Sri Lanka J. Aquat. Sci. 4 (1999): 83-90

Microsatellites as Genetic Markers for Identification of Exotic Cichlids and Their Hybrids in Sri Lanka – A Preliminary Study

M. P. K. S. K. DE SILVA[1]*, N. BAKER[2] and P.B. MATHER[2]

[1]Department of Zoology, University of Ruhuna, Matara, Sri Lanka.
[2]Department of Natural Resources Science, Faculty of Science, Queensland University of Technology, 2 George Street, Brisbane, Q 4001, Australia.

*Corresponding author

Abstract

The reservoir fishery in Sri Lanka depends mainly on exotic tilapias, especially on two species, *Oreochromis mossambicus* and *O. niloticus*. These species are known to hybridize readily and uncontrolled hybridization in some reservoirs in Sri Lanka has resulted in adverse effects on stock performance and compromised the fishery in some places. This study therefore, was designed to trial microsatellites as genetic markers for determining the relative purity of different reservoir stocks and for assessing the relative bias of hybrid stocks towards *O. niloticus* or *O. mossambicus* alleles. If variation at microsatellite loci can be used to characterize the hybrid status of stocks and relate it with external morphological characteristics, then the markers can be used to evaluate relative stock performance in different reservoirs to determine if the differences in performance are related to genotype (bias towards *O mossambicus* or *O. niloticus* alleles) or differences in local environmental conditions.

Introduction

Introduced tilapia species namely, *Oreochromis niloticus* and *O. mossambicus* account for the major part of commercial catches (De Silva 1988) and thereby contribute significantly to the reservoir fishery of Sri Lanka. Apart from the above species *Tilapia rendalli* also has established populations in Sri Lankan reservoirs. The reproductive biology (De Silva & Chandrasoma, 1980; De Silva 1986), nutritional ecology (De Silva 1985a) and fishery of *O. mossambicus* (Chandrasoma 1986; De Silva 1985b; Amarasinghe & De Silva 1992a, 1992b) in Sri Lankan reservoirs have been well documented.

Natural hybridization appears to be a common phenomenon in fishes especially in freshwater and anadromous fish (Elder *et al.* 1971; Verspoor 1988; Campton 1988). Likelihood of hybridization is determined primarily by environmental factors and geological events. Recently human activities have also influenced the potential of hybridization (Avise & Van Den Avyle 1984). Natural hybridization is generally considered to be undesirable because species, sub species or populations may be modified or extirpated through gene introgression (Moreau 1986; Elder *et al.* 1971).

Natural hybridization is common among cichlids (Macaranas *et al.* 1986: Pante *et al.* 1989; Gregg *et al.* 1997). *O niloticus* and *O. mossambicus* are known to interbreed easily (Hickling 1971) and this process occurs in Sri Lankan *O. niloticus* and *O. mossambicus* populations too (De Silva & Ranasinghe 1989; Amarasinghe & De Silva 1996).

Amarasinghe & De Silva (1996) showed that hybridization between *O. niloticus* and *O. mossambicus* resulted in a male-dominant sex ratio and a decline in fecundity which may adversely affect the long term viability of the fishery. Furthermore, Moreau (1986) reported the collapse of an existing fishery based on *C. macrochir* in lake Itasy, Madagascar due to hybrid introgression of *O. niloticus* genes into the *O. macrochir* populations.

Therefore, precise identification of hybrids where they occur is a prerequisite for determining growth performance, reproduction, fecundity and mortality in order to define appropriate fishery management strategies.

Morphological methods for identifying hybrids suffer several shortcomings. Hybrids are usually assumed to be morphologically intermediate to the parental species, but this is often not the case (Campton 1990). Furthermore, the morphological characters are often influenced to varying degrees by environmental factors.

Biochemical and molecular genetic methods have been used extensively to detect natural hybridization between congeneric taxa of fish and shell-fish. Allozymes and mitochondrial DNA (mt DNA) have commonly been used in these studies as genetic markers (Verspoor & Hammer 1991; Macaranas *et al.* 1986).

DNA microsatellites, a recently developed molecular tool have proven to be useful genetic markers for addressing questions at a variety of scales such as determining gender (Longmire *et al.* 1993; Delehanty 1995), parentage (Amos *et al.* 1993; Kellog *et al.* 1995) and genetic structure of populations (Taylor *et al.* 1994; Dallas *et al.* 1995), and for establishing relationships among species including extent of hybridization (Roy*et al.* 1994).

Microsatellite loci which are also referred to as simple sequence length polymorphisms (SSLPs), simple sequence repeats (SSRs) or short tandem repeats (STRs), are regions of DNA containing tandem repeats of short sequence motifs, which occur abundantly in all eukaryotic genomes. Loci can be scored relatively easily using a combination of Polymerase Chain Reaction (PCR) amplification followed by electrophoresis to separate alleles which differ in length as a result of differences in the number of repeat units. The aim of the present study therefore was to identify microsatellite markers for pure exotic cichlid species and for hybrids between *O. mossambicus* and *O. niloticus* present in Sri Lanka.

Materials and Methods

Fifty three individuals from different reservoirs/water bodies in Sri Lanka namely, Udawalawa, Chandrikawewa, Badagiriya, Negambo estuary, Seguwantive, Ruhuna University canal and ponds were used in this study. Fish were caught either by gillnets (mesh size 6-12 cm) or hook-and-line. Specimens were brought to the laboratory live and were kept in fibreglass tanks until used for the investigation. Morphological and meristic characters were recorded from each individual and DNA samples were extracted from white muscle tissue (100 mg) as described by Hillis *et al.* (1990).

Microsatellite loci were amplified by the PCR using 50 ng of genomic DNA, 1.5 x T^{th} reaction buffer (Biotech), 2 mM dNTPs (dCTP labelled with 32P), 3 mM $MgCl_2$, 16 pmol of each primer, 0.02U T^{th} polymerase (Biotech); and dd H_20 to a volume of 20 ml (Renwick 1997).

Primers for two microsatellite loci UNH106 and UNH146 Research Genetics Map Pair Tilapia Primers were screened. Table 1 lists the primer sets used in this study, their DNA sequences, the size of the corresponding microsatellite locus, and type of microsatellite repeat sequence (Renwick 1997). DNA samples were amplified using the following cycles: 1

min at 94°C, 1 min at 55°C and 2 min at 72°C, for 33 cycles with a 10 min extension at 72°C (Renwick1997). Following amplification, PCR products were electrophoresed through a 5% denaturing polyacrylamide sequencing gel. Gels were dried for one hour and visualized using autoradiography. Two pure *O. niloticus* stocks of different origins (Israel and Chitralda) and two pure *O. mossambicus* (Australia and Indonesia) samples were also run as references in parallel with the Sri Lankan tilapia samples. As a pilot study, DNA from 10 individuals from Sri Lankan populations and DNA from pure *O. mossambicus* (3 individuals) and pure *O. niloticus* (2 individuals) of different origins were also subjected to PCR amplification with the UNH146 and UNH106 primers.

Table 1. *Oreochromis niloticus* primer specifications.

Locus (Primer name)	Primer sequence 1: Forward sequence 2: Reverse sequence	Size (bp)	Repeat sequence
UNH146	1:CCACTCTGCCTGCCCTCTAT 2:AGCTGCGTCAAACTCTCAAAAG	122	$(CA)_{10}$
UNH106	1:CCTTCAGCATCCGTATAT 2:GTCTCTTTCTCTCTGGTCACAAG	134	$(CT)_{13}(CA)_{20}$

Results

In this pilot study, DNA was successfully amplified and polymorphisms were detected at both loci. Pure *O. mossambicus* and *O. niloticus* individuals were fixed for different alleles at both loci, while both *O. mossambicus* and *O. niloticus* alleles were evident in the Sri Lankan samples. Heterozygotes however were absent in the Sri Lankan samples at UNH106. Therefore, the UNH146 locus was selected for further studies with the goal of developing a marker for determining the percentage of individuals that were hybrids.

Table 2. Number of homozygotes of each species and number of heterozygotes present in different reservoirs/waterbodies. Om - *O. mossambicus*; On - *O. niloticus*; Tr - *Tilapia rendalli*.

Reservoir/ water body	Total number of individuals	Number of Homozygotes			Number of Heterzygotes
		Om	On	Tr	
Badagiriya	9	2	1	0	6
Udawalawa	17	6	0	7	4
Chandrikawewa	13	1	0	11	1
University pond	2	2	0	0	0
University canal	2	2	0	0	0
Negambo estuary	6	6	0	0	0
Seguwantive	4	3	0	0	1
Total	53	22	1	18	12

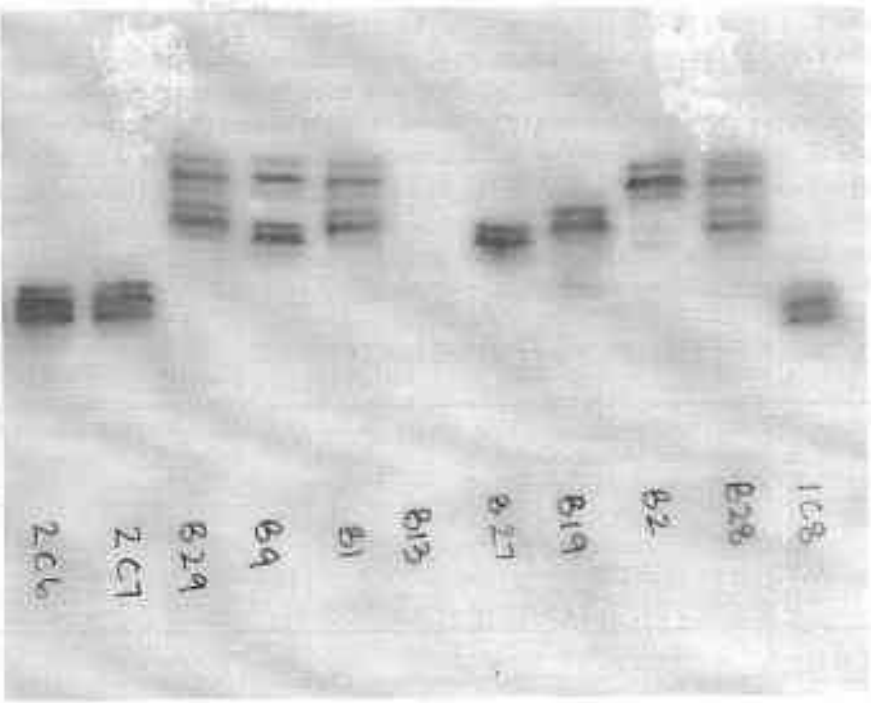

Fig. 1. Scan of autoradiograph of Locus UNH146.

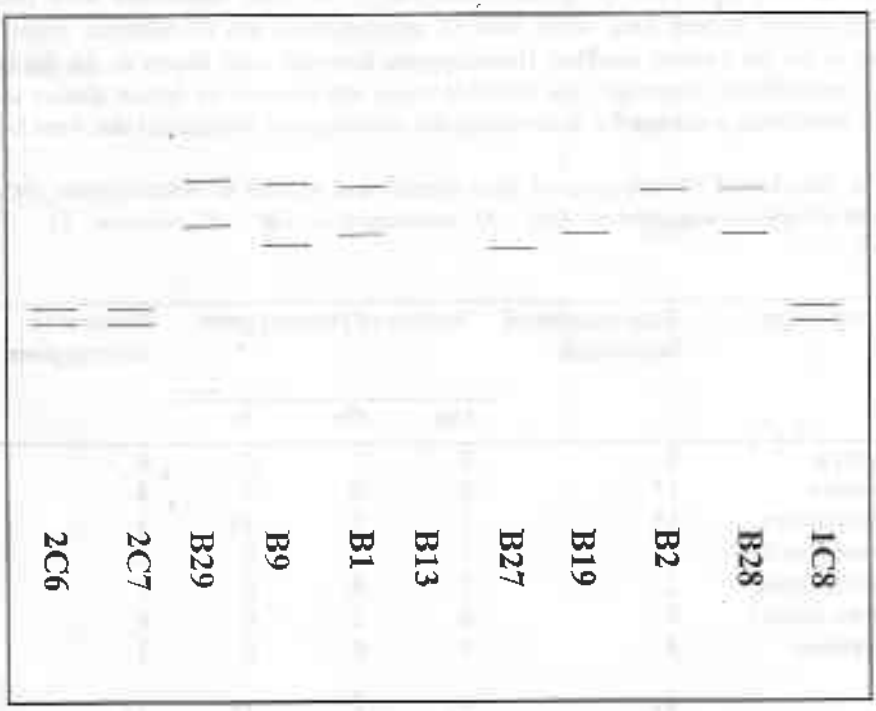

Fig. 2. Interpretation of Autoradiograph

DNA from 53 individuals representing different reservoirs/water bodies (Table 2) was amplified and all individuals were screened for allelic diversity. Although our sample size was small, seven alleles (a-g) were observed at the UNH146 locus (Figs 1 and 2). Figure 3 shows the allele frequency distributions at the UNH146 locus in the sampled Sri Lankan individuals.

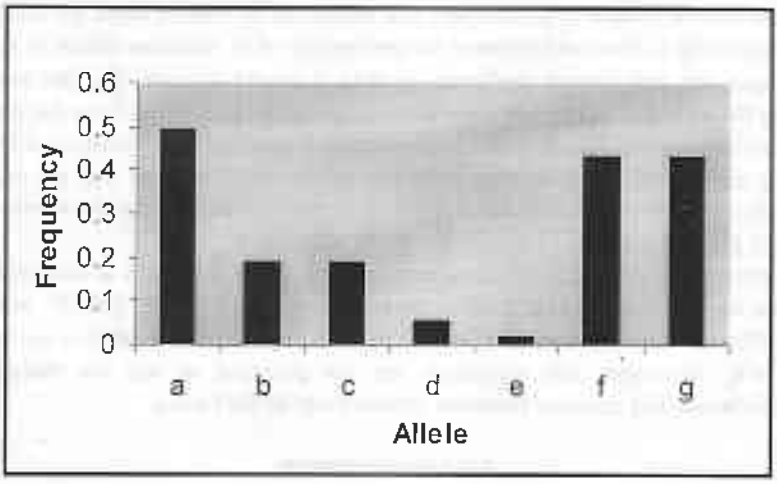

Fig. 3. Distribution of alleles in the sampled tilapia populations.

According to the autoradiographs, only one individual (B2) from Sri Lanka showed a pure *O. niloticus* phenotype (B2) while one heterozygote (3U4) had a different *O. niloticus* allele from the reference *O. niloticus* individuals. Three alleles were observed in *O. mossambicus* individuals. The other two alleles that showed comparatively slower migration than the *O. mossambicus* and *O. niloticus* alleles could be assigned to *Tilapia rendalli* after considering morphlogical features of those individuals. All *T rendalli* individuals were monomorphic. Table 2 shows the number of homozygotes and heterozygotes detected in each reservoir after screening for allelic diversity.

Discussion

O. niloticus X *O. mossambicus* hybrids are important components of the commercial catch in freshwater reservoirs in Sri Lanka. Variation in relative productivity among reservoirs may be correlated with differences in the relative 'mix' of *O. mossambicus* and *O. niloticus* alleles in the various reservoir stocks. If this proves to be true, then developing an index of the extent of hybridization and determining whether it is biased

towards *O. niloticus* or *O. mossambicus* alleles may provide an approach for managing cichlids stocks in reservoirs of Sri Lanka to improve their long term productivity.

The current study while only preliminary, examined the potential for using microsatellite allelic diversity for identifying pure and hybrid cichlids in seven Sri Lankan waterbodies. Unique microsatellite alleles at the UHN146 locus (Renwick 1997) were identified in pure *O. niloticus*, *O. mossambicus* and *T. rendalli* reference individuals. This permitted unambiguous determination of the relative number and distribution of *O. niloticus*, *O. mossambicus* or *T. rendalli* alleles in each sampled stock and thereby developing a Hybrid Index. A detailed study could therefore assess the relative ratio of *O. niloticus* to *O. mossambicus* alleles in each impoundment and relate this to relative stock performance. If a positive relationship is observed between the percentage of *O. niloticus* alleles in stocks from different reservoirs and growth performance then it would indicate that the major factor contributing for its performance is not variation in environmental conditions but genotype. If however, performance of stocks with a high relative frequency of *O. niloticus* alleles varied significantly among lake environments then this result would suggest that the major factor affecting differential performance was not genotype but variation in reservoir environmental conditions (e.g. relative levels of trophic status/productivity).

Thus, results of this pilot study confirm that allelic variation at microsatellite loci can be used to differentiate the relative percentage of *O. niloticus* and *O. mossambicus* alleles in different reservoir stocks of exotic cichlids. Provided that relative performance is correlated with genotype, this technique has the potential to aid the management of freshwater fisheries and enhance reservoir productivity in Sri Lanka.

Acknowledgements

Financial support of the Australian Centre for International Agricultural Research (ACIAR) and the Crawford Fund for International Agricultural Research, logistic support of Professor S.S. De Silva (Deakin University, Australia) and Professor U.S. Amarasinghe (University of Kelaniya, Sri Lanka) are gratefully acknowledged.

References

Amarasinghe U.S. & S.S. De Silva 1992a.
Empirical approaches for evaluating efficiencies of different fishing methods in tropical, shallow reservoirs: a Sri Lankan case study. In: Reservoir Fisheries of Asia (S.S. De Silva ed.). pp. 217-227. International Development Research Centre, Ottawa.

Amarasinghe U.S. & S.S. De Silva 1992b.
Population dynamics of *Oreochromis mossambicus* and *O. niloticus* (Cichlidae) in two reservoirs in Sri Lanka. Asian Fisheries Science 5: 37-61.

Amarasinghe U.S. & S.S. De Silva 1996.
Impact of *Oreochromis mossambicus* X *O. niloticus* (Pisces: Cichlidae) hybridization on Population reproductive potential and long-term influence on a reservoir fishery. Fisheries Management and Ecology 3: 239-249.

Amos B., C. Schotterer & D. Tautz 1993.
Social structure of pilot whales revealed by analytical DNA profiling. Science 260: 670-672.

Avise J.C. & M.J. Van Den Avyle 1984.
Genetic analysis of reproduction of hybrid white bass X striped bass in the Savannah river. Transactions of the American Fisheries Society 113: 563.

Campton D.E. 1988.
Natural hybridization and introgression in fishes. In: Population Genetics and Fishery Management. (N. Ryman & F. Utter eds). pp. 181-192. University of Washington Press, Seattle.

Campton D.E. 1990.
Application of biochemical and molecular markers to analysis of hybridization. In: Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management (D.H. Whitmore ed.). pp. 240-264. CRC Press Boca Raton, FL.

Chandrasoma J. (1986) Trends in reservoir fisheries in Sri Lanka with special reference to *Etroplus suratensis*, *Tilapia rendalli*, and *Oreochromis niloticus* In: The First Asian Fisheries Forum (J.L. Maclean, L.B. Dizon & L.V. Hossilos eds). pp. 353-356. Asian Fisheries Society, Manila.

Dallas J.F., B. Dod, P. Boursot, E.M. Prager & F. Bonhomme 1995. Population subdivision and gene flow in Danish house mice. Molecular Ecology 4: 311-320.

Delehanty D.J. 1995.
Genetic and morphological methods for gender identification of Mountain quail. Journal of Wildlife Management 59: 785-789.

De Silva C.D. & J. Ranasinghe 1989.
Biochemical evidence of hybrid gene introgression in some reservoir populations of tilapia in southern Sri Lanka. Aquaculture and Fisheries Management 20: 269-277.

De Silva S. S & J. Chandrasoma 1980.
Reproductive biology of *Sarotherodon mossambicus*, an introduced species, in an ancient man-made lake in Sri Lanka. Environmental Biology of Fishes 5: 253-259.

De Silva S.S. 1985a.
Body condition and nutritional ecology of *Oreochromis mossambicus* (Pisces, Cichlidae) populations of man-made lakes in Sri Lanka. Journal of Fish Biology 27: 31-47.

De Silva S.S. 1985b.
Status of the introduced cichlid *Sarotherodon mossambicus* (Peters) in the reservoir fishery of Sri Lanka: a management strategy and ecological implications. Aquaculture and Fisheries Management 16: 91-102.

De Silva S.S. 1986.
Reproductive biology of *Oreochromis mossambicus* populations of man-made lakes in Sri Lanka: a comparative study. Aquaculture and Fisheries Management 17: 31-47.

De Silva S.S. 1988.
Reservoirs of Sri Lanka and their fisheries. FAO Fisheries Technical Paper 298: 128 p.

Elder H.Y., D.J. Garrod & P.J.P. Whitehead 1971.
Natural hybrids of the African cichlid fishes *Tilapia spirulus nigra* and *T. leucosticta*: a case of hybrid gene introgression. Biological Journal of Linnean Society 3: 103-146.

Gregg R.E., J.H. Howard & F. Shonhiwa 1997.
Introgressive hybridization of tilapias in Zimbabwe. Journal of Fish Biology 52: 1-10.

Hickling C.F. 1971.
Fish Culture. Faber and Faber, London. 317 p.

Hillis D.M., A. Larson, K.S. Davis & A.E. Zimmer 1990.
Nucleic Acids III. In: Sequencing in Molecular Systematics (D. M. Hillis & C. Mortiz eds). Sinauer Associates Inc., Sunderland, Massachusetts.

Kellogg K.A., J.A. Markert, J.R. Stuffer & T.D. Kocher 1995.
Microsatellite variation demonstrates multiple paternity in lekking cichild fisher from Lake Malawi, Africa. Proceedings of the Royal Society, London B 260: 79-84.

Longmire J. L., M. Maltbie, R.W. Pavelka, L.M. Smith, O A. Ryder, D.E. Ellsworth & R.J. Baker 1993.
Gender identification in birds using microsatellite DNA fingerprinting analysis. Auk 110: 378-381.

Macaranas J. M., N. Taniguchi, M.J.R. Pante, J. Capili & R.S.V. Pullin 1986.
Electrophoretic evidence for extensive hybrid gene introgression into commercial *Oreochromis niloticus* (L) stocks in the Philippines. Aquaculture and Fisheries Management 17: 249-288.

Moreau J. 1986.
Inland fisheries in Madagascar. In: Reports and Papers presented at Indo-Pacific Fishery Commission Expert Consultation on Inland Fisheries of the Larger Indo-Pacific Islands, Bangkok, Thailand, 4-9 August 1986 (T. Petr ed.). FAO Fisheries Report 371 (Supplement):.72-103. FAO, Rome.

Renwick M.J. 1997.
Correlations between growth performance and microsatellite allelic variation in cultured *Oreochromis niloticus* stocks. Unpublished Honours Thesis, Queensland University of Technology, Brisbane, Queensland.

Roy M.S., E. Geffern, D. Smith, E.A. Ostrander & R.K. Wayne 1994b.
Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution 11: 553-570.

Taylor A.C., W.B. Sherwin, & R.K. Wayne 1994.
Genetic variation of microsatellite loci in a bottlenecked species: The northern hairy nosed wombat *Lasiorhinus krefftii*. Molecular Ecology 4: 277-290.

Trewavas E. 1983.
Tilapiine Fishes of the Genera *Sarotherodon, Oreochromis* and *Danakilia*. British Museum (Natural History), London. 583 p.

Verspoor E. 1988.
Widespread hybridization between native Atlantic salmon, *Salmo salar* and Introduced brown trout, *Salmo trutta*, in Eastern Newfoundland. Journal of Fish Biology 32: 327-334.

Verspoor E. & J. Hammer 1991.
Introgressive hybridization in fishes: the biochemical evidence. Journal of Fish Biology 39 (Supplement A): 309-334.