buffer systems used are described in Table 1. A total of 13 enzyme systems were screened using the enzyme-staining methods of Harris and Hopkinson (1976) and Hillis and Moritz (1990). The methods of Shaklee et al., (1990) were followed for the interpretation of gels and locus nomenclature. Statistical analysis was performed using the BIOSYS-1 programme of Swofford and Selander (1981). The statistical calculations included the following: the percentage of polymorphic loci ($P_{0.95}$), average observed ($H_O$) and expected heterozygosity ($H_E$) per locus and allele frequency deviations from expected Hardy-Weinberg proportions using the $\chi^2$-test for goodness of fit. Levene's correction for Hardy-Weinberg equilibrium was used in order to take the small population size into account (Levene, 1949).

## Results

Seven of the 20 loci analysed in *A. johnstoni* revealed polymorphism. The loci screened, enzyme commission numbers and buffer systems used for each protein analysed are listed in Table 1. The allele frequencies for polymorphic loci, percentage of polymorphic loci using the 0.95 criterion and average observed ($H_O$) and expected ($H_E$) heterozygosities are presented in Table 2. The percentage of polymorphic loci ($P_{0.95}$) was calculated at 15% and the observed heterozygosity estimate was $H_O$ = 0.050. Allele frequencies at the **EST-3**, **GPD-1** and **GPI-2** loci deviated significantly from expected Hardy-Weinberg proportions (Table 2). A deficit of heterozygotes were also observed at these loci (Table 2).

## Discussion

According to Nei (1987), one of the main objectives of population genetics is to describe the amount of genetic variation in populations and then to study the maintenance of this variation. Analysis of the

* To whom all correspondence should be addressed.
☎(015) 268-2268; fax(015) 268-2268; e-mail: swartk@unin.unorth.ac.za
*Received 12 June 2000; accepted in revised form 16 October 2000.*

**TABLE 1**
**Locus abbreviations, enzyme commission (E.C.) numbers and buffers giving the best results for each protein analysed**

| Protein | Locus | E.C. Nr. | Buffer |
|---|---|---|---|
| Adenylate kinase | AK-1 | 2.7.4.3. | A |
| Creatine kinase | CK-1, 2 | 2.7.3.2 | B |
| Esterase | EST-1, 2*, 3 | 3.1.1.- | B |
| Fumarate hydratase | FH- 1 | 4.2.1.2 | C |
| Glycerol-3-phosphate dehydrogenase | GPD- 1 | 1.1.1.8 | C |
| Glucose-6-phosphate isomerase | GPI- 1, 2*, 3 | 5.3.1.9 | C |
| Isocitrate dehydrogenase | IDHP- 1 | 1.1.1.42 | A |
| L-Lactate dehydrogenase | LDH- 1,2 | 1.1.1.27 | B |
| Peptidase: | | 3.4.-.- | |
| Substrate: Glycyl-L-leucine | PEP-C-1 | | B |
| Leucyl-tyrosine | PEP-LT- 1 | | B |
| Phosphoglucomutase | PGM- 1, 2* | 5.4.2.2 | B |
| General protein | PROT- 1 | | B |
| Superoxide dismutase | SOD- 1 | 1.15.1.1 | B |

A - a continuous Tris, citric acid (pH 6.9) buffer system (Whitt, 1970)
B - a continuous Tris, boric acid, EDTA buffer system (pH 8.6) (Markert and Faulhaber, 1965)
C - a discontinuous Tris, citric acid (gel pH 8.7), lithium hydroxide, boric acid (electrode pH 8.0) buffer system (Ridgway, et al. 1970)
* polymorphic loci ($P_{0.95}$)

**TABLE 2**
**Allele frequencies for polymorphic loci, percentage of polymorphic loci using the 0.95 criterion ($P_{0.95}$), average observed ($H_O$) heterozygosity with standard errors (SE) in parentheses and chi-square values ($\chi^2$) at loci where allele classes deviated significantly (P< 0.05) from Hardy-Weinberg expectations and estimates of heterozygote deficiency (D) for each locus for *A. johnstoni***

| Locus | Allele | Frequency | $\chi^2$ values | Heterozygotes | | D |
|---|---|---|---|---|---|---|
| | | | | Observed | Expected | |
| EST-1 | 100 | 0.980 | | | | |
| | 95 | 0.020 | | 1 | 1.000 | 0.000 |
| EST-2 | 105 | 0.040 | | 7 | 0.086 | -0.104 |
| | 100 | 0.820 | | | | |
| | 95 | 0.140 | | | | |
| EST-3 | 100 | 0.042 | 47.022 | | | |
| | 95 | 0.958 | | 0 | 1.957 | -1.000 |
| GPD-1 | 100 | 0.960 | 49.021 | | | |
| | 95 | 0.040 | | 0 | 1.959 | -1.000 |
| GPI-2 | 100 | 0.080 | 32.711 | | | |
| | 95 | 0.920 | | 0 | 3.755 | -1.000 |
| PGM-1 | 100 | 0.040 | | | | |
| | 95 | 0.960 | | 2 | 1.959 | 0.021 |
| PGM-2 | 100 | 0.840 | | | | |
| | 95 | 0.160 | | 6 | 6.857 | -0.125 |

| | |
|---|---|
| $P_{0.95}$ | 15.0 |
| $H_O$ | 0.050 (0.020) |

genetic variation of *A. johnstoni* in the present study revealed a heterozygosity value of $H_O = 0.050$ and percentage polymorphic loci ($P_{0.95}$, 15%) which compare well with the average heterozygosity values reported for other fish species (H = 0.05 and P = 15.2 %) (Nevo et al., 1984; Buth et al., 1991; Kirpichnikov, 1992).

Deviations from expected Hardy-Weinberg proportions were observed at the **EST-3**, **GPD-1** and **GPI-2** loci. The deviations were as a result of a deficit of heterozygotes at these loci. Several other factors such as the number and type of loci studied, natural selection, small effective population sizes, migrations and population bottlenecks can also contribute to Hardy-Weinberg disequilibrium (Kirpichnikov et al., 1990). The presence or absence of alleles in different populations of the same species can give an indication whether a population has experienced a population bottleneck. Population bottlenecks of short duration have little effect on heterozygosity but can reduce the number of alleles in a population (Allendorf, 1986). However, loss of a specific allele in a population may influence the fitness of that population. The degree of survival of heterozygotes and homozygotes of allozyme phenotypes varies in accordance with external environmental stimuli such as pollutants (Mitton and Grant, 1984; Nevo, 1984) because the survival of a population depends on its ability to adapt to changing environmental conditions (Lande and Barrowclough, 1987).

*Aplocheilichthys johnstoni* can adapt to a wide range of habitats and may be found in rivers and floodplains (Skelton et al., 1985). It has been suggested that most species with numerous populations or with large effective population sizes, display high levels of heterozygosity (Kirpichnikov, 1992). Genetic variability, within or between populations, can enhance fitness within a particular habitat and promote colonisation by allowing persistence across a wider range of environments (Carvalho, 1993). The levels of genetic variation observed in *A. johnstoni* may contribute to this species' ability to inhabit a wide range of habitats.

The effect of indiscriminate human interference on the river systems is clearly illustrated in a report by Kleynhans (1986). For example *A. katangae* disappeared from the Apies River as a result of habitat destruction due to the extraction of water, use of pesticides and pollution. Both *A. katangae* and *A. johnstoni*, utilise neustonic organisms and are vulnerable to the use of insecticides and other pollutants. Excessive use of insecticides will have a negative impact on the survival of these species. The decline of the species in certain river systems also implies the eradication of a valuable biological control agent. The presence or absence of *A. johnstoni* in a river system can be used as an environmental health indicator and it provides nature conservationists with a useful tool when assessing the conservation status of river systems.

The results of the current study are the first to provide information on the genetic structure of *A. johnstoni*. This information is needed to establish a genetic database which can be used to make informed management decisions especially regarding translocations of this species since Johnston's topminnow is an important biological control agent. During the current investigation only 20 loci and 25 individuals were studied. However, Gorman and Renzi (1979) concluded that heterozygosity estimates are far more affected by the number of loci sampled than by the number of individuals and even with only 20 loci, they observed no significant differences in the heterozygosity values. We recommend analysis of the genetic structure of more populations of *A. johnstoni* from different localities, including the isolated populations from the Marico, Crocodile, Notwane and Levhuvu Rivers (Limpopo River system). This can provide a better understanding of the genetic structure and future management of Johnston's topminnow populations.

## References


ALLENDORF FW (1986) Genetic drift and the loss of alleles versus heterozygosity. *Zool. Biol.* **5** 181-190.

BELL-CROSS G (1972) The fish fauna of the Zambezi River system. *Arnoldia Rhod.* **5** (29)1- 19.

BUTH DG, DOWLING TE and GOULD J (1991) Molecular and cytological investigations. In: Winfield I and Nelson J (eds.) *Cyprinid Fishes Systematics, Biology and Exploitation*. Chapman and Hall, London.

CARVALHO GR (1993) Evolutionary aspects of fish distribution: Genetic variability and adaptation. *J. Fish Biol.* **43** (A) 53-73.

GORMAN GC and RENZI J (1979) Genetic distance and heterozygosity estimates in electrophoretic studies: Effects of sample size. *Copeia* **2** 242-249.

ENGELBRECHT GD and MULDER PFS (1999) Extremely high genetic differentiation between two populations of the river goby, *Glossogobius callidus* (Smith 1937). *Water SA* **25** (1) 85-90.

HARRIS H and HOPKINSIN DA (1976) *Handbook of Enzyme Electrophoresis in Human Genetics.* North-Holland Publishing Company, Amsterdam.

HILLIS DM and MORITZ C (1990) *Molecular Systematics*. Sinauer Associates, Massachusetts.

KIRPICHNIKOV VS (1992) Adaptive nature of intrapopulational biochemical polymorphism in fish. *J. Fish Biol*. **40** 1-16.

KIRPICHNIKOV VS, MUSKE GA, SCHOLL-ENGBERTS AD, CHERNOV VD and BORCHSENIUS SN (1990) Genetic structure and allele frequency dynamics in the sockeye salmon population of Lake Dalneye, Kamchatka. *Aquaculture* **84** 13-25.

KLEYNHANS CJ (1986) The distribution, status and conservation of some fish species of the Transvaal. *S. Afr. J. Wildl. Res.* **16** (4) 135-144.

LANDE R and BARROWCLOUGH GF (1987) Effective population size, genetic variation and their use in population management. In: Soulè ME (ed.) *Viable Populations for Conservation.* Cambridge Univ. Press, Cambridge.

LEVENE H (1949) On a matching problem arising in genetics. *Annu. Math. Stat.* **20** 91-94

MARKERT CL and FAULHABER I (1965) Lactate dehydrogenase patterns of fish. *J. Exp. Zool.* **159** 319-332.

MITTON JB and GRANT MC (1984) Associations among protein heterozygosity, growth rate and developmental homeostasis. *Annu. Rev. Ecol. Syst.* **15** 470-499.

NEI M (1987) *Molecular Evolutionary Genetics.* Columbia Univ. Press, New York.

NEVO E (1984) Genetic diversity in nature. Patterns and theory. *Evol. Biol.* **17** 217-246.

NEVO E, BEILES A and BEN-SHLOMO R (1984) The evolutionary significance of genetic diversity, ecological demographic and life history correlates. Evolutionary dynamics of genetic diversity. *Lecture Notes in Biomathematics* **53** 13-213.

RIDGWAY GJ, KLONTZ GW and LEWIS RD (1970) Polymorphism in the esterase of Atlantic herring. *Trans. Am. Fish. Soc.* **99** 147-155.

SHAKLEE JB, ALLENDORF FW, MORIZOT D and WHITT GS (1990) Gene nomenclature for protein-coding loci in fish. *Trans. Am. Fish. Soc.* **119** 2-15.

SKELTON PH (1993) *A Complete Guide to the Freshwater Fishes of Southern Africa.* Southern Book Publishers, Halfway House.

SKELTON PH, BRUTON MN, MERRON GS and VAN DER WAAL BCW (1985) The fishes of the Okavango drainage system in Angola, South West Africa and Botswana: Taxonomy and distribution. *Ichthyol. Bull.* **50** (50) 1-21.

SWOFFORD DL and SELANDER RB (1981) BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72** 281-283.

WHITT GS (1970) Developmental genetics of the lactate dehydrogenase isozymes of fish. *J. Exp. Zool.* **175** 1-35.