# Aquaculture Strategy For Restoration of Threatened Lake Victoria Fishes: The case for *Oreochromis variabilis* (Boulenger, 1906) and *Labeo victorianus* (Boulenger, 1901)

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## Abstract

The endemic *Oreochromis variabilis* and *L. victorianus* are among Lake Victoria's most threatened fish species whose population sizes are on the decline. This study, carried out between August 2000 and April 2001, aimed at characterizing the 'refugia' ecosystems of *O. variabilis* and its growth performance in small water bodies (SWBs), developing artificial spawning techniques and characterizing existing morphological and genetic variation of extant populations of *L. victorianus* in order to bring the species under aquaculture as a restoration strategy. Studies of *O. variabilis* were carried out by comparing its growth performance in stocked semi-intensive and modified extensive closed systems in different ecological zones within the Lake Victoria basin. Growth in *O. variabilis* evaluated as average growth rates, was satisfactory in both systems and eco-zones, even in areas with extreme environmental gradients. The isometric characteristics of *O.variabilis* estimated by formula $W = aL^b$ were better than those of wild populations in the 'refugia' ecosystems in each respective eco-zone. Multivariate analysis of morphological data showed that there was reasonable differentiation between *L. victorianus* populations from different drainages, with the southern populations being most distinct. Majority of the variation in *L. victorianus* was within populations (91.3%), with an overall $F_{ST}$ of 0.08846 for all loci. For effective aquaculture and conservation, fish breeders should use local fish material for their stocking programs; yet ensure that different age classes form part of their brood-stock. *L. victorianus* was spawned artificially using intramuscular injection of *Clarias gariepinus* pituitary extracts (C.g.PE) and Human chorionic gonadotropin (HcG) to induce ovulation. Successful inducement of ovulation occurred only in trials with C.gPE. Fertilization rates in breeding experiments for *L. victorianus* averaged 86% and hatching percentages 70%. This study indicates the viability of the two species for culture in the basin. More hope is therefore raised for expansion on the farming practices in the basin. Such a fisheries production can provide 75% of the animal protein requirements of the poor rural households and guarantee continued survival of the species within the basin. Stocking of small water bodies for increased fish production enhances further, the integrated resource use and management of the endemic but threatened Lake Victoria fish stocks.

**Key words:** *Oreochromis variabilis*, *Labeo victorianus,* genetic variation, restoration, ecology, small water bodies.

## Introduction

Throughout all known fisheries history, both open-access and culture fisheries, native food fishes evolve to become delicacies and part of daily diet for the resource adjacent communities. Cultivation of endemic fishes will, however, require technologies and strategies that utilise or maximise environmental productivity in order to guarantee sustainability by the often resource-poor rural human populations.

*Oreochromis variabilis* is presently listed in the World conservation union (IUCN) Red Book of endangered species (Maithya, 1998: Kaufman, 1992). Similarly the riverine *Labeo victorianus* (Pisces: Cyprinidae), locally known as '*ningu',* from Lake Victoria has recently experienced serious declines in population size (Whitehead, 1958; Cadwallad, 1965; Benda, 1979; Ogutu-Ohwayo, 1990). Both fish species are endemic to Lake Victoria. Their disappearance from commercial and subsistence landings of the Lake Victoria fishery has been explained by several authors as due to predation by Nile Perch (*Lates niloticus*), competition for food by Nile tilapia (*O. niloticus*) environmental degradation including pollution, over-fishing and destructive fishing methods (Cadwallad, 1965; Balirwa and Bugenyi, 1980; Ogutu-Ohwayo 1990; Greboval and Mannini 1992). This study is a concerted effort to bring the species under culture practice and restore them to the market place. While *O. variabilis* breeds easily under culture practice, the *L. victorianus* suffers a major constraint due to its inability to spawn naturally in captivity. Development of artificial spawning techniques, production of the fingerlings and their subsequent stocking in small water bodies and other larger water masses including Lake Victoria is the only opening likely to bring about their restoration.

Successful aquaculture of any fish species will depend on the existence of genetically diverse stocks since long-term adaptability of populations is dependent upon a base of genetic variation with which to respond to environmental or biotic novelties (Fisher, 1930). The use of information on morphological and life history variability, on distributions and on stock structure, enables proper utilization and management of fish during the culture period. This study aimed at characterizing the different ecosystems playing 'refugia' to *O. variabilis* and its growth performance in small water bodies (SWBs) and characterizing existing morphological and genetic diversity of extant populations of *L. victorianus* in Lake Victoria basin and to develop artificial spawning techniques in order to bring the species under aquaculture. This study was conducted between August 2000 and April 2001 after a successful reconnaissance mission.

## Materials and methods

### Reconnaissance studies for characterization process

For identification and selection of study sites, a 10 days' reconnaissance study was carried out in Lake Victoria and its basin from 4$^{th}$ to 14$^{th}$ August 2000. Evaluation of physical, chemical and biological integrity of water bodies was done in the field. Physical and chemical parameters were obtained by use of relevant field equipment at each sampling site before netting for fish activity started. Small water bodies were studied through observation of water mass characteristics and collection and identification of aquatic organisms. To establish the existence or otherwise of *O. variabilis* and *L. victorianus* in Lake Victoria, small water bodies and in the rivers, electro-fishing, gill netting (at least 6 hours minimum) and beach seining was done using nets of 30 mm mesh size (Knot to knot) or 30 mm code-end mesh size. The study area was within the Kenyan part of Lake Victoria basin and is shown in figure 1.

a) *Oreochromis variabilis*

Ecological studies were conducted monthly in each research site. SWBs were used as experimental plots. Sampling units were created by employing 20 x 30m stratified transects in randomized complete block design. Samples were collected monthly from September 2000 to April 2001 using gillnets of mesh sizes 30-230 mm nominal bar length. A sub-sample was randomly selected from the total sample. For each sub-sample length and weight of each individual fish were measured immediately after capture. Total length was measured to the nearest mm and total wet weight to the nearest g. The parameters *a* and *b* of the length- weight relationship ($W = aL^b$) were computed using the Least Square method (Draper and Smith, 1966) and the method of Ricker (1975) and Pauly (1984) for all sub-samples of *O. variabilis* for each water body. This enabled estimation of isometric characteristics of the populations. The sex, fecundity, maturity stage, stomach fullness and contents were determined. Size classes per capture were also determined. Environmental parameters pH, dissolved oxygen, temperature, water hardness and turbidity were measured *in situ.* The population structure and environmental gradients were then analyzed and compared with those from stocked water bodies in each eco-zone.

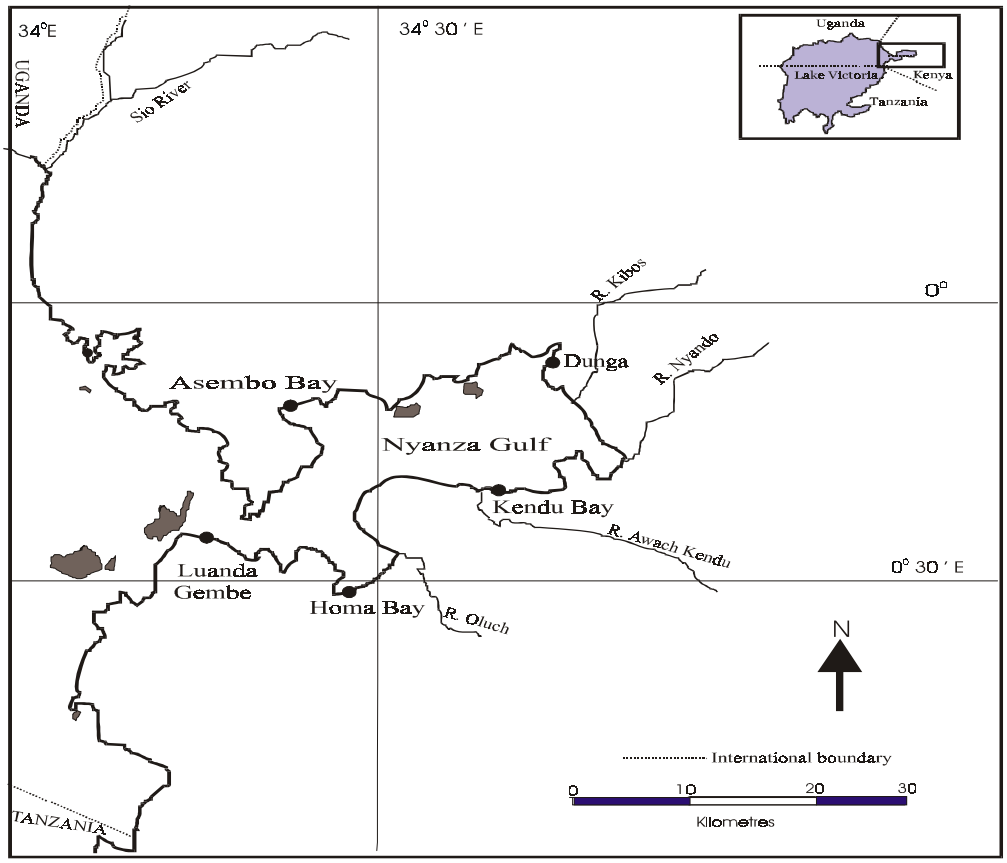


Figure 1. Map of Nyanza Gulf, Lake Victoria showing sampling sites

Fish seed for stocking ponds and SWBs were sourced from hatchery ponds at KMFRI Sangoro laboratory. Stocking of experimental plots started in October 2000 and ended in

November 2000. Fingerlings of *O. variabilis* used for stocking were 3.9 $\pm$ 1.25 SD g average weight and 4.7 $\pm$ 2.16 SD cm mean length. The experimental design was aimed at determining production levels in pond model and SWB model in terms of growth rates, condition and maximum attainable size (g) of *O. variabilis* at each recruitment size. SWB culture systems were selected on the basis of ecological zonation to represent effect of climatic regimes. Experimental stocked plots include Oki SWB, Maranda and Ochot SWBs (low altitude), Lorida Fish Farms and Suka SWB (mid altitude) and Wanyonyi Fish Farm (high altitude). Stocking densities ranged from 240 to 10,380 fingerlings to represent a rate of 1-2 fish per $m^2$ depending on size of the culture system. Size of experimental SWBs ranged from 900$m^2$ - 16000$m^2$ while experimental ponds are 150 – 600 $m^{2.}$ The stocked pond populations were fed on a starter diet (prepared according to the method of MacGrath, 1976 and Dupree, 1976) for one month while those in SWBs relied on natural productivity of the water mass. SWBs relied entirely on catchment area for fertilization and renewal of plankton growth. Ponds were fertilized with cattle dung at the rate of 3 kg and 5 kg per pond fortnightly.

To investigate the production potential of *O. variabilis* in SWBs random samples were taken from stocked SWBs through monthly harvests with replacements. Total length and wet weight of each fish individual in the samples were determined. Drag netting using mosquito mesh size nets was used for sampling the stocked plots.

**a) *Labeo victorianus***

Morphological and genetic studies

*L. victorianus* samples for genetic studies were obtained from rivers Nzoia, Sondu-Miriu, Kuja, Yala and Nyando, which are shown in Figure 1. A total of 133 ningu samples from eight sites were chosen for this study. From each specimen, morphometric and meristic characters were recorded. These included standard length, total length, maximum body depth, head length, head width, eye diameter and number of scales on lateral line and fin ray counts on dorsal and anal fins. Fish were dissected on-site and organs transported to the laboratory in dry ice and stored at $20^0$ C until analysis. Liver and muscle extracts were prepared by grinding the tissue and zymograms obtained by migration on 11% starch gels using horizontal gel electrophoresis. The following enzymes were examined: lactate dehydrogenase (LDH), aspartate aminotransferase (AAT), superoxide dismutase (SOD), alcohol dehydrogenase (ADH), esterase (EST), acid phosphatase (ACP), alkaline acid phosphatase (ALP), phosphoglucomutase (PGM), malate dehydrogenase (MDH), mannose-6-phosphate isomerase (MPI), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fructose-6-diphosphate (FDP), glycerol-3-phosphate dehydrogenase (G3PDH), creatine phosphokinase (CPK), glucose-6-phosphate isomerase (GPI), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), isocitrate dehydrogenase (IDH), glutamate dehydrogenase (GLUDH), sorbitol dehydrogenase (SDH), and catalase (CAT). Buffer systems and enzyme stain recipes followed Murphy *et al.,* (1990), Shaw and Prasad (1970), and Pasteur *et al.,* (1988) with slight modifications where necessary.

Fish from different populations differed in total length (one-way analysis of variance, $F_{7, 181} = 10.07$, p=0.000). In morphometric studies, populations should be compared in shape variables without the effects of variation in size (Reist, 1986). The effects of size on morphometric characters such as head length, head width, eye diameter and body depth were therefore removed by calculating linear regressions for each character against standard length (Reist, 1986). The regression residuals (shape variables) were then used as response variables in a principal component analysis (PCA) and multivariate linear discriminant analysis with the software program MINITAB® for Windows (version 12.21). At first, sites were used as groups, later sites were lumped together into a northern group consisting of Yala and Nzoia, a central group consisting of Miriu, Awach and Machine and a southern group consisting of Ranen, Riana and Migori as shown in Figure 1. Grouping of sites in the northern and central groups was based on proximity to each other except for Awach and Machine, which are both tributaries of river Nyando. Tests of genetic differentiation between populations and pair-wise $F_{ST}$ values were calculated using the internet-based program GENEPOP (Raymond and Rousset, 1995). A hierarchical analysis of molecular variation (AMOVA) was done using the Arlequin program (Schneider *et al.,* 1997), sites being grouped according to drainages and proximity to each other as done for the morphological studies above.

**Breeding studies**

Mature females for breeding experiments were selected by observing their abdomen. Females that had swollen and soft abdomen, fully distended ovipositors and which released eggs on applying gentle pressure on their abdomens were selected for the experiments while males were selected based on their release of white milt when gentle pressure was applied on their abdomen. Females were injected intramuscularly either once or twice with pituitary extracts of the African catfish *Clarias gariepinus* (C.g.PE) or Human chorionic gonadotropin (HcG). Controls were injected with 2 ml of normal saline. The second injection when applied was administered 12 or 13 hours later. Fish were observed for signs of ovulation after every two hours. At appropriate time eggs were stripped into dry enamel bowls and fertilized with milt pooled from three mature running males. Eggs were incubated in trays placed in cages in a slow moving (0.1 m/s) part of the river (Charo and Oirere 2000). The appropriate incubation densities were determined by recording the maximum egg diameters reached 1 hour after incubation (Table 14). These were incubated at very low densities to ensure that they had enough space for expansion. Fertilization success was determined as the percentage of eyed eggs and hatching success as the percentage of eggs that hatched.

## Results and discussion

### Reconnaissance study

A total of 250 dams and several ponds and rivers were visited. Major dams characterized included Ongoro, Oki, Oyombe, Kosiga, Marrum, Mukuyu, Suka Ebiruga, Winjo, Tito Kochere, Nyakisese, Komondi, Nyabuhanze, Nyansiongo, Kijauri, Emityot, Gesebei, Mamboleo, Ndubai, Kaparuso, Maranda, Ochok, Tinga- Mwer, Ulanda (Kaugage) Kalenjuok, Ochilo, Mauna, Yenga, Chwele, Marinda, Kewa, Baharini and Kesses dams. The rivers visited included Nzoia, Sondu-Miriu, Kuja, Yala, Oluch, Awach, Sio and Nyando.

A checklist of the potential food items found in the water column is presented in table 7. Most of the plankton populations found in the water column form major food items for *O. variabilis* and *L. victorianus*. This means that the propagation of the two species in SWBs (including rivers) and the main lake will require minimal supplemental feeding. Rural communities who earn their livelihood from these SWBs will require low inputs to achieve high yields. Restoration of the two species is therefore less costly after stocking with fingerlings.

Table 1 A comparison between condition of reared and wild population of *O. variabilis* in different ecozones of Lake Victoria basin and refugia ecosystem of the lake.

| **Stock Type** | **Ecozone/Aquazone/L. Refugium** | **Condition (b coefficient)** |
|---|---|---|
| Reared | Low altitude | 2.71 - 2.91 |
| wild | | 2.27 - 3.54 |
| Reared | Mid altitude | 2.32 - 3.38 |
| wild | | 2.12 - 3.80 |
| Reared | High altitude | 2.14 - 2.75 |
| wild | | 2.22 - 3.15 |
| Reared | Lake refugia | - |
| wild | | 2.10 - 3.78 |

Table 2 Growth rates after one month of stocking

| **Experimental plot** | **Eco-zone** | **Growth rate (g/day)** |
|---|---|---|
| Wanyonyi unit | High altitude | 0.43 |
| Suka unit | Mid altitude | 0.51 |
| Lorida units | | 0.54 |
| Maranda and Ochok units | Low altitude | 0.65 |
| Oki unit | | 0.78 |

Ecological studies in refugia systems showed that males of *O. variabilis* were found to be more numerous than females with sex ratio of 4:1 respectively. Sex ratios in *L. victorianus* differed within different river systems with generally more males than females. Average sex ratio was 3:2 (males to females respectively). First maturity in males occurred at 14.2 cm average length while size of first brooding females ranged from 15.1 - 19.0 cm with fecundity range of 51 to 4294. Length at first maturity for *L. victorianus* averaged 7.3 cm for females and 7.9 cm for males while fecundity ranged from 20,712 to 65, 955. Shallow and rocky inshore areas where primary productivity was very high provided major refugia habitats for *O. variabilis* in Lake Victoria while *L. victorianus* occurred mainly in rocky and stony parts of the rivers with periphytonic algae where they foraged.

Table 3 Growth rates after four months of stocking

| Experimental plot | Ecozone | Growth rate (g/d) |
|---|---|---|
| Wanyonyi unit | High altitude | 0.33 |
| Suka unit | Mid altitude | 0.48 |
| Lorida units | | 0.33 |
| Maranda and Ochok units | Low altitude | 0.50 |
| Oki unit | | 0.63 |

**Growth studies on *Oreochromis variabilis***

Differential trends in length and weight were determined by comparing the condition of stocked and wild populations in the refugia ecosystems in the three aquazones. The results are shown in Table 1. It is observed that there is better isometric condition in the stocked than the wild populations in the low and middle altitude of Lake Victoria catchment. The condition of the stocked population is also better than that of the lake refugia population (Table 1) *O. variabilis* tends to assume allometric growth condition as the catchment altitude increases. The condition is significantly higher in low and mild altitude than in higher altitude (P=0.05). Tables 2 and 3 show growth rates in two-month stepwise analysis between period of stocking and last period of evaluation. These growth rates were used as the principal determinants of differential trends in growth patterns due to effect of aquazonation. Overwhelming evidence indicate that lower altitude stocked populations attained higher growth rates (g/day) than those in mid and higher altitude. The difference between growth rates in mid and higher altitude is not significant (t=44.083; p=0.05).

Environmental gradients driving the ecozonations are shown in Table 5. It is shown that temperature and dissolved oxygen are limiting environmental gradients in aquazones and fish growth. Table 6 shows the phytoplankton food of *O. variablilis*. It has been demonstrated that this fish species feeds on a great variety of food plankton. *Anabaena*

*spp*, diatoms and green algae are more common food items in all ecological regions. Aquaculture zonation as demonstrated in this study is necessary for describing the interplay between environments, species and system choice. This is in conformity with West (1996) who reports that the degree of success in achieving full potential is determined by the prevailing ecological conditions.

Table 4. A comparison between population of stocked and wild populations of *O. variabilis*

| ECOZONE | EXPERIMENTAL PLOT | STOCK TYPE | RECRUITED SIZE CLASS (CM) | ATTAINED AVERAGE WET WEIGHT (g) ± SD |
|---|---|---|---|---|
| Low altitude | Oki farm | Stocked | 10.0-15 | 42.7 ± 13.08 |
| | | | 15.1-20.0 | 72.5 ± 30.19 |
| | Oele beach | Wild | 10.0-15.0 | 54.45 ± 11.88 |
| | | | 15.1-20.0 | 94.8 ± 9.83 |
| | Yenga dam | Wild | 10.0-15.0 | 46.67 ± 10.37 |
| | | | 15.1-20.1 | 99.17 ± 25.71 |
| Medium altitude | Suka farm | Stocked | 10.0-15.0 | 51.36 ± 14.17 |
| | | | 15.1-20.0 | 89.17 ± 32.14 |
| | Komondi dam | Wild | 10.0-15.0 | 55 ± 14.91 |
| | | | 15.1-20.0 | 121 ± 20.61 |
| High altitude | Wanyonyi farm | Stocked | 10.0-15.0 | 38.95 ± 20.24 |
| | | | 15.1-20.0 | - |
| | Wabukhonyi dam | Wild | 10.0-15.0 | 43 ± 11.69 |
| | | | 15.1-20 | 85 ± 26.14 |

*Labeo victorianus*

(i) **Morphological and genetic variation**

The principal component analysis resulted in four components with eigen values of more than 1.00. The first three components explained over 50% of all morphological variation (Table 8). The first principal component (PC1) was composed of body depth and eye diameter, the second principal component (PC2) was composed of head length, head width and weight while the third component (PC3) consisted of number of rays on dorsal and anal fins. PC4 consisted of number of scales on lateral line. However, analysis of variation of the regression residuals was non-significant: body depth, eye diameter ($F_{7,159}$=0.00, P= 1.000), head length, head width ($F_{7,159}$=0.01, P= 1.000), and weight ($F_{7,159}$=0.002, P=1.000). The discriminant analyses of morphological data results are shown for populations and groups in Table 9 and 10 respectively. In the between population analysis, the classification accuracy of the discriminant function was low with the proportion of correctly assigned individuals ranging from between 0% for Yala and Miriu

to 55% for Ranen with a mean of 21.9% for the entire sample. The within group classification was low for populations in the central group (19.1%) and for the northern group (38.2%) but was relatively high (67.2%) for the southern group, with a mean of 40.6% for the three groups.

Table 5 Characterization of major environmental gradients influencing growth and population characteristics of *O. variabilis* in Lake Victoria catchment

| **Ecozone** | **Environmental** | **Gradient** |
|---|---|---|
| Low altitude | Temperature | 26-30.6$^0$c |
| | Dissolved oxygen | 6.5-9.8mg/l |
| | PH | 6.92-8.8 |
| | Water hardness | 50.1-130mg/l $CaCO_3$ |
| | Turbidity | 72.9-375.0 NTU |
| Middle altitude | Temperature | 20.1-26.8$^0$c |
| | Dissolved oxygen | 5.1-7.6mg/l |
| | PH | 5.0-7.8 |
| | Water hardness | 40.0-86mg/l $CaCO_3$ |
| | Turbidity | 53.2-188.7NTU |
| High altitude | Temperature | 18.5-24.9$^0$c |
| | Dissolved oxygen | 4.2-7.2mg/l |
| | PH | 5.4-7.4 |
| | Water hardness | 75.0-256mg/l $CaCO_3$ |
| | Turbidity | 69.7-367NTU |
| Lake Victoria refugia systems | Temperature | 27.5-30.7$^0$c |
| | Dissolved oxygen | 5.3-7.2mg/l |
| | PH | 7.4-8.3 |
| | Water hardness | 86.0-92.3 mg/l $CaC0_3$ |
| | Turbidity | 69.4-100.8NTU |

Studies on species of *Labeo* have shown that there is considerable overlap in meristic and morphological variables across species (Reid, 1985). For example *Labeo umbratus* and *L. capensis* from two localities (Van Vuuren *et al*., 1990), showed a great deal of overlap in body shape variables within and between both species. This overlap in characters was also evident in this study. Nevertheless, body depth; eye diameter and head parameters could explain most of the morphological variation. Although body depth may reflect fecundity in female fish, there was no significant variation in fish from different populations and therefore the observed value in the PCA reflects true morphological variation. In Africa and Asia, species of *Labeo* that live in swift waters have characteristic features, which include small eyes (Reid, 1985). Thus observed eye diameter differences may be more reflective of environmental rather than genetic differences although according to Reid (1985), small eyes do not confer any immediate

hydrodynamic advantages. Head morphology reflects the feeding habits of a fish species (Pakkasmaa and Piironen, 2001), and may indicate subtle differences in diet of fish from

Table 6: Phytoplanktonic food of *O. variabilis*

| **Ecozone/Ecosystem** | ***Commonly identified food item*** |
|---|---|
| Lake refugia | **Aulacosira** |
| | *Microcystis sp* |
| | *Melosira* |
| | *Anabaena* |
| | *Oscillatoria* |
| | *Scenedesmus* |
| Low altitude | *Ankistrodesmus* |
| | *Euglena* |
| | *Microcystis* |
| | *Anabaena* |
| | *Oscillatoria* |
| | *Nitzschia* |
| | Discoid diatoms |
| Mid altitude | Diatoms |
| | *Oscillatoria* |
| | *Chlorococcus* |
| | *Euglena* |
| | **Anabaena** |
| | *Scenedesmus* |
| High altitude | *Anabaena* |
| | *Oscillatoria* |
| | Euglenoid flagellates |
| | *Selanastrum* |

different sites. So far, studies on the feeding habits of *L. victorianus* have indicated that its diet consists of algae and detritus material (Cadwallad, 1965, Ochumba and Manyala, 1992); but since no attempt has been made to identify the algal species involved, it is now impossible to relate different populations to particular diets, although such a relationship is likely. To answer this question, further study on foraging behavior and diet may be necessary.

In the investigation of allozyme variation, 12 enzymes coding for 26 presumptive loci could be scored (Table 11). Of these, 8 were found to be polymorphic and their allele frequencies are presented in Table 12. A hierarchical analysis of molecular variance (AMOVA) in which sites were grouped into the three groups: southern, central and northern, indicated that 91.15% of the total genetic variability was due to variation within sites with an overall $F_{ST}$ of 0.08846 for all

loci. A matrix comparison of genetic and morphological distances showed a positive but non-significant correlation (r = 0.334, $P$ = 0.089).

ii) **Artificial breeding of *Labeo victorianus***

Successful inducement of ovulation occurred in all trials involving the use of C.g.PE but did not occur in the case of HcG or in the controls. In C.g.PE, it took a total of 20 hours for the fish to completely ovulate. The amount of hormones administered, weight of fish, fertilization and hatching percentages are presented in Table 13. Fertilization rates ranged from 75-95%, which are acceptable considering that some of the males may have been unripe since they were not treated with hormones.

Hatching began at 39 hours after fertilization with live embryos occurring only where two injections of C.g.PE were administered. This time after hatch (39 hours) is consistent with hatching times for *Labeo umbratus* as reported by Bok (1987) but conflicts with Fryer and Whitehead (1959) who reported a time of first hatch of 45 hours after fertilization. The water temperature during incubation was 21.8 to 23.6°C. Temperature range for experiments by Fryer and Whitehead (1959), who incubated *L. victorianus* eggs collected from the wild in irrigated troughs, was between 23 and 25°C, which is higher than the present study. Since temperature under normal circumstances reduces first time of hatch, it is necessary that specific experiments be done to understand the effect of temperature on hatching times of *L. victorianus* eggs.

The hatching percentages of eggs incubated in river water ranged from 50 to 90% with an average of 70%. These were more consistently high when compared with findings by other studies on egg incubation of cyprinids. For example Mills (1980) found 0% hatching rates when he incubated Dace, *Leuciscus leuciscus* eggs in river water probably due to siltation but got an average of 94% hatching successes in re- circulated spring water. Although ovulation occurred in females injected once with C.g.PE, none of the eggs hatched successfully. A number of eggs developed to the stage where optical vesicles and notochord appeared but did not elongate even though they hatched after 48 hours. The second injection appears to be necessary for hatching of live fully formed fry.

Egg diameters before induction of ovulation, and one hour after treatment are shown in table 14. It has been suggested that a single layer of eggs with little egg contact is ideal for hatching. The number of eggs that can fit into an incubation tray should be calculated based on the maximum diameter of the eggs one hour after incubation because by this time the eggs will have reached their maximum diameters. Based on the egg diameters the best incubation diameters are approximately 3.5 $mm^2$/egg i.e. to incubate 100 eggs on a tray will require at least 18.7 x 18.7 mm space. Higher egg densities during incubation

Table 7. Checklist of common phytoplankton found in the small water bodies

| Species name | |
|---|---|
| *Anabaena circinalis* | *Microcoleus subtorulosus* |
| *Anabaena flos-aquae* | *Microcystis aeruginosa* |
| *Anabaena spiroides* | *Microcystis marginata* |
| *Ankistrodesmus falcatus* | *Microcystis viridis* |
| *Aphaizomenon flos-aquae* | *Nitzchia holsatica* |
| *Asterionella formesa* | *Nitzchia sigmoidea* |
| *Asterionella gracillina* | *Nostoc kihlmani* |
| *Bacillus* | *Nostoc linckia* |
| *Beggiatoa sp.* | *Oscillatoria tenuis* |
| *Bulbochaeta intermedia* | *Oscilltoria putrida* |
| *Chodatella armata* | *Peritriches* |
| *Chromatium okenii* | *Phacus caudatus* |
| *Cylindrospermum stagnale* | *Phacus pleuronectes* |
| *Cymbella caespitosa* | *Plectonema tomasiniamana* |
| *Cymbella cistula* | *Pseudomonas hylina* |
| *Epithemia turgida* | *Rivularia sp.* |
| *Euglena oxyuris* | *Siderocapsa major* |
| *Euglena tripteris* | *Siderocapsa treubii* |
| *Flagilaria crotonensis* | *Sideromonas confervarum* |
| *Fragilaria constuens* | *Sphacrotilus natans* |
| *Gallionella ferruginea* | *Spirillum granulatum* |
| *Gloeocapsa turgida* | *Surirella biseriata* |
| *Gloeotila contorta* | *Synedra actinastriodes* |
| *Lamprocystis rosea-persicina* | *Synedra acus* |
| *Lamprosystis roseo-persicina* | *Thiobacillus thioparus* |
| *Leptothrix crassa* | *Thiocystis violacea* |
| *Leptothrix ochracea* | *Thiodictyon elegans* |
| *Leptothrix sideropous* | *Thiopedia rosea* |
| *Lyngbya nyassae* | *Thiophysa macrophysa* |
| *Melosira ambiqua* | *Tolypothrix lanata* |
| *Melosira italica* | *Trachelomonas rugulosa* |
| *Merismopedia glauca* | *Uroglena volvox* |
| *Merismopedia tenuissima* | *Zoogloea ramigera* |

appear to have a negative effect on the realized hatching successes by reduction of aeration and accelerated fungal attacks.

Table 8. Principal Component analysis for all the variables

| Variable * | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Maximum body depth | **-0.668** | -0.134 | 0.090 | -0.068 |
| Head length | 0.156 | **-0.511** | 0.362 | -0.264 |
| Head width | 0.116 | **-0.544** | 0.424 | 0.060 |
| Eye diameter | **-0.669** | -0.111 | 0.134 | -0.104 |
| Weight | 0.100 | **-0.427** | -0.159 | 0.575 |
| Scales on lateral line | -0.215 | 0.069 | 0.062 | **0.739** |
| Rays on dorsal fin | 0.108 | 0.244 | **0.541** | 0.171 |
| Rays on anal fin | 0.027 | 0.406 | **0.585** | 0.074 |
| Eigenvalue | 2.050 | 1.432 | 1.085 | 1.044 |
| % variance | 25.6 | 17.9 | 13.6 | 13.0 |
| Cumulative % variance | 25.6 | 43.5 | 57.1 | 70.1 |

*Analysis for the size dependent variables is based on the correlation matrix. A total of eight Principal Components were computed, the components shown here are those with eigenvalues higher than 1. The largest correlation coefficients for each variable are in bold

The use of common carp pituitary (CPE) and Human chorionic gonadotropin (HcG) to induce ovulation in cyprinids, although successful, has several drawbacks (Lin and Peter, 1991). These drawbacks include their high costs and for HcG, the problems of storage. In our case, HcG could not induce ovulation even after increasing the hormonal strength to 5000 iu and may be useful only after solving the problem of dosage. The use of pituitary gland from locally available and more abundant fish like *C. gariepinus* is much more cost effective and can effectively replace CPE. C.g.PE could be developed into an important source for hormone for *L. victorianus* and other related cyprinids in the region just as is the case for common carp pituitary extracts.

The hatched *L. victorianus* fry have shown very high survival rates of up to 70% in the first 80 days post-hatch being fed solely on phytoplankton. Given its high fecundity of up to 162,000 and its high growth rate (Cadwallad 1965, Fryer and Whitehead 1959), ningu like all other *Labeos* is likely to do well under aquaculture conditions. The role of captive breeding in the conservation and aquaculture of this species is crucial. Since *in situ* breeding programmes that do not remove fish from their natural environment are more desirable than those that introduce un-natural sources of physical stress (Vrijenhoek,

1998), the successful *in situ* trials give an appropriate option for carrying out restocking programmes.

Table 9. Discriminant analysis showing proportion of individuals correctly assigned to each population. Average proportion for individuals correctly assigned for the entire sample is in bold.

| Population | Awach | Machine | Miriu | Migori | Ranen | Riana | Nzoia | Yala |
|---|---|---|---|---|---|---|---|---|
| Awach | 6 | 4 | 8 | 3 | 4 | 6 | 1 | 3 |
| Machine | 1 | 10 | 7 | 3 | 4 | 4 | 2 | 4 |
| Miriu | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Migori | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| Ranen | 3 | 7 | 6 | 6 | 11 | 7 | 3 | 7 |
| Riana | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |
| Nzoia | 4 | 5 | 6 | 1 | 1 | 6 | 6 | 5 |
| Yala | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 14 | 26 | 28 | 14 | 20 | 24 | 12 | 22 |
| Correct | 6 | 10 | 0 | 1 | 11 | 1 | 6 | 0 |
| Proportion | 0.429 | 0.385 | 0.000 | 0.071 | 0.550 | 0.042 | 0.500 | 0.000 |
| **Total correct proportion** | **21.9%** | | | | | | | |

The results of this study indicated low genetic differentiation among different drainages yet showed an appreciable amount of morphological variation *in L. victorianus*, which could be used as a basis for management and aquaculture. The genetic structure observed, although weak, implies that for successful breeding and conservation, fish breeders should use local fish material for their stocking programs, yet ensure that different age classes form part of their brood stock. This study has shown that more studies involving other molecular techniques, a larger and more widespread samples collected over several years are advisable. Furthermore there is reason to carry out comparative growth studies on fish from the three morphologically distinct groups to investigate if this distinctiveness may be genetic.

## The potential of small water bodies as restoration sites

Potential for small-scale fisheries and for restoration of *O. variabilis* and *L. victorianus* in and around SWBs in the Lake Victoria basin is greatly unrecognised and undervalued. SWBs could provide over 75% of the animal protein requirements of the rural households (Nasser, 1999). It is therefore important that these water bodies are well stocked and their fisheries managed and that developmental activities explicitly consider their impact on the overall inland fisheries. This is a little studied fishery resource, which could contribute significantly to the fishery of Kenya's inland waters. The number of species of plankton realised from these water bodies is a clear indication that small water bodies can

be useful production units for the two species. The higher growth condition values observed in the smaller water bodies may suggest that they have comparatively higher primary productivity that can support a higher biomass of *O. variabilis.*

Table 10. Discriminant analysis showing proportion of individuals correctly assigned to each group. Average proportion for individuals correctly assigned for the three groups are in bold.

| Group | Central | Southern | Northern |
|---|---|---|---|
| Central | 13 | 11 | 8 |
| Southern | 33 | 39 | 13 |
| Northern | 22 | 8 | 13 |
| Total (n) | 68 | 58 | 34 |
| N correct | 13 | 39 | 13 |
| Proportion | 0.191 | 0.672 | 0.382 |
| **Total correct proportion** | **40.6%** | | |

Table 11. Enzymes that had sufficient activity, their enzyme commission numbers, tissue they were stained from and the buffer, which gave clearest resolution. In the tissue column, L refers to liver and M refers to muscle. Buffers: TCE (Tris citrate EDTA. Electrode/gel pH 7), Tris HCl-(electrode pH 8.2/ gel pH 8.5), RW(Tri-lithium-citrate borate- electrode pH:8.1/ gel pH 8.3).

| Protein | Locus | E.C. No. | Tissue | Buffer |
|---|---|---|---|---|
| Esterase | EST | 3.1.1.1 | L | Tris HCl |
| Glyceraldehyde-3-phosphate | GAPDH | 1.2.1.12 | M | TCE |
| dehydrogenase | LDH | 1.1.1.27 | M | Tris HCl |
| Lactate dehydrogenase | GLUDH | 1.4.1.2 | L/M | RW |
| Glutamate dehydrogenase | G3PDH | 1.1.1.8 | L/M | RW |
| Glycerol-3-phosphate dehydrogenase | ADH | 1.1.1.1 | L | RW |
| Alcohol dehydrogenase | GPI | 5.3.1.9 | L/M | RW |
| Glucose-6-phosphate isomerase | PGM | 5.4.2.2. | L/M | Tris HCl |
| Phosphoglucomutase | ME | 1.1.1.40 | M | RW |
| Malic enzyme | MDH | 1.1.1.37 | M | RW |
| Malate dehydrogenase | G6PDH | 1.1.1.49 | L/M | RW |
| Glucose-6-phosphate dehydrogenase | ACP | 3.1.3.2 | M | RW |
| Acid phosphatase | | | | |

## Conclusion

SWBs in Kenya are numerous and an under - utilized resource for fish production. Culture of indigenous food fishes such as *O. variabilis* in these ecosystems can play an important role in improving the food and nutrition security of rural population. The most appropriate and available techniques to achieve full aquaculture potential should include species introductions and stockings into small water bodies and new

Table 12. Allele frequencies for polymorphic loci across 8 sites in five rivers in Lake Victoria basin.

| | | **Nyanza Gulf** | | | | **River Kuja** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Northern open lake** | | | | | | | | | |
| Site | Awach(1) | Machine (2) | Miriu (3) | Migori (4) | Ranen (5) | Riana (6) | Nzoia (7) | Yala (8) | |
| n | 14 | 26 | 28 | 14 | 20 | 24 | 12 | 22 | |
| | Allele | | | | | | | | |
| *GAPDH-2* | 100 | 0.893 | 0.942 | 0.857 | 0.929 | 0.611 | 0.938 | 0.917 | 1.000 |
| | 90 | 0.107 | 0.058 | 0.143 | 0.071 | 0.389 | 0.062 | 0.083 | 0.000 |
| *MDH-1* | 110 | 0.357 | 0.750 | 0.796 | 0.214 | 0.775 | 0.604 | 0.792 | 0.864 |
| | 100 | 0.643 | 0.250 | 0.204 | 0.786 | 0.225 | 0.396 | 0.208 | 0.136 |
| *LDH-1* | 100 | 0.857 | 0.904 | 0.750 | 0.964 | 0.825 | 0.875 | 0.750 | 0.659 |
| | 90 | 0.143 | 0.096 | 0.250 | 0.036 | 0.175 | 0.125 | 0.250 | 0.341 |
| *EST-1* | 100 | 0.857 | 0.769 | 0.679 | 0.929 | 0.700 | 0.771 | 0.833 | 0.786 |
| | 95 | 0.143 | 0.231 | 0.321 | 0.071 | 0.300 | 0.229 | 0.167 | 0.214 |
| *EST-2* | 100 | 0.286 | 0.077 | 0.232 | 0.179 | 0.375 | 0.326 | 0.125 | 0.300 |
| | 95 | 0.714 | 0.827 | 0.768 | 0.821 | 0.625 | 0.674 | 0.875 | 0.675 |
| | 90 | 0.000 | 0.096 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 |
| *GPI-2* | 110 | 0.714 | 0.846 | 1.000 | 0.857 | 0.950 | 0.667 | 0.667 | 0.659 |
| | 100 | 0.286 | 0.154 | 0.000 | 0.143 | 0.050 | 0.333 | 0.333 | 0.341 |
| *PGM-1* | 105 | 0.643 | 0.404 | 1.000 | 1.000 | 0.425 | 0.750 | 1.000 | 0.614 |
| | 100 | 0.143 | 0.385 | 0.000 | 0.000 | 0.225 | 0.250 | 0.000 | 0.227 |
| | 95 | 0.214 | 0.212 | 0.000 | 0.000 | 0.350 | 0.000 | 0.000 | 0.159 |
| *ADH* | 100 | 0.607 | 0.615 | 0.714 | 0.964 | 0.650 | 0.833 | 0.458 | 0.477 |
| | 85 | 0.393 | 0.385 | 0 .286 | 0.036 | 0.350 | 0.167 | 0.542 | 0.523 |
| *Ho* | | 0.091 | 0.102 | 0.087 | 0.059 | 0.091 | 0.092 | 0.105 | 0.099 |
| *He* | | 0.129 | 0.119 | 0.126 | 0.065 | 0.144 | 0.093 | 0.104 | 0.148 |

Number beside site name refers to the sites as indicated on figure 1., n = number of individuals sampled at each site; Ho = observed heterozygosity.

impoundments. Economically viable aquaculture production however, is dependent on the equilibrium between environment quality and choice of culture system. Using *Oreochromis variabilis* as the test species SWBs in the Lake Victoria basin have proved viable extensive culture systems.

Growth performance of stocked *O. variabilis* in semi-intensive and modified extensive closed systems in different ecological zones within the Lake Victoria basin was satisfactory in both systems, even in areas with extreme environmental gradients. This indicates the viability of the species for culture in the basin. The successful artificial breeding of *L. victorianus* together with the acquired information on genetic and morphological variation is an important step towards the achievement of sustainable utilization and management of the species. The fact that *L. victorianus* and *O. variabilis* are both herbivores and the high primary productivity of the small water bodies shows that there is little requirement for supplemental feeding if these water bodies are fertilized. More hope is therefore raised for expansion on the farming practices in the basin. The small water bodies can be transformed into extensive culture systems with fingerlings of *O. variabilis* and *L. victorianus* being stocked into SWBs to support a diversified inland fisheries production. Such a fisheries production can provide 75% of the animal protein requirements of

Table13. Hormonal treatment applied, fertilization rates and hatching percentages in ningu eggs incubated in river water.

| | Hormonal application | | Fertilization rates (%) | Hatching success (%) |
|---|---|---|---|---|
| | Treatment 1 (6.30 p.m.) | Treatment 1 (7.00a.m) | | |
| Group 1 | C.g.PE, | C.g.PE, | 86 | 70 |
| Group 2 | C.g.PE, 1.8mg | - | 80 | 0 |
| Group 3 | Hcg | HcG | 0 | 0 |
| Group 4 | HcG | - | - | - |
| Group 5 | Saline (controls) | Saline | - | - |

Table 14. Egg sizes of *Labeo victorianus* at different stages of induced ovulation and incubation.

| | Egg diameters | | | |
|---|---|---|---|---|
| | Before induction | After 1st treatment | After 2nd treatment | 1hour after incubation |
| Mean egg size | 0.67 | 0.67 | 0.68 | 1.85 |
| Std deviation | 0.05 | 0.05 | 0.04 | 0.37 |
| Minimum size | 0.6 | 0.62 | 0.64 | 1.2 |
| Maximum size | 0.7 | 0.7 | 0.72 | 2.4 |

the resource-poor rural households as well as well as guaranteeing continued survival of the species. Since "necessity is the mother of invention," the innovative evolutionary progression in aquaculture should have more emphasis on diversification of farming practices. Man-made impoundments for water storage for domestic, irrigation, industrial and livestock uses are rapidly becoming a more commonplace feature in Kenya. Stocking of these water bodies for increased fish production of threatened and endangered fish species enhances further the integrated resource use and management of the Lake Victoria ecosystem.

**References**


Allendorf, F.W., and S. R. Phelps, (1981). The use of allelic frequencies to describe population structure*Canadian Journal of Fish and Aquatic Sciences* **38**: 1507−1514.

Balirwa, J.S., and F. B. B. Bugenyi, (1990). Notes on the fisheries of the River Nzoia, Kenya. *Biological Conservation* **18**: 53−58.

Benda, L.C. (1979). Analysis of data from 1968-1976 from nine fish landings in the Kenyan waters of Lake Victoria. *Journal of Fish Biology* **15**: 385−387.

Bok, A.H. (1987). Induced spawning of moggel, *Labeo umbratus* (Smith) (Pisces: Cyprinidae). J. Limnol. S. Africa **13**: 23-24.

Cadwallad, D.A. (1965). Notes on the breeding biology and ecology of *Labeo victorianus*. *Review of Zoology and Botany of Africa* **72**: 109 −134.

Charo, H. and W. Oirere, (2000). River-based artificial propagation of The African catfish *Clarias gariepinus:* an option for the small fish farmer. Naga, ICLARM Q**. 23**(1): 14-16.

Cummings, S.A., E. L. Brannon, K. J. Adams, and G. H. Thorgaard, (1997). Genetic analyses to establish captive breeding priorities for endangered snake river sockeye salmon. *Conservation Biology* **11**: 662**–**669.

Draper, N., and H. Smith, (1966). *Applied regression analysis.* John Wiley sons inc. New York.

Dupree, H. K., (1976). Studies on nutrition and feeds of warm water fishes. *Aqua. Nutri.*

Fisher, R.A., (1930). *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford.

Fryer, G. and P. J. P. Whitehead, (1959). The breeding habits embryology and larval development of *Labeo victorianus* Boulenger (Pisces Cyprinidae). *Review of Zoology and Botany of Africa*. **59**: 33–49.

Greboval, D. and P. Mannini, (1992). The Fisheries of Lake Victoria: Review of Basic Data. *UNDP/FAO Regional Project. Inland Fisheries Planning.* RAF/87/099/WP/16/92. FAO, Rome.

Greenwood, P. H., (1966). *The fishes of Uganda*. Uganda Society, Kampala.

Hartl, D. L. and A. G. Clark, (1997). *Principles of population genetics*. 3rd Edition. Sinauer Associates Inc, Sunderland, Massachusetts.

Hauser, L., G. R. Carvalho, and T. J. Pitcher, (1998). Genetic population structure in the Lake Tanganyika sardine *Limnothrissa miodon*. *Journal Fish Biology* **53**:413–429.

Holopainen, I. J.; J. Aho, M. Vornanen, and H. Huuskonen, (1997). Phenotypic plasticity and predator effects on morphology and physiology of crucian carp in nature and in the laboratory. *Journal Fish Biology* **50**: 781–798.

Jorde, P.E., and N. Ryman, (1995). Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics* **139**: 1077–1090.

Kaufman, L. (1992). Catastrophic changes in species rich fresh water ecosystems. *Bioscience*. **42** (ii) pp846-858

MacGrath, W. S. Jr. (1976). The role of the feed industry in developing formulated feeds for aquaculture. *Aqua. Nutri*. pp 119-123

Maithya, J., (1998). A survey of the Itchthyofauna of Lake Kanyoboli and other small water bodies in Kenya: Alternative Refugia for Endangered Fish species *NAGA* .ICLARM. Quarterly Jan-March 1998, pp54-56.

Mills, C. A., (1980). Spawning and rearing eggs of the Dace, *Leuciscus leuciscus* (L.). *Fish Management* **11**(2): 67-72.

Murphy, R. W., J. W. Sites, D. G. Buth, and C. H. Haufler, (1990). Proteins 1: Isozyme electrophoresis. – In: Hillis, D.M., & Moritz, C., (eds). *Molecular Systematics.* Sinauer Associates, Sunderland.

Nasser, A. K., (1999). Length-weight relationship of Tuna Baitfish from the Lakshad weep Islands, INDIA *Naga,* the ICLARM QUARTERLY,Vol. **22**.No. 4

Ochumba, P. B. O. and J. O. Manyala, (1992). Distribution of fishes along the Sondu-Miriu River of lake Victoria, Kenya with special reference to upstream migration, biology and yield. *Aquaculture and Fish Management***. 23**:701–719.

Ogutu-Ohwayo, R., (1990). The decline of native fishes of L. Victoria and Kyoga (E.Africa) and the impact of introduced species the Nile perch, *Lates niloticus* and Nile tilapia, *Oreochromis niloticus. Environmental Biology of Fish* **27**: 81–96.

Pakkasmaa, S. And J. Piironen, (2001). Morphological differentiation among local trout (*Salmo trutta)* populations. *Biological Journal of the Linnaean Society* **72**:231–239.

Pasteur, N., G. Pasteur, F. Bonhomme, J. Catalan, and J. Britton-Davidian, (1988). *Practical Isozyme Genetics.* Ellis Horwood Limited, Chichester.

Pauly, D. (1984). *Fish population dynamics in tropical waters: a manual for use with programmable calculators.* ICLARM. Stud. Rev. pp 65-74

Raymond, M., and F. Rousset, (1995). GENEPOP version 1.2: Population genetics software for exact tests and ecumenicism. *Journal Heredity* **86**:248–249.

Reid, G.M. (1985). *A revision of African species of Labeo (Pisces: Cyprinidae) and a re-definition of the genus.* Braunschweig Verlag Von J. Cramer, pp.322.

Reist, J. D. (1986). An empirical evaluation of coefficients used in residual and allometric adjustment of size variation. *Canadian Journal of Zoology* **64**:1363–1368.

Ricker, W. E., (1975). Computation and interpretation of biological statistics of fish populations. *Bul. Fish. Res. Board Can.* 191. Ottawa, 282p.

Schneider, S., J. M. Kueffer, D. Roessli, and L. Excoffer, (1997). *Arlequin vers. 2.0. A software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva.

Shaw, C.R. and R. Prasad, (1970). Starch gel electrophoresis of enzymes- a compilation of recipes. *Biochemical Genetics* **4**: 297–320.

Van Vuuren, N. G., P. F. S. Mulder, J. T. Ferreira, and F. H. Van der Bank, (1990). A morphometric and electrophoretic analysis of *Labeo capensis* and *Labeo umbratus* from two localities in southern Africa. Water *SA* **16**:135–146.

Vrijenhoek, R.C. (1998). Conservation of freshwater fish. *Journal of Fish Biology* **53**:394-412.

Weir, B.S. and C. C. Cockerham, (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**:1353–1370.

West, W. Q. B., (1996). The status of aquaculture in Africa. Aquaculture in Africa. *Proceed. 4th inter-Africa committee on Oceanography.* Pp. 42 – 70.

Whitehead, P. J. P., (1958). Indigenous river fishing methods in Kenya. *East African Agricultural Journal* **24**:111.