THE REPRODUCTIVE BIOLOGY AND ARTIFICIAL BREEDING OF NINGU *LABEO VICTORIANUS* (PISCES: CYPRINIDAE)

SUBMITTED IN FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY

OF

RHODES UNIVERSITY

By

Justus Rutaisire

FEBRUARY 2003

Sexually mature male *Labeo victorianus* from; A, Kagera River and B Sio River

DEDICATION

To all those who endeavour to make the world a better place to live in.

TABLE OF CONTENTS

SHORT TITLES OF TABLES

SHORT TITLES OF FIGURES

ACKNOWLEDGEMENTS

The completion of this thesis would not have been possible without the contributions of many individuals and organisations who unreservedly gave their time, knowledge, moral and financial support. Although not all are mentioned here, they are not less acknowledged. I am particularly grateful to my supervisor Dr Tony Booth for his constructive criticism from the inception of the research project to the last sentence of this thesis. His ingenuity and desire for perfection guided me through the unknown. I am short of words to express my gratitude to him; all I can say is thank you. Dr Ruth Muwazi is greatly acknowledged for overseeing my work in Uganda and Dr Horst Kaiser is thanked for his academic advice and logistical support.

I am grateful to Makerere University for granting me study leave and for supporting my study at Rhodes University. The Andrew Mellon Foundation, administered through Rhodes University, and the Lake Victoria Environmental Management Project through Fisheries Resources Research Institute provided financial support. The following people provided invaluable administrative support; Prof. Eli Katunguka, Dean of the Faculty of Veterinary Medicine, Prof. John Opuda-Asibo Director, School of Postgraduate Studies and Dr Christine Dranzoa, Head of Department, Wildlife and Animal Resources (all from Makerere University), and Prof. Peter Britz, Head of Department, Department of Ichthyology and Fisheries Science and Mr John Gillam, Financial Aid Officer (from Rhodes University). I am also grateful for the moral support I received from my colleagues at the Department of Wildlife and Animal Resources Management who include, but are not restricted to,

Irene Naigaga, Michael Ocaido, Ludwig Siefert, Godfrey Kamugisa and Kenneth Okello.

Mr Godfrey Kityo, Kajansi Aquaculture Research Station, and Ms Solome Namirimu provided additional technical support, while Ms Shirley Pinchuck, and Mr Robin Cross guided me through the electron microscopy process. Maretha Potgieter and Dr Irene Naigaga proof-read the thesis.

I have greatly appreciated the company of the following postgraduate students at Rhodes University: Emmanuel Kaunda, Imran Shiran, Daniel Babikwa, Monica Mwale, Irene Naigaga, Sloans Chimatiro, Mark Simpson, Warren Potts, John Radull, Oscar Mwangi, Carol Simon, Niall Vine, Christine Kisamba, Russel Chalmers, Moses Rotich, Alister Munthali. Your jokes and advice made my stay at Rhodes worthwhile. The services of Ms Phumeza, library of the South African Institute of Biodiversity and Ms Faith Faithful, the DIFS, are also gratefully acknowledged.

Special thanks go to my wife Milly and my children Enock and Esther for their support and for bearing with me during the many times I was away from home to pursue this study programme.

Above all is the Almighty God; I give thanks to him for it is he who has brought me thus far.

ABSTRACT

Lake Victoria, the largest tropical lake, has faced an unprecedented assault on its ecosystem through a variety of anthropomorphic causes that include the introduction of Nile perch *Lates niloticus* and over-fishing. As a result there have been species extinctions and declining fish population trends. This thesis explores options for reversing these declining population trends and the restoration of collapsed fisheries based on indigenous non-cichlid species. A candidate species was chosen - the cyprinid fish *Labeo victorianus*.

This thesis studied various aspects of *L. victorianus*' reproductive biology in two geographically distant populations. These included general reproductive patterns in relation to proximate environmental conditions, gonadal development and recrudescence, sex development, characterisation of genetic and morphological diversity, and induced spawning. Fish from both populations were found to be potamodrometic, and highly fecund, iteroparous spawners. *L. victorianus* was also shown to be an undifferentiated gonochorist, where all individuals pass through an intersexual juvenile stage prior to differentiation, and maturation to either sex. Sexual maturity was attained at a significantly larger size within the Kagera River than that of Sio River fish – possibly as a response to genotypic and/or phenotypic differences. Spawning seemed to be synchronised with rainfall in Kagera River - a pattern that was not strictly adhered to with the Sio River. Thorough microscopic investigation of recrudescence patterns indicated there was uninterrupted spawning in fish from the Kagera River followed by Type I oocyte atresia. In contrast, there was a 90% spawning failure, as characterised by Type II oocyte atresia, within the Sio River population. Aspects of spermatogenesis and sperm ultrastructure using light- and ultramicrotomic methods are described. Although the populations had varying reproductive biology parameters and were morphologically distinct, they remained undifferentiated at the mitochondrial level. Both populations were characterised by low nucleotide diversity – a feature attributed to a bottleneck event. The option of captive breeding was explored by conducting induced spawning experiments. Success was only achieved with a decapeptide Gonadotropic Releasing Hormone ([D-Arg^6,

Pro^{9}-NEt])-sGnRH) in combination with a water-soluble dopamine receptor antagonist metoclopramide.

This thesis stresses the importance of a research-oriented approach in the conservation of Lake Victoria's indigenous fish resources. It was concluded that information needed for the development of management policies can be generated within a reasonably short time period, of approximately three years, with modest levels of funding support.

CHAPTER ONE

General Introduction

The fisheries resources of Uganda

Lake Victoria and other fresh water bodies provide a large resource base for Uganda. Of the 241 500 km^2 total surface area of the country, 36 280 km^2 (*ca* 15%) is open water and 5 180 km^2 (*ca* 2.2%) occupied by wetlands. This freshwater resource supports a socio-economically important fisheries sub-sector contributing $ 45 million in 1996 to the Ugandan Gross Domestic product (NEMA, 1998). It is estimated that 75,000 people are directly employed in this sub-sector with *ca* 500,000 people indirectly employed in fisheries related activities, (MAAIF, 1995). Of all the water bodies in Uganda, the fisheries sub-sector heavily depends on Lake Victoria, which contributes nearly half of the total national fish catch (Table 1.1).

Table 1.1 Ugandan annual fish catch by water body (1995 - 1999). Data from the Fisheries Department, MAAIF, Statistical Abstract (2000).

	Catch ('000 tonnes)				
Water body	1995	1996	1997	1998	1999
Lake Victoria	103.0	106.4	106.6	105.2	111.4
Lake Albert	16.4	21.9	19.1	19.1	19.5
Albert Nile	4.7	4.6	3.4	3.5	3.7
Lake Kyoga	80.2	80.6	80.1	80.2	79.3
Lake Edward, Lake George and Kazinga Channel	5.2	4.8	6.4	5.6	5.8
Other waters	3.7	3.7	3.7	3.5	3.7
Total	213.2	222.0	219.3	217.1	223.4

The main economic activities of the communities around Lake Victoria, and its catchment, are small-scale agriculture and fishing with fish contributing significantly to protein security. According to NEMA (1998) fish was the highest animal protein consumed per capita at 13 kg, and provides 50% of the country's total animal protein

intake. On the whole, however, animal proteins are not adequate and there is widespread malnutrition especially among children. According to NEMA (1998), 45% of the children below three years of age are stunted, 23% underweight and 2% are wasted due to chronic food insecurity. Fish is a high biological value animal protein that has the potential to supplement the nutritive value derived from starchy meals common among the African rural poor.

Lake Victoria

Lake Victoria, the world's largest tropical freshwater lake, provides a source of livelihood for over 30 million people through fishing, agriculture and transport industries (Ochumba, 1994). Thirteen fish families inhabit the lake and can be divided into six cichlid and fifteen non-cichlid genera (Ogari, 1992). At the turn of twentieth century, the lake had a high species diversity, with over 300 species of endemic cichlid species (van Oijen *et al*., 1981; Goldschmidt *et al*., 1993; Witte *et al.*, 1992a; 1992b). Almost all the trophic levels were occupied, and each habitat was considered occupied with each to having a unique haplochromine community (van Oijen *et al*., 1981; Witte *et al*., 1992b).

During the second half of the twentieth century – concomitant with the introduction of Nile perch *Lates niloticus* – the lake ecosystem was disrupted resulting in considerable changes in limnological parameters and ichthyofaunal composition. Studies noted dramatic declines in fish diversity (Ogutu-Ohwayo, 1990; Witte *et al.*, 1992b; Gophen *et al*., 1995). There has also been increased inflow of nutrients from the catchment, primarily from fertilizers, resulting in artificially elevated primary productivity (Ochumba and Kibara, 1989; Mugidde, 1993). The bottom layers of the

lake are largely anoxic compared to the 1960s (Talling, 1965; 1966; Hecky, 1993). Plankton composition has also changed. Diatoms have been replaced by the blue-green cyanobacteria, while the previously calanoid copepod dominated zooplankton has been replaced by cyclopoid copepods (Hecky, 1993). Turbidity has also increased with the Secchi transparency index having declined from five meters in the 1930s to less than one meter in the 1990s (FAO, 1995).

The most dramatic and unfortunate changes have been in the indigenous fish stocks (Table 1.2). There has been a reduction in abundance with more than two hundred indigenous haplochromines now considered extinct (Witte *et al*., 1992a and b; Kaufman, 1996). Work by Okaranon (1994) has also indicated that the African lungfish *Protopterus aethiopicus*, and the catfishes *Clarias gariepinus* and *Bagrus docmak*, were disappearing from the catches. The commercial fishery on the lake that was previously multispecific in composition is now oligospecific based on two alien species *L. niloticus*, *Oreochromis niloticus*, and a small native cyprinid *Rastrineobola argentea* (Acheng, 1990; Okaronon, 1994; According to the National Environmental Management Authority, NEMA, (1998), there has been a fish species diversity reduction from 24 taxa in 1969 to 14 in 1995 representing a 42% decrease over this period. Haplochromines and other indigenous fish species originally constituted approximately 83% of the catches by weight have now dropped to about 0.6% (NEMA, 1998). Although there are reports of recovery of some haplochromines (Witte *et al*., 2000) *L. niloticus* still comprises 96.5% of the catch by weight from the lake, while the other dominant alien fish *O. niloticus* comprises 2.9%.

Table 1.2 Estimated fish catch (tonnes) for Lake Victoria between 1965 and 1988. Data from MAAIF, Fisheries Department, Uganda.

Year	*Lates*	Tilapiines	*Bagrus*	*Barbus*	*Protopterus*	*Clarias*	*Synodontis*	Haplochromines	*Labeo*	*Mormyrus*	*Rastrineobola*	Others	Total
1965	3	20985	1035	688	544	628	2	-	-	499	-	-	24384
1966	6	20610	2739	597	967	1731	-	851	-	513	-	6	28020
1967	2	14883	10552	1028	4286	2742	243	3058	107	457	-	522	38180
1968	3	6378	6071	1140	15612	3618	19	7594	-	74	-	-	40509
1969	600	19844	7930	1725	5974	5233	511	2288	204	1709	-	255	46273
1970	620	17760	11140	430	6990	3310	220	1100	-	-	-	160	41730
1971	728	14190	11268	536	6186	3345	400	1050	105	1000	-	1	38809
1972	840	10080	11020	601	5836	3323	558	1866	188	986	-	3	35301
1973	975	7490	10368	540	6500	3110	475	1845	248	950	-	-	32501
1974	1086	6465	8987	590	3306	2757	180	1780	85	250	15	-	25501
1975	250	7000	2930	260	195	1290	70	1690	10	40	10	-	13745
1976	540	1850	4380	130	1800	1320	40	1000	10	20	10	-	11100
1977	460	3110	4910	530	2270	1920	570	1560	30	240	-	-	15600
1978	460	3110	4900	530	2300	1900	540	1560	30	240	-	-	15570
1979	190	1650	6530	360	1370	2330	2540	1550	40	130	70	-	16760
1980	129	2,302	3845	62	365	2376	735	93	-	92	-	-	9999
1981	785	6570	3877	61	2795	2310	843	87	-	72	-	-	17000
1982	1947	460	3,907	-	5459	265	890	73	-	-	-	-	13000
1983	13980	382	2141	58	51	181	110	61	-	40	-	-	17004
1984	23927	2279	17633	58	108	237	521	-	-	29	-	-	44732
1985	37386	1268	15017	10	71	573	223	-	5	25	-	-	54578
1986	41000	5750	9288	104	263	125	-	-	-	296	-	-	56828
1987	76617	5794	7058	66	354	1185	55	5	2	13	2001	14	93164
1988	92031	11570	206	30	315	429	6	416	3	22	2033	30	107091

Several authors have proposed reasons for this dramatic decline within a period of less than 50 years. Okedi (1970), Ochumba (1994 and Goldshmidt *et al*. (1993) have all implicated the introduction of *L. niloticus* into the lake as the cause of the reduction of about 65% of the endemic haplochromine ichthyofauna. *L. niloticus* was introduced in to the lake in the late 1950s (Hughes, 1992; Gee, 1964; Oguto-Ohwayo, 1990) but the first catches were only recorded in 1960 (Hambalyn, 1960). For almost twenty years following its introduction, *L. niloticus* constituted a very small proportion of the lake's fish total catch until 1978 when a population explosion was noticed – evident in huge catches (Kudhongania and Cordone, 1974; Ogari, 1985; Ogutu-Ohwayo, 1990). Reported limnological changes have been attributed to the disappearance of phytoplanktivorous and detritivorous haplochromines – as a direct result of predation by *L. niloticus* – a situation that has been further exacerbated by increased nutrient load from the catchment (Ochumba and Kibara, 1989; Goldshmidt *et al*., 1993; Hecky, 1993; Gophen *et al*., 1995).

L. niloticus cannot be completely exonerated as there is ample evidence that it may have expedited the decline of indigenous fish stocks. Catches of *O. esculentus*, which was once a major commercial species, started to decline more than 20 years prior to the introduction of *L. niloticus* (Ogutu-Ohwayo, 1990). In the case of *Labeo victorianus,* a decline in catches was noticed as early as 1953 (Cadwalladr, 1965a). The pre-*L. niloticus* decline of many indigenous fishes can, therefore, be attributed to overfishing, a practice that has since persisted.

The sustainability of the fisheries sub-sector is, therefore, uncertain. Reasons underlying this uncertainty relate to ecological changes. It is, therefore, uncertain whether fish production that is primarily based on alien species such as *L. niloticus* within a eutrophic

environment will be sustainable. It is known that lake systems that are highly eutrophic and oligospecific, and dominated by alien species, deliver production in bursts for a few years but must be reconfigured through intense manipulation (Kaufman, 1993). According to NEMA (1998) a national fish production deficit of 82,800 metric tonnes is predicted by the year 2010. At the beginning of the 20th century fish requirements of the rural communities were provided by small-scale artisanal fishers who exploited indigenous fish stocks. With the current decline in fish diversity, artisanal fishers operate on diminishing returns. The *L. niloticus* fishery is operated by wealthy urban businessmen who own the expensive gear and craft necessary for harvesting this large species. Several fish processing plants have been developed along the lake's shoreline targeting the *L. niloticus* for the export market. Ugandans who cannot afford *L. niloticus* fillets have recently resorted to feeding on the remaining axial skeleton after the filleting process. Bony parts are now fried or smoked and sold in trading centres and drinking places throughout the country. The local process of frying and smoking *L. niloticus* requires large amounts of wood fuel and has been associated with deforestation on a number of islands in the lake (Ligtvoet, 1989; Reidmiller, 1994). While the *L. niloticus* fishery is important for the export market, it was the indigenous fish stocks that directly benefited the local communities. The need for intervention in conservation of these fish resources was realised way back in the mid-1980's, during the Fifth Congress of European Ichthyologists held in Stockholm (Balon and Bruton, 1986). Human activities and the consequent ecological changes in the lake in the recent past have, therefore, placed the continued survival of indigenous fishes in jeopardy.

Fish conservation strategies on Lake Victoria

Global flora and fauna are disappearing at a rate greater than any fossil record (Reid, 1997; Riciardi and Rasmussen, 2000) with loss of species diversity, and consequently genetic

diversity, expected to continue (Wilson, 1985). Aquatic ecosystems are continuously subjected to ecological assaults and according to Kaufman (1993: 447) "the shattering of lake ecosystems has placed the machinery of extinction on public exhibit". The need to understand African lake ecosystems and their flora and fauna, to prevent further loss of genetic diversity, is therefore imperative.

Interventional measures have revived populations of species that were on the verge of extinction (IUCN, 1987). Scientists are, however, opposed to human intervention. Indecisiveness and a *laissez faire* attitude by scientists could be partly responsible for failure to avert extinctions. The irony is that "nature" is not really left alone, alien species introductions, intense exploitation of natural resources, and the pollution of ecosystems will always continue to threaten the survival of flora and fauna without any remedial measures from the sceptical scientists. Bruton (1995) laments the extinction of vertebrates that has occurred in Lake Victoria without any ameliorative measures, and attributed it to general apathy towards the threats facing aquatic fauna. He was also critical of captive propagation as a remedial action. He rebuts the preposition for intervention to save indigenous cichlids and urges ecologists and taxonomists to take advantage of the opportunity provided by the lake to undertake detailed evolution studies (Bruton, 1990; 1995). He does not, however, consider the socio-economic impacts on the riparian communities that would result from relegating their source of livelihood to evolution students as "a kind of a laboratory".

Whereas it is acknowledged that the Lake Victoria ecosystem cannot be restored to its original status, advocating a no-intervention attitude is fallacious. A deeper analysis of the cause of the massive vertebrate extinction in Lake Victoria will reveal that it is inextricably woven into the fabric of Ugandan society. The introduction of the *L. niloticus* was because

of the human need for recreational fishing, and later the provision of food that could not be met by the then abundant, but largely unknown and yet traditionally important, haplochromine cichlids (Graham, 1929). The role of haplochromines in the maintenance of a healthy ecosystem of the lake was obscure to the riparian communities. Unfortunately, scientists that have worked on the lake during the last 15 - 20 years have mainly focused on the haplochromines, without due consideration of catch decline of other traditionally important food fish species such as *O. esculentus*, *O. variabilis*, *Bagrus docmak*, and *L. victorianus*. It is not surprising that recommendations to conserve haplochromines through control of the *L. niloticus* population (Balon and Bruton, 1986; Barrel and Witte, 1986) have not been implemented.

Conservational strategies for the lake that do not implicitly address fundamental human requirements are deemed to achieve few tangible results. A more suitable approach would be the development of a comprehensive management plan that considers both the effects of past anthropological mistakes and addresses the expectations of the riparian communities. Such a management plan would also need to address the largely overlooked, yet equally threatened, non-cichlid ichthyofauna. Such a plan would address the conservation of flora and fauna through scientifically evaluated means without necessarily being utilitarian. Scientific evaluation of remedial plans would therefore depend strongly on the availability of biological knowledge of the species of interest. Several options within this plan would be available with the most plausible being the introduction of a fishing ban during spawning seasons in spawning areas, and captive breeding for both conservation and to satisfy market demands.

Identification of a suitable candidate species - *Labeo victorianus*

The genus *Labeo* occurs in Africa and Asia but is absent in Europe and the Americas. According to Reid (1985), the genus originated after events that separated the old-world tropics from North America and Europe, but before the separation of Africa and Asia. Fossil records indicate that the labeines inhabited the African continent as far back as three to five million years ago (Greenwood, 1972; Reid, 1985). The genus is widely distributed, with at least 80 species, on the African continent and contributes at least 16.4% of the African cyprinid fauna (Skelton *et al*., 1991). One species *L. victorianus* Boulenger, locally known as ningu, is limited in its distribution to the Lake Victoria basin (Greenwood, 1966).

At the start of the 20th century, fishing on Lake Victoria was subsistence-based (Garrod, 1961). Gears were rudimentary-basket traps, papyrus nets, hooks and harpoons. In 1905, traditional fishing gears were replaced with flax gillnets, and replaced in 1952 by more efficient and longer lasting synthetic fibre gillnets (Cadwalladr, 1965a; Ogut-Ohwayo, 1990). These gear improvements marked the start of a dramatic decline in the *L. victorianus* fishery. Three years after introduction of the synthetic gill nets, the catch per net of *L. victorianus* from Kagera River dropped by 64.3% from 11.2 fish net^{-1}night^{-1} in 1951 to 4.0 fish net^{-1}night^{-1} in 1953 (Fig. 1.1).

Information pertaining the life history of *L. victorianus* is that, as with other members of the genus (Reid, 1985; Skelton *et al*., 1991; Weyl and Booth, 1999), is potamodrometic and moves into affluent rivers to spawn in vegetated flooded pools (Fryer and Whitehead, 1959). Being a local delicacy, and coupled with its predictable migratory habits, considerable fishing pressure was directed at this species to satisfy a high consumer demand. By 1969, the imminent collapse of *L. victorianus* fishery prompted scientists to

recommend the prevention of fishing during spawning migrations (Cadwalladr, 1969). Advice was ignored and the populations continued to decline (Table 1.1). Over the last 20 years there has been hardly any *L. victorianus* recorded in the fish catches from the lake (MAAIF, 1995). Such data and information from fishers caused obvious concern that the species may be reduced to dangerously low levels. Recent surveys have only found two allopatric populations; one inhabiting the Sio River (0° 13′53″ N, 34° 00′ 30″E) on the Uganda-Kenya border, and another in the Kagera River (0° 56′ 28.1″ S, 31° 46′ 18″E) on the Uganda-Tanzania border (Rutaisire, unpublished data). Given the recent record of fish extinction in Lake Victoria, and its disappearance from former habitats, the threat of extinction cannot be taken lightly.

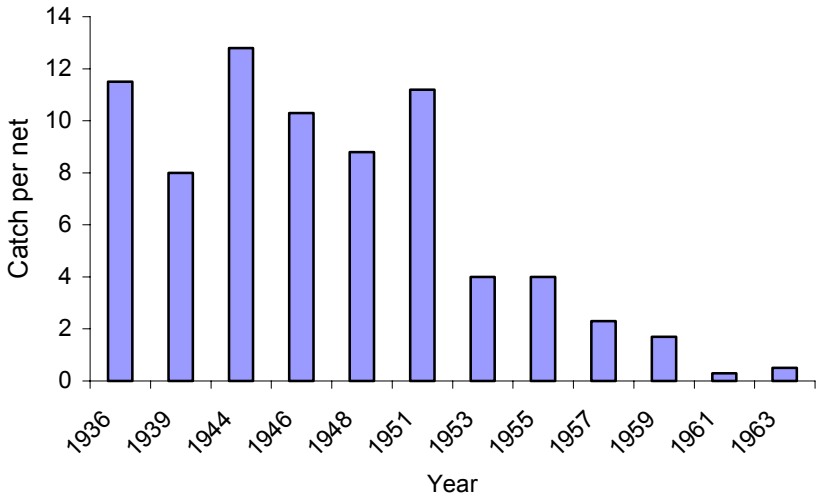

Figure 1.1 Annual catch per net of *Labeo victorianus* from river Kagera 1936-1963. Data from Cadwalladr (1965a).

Banning of fishing during spawning seasons in spawning grounds

A fishing ban would be the fastest and possibly the cheapest control measure that would afford immediate protection to *L. victorianus* adults migrating upstream prior to spawning. This method would be the least opposed by conservative biologists who are always sceptical of measures of intervention. The major drawback to this approach is its implementation. There is a history of poor law enforcement in East Africa with fisheries managers being ineffective in enforcing fisheries regulations. In some cases managers have yielded to pressure from fishers and reviewed rules accordingly (Ogutu-Ohwayo, 1990). In the case of *L. victorianus,* banning of fishing during the spawning season was recommended 30 years ago (Cadwalladr, 1969) but has yet to be implemented. There is a tendency among fisheries officers in Uganda to ignore fishes whose catches have dwindled and concentrate on commercially important species. This is presumably because of monetary gains. Personal observation at Kasensero landing site in Rakai district indicated that catch statistics of *L. niloticus* were daily collected but there was no record at all of the nearby multispecific riverine fishery at the mouth of Kagera River. According to Balon and Bruton (1986), there is a serous communication gap between the scientific community, administrators and managers of natural resources, such that scientific advise is not sought and if sought is not heeded. This is especially true in developing countries where lack of resources hinder information dissemination and effective law enforcement.

On the other hand a ban on fishing is to advocate for the "do not touch approach" without offering alternatives. This approach, when enforced without community education, can be detrimental to natural resources. It creates animosity between the users and management. In most cases local communities are more knowledgeable about fish behaviour than the ill-trained and poorly equipped fisheries personnel. The skilled fishers within the community

take advantage of this situation to continue exploiting the resources when there are highly vulnerable as in the case of *L. victorianus*. If a fishing ban is to succeed, the communities have to be made aware that they are the custodians of the resource, and that conservation is for their long-term benefit. At the moment the level of community sensitisation is low and coupled to a situation where protein sources are scarce, it would be untenable to expect population recovery through law enforcement.

Captive propagation for culture

Cyprinids have been cultured for centuries (Pillay, 1990; Jolly and Clonts, 1993), and comprise the largest group of cultured fishes (Chamberlain, 1993). Although *L. victorianus* has not been cultured, it has potential. A member of the same genus rohu, *L rohita*, is widely cultivated in India (Jhigran and Pullin, 1985; Hadler *et al*., 1991). In Malawi two African labeines *L. mesops* and *L. cylindricus* are being developed for small-scale aquaculture (Hiroyuki *et al*., 1999). Previous efforts in Uganda have focussed on the alien common carp *Cyprinus carpio* because its biology is well understood. The culture of such alien species has not been successful because of high fry predation and mortality (Rutaisire, 1997). The culture of alien cyprinids has clearly not been able to relieve fishing pressure on indigenous cyprinids. An alternative approach that prioritises development of indigenous fish species for culture is required. Through mass juvenile production and culture, environmental education can be conducted and the gap between demand and supply of the fish narrowed. Additional employment may also be possible.

Captive propagation for conservation

Captive breeding of endangered species is becoming a viable, and in some instances, the only option for conservation. The International Union for Conservation of Nature (IUCN)

recognises the principle of captive propagation and considers it part of the global strategy to conserve biodiversity (Ribbink, 1987). Captive propagation and reintroduction were among the recommendations adopted for the conservation of Lake Victoria cichlids at the Fifth Congress of the European Ichthyologists (Balon and Bruton, 1986). However, some scientists have since expressed their reservations about its promulgation.

Considering that the remaining *L. victorianus* populations are about 500 km apart, they could be described as insular, despite occurring in the same water body. There is a possibility that these populations are already genetically bottlenecked. According to Meffe (1987) isolation increases population differentiation and leads to genetic decay. This problem makes it impossible for isolated populations to recover without intervention. The threat to these small populations would be a loss of genetic variability, which alone may lead to extinction or at best weaken the heterozygotic base from which to launch a recovery (Ribbink, 1987). Small populations are less adapted to cope with environmental challenges and are vulnerable to extinction (Mayr, 1963). Fortunately there are more than 500 individuals required to constitute an effective population size for minimisation of loss of variance and fitness through genetic drift and inbreeding (Franklin, 1980; Soule and Wilcox, 1980). It is, therefore, unlikely that the two populations are too severely bottlenecked and adequate genetic variation may still exist. However, the remaining populations may be of similar haplotypes and have a low genetic diversity. It is, therefore, important that prior to the implementation of a captive breeding programme the genetic status of the populations of interest are characterised. This would ensure that reintroductions do not cause undesired hybridisation. Prudent conservation strategies will, therefore, have to involve genetic analysis and determination of variance within and between populations.

Objectives of the study

The measures discussed above all require reproductive biological information. Life-history parameters such as gonadal development, sex differentiation, size-at-50%-maturity, spawning patterns, fecundity and recrudescence patterns have to be determined before positive interventional measures can be instituted. The reproductive biology of indigenous cyprinids in Lake Victoria, including *L. victorianus*, are unfortunately poorly known. Only two studies have investigated the reproductive biology of *L. victorianus*. Cadwalladr (1965b) provided a general overview on breeding biology and growth, whereas Fryer and Whitehead (1959) reported on the breeding habits and hatched fertilised eggs collected from natural habitats. Their attempts to induce spawning and artificially fertilise the eggs were, however, unsuccessful. Several other unsuccessful attempts have been made to breed *L. victorianus* in captivity (Unpublished Kajjansi Aquaculture Research Centre records). Failure of *L. victorianus* to breed in captivity, and the lack of gametes have therefore curtailed any captive propagation programmes. This thesis has been structured to provide the required fundamental knowledge on the reproductive biology of the species, and apply it to the development of appropriate technologies for induced spawning. Due to the insular nature of the existing populations a genetic investigation was also initiated**.**

The overall objective of the study was to investigate aspects of the reproductive biology of *L. victorianus* and develop appropriate technologies for is captive breeding. The thesis has been structured into eight chapters. The study area and general methods used are outlined in Chapter 2. Chapter 3 investigates the reproductive biology of the two populations. Detailed testicular and ovarian structure, together with a histomorphological description of recrudescence, is investigated in Chapter 4. In Chapter 5, sex differentiation is histomorphologically investigated. Morphological and genetic characterisation of the two

populations is described in Chapter 6, and in Chapter 7, data obtained in Chapters 3 and 4 are used to induce spawning in the species. Finally, Chapter 8 discusses the overall findings of the thesis within the context of fisheries and aquaculture management in Uganda.

CHAPTER TWO

Study area and sampling methods

Study Area

The study was conducted in the Kagera and Sio River systems and Lake Victoria (Fig. 2.1). These sites comprise the known current range of *L. victorianus* on the Ugandan side of the Lake Victoria basin.

The Kagera and Sio River systems

The Kagera River, also known as the Akagera in Rwanda, is the most remote headstream of the Nile River and largest inflow into Lake Victoria. It is formed at the confluence of two headstreams; the Nyabarongo and the Ruvubu/Ruvuvu river systems, which in turn arise from Rwanda and the Burundi highlands, respectively. The Kagera River follows the boundary of Rwanda and Tanzania northward before turning along the boundary of Uganda and Tanzania. It then enters Tanzania for about 150 km and drains into the western side of Lake Victoria at Kasensero in Uganda. The Sio River, which partly forms the border between Kenya and Uganda, arises on the southern slopes of Mount Elgon in Kenya and drains into the North Eastern part of Lake Victoria.

The Sio River is small, about six to ten meters wide and less than five meters deep. The Kagera River is a larger and deeper river with a depth of over 12 meters and a width of over 20 meters. Associated with these river systems is fringing vegetation with a wide diversity of aquatic flora including *Cyperus, Phragmites, Typha, Lemna, Azolla, Pistia, Vossia* spp. The alien water hyacinth *Eichhornia crassipes* is the

dominant species on the fringes of the river mouths. Opposite to the mouth of Kagera River are the rocky Busungwe Islands. Similarly located but smaller islands occur offshore of the Sio River. *L. victorianus* was found to inhabit the two river systems and the main lake especially around the Islands.

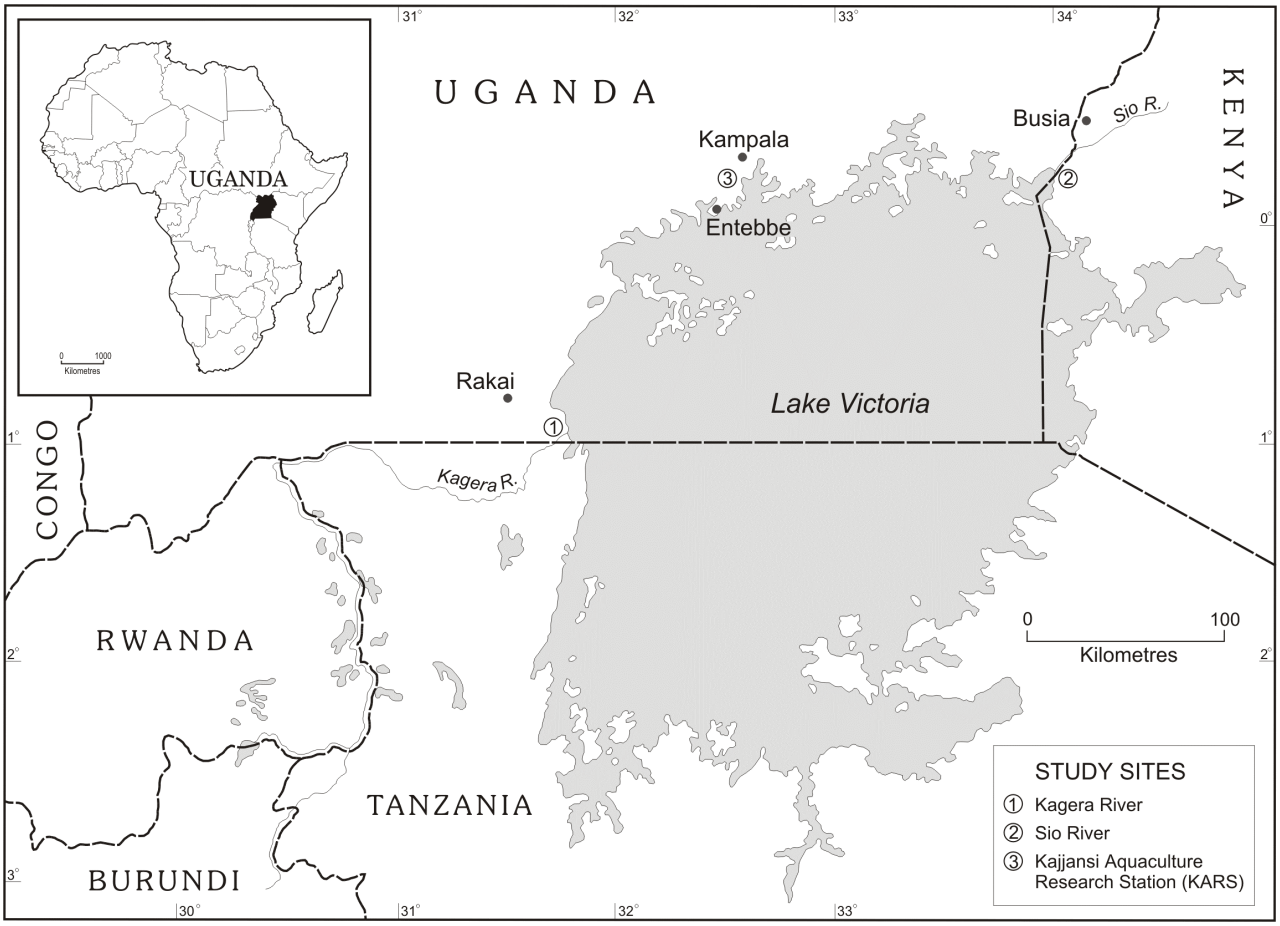

Figure 2.1. Map of the Lake Victoria basin showing the study sites.

Lake Victoria

Lake Victoria, also referred to as Victoria Nyanza, is the largest lake in Africa and the second-largest freshwater lake in the world. The lake occupies an area of approximately 68 457 km^2 (NEMA, 1998) with 3 440 km of coastline. The lake is situated between the Western and Eastern Rift Valleys along the great plateau. It is 1

134 m above sea level and reaches a depth of 79 metres with a mean depth of about 40 m.

Most of the lake is surrounded by Precambrian bedrock with the exception of the Kavirondo Gulf, which is dominated by recent alkaline, volcanic and sedimentary units (Johnson *et al.*, 2000). The lake arose as a result of uplift along the western branch of the East African rift valley in the late Pleistocene time and back flowing of the rivers that previously drained westwards (Bishop and Trendall, 1967). Based on seismic reflection profiles and six piston cores Johnson *et al*. (1996) showed that Lake Victoria dried up completely during the late Pleistocene approximately 14 000 years before present.

As the pre-existing lake dwindled in size, a few species found refuge up the rivers flowing into the basin and later returned into the lake as the basin refilled (Johnson *et al.*, 2000). *L. victorianus* is one of the species indigenous to the lake basin. Records of the fish's distribution indicate its occurrence in all the five countries of the Lake Victoria basin (Table 2.1).

L. victorianus specimens used in this study were obtained from the two rivers systems and the islands in the lake. Samples collected in Chapters 3, 4 and 5 were obtained at Maddwa (0º 13′53″ N, 34º 00′ 30″E) on the Sio River and Mubanzi (0° 56′ 28.1″ S, 31° 46′ 18″E) on the Kagera River (Fig. 2.1). Induced spawning experiments were conducted in tributaries of Sio River, Sunfish farm and the Kajjansi Aquaculture Research Station.

Kajjansi Aquaculture Research Station and Sunfish Farm

Kajjansi Aquaculture Research Station (KARS) and the Sunfish Farm are located eight kilometres south of Kampala (Fig. 2.1). KARS is a government station under the authority of the National Agricultural Research Station, while Sunfish Farm is a private property. The two facilities are adjacent to each other. Kajjansi area is drained by small streams and rivers that flow into the nearby ecotone of Lake Victoria. A protected stream supplies the Sunfish Farm, while the Kajjansi River supplies the KARS. The soils at KARS and Sunfish farm are loamy-clay which are suitable for construction of earthen fishponds.

Table 2.1 Some of the recorded occurrences of *Labeo victorianus* within the Lake Victoria basin. Data from Froese and Pauly (2002).

Country	Location	Year collected	Sources of information
Burundi	Lake Rweru	1981	Anon, 1997
Burundi	Ruvubu River	1981	Anon, 1997
Rwanda	Lake Bilira	1985	Anon, 1997
Rwanda	Lake Hago	1986	Anon, 1997
Rwanda	Lake Ihema	1986	Anon, 1997
Rwanda	Lake Mirayi	1985	Anon, 1997
Rwanda	Lake Mpanga	1986	Anon, 1997
Rwanda	Lake Mugesera	1985	Anon, 1997
Rwanda	Lake Rwanyakizinga	1986	Anon, 1997
Rwanda	Lake Sake	1985	Anon, 1997
Rwanda	Lake Rweru	1986	Anon, 1997
Rwanda	Nyabarongo River	1986	Anon, 1997
Rwanda	Akanyaru River	1986	Anon, 1997
Tanzania	Kagera River	1992	Anon, 1997
Uganda	Lake Victoria	1952	Anon, 1997
Uganda	Kommi Island	1906	Anon, 1999
Uganda	Lake Kyoga	1929	Anon, 1999
Uganda	Malaba River	1962	Anon, 1999
Uganda	Aswa River	1961	Anon, 1999
Uganda	Victoria Nile River	1911	Anon, 1999
Kenya	Dunga bay, L. Victoria	1991	Anon, 1997
Kenya	Nzoia River	1966	Anon, 1999
Kenya	Kendu bay, Kibuon River	1993	Anon, 2000
Kenya	Nyando River	1997	Anon, 2000

Sampling procedure

L. victorianus samples from Chapters 3 and 4 were obtained by gill netting in the Kagera and Sio Rivers. A fleet of eight 27-75mm stretched mesh and 45 m were set for 12 hours twice a week for 12 months. Caught fish were removed from the nets, various measurements taken, samples labelled and preserved according to the intended study. Fixed samples were processed in the histology laboratory at the Faculty of Veterinary Medicine, Makerere University in Uganda.

In the laboratory histological samples were trimmed to pieces of approximately $1cm^3$ before loading in cassettes. The sample-loaded cassettes were placed into an automatic tissue processor where dehydration of the tissues in ascending grades of alcohol occurred. The samples were cleared in two changes of xylene and impregnated in four changes of molten wax. The tissues were then removed from the automatic processor and embedded in molten paraffin wax with a 60^oC melting point. The wax embedded tissues were attached to wooden blocks, trimmed, frozen and sectioned with a well-sharpened microtome knife. The sections were floated onto glass slides in a warm water bath, oven-dried and stained (Luna, 1968). Samples for ultrastructure studies were processed and embedded in pure epon with 2% accelerator at 60^oC at Makerere University before delivery to the Electron Microscopy Unit, Rhodes University, South Africa for further processing and observation under a Joel Transmission electron microscope.

Samples for genetic and morphometric characterisation of the two populations were actively fished by drift netting and basket trapping at various sites along the rivers,

ranging from river mouths to five kilometres upstream. Fresh pieces of muscle tissue, excised from the nape region of the fish, were immediately stored in cryovial tubes containing 25% dimethylsulfoxide (DMSO) saturated with a NaCl solution (Amos and Hoezel, 1991). DNA extraction, analysis and manual nucleotide sequencing was conducted at the Molecular Biology Laboratory, Makerere University Institute of Environment and Natural Resources.

Induced breeding experiments were conducted using broodfish captured on their upstream migration at Sitengo on the Sio River. The fish were purchased from fishers who caught them in reed enclosures set across the Sio River. It was not possible to set such traps in the Kagera River because of the depth and the force of the water flow that washed away traps. Induced spawning trials were conducted first at Sunfish Farm, and then later in the tributaries of the Sio River at Maddwa and in ponds at the KARS.

Juvenile *L. victorianus*, used in Chapter 5, were obtained from both the induced spawning experiments and beach seining at the mouth of the Sio River using a pond seine net. It was not possible to obtain extremely young fish from the Kagera River because of gear limitations. The pond seine net used in the Sio River could not withstand the water flow of the Kagera River. Gonadal development was followed in young fish from the induced spawning experiment reared in ponds at the KARS and bimonthly sampling in the Sio River.

CHAPTER THREE

Spatial patterns in the reproductive biology of *Labeo victorianus*

Introduction

Knowledge regarding aspects of the reproductive biology of a harvested or cultured fish is important for management. For example policies developed to assist with harvesting rates are usually based on information of the species' reproductive biology such as size at sexual maturity, duration and periodicity of spawning, and even sex change in reef dwelling species (Shapiro, 1984; Wootton, 1984). From an aquaculture perspective, market forces demand well-planned production of gametes and offspring to ensure year-round production of fish (Bromage, 1995; Patino, 1997). Manipulation of a fishes reproductive system under culture conditions requires an understanding of natural spawning patterns and other influential factors.

Munro (1990) reviews the environmental factors that influence teleost reproduction and has categorised them as either ultimate or proximate. Ultimate factors relate to offspring survival and growth, while proximate factors are concerned with coordination of gonadal cycles with the environment so as to maximise an individual's potential fecundity and reproductive fitness. Teleosts have inherent mechanisms for correlating gonadal structure and function with environmental factors. This results in temporal and spatial spawning seasons. This strategy allows for spawning when conditions are optimal for survival and growth of the progeny (Bye, 1984; Munro, 1990). The correlation between reproduction and the environment is mediated through the neuroendocrine system, which perceives environmental cues and transduces signals that influence gonadal structure and their function (Peter and Yu, 1997).

The reproductive biology of *L. victorianus* is still largely unknown. Whereas published notes by Cadwalladr (1965b) provide an overview of its breeding biology and ecology, information on important reproductive biology aspects such as oogenesis, histological staging, lengths-at-sexual maturity, and sex ratio by season are still lacking. Other information available includes observed larval development and hatching of eggs collected from the wild and a description of spawning areas (Fryer and Whitehead, 1959). Unfortunately no reproductive data have been collected in the last three and half decades. During this period, the fish's population has crashed to historically low levels and the water environment radically modified (Talling, 1965; 1966; MAAIF, 1988; Hecky, 1993).

This Chapter contributes to the knowledge of *L. victorianus*' reproductive biology by investigating, and contrasting, two extant populations in Uganda. The reproductive aspects investigated are reproductive seasonality, sexual maturity, fecundity and sex ratios during spawning migrations.

Materials and methods

Sampling procedure

A total of 137 and 188 female fish were caught from the Kagera and Sio Rivers respectively during the period between September 1999 and August 2001. Details regarding the collection procedure are described in Chapter 2. After capture, fresh fish were measured. Information recorded for each specimen included measurements for fork length (FL) and standard length (SL) to the nearest 0.1 mm, and total mass (W) to the nearest 0.01g. The mass of the excised ovary, liver, mesenteric fat and eviscerated fish (We) were later recorded.

Each tissue's relative mass to the eviscerated mass was calculated. Indices included a gonadosomatic (GSI), and a mesenteric fat (MFI) index. Due to the absence of a true stomach and the looping nature of labeine intestines, contents were not easily squeezed out of the gut. A feeding index (FI) as employed by Gonçalves and Almada (1997) for *Salaria pavo* was, therefore, calculated as that proportion of the total weight of entire gut to the eviscerated weight. The sex of each specimen was recorded and gonads macroscopically staged (Table 4.3). Later histological examination of the gonads validated field observation (see Chapter 4). An eviscerated condition factor was calculated as $K_e = 1000 \times We/L^3$. The derived indices were compared among months using Analysis of Variances (ANOVA) if the data passed tests for normality and homoscedasticity. A Kruskal-Wallis test was used where the transformed data did not fulfil parametric assumptions. Rainfall data was obtained from the meteorology department, while hydrological data was from the Water Department, Uganda.

Reproductive seasonality

Cyclic gonadal recrudescence was described by a periodic regression of observed GSI on the sine and cosine of the angular transformation of the independent variable, i.e. month of the year. The multiple linear regression model is of the form

$$Y = \beta_0 + \beta_1 SinR(X_1) + \beta_2 \cos R(X_1) + \beta_3 \sin R(X_2) + \beta_4 \cos R(X_2)$$

where β_0 is the mesor (or amplitude), $R(X_1)$ the angular transformation of month of the year (MOY), $R(X_2)$ angular transformation of half month of the year (HMOY). $\beta_1, \beta_2, \beta_3, \beta_4$ are the regression coefficients. The angular location of peaks (P) of GSI was calculated as $P = t_0 + \delta$ where t_0 is the nominal zero of the cycle, and δ the arctan of the

ratio of the respective coefficients. Maximum positive or negative departure of predicted GSI from the mesor is calculated as $A = \left(\beta_1^2 + \beta_2^2\right)^{1/2}$.

Sexual maturity

Length-at-maturity, that length when 50% of the sampled fish in a size class are sexually mature, was calculated by fitting a logistic ogive to the proportion of reproductively active fish during the spawning season in centimetre size classes. The two-parameter ogive is described by the equation $P_L = (1+\exp(L - L_{50}))^{-1}$ where P_L is the percentage of mature fish at length L, L_{50} the length-at-maturity. The model parameters were estimated by non-linear minimisation of a negative binomial log -likelihood of the form

$$-\ln L = \sum_L y_L \ln\left(\frac{P_L}{1-P_L}\right) + n_L \ln(1-P_L)$$

where y_L is the observed numbers of fish mature in n_L fish sampled in length class L.

Fecundity and egg size

For the determination of fecundity and egg size, only fish in the "ripe- and- running" stages were considered. Histological validation outlined in Chapter 4 showed that it was these maturity stages that had yolked eggs (Cambray, 1982). Fresh ovaries were fixed in buffered 10% formalin for 12 hours and stored in 70% ethanol. Ovary mass was recorded and all the eggs from the left ovary emptied into a beaker of water and shaken gently to cause uniform mixing. Volumetric subsamples of 1-2 ml was pipetted from the mixture and transferred to a petri dish where all ova were counted using a tally counter. Fecundity was calculated as

$$F = n \times (V/v) \times (W/w)$$

where n is the number of ova in the sub sample, V is the volume of ova and water, v the volume of subsample, W the weight of both ovaries and w the weight of the ovary whose eggs were counted. A second sample of ova was obtained from the ovary that was not used for fecundity studies, and their diameter measured along their median axis with a calibrated eyepiece micrometer fitted on a dissecting microscope at ×20 magnifications.

Absolute fecundity and egg size were plotted against eviscerated mass and fork lengths and the least-squares regression parameters estimated from the base-10 logarithm transformed data. Elevations and slopes of the regression of the two populations were compared by analysis of interactions in the multiple regression models.

Results

Reproductive seasonality

The gonadosomatic indices of females varied significantly between months for both the Kagera River ($H = 35.7$, $n = 137$, $P < 0.01$) and Sio River ($H = 58.4$, $n = 188$, $P < 0.01$) populations. The minimum observed GSI were in June (10.44±2.8%) and July (2.09±0.76%), while the maximum was reached in March for both the Kagera (10.64±1.74%) and Sio River (30.27±4.71%) populations, respectively. Gonadosomatic index variation followed a bimodal pattern, with maxima in January to February and September-October for both populations (Fig. 3.1). The same pattern was exhibited for rainfall and water levels in the two study sites (Figs. 3.2 and 3.3.) The calculated peak of GSI from the coefficients of periodic regression of GSI on the angular transformed month of the year (RX) corresponded to a primary peak in February, and a secondary peak in October for both populations (Table 3.1).

Table 3.1 Summary of periodic relationships between *Labeo victorianus* gonadosomatic index and month of the year. Results are summarised for two populations inhabiting Lake Victoria, Uganda with samples collected between January and December 2000.

River	Equation	R^2	P
Kagera	$Y = 7.20 + 0.007\sin R(MOY) + 3.55\cos R(MOY) + 0.941\sin R(HMOY) - 1.58\cos R(HMOY)$ $p = 0.09 \quad p<0.01 \quad p = 0.02 \quad p < 0.01$ First peak 1st February, Amplitude 3.55 Second peak 28th October, Amplitude 1.84	0.96	<0.01
Sio	$Y = 21.6 + 2.49\sin R(MOY) + 6.67\cos R(MOY) + 2.32\sin R(HMOY) - 3.97\cos R(HMOY)$ $p = 0.02 \quad p < 0.01 \quad p = 0.05 \quad p < 0.01$ First peak 19th February, Amplitude 7.12 Second peak 30th October, Amplitude 4.59	0.93	<0.01

Table 3.2 Monthly data on *Labeo victorianus* samples processed for histology from the Kagera and Sio Rivers.

	Kagera River			Sio River		
Month	Male	Female	Total	Male	Female	Total
Jan	26	18	44	28	22	50
Feb	34	17	51	26	24	50
Mar	36	11	47	5	11	16
Apr	21	9	30	31	14	45
May	24	13	37	4	13	17
Jun	10	11	21	16	11	27
Jul	6	4	10	27	21	48
Aug	16	6	22	22	12	34
Sep	11	7	18	18	13	31
Oct	37	16	53	14	25	39
Nov	21	10	31	8	13	21
Dec	12	15	27	18	9	27
Total	254	137	391	217	188	1187

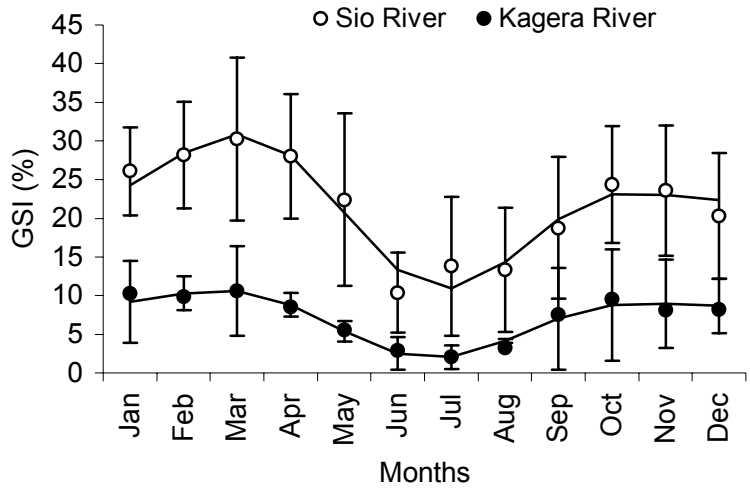

Figure 3.1 Monthly observed mean (± standard deviation) and predicted female *Labeo victorianus* gonadosomatic indices from samples collected in the Kagera and Sio Rivers between January and December 2000.

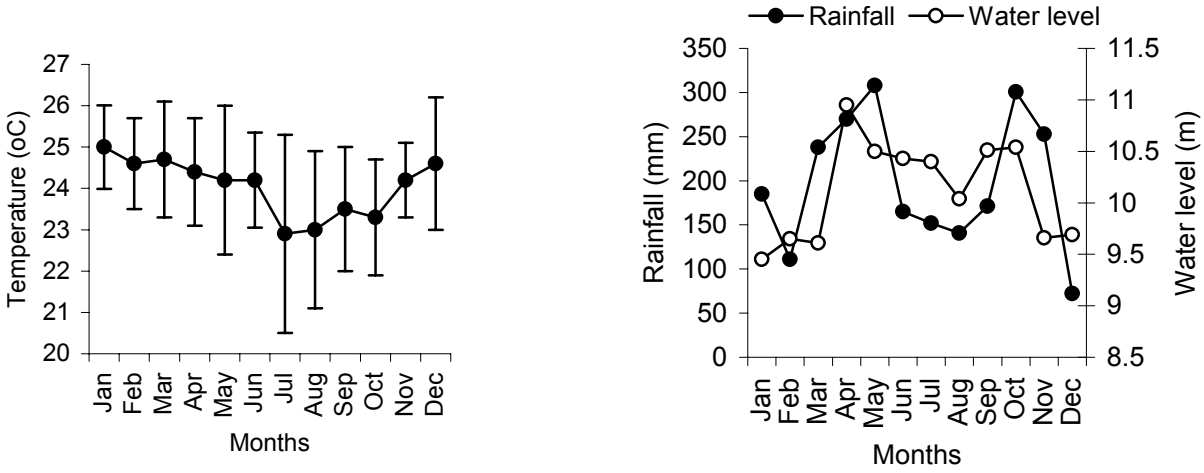

Figure 3.2 Variations in water temperature, rainfall and water levels in the Kagera River, Uganda between January and December 2000.

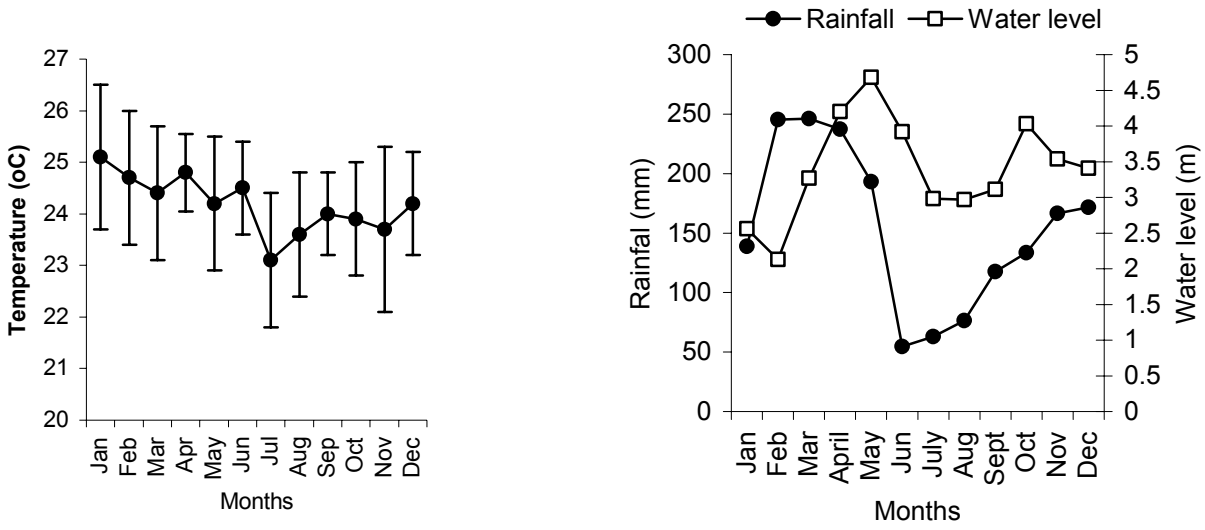

Figure 3.3 Variations in water temperature, rainfall and water levels in the Sio River, Uganda between January and December 2000.

Additional data relating to reproduction seasonality was inferred from the relative monthly proportions of the various oocyte stages and is described in Chapter 4. The highest proportion of "spent" gonads were in March, April, May, October and November samples from the Kagera River population. In the Sio River population the first breeding season corresponded to that of the Kagera River, but the second spawning period started in August, a month earlier than in the Kagera River. Freshly "spent" fish were sampled in August from Sio River populations (Fig. 3.4) when all the fish from Kagera River had ovaries that were still in the "developing" stage. The Kagera River samples displayed a clear distinct pattern of gonadal maturation with no "ripe" ovaries sampled in July. In contrast, fish from Sio River population had more than half of the "ripe" fish in the same month.

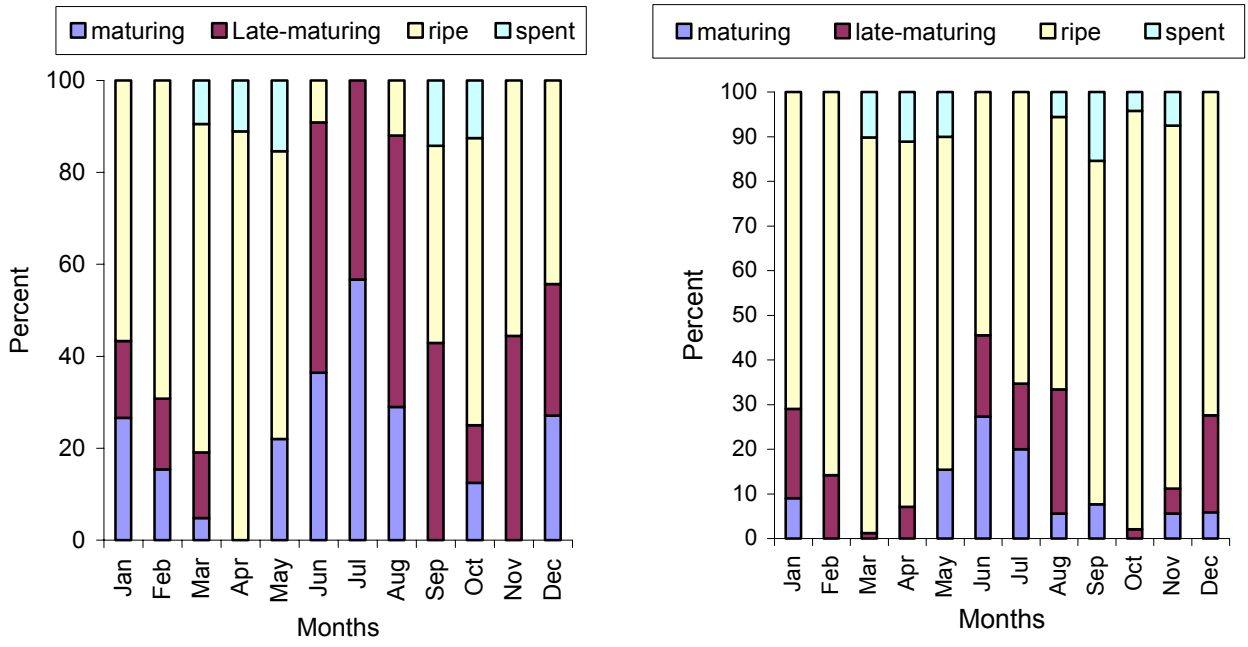

Figure 3.4 Monthly maturity stages for female *Labeo victorianus* sampled from the Kagera (left panel) and Sio Rivers (right panel) between January and December 2000

Length-at-maturity was estimated at 21.9 cm FL for the Kagera River population and 11.8 for the Sio River population (Fig. 3.5). A likelihood ratio statistic showed a significantly different L_{50} estimates between populations ($P < 0.05$) (Table 3.3). The rate of sexual maturity, the parameter δ, did not differ significantly ($P > 0.05$) between the two populations.

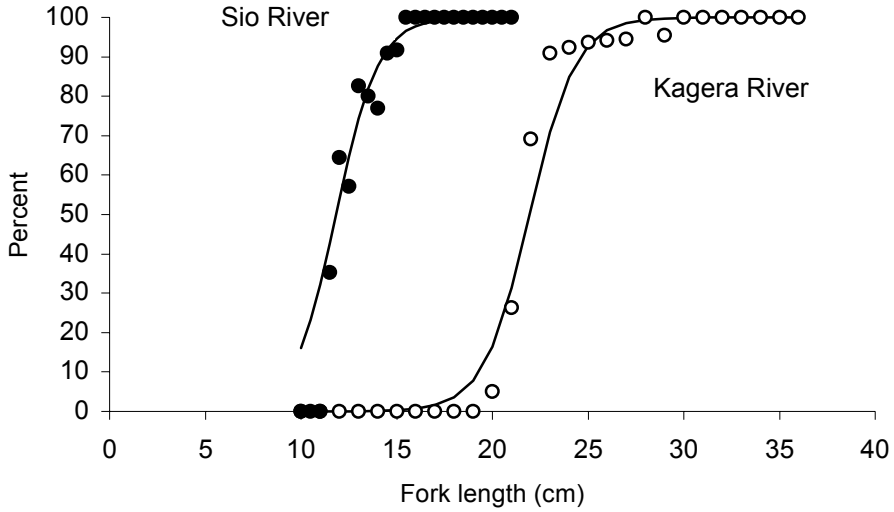

Figure 3.5 Observed and predicted percent sexually mature female *Labeo victorianus* sampled between September 1999 and August 2001 from the Kagera and Sio Rivers, Uganda.

Table 3.3 Female *Labeo victorianus* logistic ogive parameter estimates and likelihood ratio tests for fish sampled in the Kagera and Sio Rivers, Uganda between January and December 2000. The likelihood ratio tests, twice the difference between the log-likelihoods, test the hypothesis that specific combinations of the ogive parameters are equivalent.

			Null hypothesis		
	Parameter	All different	L_{50} equal	δ equal	L_{50} and δ equal
Kagera River	L_{50}	21.93	21.93	21.94	6.94
	δ	1.19	1.19	1.16	10.04
Sio River	L_{50}	11.83	21.93	11.78	6.94
	δ	1.10	>10.00	1.16	10.04
λ			104.7	0.08	137.22
P ($\lambda \leq \chi^2$)			<0.01	0.77	<0.01

In the male fish, the gonadosomatic indices also varied significantly between months in both the Kagera River ($F = 4.65$, $P < 0.01$, $n=254$) and Sio River ($H= 36.6$, $n = 217$, $P < 0.01$) populations. Both populations exhibited an annual bimodal trend. No significant periodicity was noted in either population ($P > 0.05$). "Spent" fish were rare, with only a few specimens sampled in May-June, October and November in the Kagera River, and in May- August, October-November from the Sio River population

(Fig. 3.6). Fish with testes in the "maturing" stages were the most prevalent in both populations.

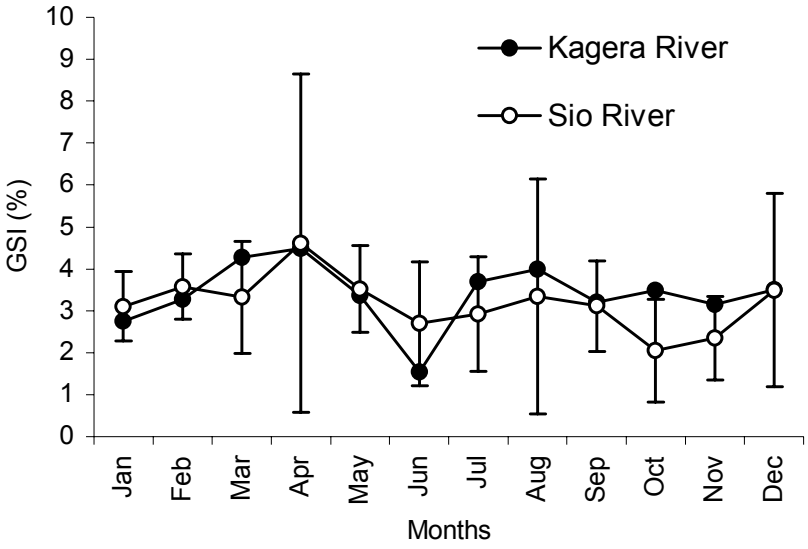

Figure 3.6 Monthly observed mean (± standard deviation) and predicted male *Labeo victorianus* gonadosomatic indices from samples collected in the Kagera and Sio Rivers between January and December 2000.

Male length-at--maturity was estimated at 22.1 cm and 12.8 cm FL for the Kagera and Sio River populations, respectively (Fig. 3.7; Table 3.4). Only the L_{50} parameter was significantly different between both populations ($p < 0.05$).

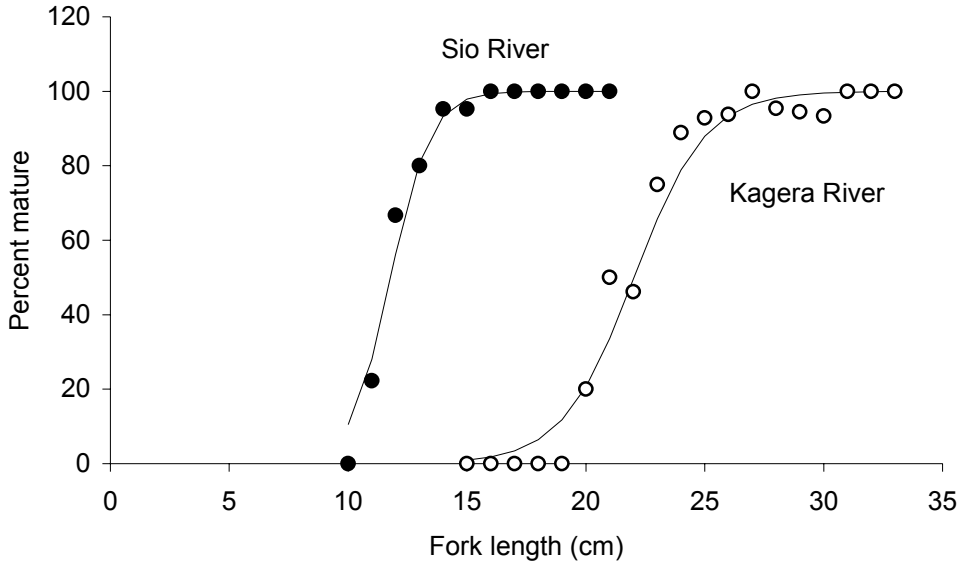

Figure 3.7 Observed and predicted percent sexually mature male *Labeo victorianus* sampled between January and December 2000 from the Kagera and Sio Rivers, Uganda.

Table 3.4 Male *Labeo victorianus* logistic ogive parameter estimates and likelihood ratio tests for fish sampled in the Kagera and Sio Rivers, Uganda between January and December 2000. The likelihood ratio tests, twice the difference between the log-likelihoods, test the hypothesis that specific combinations of the ogive parameters are equivalent.

			Null hypothesis		
	Parameter	All different	L_{50} equal	δ equal	L_{50} and δ equal
Kagera River	L_{50}	22.02	12.18	22.08	14.65
	δ	1.50	8.48	1.36	5.91
Sio River	L_{50}	11.79	12.18	11.19	14.65
	δ	0.84	0.77	1.36	5.91
λ			125.70	3.62	177.00
P ($\lambda \leq \chi^2$)			<0.01	0.06	<0.01

Fecundity

The regression of fecundity on somatic mass revealed a linear relationship, and a curvilinear relationship when fecundity was regressed on fork length and eviscerated weight for both the Kagera River and Sio populations, respectively. Regression equations for the relationships of fecundity on fish size are summarised in Table 3.5.

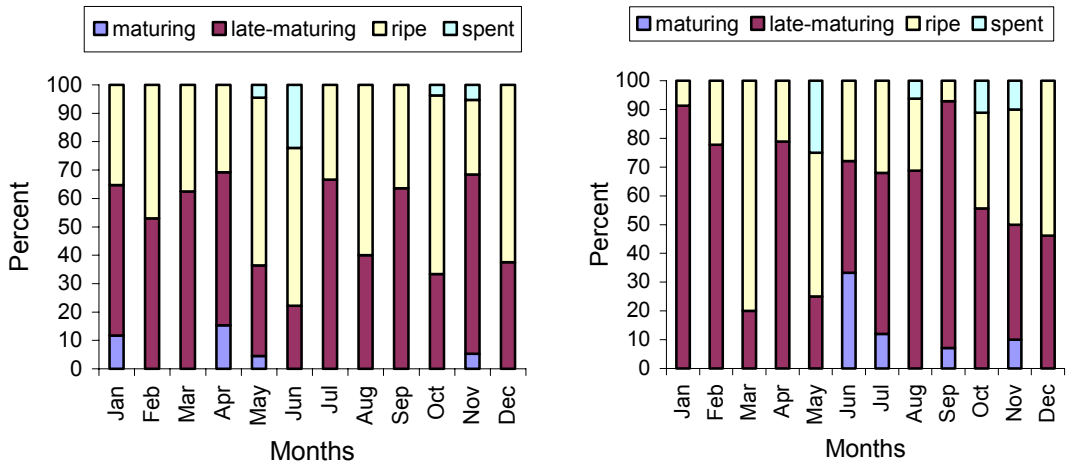

Figure 3.8 Monthly maturity stages for male *Labeo victorianus* sampled from the Kagera (left panel) and Sio Rivers (right panel) between January and August 2000.

Table 3.5 Regression relationship between fecundity on fork length and eviscerated weight for two populations of *Labeo victorianus*.

Population	Regression equation	R^2	n
Kagera River	$Log_{10}F = 0.28 + 3.16\ Log_{10}FL$	0.73	
	$Log_{10}F = 2.49 + 0.914\ Log_{10}We$	0.78	220
Sio River	$Log_{10}F = 0.07 + 3.191\ Log_{10}FL$	0.93	
	$Log_{10}F = 2.05 + 1.071\ Log_{10}We$	0.93	349

Table 3.6 Regression relationship between oocyte size and eviscerated weight for two populations of *Labeo victorianus*.

Population	Regression equation	R^2	n
Kagera River	Log_{10} oocyte diameter = $-0.47+0.17\ Log_{10}We$	0.55	220
Sio River	Log_{10} oocyte diameter = $-0.16+0.05\ Log_{10}We$	0.10	349

There were no significant differences in the relative rates of change of fecundity per unit increase in eviscerated weight or the mean fecundity adjusted to common body weight ($F_{3,128}$, $p > 0.05$, $R^2 = 0.98$).

Oocyte diameter

Least-square regression of yolked oocyte diameter on fish weight indicated that ova size increased with weight but with a low coefficient of determination (Kagera River :

R^2 = 0. 55; Sio River : R^2 = 0.10). The regression equations defining the relationship between egg size and eviscerated fish mass are summarised in Table 3.6.

Multiple regression analysis of oocyte size on eviscerated fish weight and site-by-eviscerated weight interaction indicated significantly different elevations ($F_{3,128,}$ P < 0.01, R^2 = 0.56) but the coefficient for site-by-eviscerated weight interaction was not significant (P > 0.05).

Feeding indices

There was significant variation in the feeding index between months for female fish from both the Kagera River (H = 40.5, n = 137, p < 0.01) and Sio River (H = 65.6, n = 188, P < 0.01) populations. Variation in the feeding index was also significant in male fish (Kagera: H=49.4, n = 243, P < 0.01; Sio: H = 25.7, n = 217, P < 0.01). Variation in the feeding index in female fish inhabiting the Kagera River followed a bimodal pattern with the maxima in June-July and December-January. In males, a sharp increase was observed between July and August followed by a decline from August to September. In the River Sio population, females showed a unimodal peak in June, while in males the index oscillated between months except between March and April where there was a sustained decline (Fig. 3.9).

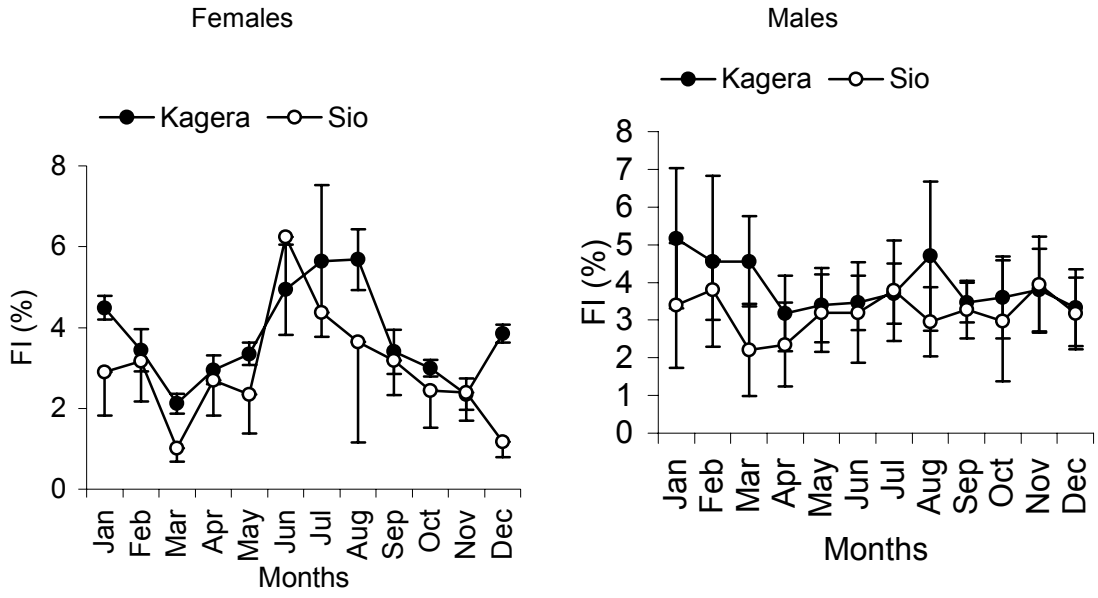

Figure 3.9 Monthly observed mean (± standard deviation) and predicted male and female *Labeo victorianus* feeding indices from samples collected in the Kagera and Sio Rivers between January and December 2000.

Mesenteric Fat Index

Deposition of fat in the mesentery of the female fish varied significantly between months in both populations (Kagera: $H = 48.1$, $P < 0.01$; Sio: $H = 75.4$, $P < 0.01$). In the Kagera River samples, there was a sharp rise in fat deposition from May reaching a peak in June before declines (Fig. 3.10). A similar pattern was observed in fish inhabiting the Sio River except that a longer peak between June and August was observed (Fig. 3.10). Field observations indicated that fat deposition in the mesentery was mainly in "maturing" and "late-maturing stages". In the "ripe-running" and "spent" stages, the fat deposits were already depleted. In males, the MFI varied significantly between months in the two populations using a Kruskal-Wallis test (Kagera: $H = 103.3$, $P < 0.01$; Sio: $H = 108.2$, $P < 0.001$) with variation exhibiting a bimodal pattern. In the Kagera River population, the MFI was lowest from April to June and during October-November period, while in the Sio River population the decline was only for the months April and November.

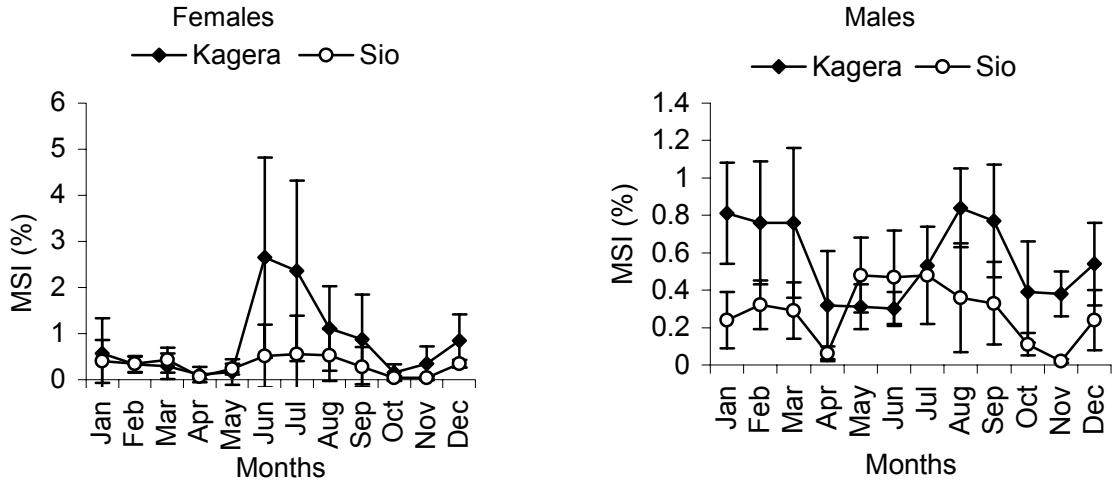

Figure 3.10 Monthly observed mean (± standard deviation) and predicted male and female *Labeo victorianus* mesenteric fat indices from samples collected in the Kagera and Sio Rivers between January and December 2000.

Hepatosomatic index

In females, there was no statistically significant differences in hepatosomatic indices within months (Kagera: $F = 1.73$, $P > 0.05$, $n = 137$; Sio: $H = 5.33$, $P > 0.05$, $n = 188$). Similar results were obtained for male fish (Kagera: $H = 1.05$, $P > 0.05$, $n = 254$; Sio; $H = 19.2$, $P > 0.05$, $n = 215$) in both populations.

Eviscerated condition factor

Female eviscerated condition factor varied significantly between months in the Kagera River ($H = 25.8$, $n = 137$, $P < 0.01$) and Sio River ($H = 51.7$, $n = 188$, $P<0.01$) populations, respectively. Condition peaked in the Sio River population in May, and lasted up to July but declined thereafter. A slight increase in the mean condition factor was noticed between September and October followed immediately by a decline (Fig. 3.10). Significant differences in male mean monthly eviscerated condition factor were also noted between months for the two populations (Kagera: $H = 25.3$, $p < 0.01$, $n =$

243; Sio: H = 49.6, p < 0.01, n=217). Both populations were in their "best" somatic condition between June and August (Fig. 3.11).

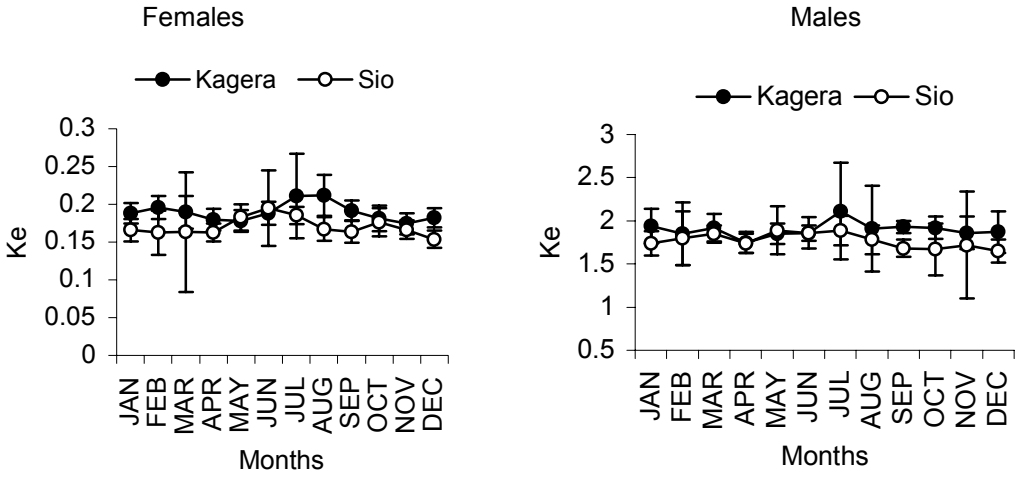

Figure 3.11 Monthly observed mean (± standard deviation) and predicted male and female *Labeo victorianus* eviscerated condition factors calculated from samples collected in the Kagera and Sio Rivers between January and December 2000.

Table 3.7 Monthly data on sex distribution of *Labeo victorianus* in the Kagera and Sio Rivers. The Binomial probability that there is a departure from and expected 1:1 sex ratio is provided for each month and river.

	Kagera River			Sio River		
Month	Male	Female	P	Male	Female	P
Jan	26	18	0.06	27	22	0.08
Feb	34	17	<0.01	26	24	0.10
Mar	45	43	0.08	35	34	0.09
Apr	39	41	0.07	39	33	0.07
May	24	13	0.02	24	23	0.11
Jun	10	5	0.09	26	21	0.09
Jul	6	4	0.20	28	26	0.10
Aug	16	5	0.01	26	23	0.10
Sep	12	7	0.09	33	38	0.07
Oct	37	32	0.08	43	39	0.08
Nov	21	20	0.12	48	43	0.07
Dec	19	15	0.10	29	23	0.08

Monthly sex ratios and catch rate

Sex ratios for the months of February, May and August samples from the Kagera River were male biased. Fish caught during the rest of the year did not deviate significantly

from unity (Table 3.6). In the Sio River population, there were no male or female biased sex ratios.

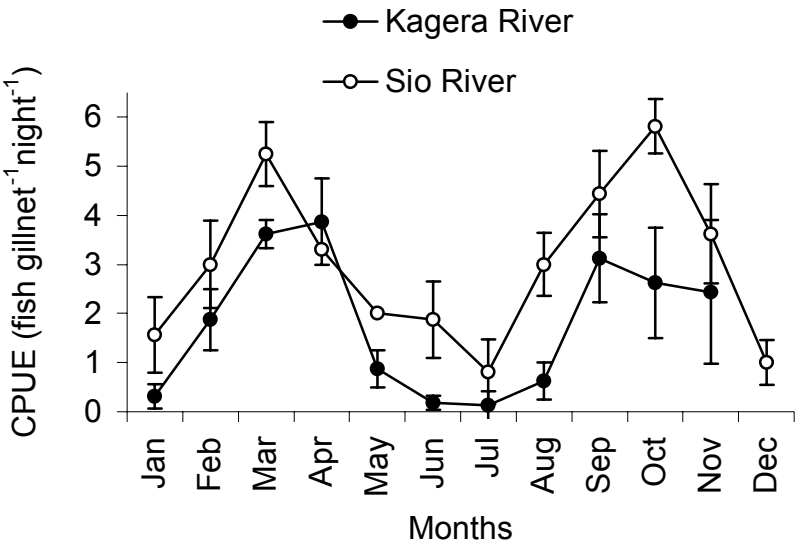

Figure 3.12 Mean monthly gill net catch-per-unit-effort (± standard deviation) for *Labeo victorianus* in the Sio and Kagera Rivers, Uganda between January and December 2000.

Catch-per-unit-effort was generally higher in the Sio River than the Kagera River (Fig. 3.12). Highest catch-per-unit-effort was during March- April and September-November for both populations. Lowest catch-per-unit-effort was noted during June-July.

Discussion

Reproductive similarities and differences between the study populations of *L. victorianus* were clearly noticeable. Reproduction seemed to be generally synchronised with both rainfall seasons in both populations, a finding confirming previous observations by Fryer and Whitehead (1959) and Cadwalladr (1965b). Synchronisation of sexual maturation and reproduction with onset of rainfall has also been documented in other African labeines (Anon, 1965; Jackson and Coetzee, 1982, Skelton *et al*., 1991; Weyl and Booth, 1999).

Non-breeding months were characterised by samples with completely full guts and high mesenteric fat deposits; a probable indication of feeding and accumulation of fat reserves prior to upstream migration to spawn. The annual peak in the gut fullness and mesenteric fat indices in both populations was approximately between June and July. The peaks were stable and longer in the Kagera River population, from June to August. In the Sio River population, a sharp rise in the feeding index from May to June was followed by drastic fall in July. June was characterised by intense feeding by females in the Sio River whereas males, in contrast, did not exhibit any seasonality in their feeding patterns. In the Sio River population, reproduction did not seem to affect feeding as it did in the Kagera River population. The breeding period in the second half of the year was longer in the Sio River population than in the Kagera River population – as indicated by the presence of "spent" ovaries. Spawning lasted for four months, between August and November, in the Sio River, while in the Kagera River it lasted for two months between September and October. Sio River females appeared to feed rapidly in the adjacent lake and moved into the river to spawn shortly thereafter. This was noticed by the capture of freshly "spent" females in August in the Sio River, a month prior to when fish in the same gonadal stage could be caught in Kagera River. The Sio River population could, therefore, have adjusted their feeding regime as to maximise chances of spawning at the earliest availability of suitable conditions.

The presence of both sexes in the Sio River throughout the year was of interest. The increase in male GSI ahead of females, together with the predominance of "maturing" and "late maturing" stages was possibly due to faster onset of recrudescence. The significantly higher male sex ratio in February and August in Kagera River suggests that a sex specific upstream migration of males ahead of females prior to spawning occurred. No difference

in sex ratio was observed in Sio River population, which might imply no sex specific upstream migration.

The period between June and July was characterised by intense feeding and replenishment of energy reserves for spawning in both populations. This feeding period coincides with the complete mixing of the lake. It is known that complete mixing of Lake Victoria occurs during this period when the established thermocline breaks down under the seasonal onset of the south-east trade winds causing the lake to become isothermal with respect to depth (Talling, 1966). It is therefore possible that mixing results in increased availability of detritus, epilithic algae, diatoms and crustaceans on which *Labeo* species are known to feed (El Moghraby and Rahman, 1984; Khumalo, 2001). Though *L. victorianus* had two non-spawning seasons, June and July is the only period when feeding and mesenteric fat indices were the highest and fish were considered to be in their best condition as defined by a high mass to length relationship. These indices started to decline shortly before the spawning seasons and indicated cessation of feeding and the subsequent utilisation of the deposited fat reserves during spawning. Cessation of feeding during the spawning season was also observed in *L. cylindricus* (Weyl and Booth, 1999), and could be common behaviour in the African lineage.

Utilisation of tissue reserves during oogenesis and subsequent reduction of deposits at the onset of spawning has been reported in several fish species including; sockeye salmon *Oncorhynchus nerka* (Idler and Bitners, 1959), American plaice *Hippoglossoides platessoides* (Mackinnon, 1972), northern pike *Esox lucius* (Medford and Mackay, 1978), European plaice *Pleuronectes platessa* (Dawson and Grimm, 1980) and rainbow trout *Oncorhynchus mykiss* (Nassour and Léger, 1989). In the blenniid fish *Salaria pavo*, reduction in feeding opportunities was reported to be the major cost of reproduction

(Gonçalves and Almada, 1997). According to Fouda *et al*. (1993) nutrients foraged in one part of the year or area are expended at another time or place when optimal conditions for reproduction occur. The liver, muscle tissue and mesenteric fat have been reported to be the organs that store energy reserved for reproduction in the some fish species. Clearwater and Pankhurst (1994) attributed the increase of hepatosomatic index in red gurnard *Chelidonichthys kumu* prior to spawning, to the liver being the storage organ in that species. In *P. platessa* the main source of protein reserves was the carcass (Dawson and Grimm, 1980). Okuda (2001) attributed a decline in the somatic condition, hepatosomatic and fat body indices in the cardinalfish *Apogon notatus* to reproductive costs during spawning. In *L. victorianus,* reproduction does not seem to exert energy costs on the liver as revealed by no significant variation in the monthly hepatosomatic index of both populations. Similar findings are reported in *O. mykiss* where the liver plays little role in lipid storage (Nassour and Léger, 1989). MacFarlane *et al*. (1993) investigated the role of accumulated lipids in the mesentery of the yellowtail rockfish *Sebastes flavidus* and showed that a greater proportion was incorporated in the developing ovaries. The sharp decline in the mesenteric fat index in *L. victorianus* during spawning periods suggests that the mesenteric fat deposits are the major source of energy for migration and reproduction. Accumulation of mesenteric fat as reported in some fishes (MacFarlane *et al*., 1993; Okuda, 2001) is important since lipids are known to play a number of roles such as formation of vitellogenin, insulation of organs, buoyancy and several other physiological functions (Weigand, 1996). Mesenteric fat may, however, not be adequate in satisfying reproductive demands in *L. victorianus* and could be supplemented by somatic resources - hence the decline in condition during spawning seasons. The short spawning period in the Kagera River allowed for a longer time to replenish non-somatic energy reserves, such as mesenteric fat, resulting in a reduced condition index. Weyl and Booth (1999) attributed

growth checks in *L. cylindricus* to physiological stress due to spawning, a factor that may have contributed to the decline in condition factor during spawning months.

Reproductive patterns in Kagera River population conformed to the "norm" of synchronisation of spawning with rainfall. Deviations from this pattern, were, however observed in the Sio River population. Spawning occurred prior to onset of rainfall. Further deviation from the "norm" was inferred from the feeding patterns and catch-per-unit-effort. It is known that reproductive strategy of a species will be the summation of a suite of traits that enable an individual to produce the maximum number of offspring. These traits include age and size at first reproduction, size and age specific fecundity schedules, reproductive effort and the timing of spawning (Mills, 1991).

Reproductive effort also entails a cyclical demand for energy and material from the body, with bioenergetic expenditures needed for reproduction being closely related to other metabolic requirements. (Miller, 1984). Individuals must reach a threshold size before they are capable of distributing energy resources between somatic growth and gametogenesis in response to appropriate environmental cues (Munro, 1990). The "best" reproductive strategy is, therefore, a trade off between a short generation time (r-selection) and enhanced survival through increased competitive ability (K-selection). Species that are exposed to unpredictable environments - like the Sio River population - tend to be generalist (*sensu* r-selected) and will favour a short generation interval and allocation of available energy resources to reproductive activities (Gunderson, 1980).

The Kagera and Sio Rivers provide different environments; the Sio River is shallow (*ca* one to four metres deep), while Kagera River is deep (*ca* 10 meters in depth) and fast flowing. It was observed that fishers take advantage of the shallow Sio River to set

barriers that not only effectively target adult fish, but also impede upstream migration of those that survive the "fishing gauntlet". Seining was also observed during this study. Such indiscriminate fishing methods are not used in the Kagera River because of depth and high water flow that pose a risk factor to the fishers. It is clear that the Sio River population is under intense fishing pressure and inhabiting an environment that is not adequately protective. The observed deviations, like spawning when there was no rainfall and the attainment of sexual maturity at significantly lower sizes than the Kagera River population, could be differing phenotypic responses to overexploitation and a variable environment. A deviation from rainfall synchronised spawning has been previously reported in other African labeines. Mitchell (1984) noted that spawning in *L. umbratus* was not necessarily flood dependent as previously described by Jackson and Coetzee (1982) but rather breeds in the most suitable site available thus fitting into Balon's (1975) intermediate guild description. A deviation from the typical flood spawning pattern in the African labeines was also reported by Cambray (1985) after observation of spawning of *L. capensis* in the Orange River without any rainfall and flooding. Apparently *L. victorianus* in the Sio River is on a similar plastic trajectory that involves spawning events that are not necessarily synchronised with rainfall and at a smaller size-at-sexual maturity.

The fecundity of the two populations was also investigated since it is known to vary among populations, and at times, between strains of fish species (Bromage *et al.*, 1990; Jonsson and Jonsson, 1999). The determination of actual numbers of eggs produced would be more useful for analysis of stock dynamics (Mason, 1985). Consideration of the actual number of oocytes produced would, therefore, account for incomplete spawning or interrupted maturation of cells during trophoblastic growth followed by resorption (Foucher and Beamish, 1980), but is difficult in fishes that produce tens of thousands of eggs under lotic spawning conditions. Mason (1985) introduced the term "Apparent

Fecundity" to refer to the estimated number of oocytes undergoing trophoplasmic growth leading to cell maturity. In this Chapter, fecundity was determined following Cambray (1982)'s definition of absolute fecundity as the number of yolked ova (mature and immature) just prior to spawning. Increases in fecundity with weight found are in general agreement with findings of other workers on teleost fishes (Bromage *et al.*, 1990; Barbini and McCleave, 1997; Jonsson and Jonsson, 1999). This Chapter also found no significant differences in elevations and slopes of regressions of fecundity on eviscerated weight and weight-site interaction, suggesting no environmental effects on the rate of increase of fecundity with weight in the two populations.

Reproductive fitness in teleosts is not only affected by fecundity but also by egg size and the size at which maturity is attained. It has been demonstrated that egg size effects larval survival in teleost fishes (Bagenal, 1969; Pitman, 1979; Wallace and Aasjord, 1984; Springate and Bromage, 1985). Egg size in fish is assumed to be genotypically determined but is also affected by other factors like the size of the female parent (Springate and Bromage, 1985). Ova size within the Kagera River population was significantly larger than those of the Sio River population. The relative rate of increase of yolked oocyte size with weight-site interaction was not, however, significant. It is, therefore, possible that larger Sio River fish would have same ova size as those of the Kagera River fish.

In conclusion, this Chapter has shown that *L. victorianus* is a highly fecund synchronous fish with two spawning seasons. Disparities in reproductive patterns could be due to adaptive reproductive tactics in response to the uncertain environments or simply due to underlying genetic differences between the two populations - a factor that will be investigated in Chapter 6.

CHAPTER FOUR

Gonadal structure and histological description of recrudescence in *Labeo victorianus*

Introduction

Fisheries management uses information pertaining to the size at sexual maturity, patterns of gonadal recrudescence, spawning seasonality and synchronicity and fecundity. These data are, however, only available for a small number of teleost species of commercial importance (Tyler and Sumpter, 1996).

In labeines, reproductive studies have been primarily macroscopic in focus (Cadwalladr, 1965b; Siddiqui *et al*., 1976; Gaigher, 1983; Van Zyl *et al.,* 1995; Weyl and Booth, 1999) with only two studies investigating ovarian histomorphology (van der Merwe *et al*., 1988; Booth and Weyl, 2000). The only other study to investigate spermatogenesis in African labeines (Booth and Weyl, 2000) was generally superficial as it concentrated on ovarian recrudescence. It is acknowledged that histological studies provide precise information on oocyte development but are unfortunately slow to undertake and are expensive because they involve complex laboratory techniques (West, 1992). The importance of histological description of gametogenesis was emphasised by Booth and Weyl (2000) who noted that macroscopic staging must be validated if errors in the estimation of maturity and reproductive seasonality are to be minimised. In male fish, knowledge of spermatozoon ultrastructure has been used in systematics (Mattei, 1991) and was found to be important in the assessment of milt quality (Billard, 1978). Structural variations have been reported in several fish taxa (Mattei, 1991; Lahnsteiner *at al*., 1995) including those within the Cyprinidae (Baccetti *at al*., 1984).

Aspects of the histomorphology of *L. victorianus* from the Sio River were described by Cadwalladr (1965b), but have not since been validated. In addition, no studies on the spermatozoa ultrastructure and milt production have been conducted with the result that gonadal recrudescence is poorly understood in this species. This chapter provides a detailed description of gametogenesis in two *L. victorianus* populations and provides the first in-depth description of testes histomorphology and ultrastructure within the African *Labeo* lineage.

Material and methods

Tissue subsections were taken from rostral, middle and caudal regions of the ovary and fixed in Bouin's fluid for 24 hours prior to transfer to 70% ethanol. Other microtomy details are detailed in Chapter 2. Structural measurements were made on scanned sections from at least five fish per microscopic stage using Sigma Scan Pro Image Analysis Software.

Specimens for transmission electron microscopy were obtained from fish that were transported live to the laboratory. Small blocks of testicular tissue were immersed in 2.5% gluteraldehyde in phosphate buffer pH 7.2-7.4. Tissue samples were post fixed in 1% osmium tetroxide and processed for ultramicroscopy following standard techniques (Cross *et al*., 2001). Ultra-thin sections were contrasted with Uranyl acetate and examined under a Joel 1210 transmission electron microscope. Because of the long distance travelled between the field sites and laboratory facilities, together with the low prevalence of "spent" male fish, electron microscopy (EM) sections were taken from "developing" and "ripe" testes only (Table 4.3).

Sperm count and motility were examined using live fish that were anaesthetised with 2-phenoxyethanol (Fluka-Chemie-Sigma Aldrich) and their urogenital pore area cleaned with soft towel. Gentle pressure was applied on the belly and the exuded milt collected in a 1 ml sterile syringe. Care was taken to avoid contamination with water and excreta. The milt was diluted 1:1000 in 40 mM KCl, 85 mM NaCl, 30mM Tris-HCl pH 7.8. Sperm counts were made on a haemocytometer chamber (chamber volume 0.1µl) under a phase contrast microscope at × 400 magnification. During investigation of sperm motility, the following diluents were tried: riverine water from 0.9%Nacl solution buffered to pH 8.2 with 10 $mmol1^{-1}$, TRIS base and $25mmol1^{-1}$ glycerine following Baynes and Scott (1987) and 0.5% NaCl solution pH 7.2. Duration of progressive motility at dilution of 1:1000 for each diluent was observed under a phase contrast microscope under x400 magnification.

Results

Macromorphological description of the ovary

The ovaries of *L. victorianus* are paired organs situated in the peritoneal cavity and suspended on either side of the mid-line by a mesovarium (Fig. 4.1).

The size and shape of the ovaries varied with the stage of development (Table 4.1). In "spent" fish, the ovaries were thin straight and translucent and as oogenesis progressed, they enlarged to become lobed, sac-like, greenish cream organs filling the abdominal cavity (Table 4.1).

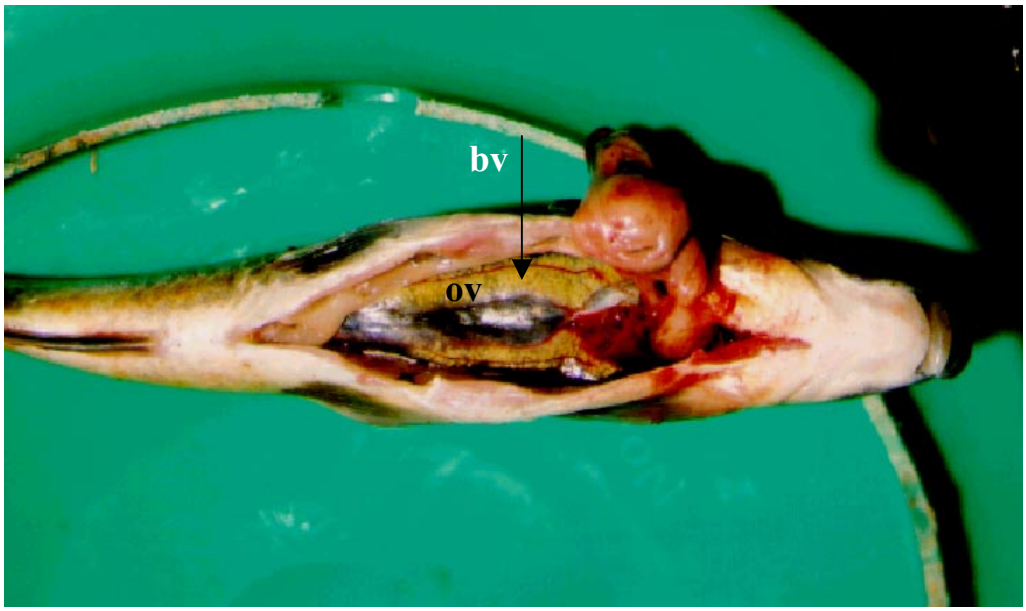

Figure 4.1 Location and macroscopic appearance of maturing ovaries (ov) in *Labeo victorianus* with blood vessel (bv).

Table 4.1 Macroscopic and histological description of various stages of ovarian recrudescence in *Labeo victorianus*. Modified from Booth and Weyl (2000).

Stage	Macroscopic appearance	Histological equivalent
1. Maturing (Includes virgins and recovering spent fish)	Ovary straight. Ova visible through the capsule. Ova white in colour.	Oogonia, chromatin nucleolar and perinuclear oocytes dominated the ovary. Cortical alveoli start to appear in few oocytes.
2. Late-maturing	Ovary increases in size, forms lobes and is the largest organ in the abdominal cavity. Ova greenish cream in colour.	Ovaries dominated by late perinuclear oocytes and primary yolk vesicle. Few secondary yolk vesicle oocytes present.
3.Ripe-running	Ovary fully distended and fills the abdominal cavity. Oocyte olive green and easily shed on application of slight pressure on the ovary.	Ovary dominated by tertiary oocytes. Few pre vitellogenic stages begin to grow for the subsequent season.
4. Spent	Ovary flaccid and often haemorrhagic if spawning was successful. Few oocyes visible giving the ovary a speckled appearance.	Post-ovulatory follicles and Type I and II atretic oocytes.

A capsule, the tunica albuginea, composed of dense regularly arranged collagen and elastic fibres mixed with a few smooth muscle cells enclosed the ovaries. The capsule gave off connective tissue septa that invaded the ovary to form the ovigerous lamellae that projected towards the ovarian lumen (Fig. 4.2). Germ cells and follicle cells were contained in the ovigerous lamellae. The follicle cells were spindle shaped in the pre-vitellogenic ovary and squamous in when vitellogenic. An ovarian artery and vein were macroscopically visible along the entire length of both ovaries (Fig. 4.1). Histological sections confirmed the high level of vascularisation of ovarian capsule and ovigerous lamellae.

Light microscopic description of oogenesis

The process of oogenesis was classified according to oocyte location and size, staining characteristics, number of nucleoli, presence of the follicular layer, and the distribution of cytoplasmic inclusions. According to these criteria oogenesis was found to proceed through six stages namely; oogonia, chromatin nucleolus, perinuclear oocytes, primary yolk vesicle oocytes, secondary yolk vesicle oocytes and tertiary yolk vesicle oocytes.

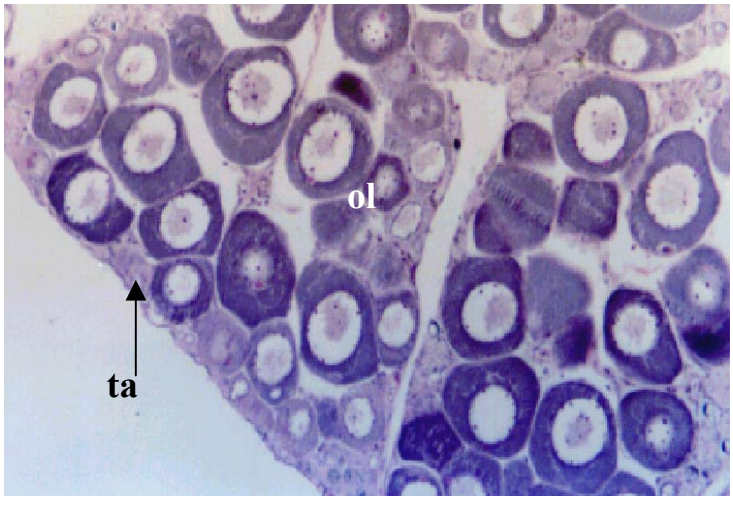

Figure 4.2 Transverse section through the ovary illustrating oogenesis. Ovigerous lamellae (ol) from the tunic albuginea (ta), H&E × 400.

Oogonia

Primary and secondary oogonia were discernable in ovarian nests where they occurred together with follicle cells. Primary oogonia were the smallest germ cells noticeable and were 10.4±0.6µm in diameter. Primary oogonia were characterised by a large nucleus:cell diameter ratio (Fig. 4.8), chromatin granules on the nuclear envelope and one distinct nucleolus (Fig. 4.3). Secondary oogonia also had high nucleus cytoplasm ratio and one nucleolus but were larger than primary oogonia and their nucleus was filled with basophilic chromatin threads (Fig. 4.3).

Chromatin nucleolus stage

Chromatin nucleolar oocytes were characterised by a large centrally located nucleus compared to the cell size with a clumps of basophilic chromatin on the nuclear wall and surrounded by a light basophilic cytoplasm (Fig. 4.3) and had a high nuclear:cell diameter ratio (Fig. 4.8).

Peri-nucleolar stage

Growth of the chromatin nucleolar oocytes to the peri-nucleolar oocyte stage was accompanied by migration out of the germ cell nests. Three types of peri-nucleolar oocytes were recognised. Pre-perinucleolar oocytes were close to the nests, polygonal in shape, and contained multiple nucleoli of varying sizes in the nucleus. Their cytoplasm was basophilic. Early peri-nucleolar oocytes were also polygonal in shape but had three to four large nucleoli and several smaller ones. Increase in the size of these cells was accompanied by the cells becoming spherical and less basophilic in Haematoxylin and Eosin (H&E). The late peri-nucleolar oocytes were the least basophilic and spherical in shape. Late peri-nucleolar

oocytes were characterised by numerous nucleoli neatly arranged on the nuclear wall (Fig. 4.4).

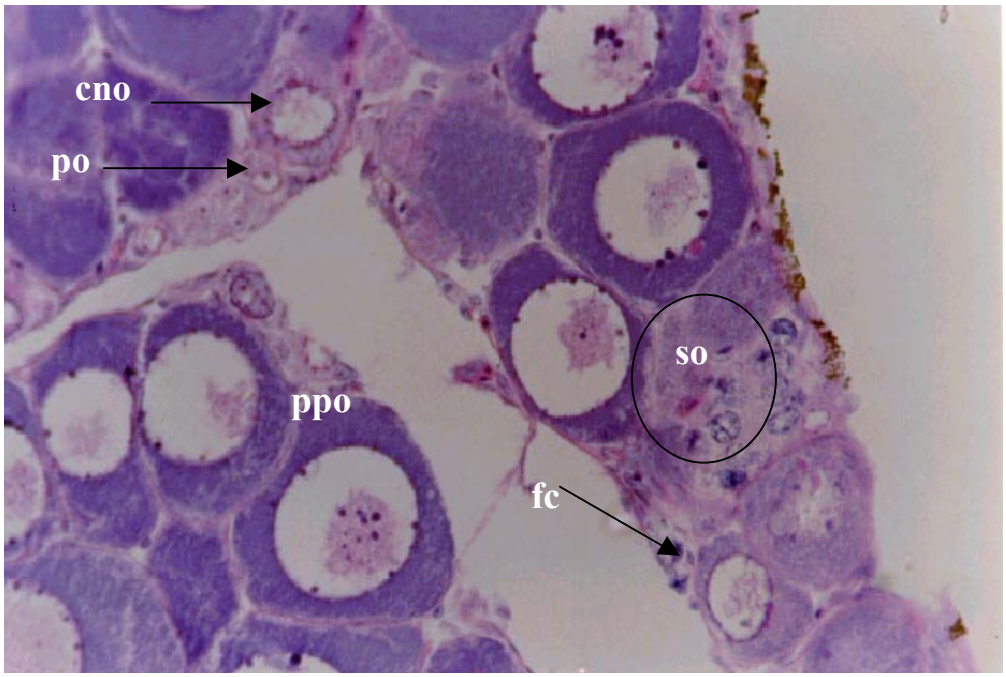

Figure 4.3 Primary oogonia (po), Secondary oogonia (so) and chromatin nucleolar oocytes (cno) occurred in ovarian nests together with follicle cells (fc). Pre-perinucleolar oocytes (ppo) were outside the nests H&E × 400.

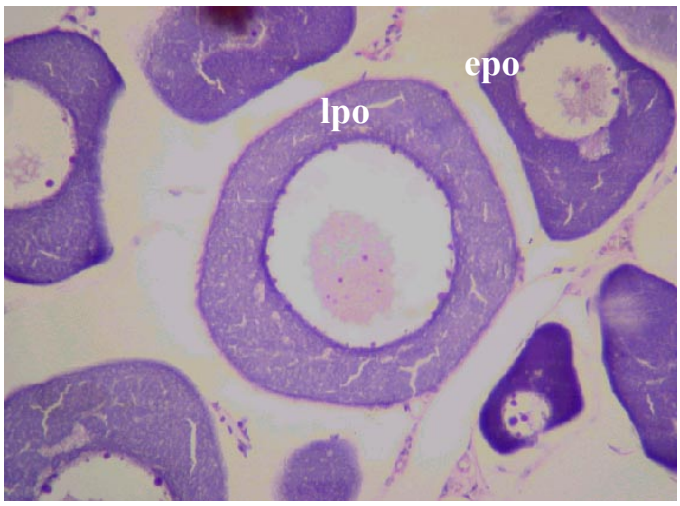

Figure 4.4 Late peri-nuclear oocyte (lpo), early perinuclear oocyte (epo) was less ovoid, H&E × 400.

All peri-nucleolar stage oocytes had acellular zona radiata and were surrounded by two follicle layers - a theca and a granulosa. Yolk vesicles, or cortical alveoli, started to appear in the late peri-nucleolar oocytes marking the end of the primary growth phase.

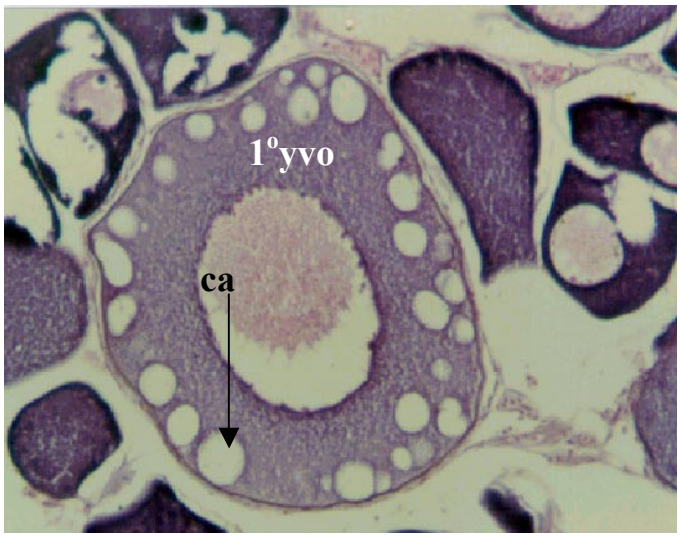

Figure 4.5 Cortical alveoli (ca) in the primary yolk vesicle oocyte (1°yvo), H&E × 400.

Primary yolk vesicle stage

During this stage the cortical alveoli formed at the periphery of the oocyte and increased in number to fill the whole of the cytoplasm. The zona radiata and the follicular layer increased in thickness together with an increase in the number of nucleoli (Fig. 4.5).

Secondary yolk vesicle oocyte stage

Oocytes in this stage were characterised by the initial appearance of acidophilic yolk granules, staining red in H&E, in the cytoplasm (Fig. 4.6). The prevalence of oocytes in this "development" stage was low when compared to other stages. Secondary yolk vesicle oocytes had similar follicular layers as the primary yolk vesicle oocytes.

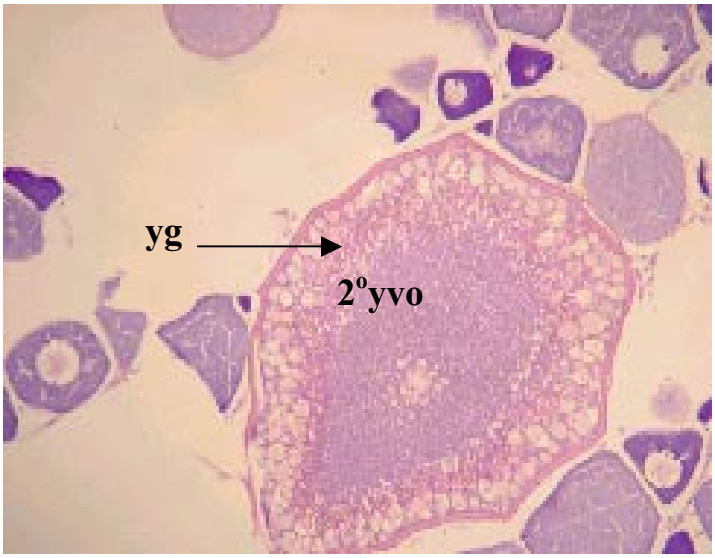

Figure 4.6 Yolk granules (yg) and the cortical alveoli in secondary yolk vesicle oocyte (2^{o}yvo), H&E × 100.

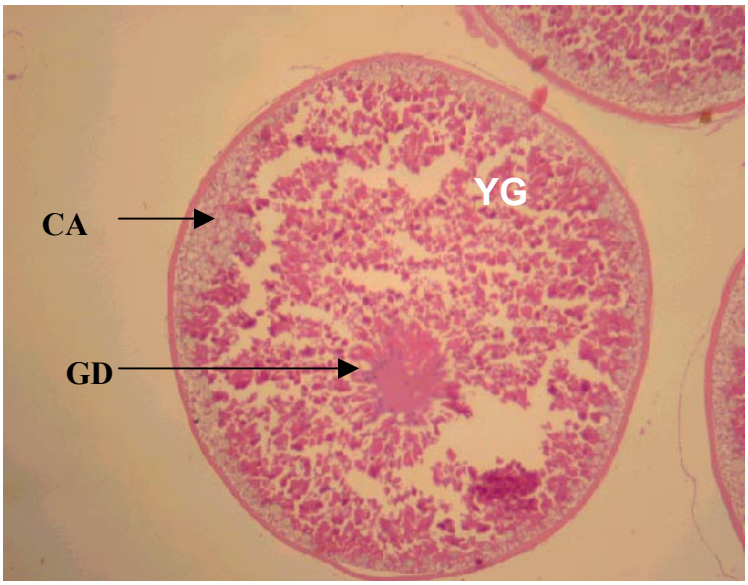

Figure 4.7 Transverse section through a tertiary yolk vesicle illustrating the central location of the yolk globules (YG), cortical alveoli (CA) and an eccentric germinal disk (GD). H&E × 100

Tertiary yolk vesicle oocyte stage

In tertiary oocytes, the yolk granules that were initially at the periphery of the cytoplasm and increased in size to form globules that occupied the entire central section of the

cytoplasm. Cortical alveoli were only at the periphery of the cytoplasm, forming a ring around the ooplasm. The nucleus at this stage was centrally positioned and irregular in shape with several nucleoli on its membrane. The chromatin in the nucleus was no longer visible at this stage. As the oocyte developed further, the nucleus migrated from the centre to the periphery of the cell (Fig. 4.7). Nucleus: cell ratio of the various gamete stages is indicated in Figure 4.8

Post-spawned Ovaries

In the post-spawned ovary, cohorts of basophilic developing oocytes were visible in "spent" ovaries (Fig. 4.9a). The theca and granulosa layers remained and hypertrophied to form the post-ovulatory follicles. The granulosa cells that were squamous prior to ovulation became cuboidal to columnar in structure with ovoid basophilic heterochromatin. The thecal layer became increasingly vascularised (Fig. 4.9b). The post-ovulatory follicles were later invaded by macrophages to form melano-macrophage centres (Fig. 4.10). The sizes of the various female gamete cells are summarised in Table 4.2.

Table. 4.2 Mean diameter (±standard error) of various oocytes stages from fixed ovaries of *Labeo victorianus*.

Stage	Cell diameter (µm)	Nuclear diameter /cell diameter (µm)	Number of nucleoli	Number of cells measured
Primary oogonia	10.42±0.6	7.13±0.5	1	29
Secondary oogonia	14.23±1.2	10.6±1.0	1	35
Perinuclear oocytes				
a) Pre perinuclear oocyte	65.29±2.9	44.21±2.4	8.3±1.96	32
b)Early perinuclear oocyte	127.28±0.5	69.06±0.4	13.1±1.05	34
c) Late perinuclear oocyte	216.78±20.5	114.67±9.9	25.3±1.58	44
Primary yolk vesicle oocyte	294.O4±15.9	121.39±14.9	22.6±1.76	36
Secondary yolk vesicle oocyte	497.02±0.40	178.06±13.6	32.25±2.02	26
Tertiary Yolk vesicle oocyte	920.27±27.23	204±7.8	-	66

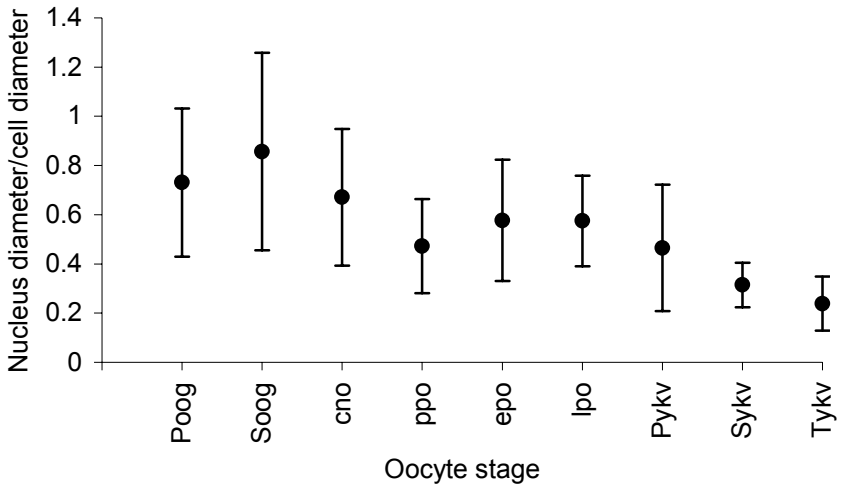

Figure 4.8 Oocyte nucleus diameter:cell ratio (mean ± standard deviation) of the various stages of oogenesis in *Labeo victorianus*. Poog = primary oogonia, Soog = secondary oogonia, cno = chromatin nucleolar oocyte, ppo = pre perinuclear ocyte, epo = early perinuclear oocyte, lpo = late perinuclear oocyte, Pykv = primary yolk vesicle oocyte, Sykv = secondary yolk vesicle oocyte. Tykv = tertiary yolk vesicle oocyte.

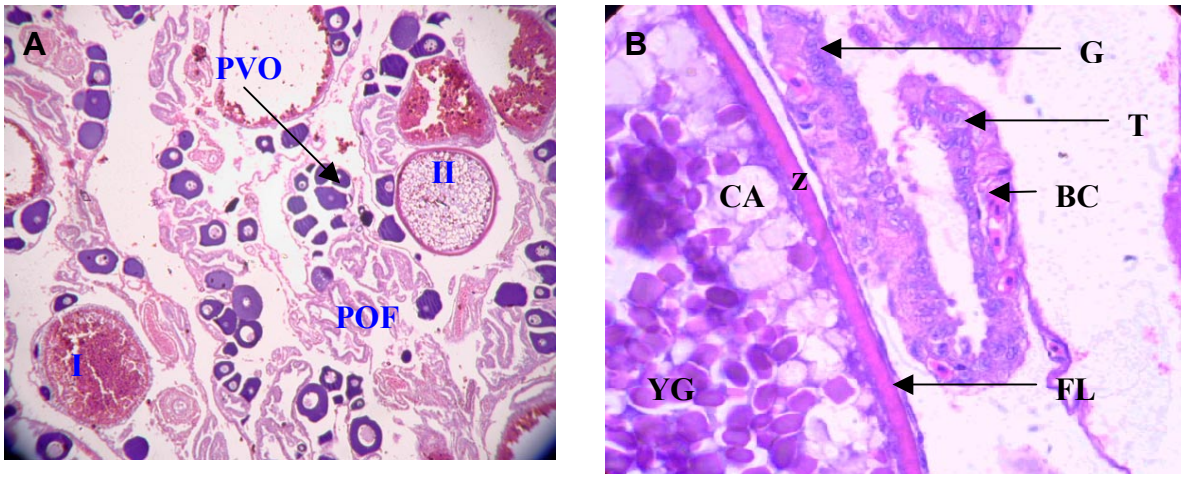

Figure 4.9 Transverse section through the ovary of *Labeo victorianus* illustrating post-ovulatory changes. A. Post ovulatory follicles (POF), atretic oocytes Type (I &II) and a cohort of previtellogenic oocytes (PVO) H&E x 100; B. Yolk globules (YG), cortical alveoli (CA), Zona radiata (Z), follicular layer (FL) from a section of a tertiary oocyte that did not ovulate and theca (T) & Granulosa (G), blood capillaries (BC) of a post-ovulatory follicle, (H&E) ×1000.

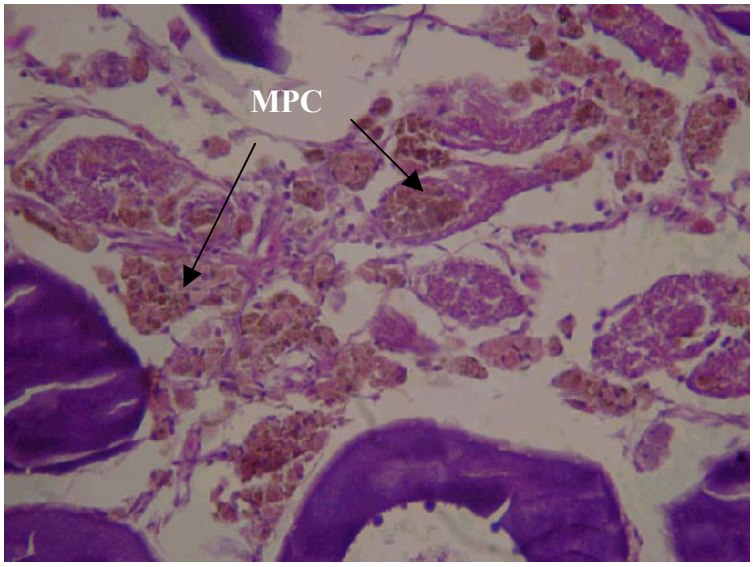

Figure 4.10 Transverse section through a spent ovary showing melanomacrophage centres (MPC) in a "spent" ovary, (H&E) ×1000.

Oocyte atresia

Oocyte atresia was common in the "spent" fish and three forms were noted. Type I atresia was characterised by fast fragmentation of the zona radiata and dissolution of the cytoplasm contents (Fig. 4.11). This type of atresia was common in tertiary oocytes of successfully spawned ovaries.

Type II atresia also occurred in vitellogenic oocytes and was characterised by the breakdown of yolk globules, to smaller granules, together with vacuolar degeneration of the cytoplasm with an intact zona radiata (Fig. 4.12b). In some samples from the Sio River, spawning was partial, as indicated by few post-ovulatory follicles among retained tertiary oocytes, while in others there were no post-ovulatory follicles. The presence of Type II atresia indicated failed spawning. In both spawning scenarios, the ovaries were cleared of the matured oocytes to facilitate gonadal recrudescence. In

this case, ovigerous lamellae with the germ cells surrounded, and formed a ring of pre-vitellogenic stages around, the "unspawned" oocyte (Fig. 4.12a) prior to onset of Type II atresia.

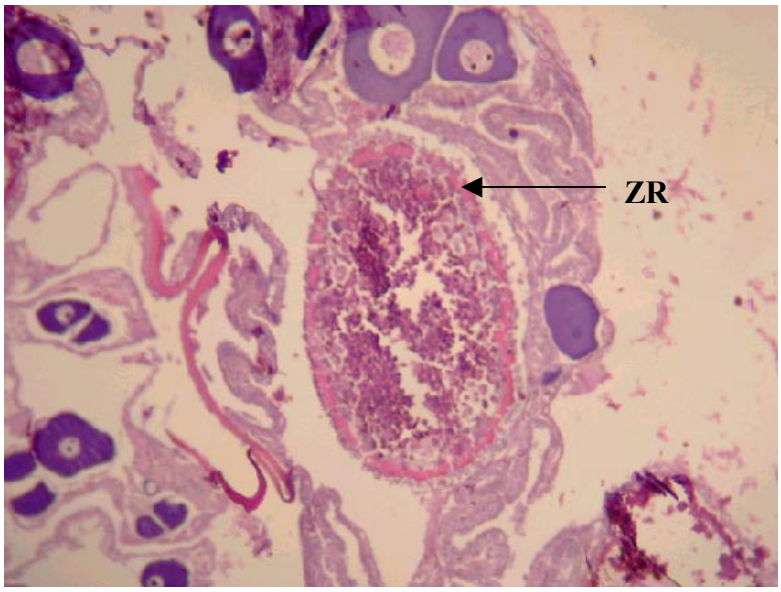

Figure 4.11 Transverse through a "spent" ovary showing disorganisation and disintegration of the zona radiata (ZR) and cell contents, H&E × 400.

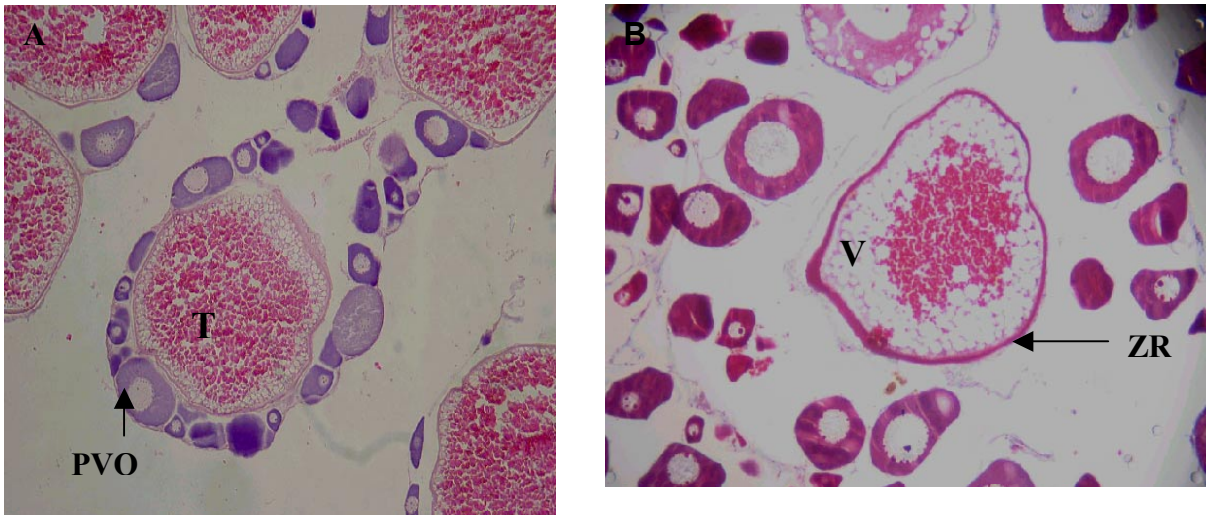

Figure 4.12 A. Encircling of a tertiary oocyte (T) by previtellogeneic oocyte (PVO) at the start of Type II atresia. (H&E) x 400. B. Vacuoles (V) in Type II atretic oocyte with zona radiata (ZR) H&E × 400

The follicular layer of "unspawned" tertiary oocytes detached from the zona radiata and fragmented into small pieces accompanied by karyolysis. The yolk globules in the oocyte cytoplasm fragmented into small granules followed by vacuolar degeneration of the oocyte and hyaline degeneration of zona radiata (Fig. 4.13). Type II atresia occurred in 29 of the 32 "spent" ovaries of fish captured in the Sio River (90.6%) and was not observed in Kagera River population. It was noted that it took over four months to resorb an oocyte, after which time, the younger oocytes had reached the primary yolk vesicle stage.

Type III atresia was observed in samples from both populations and was characterised by cloudy swelling degeneration of the pre-vitellogenic oocytes. Variations of ovarian changes and gonadosomatic indices are summarised in Figure 4.14.

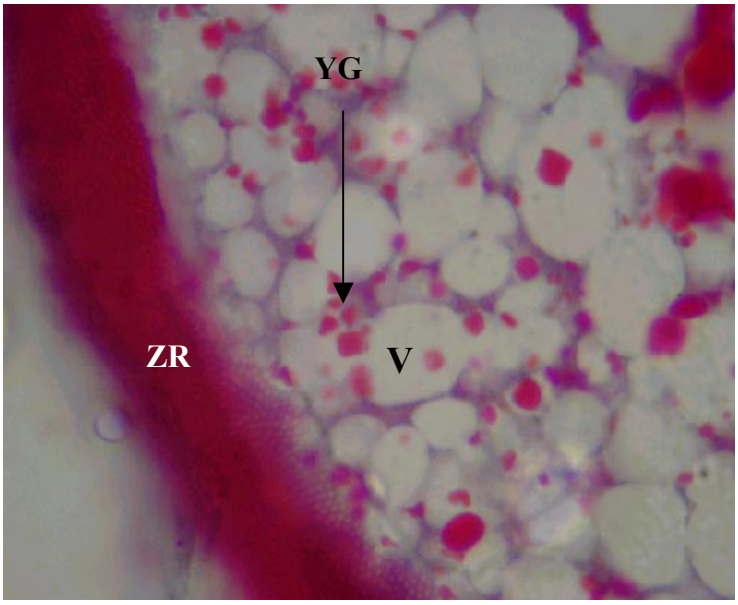

Figure 4.13 Degeneration of zona radiata (ZR) and advanced Type II atresia. Yolk globules (YG) are almost completely replaced by vacuoles (V) and the zona radiata (ZR) is undergoing hyaline degeneration. (H&E) x 400

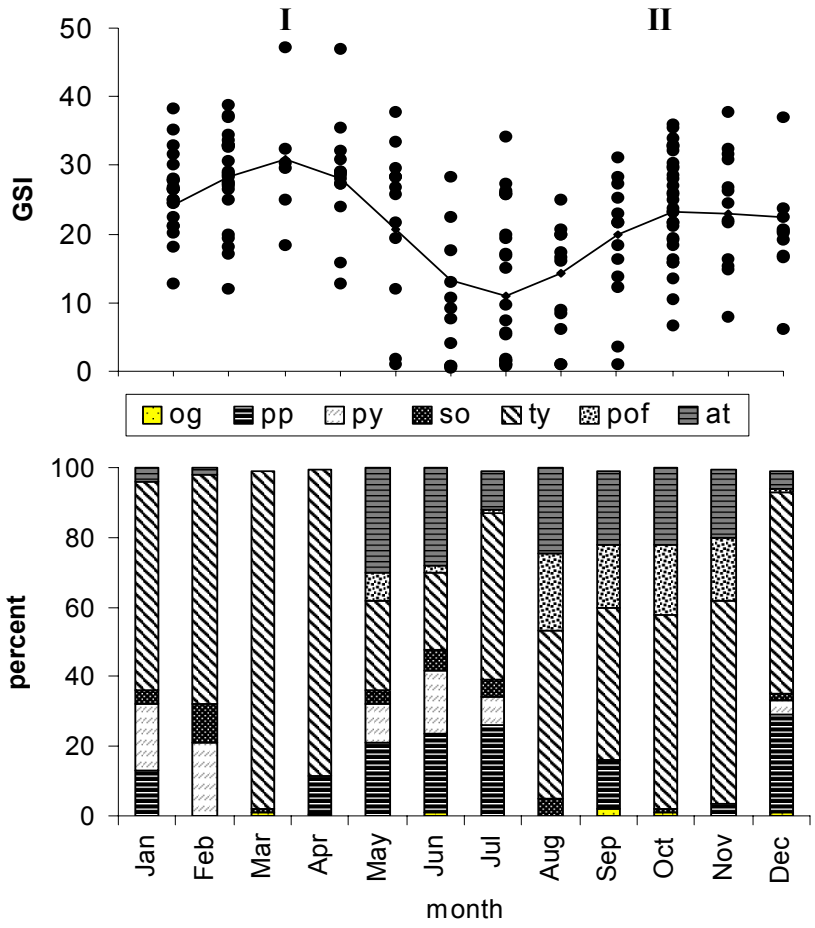

Figure 4.14 Illustration of the relationship between variation of gonadosomatic indices and cyclic changes in the ovary of *Labeo victorianus* from Sio River. Top panel - individual gonadosomatic indices showed two spawning peaks (I and II). Bottom panel - Spawning was indicated by presence of high percentage of tertiary yolk vesicle stages and fresh post-ovulatory follicles (og = oogonia, pp = pre perinucloelus oocytes, so = secondary yolk vesicle oocytes, ty = tertiary yolk vesicle oocytes, pof = post-ovulatory follicles, at = atretic oocytes, n = 188 for both panels.

Macromorphological description of the testes

L. victorianus testes are paired elongated structures, suspended by a mesorchium that extends from one testis to the other, passing over the ventral aspect of the swim bladder. In sexually immature individuals, the testes were thread-like translucent

structures. Sexual maturation was characterised by enlargement, convolution and change in colour with the testes turning white. Testes occupied the entire length of the peritoneal cavity when "ripe". After spawning, testicular whiteness receded craniocaudally and the testes straightened out. The right and left testes were separate along their lengths with main sperm ducts visible on the medial aspect. The two sperm ducts merged to form the common sperm duct that became confluent with the urinary duct. An artery and vein ran on the medial aspect with branches ramifying into the testicular parenchyma. *L. victorianus* testes were categorised into stages based on macroscopic appearance and cytological changes (Table 4.3).

Light microscopic description of spermatogenesis

A tunica albuginea, 20.4±2.1µm thick, consisting of dense irregular connective tissue rich in collagen fibres, fibroblasts and smooth muscle fibres, covered the testes. The tunica albuginea invaginated into the testicular mass to subdivide it into lobules (Fig. 4.15). Continuity of the germinal epithelium was lost during "maturing and late-maturing stages" resulting into a network of interconnected lobular compartments that emptied directly into the main sperm duct (Fig. 4.16a). The connective tissue of tunica albuginea evaginated to form the wall of the main and common sperm ducts.

The main sperm duct was lined by simple columnar epithelium that was low in the rostral and middle sections but became progressively higher caudally. The epithelial cells contained large oval nuclei and rested on a basement membrane under which was dense connective tissue composed of collagen fibres, smooth muscle fibres, fibroblasts and blood capillaries. In the common spermatic duct, the epithelium was

supported by delicate sub-epithelial connective tissue that formed several folds projecting into the lumen. The sub-epithelial connective tissue (lamina propria) blended with the dense irregular connective tissue (sub-mucosa) that formed the bulk of the spermatic duct wall.

Table 4.3 Macroscopic and histological descriptions of *Labeo victorianus* testes at various stages of gonadal recrudescence (Modified from Taylor *et al*., 1998).

Stage	Macroscopic appearance	Microscopic equivalent
1. Early recovery	Testes thick, straight, translucent, strap like and covered by mesenteric fat.	Type B spermatogonia dominate with a few Type A spermatogonia present. Dividing spermatogonia visible at the end of this stage. No spermatocytes, spermatids or spermatozoa. Somatic cells still visible. Cytoplasmic extension of the Sertoli celss enclosing 1-3 Type A spermatogonia. Leydig cells visible.
2. Active recovery	Testes enlarged, begin to turn white but still straight and covered by mesenteric fat.	All germ cell types visible. The lumens of the lobules and ducts are partially filled by spermatozoa from ruptured cysts.
3. Ripe	Testis white and start to form lobes. Mesenteric fat layer less than in Stage 2.	Spermatozoa become the dominant cells in the lobules filling more than 50% of the lumen. Main sperm duct filled with spermatozoa from ruptured cysts
4. Running	Testis white convoluted and is the largest organ in the abdominal cavity. Mesenteric fat is scarce.	Lobules and the sperm duct filled with spermatozoa and resting spermatogonia only, (discontinuation of the germinal epithelium).
5. Spent	Testes appeared as straight, thin, translucent strap on either side of the swim bladder ventral to the kidney.	Testes dominated by Type A spermatogonia. Disintegrating Spermatozoa and spermatids visible in the lumen of the lobules. No other normal germ cells are present. Somatic cells become clear under light microscope.

In the “ripe and running” testes, valve-like structures projected into the lumen of the main sperm duct at intervals of 591.1±246.3µm. These structures consisted of dense connective tissue that was continuous with that of the sperm duct wall (Fig.4.16b).

Valves measured 330.2± 23.1µm in length in the middle section of the testes, but their height decreased craniocaudally.

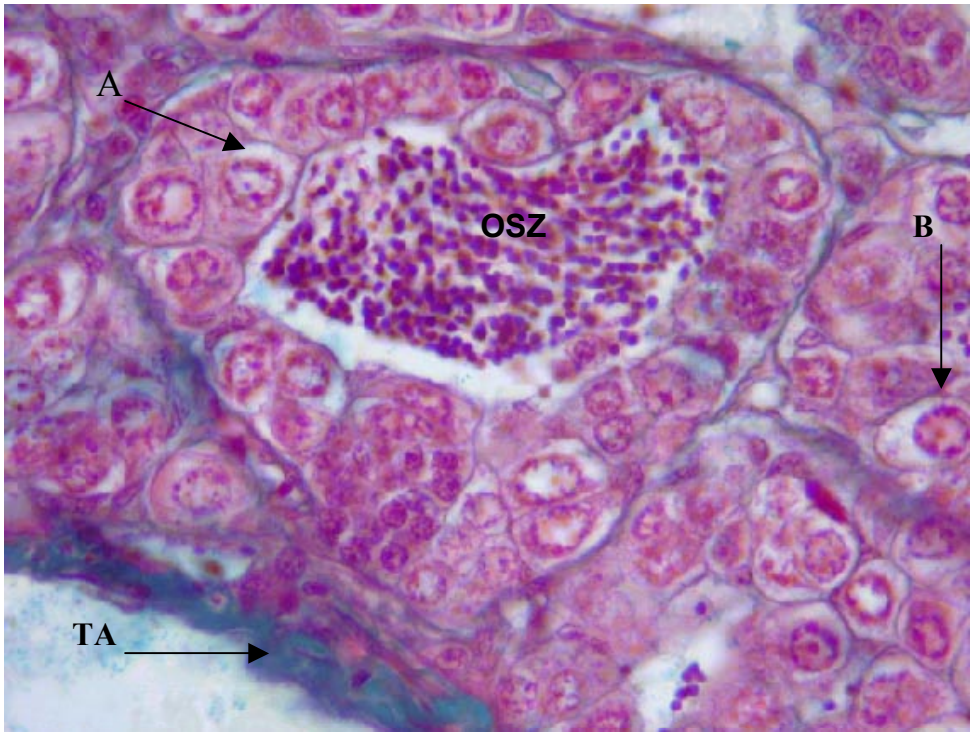

Figure 4.15 Transverse section through a spent testis; Connective tissue strands form tunica albuginea (TA) subdivide the testis into lobules; Type A spermatogonia (A) replaced old spermatozoa (OSZ), Type B spermatogonia were in the more spent lobules, MT × 1000.

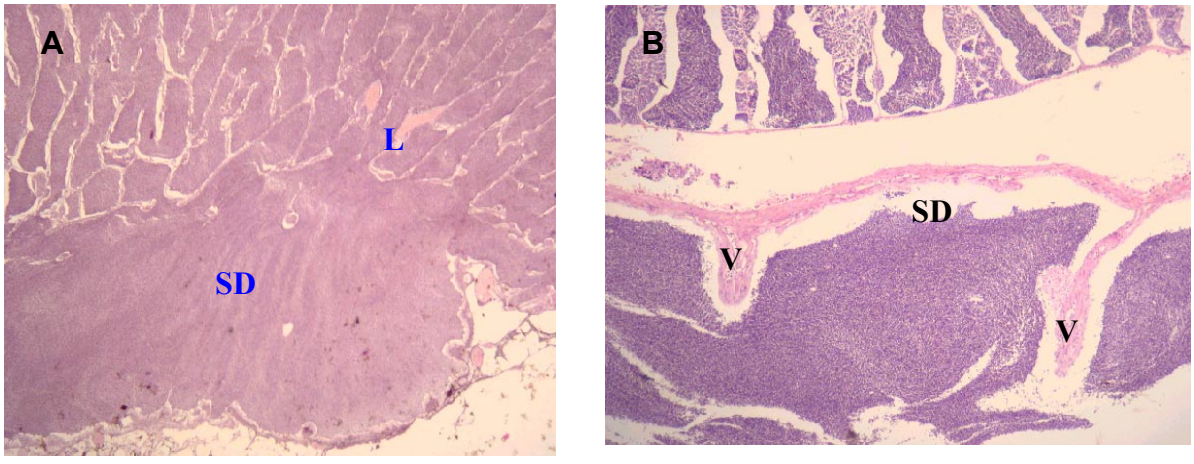

Figure 4.16 Longitudinal section through the testis showing A; lobules (L) opening into the main sperm duct (SD) and B, Valve-like structures (V) projecting into the main sperm duct (SD), H&E ×100.

Sperm ducts from both testes merged to form the common sperm duct that ran caudally to merge with the urinary duct. Prior to confluence, the walls of the two ducts united and formed a papilla that projected into the lumen of the common spermatic duct. Further caudally, the papilla was reduced to a narrow bridge of connective tissue between the urinary and the spermatic ducts. The two ducts merged and opened at the urogenital pore (Fig. 4.17). Both the urinary and sperm ducts were lined by simple columnar epithelium. The connective tissue surrounding the urinary duct was, however, denser with collagen fibres and smooth muscle fibres. The common sperm duct wall was less collagenic being dominated by elastic fibres.

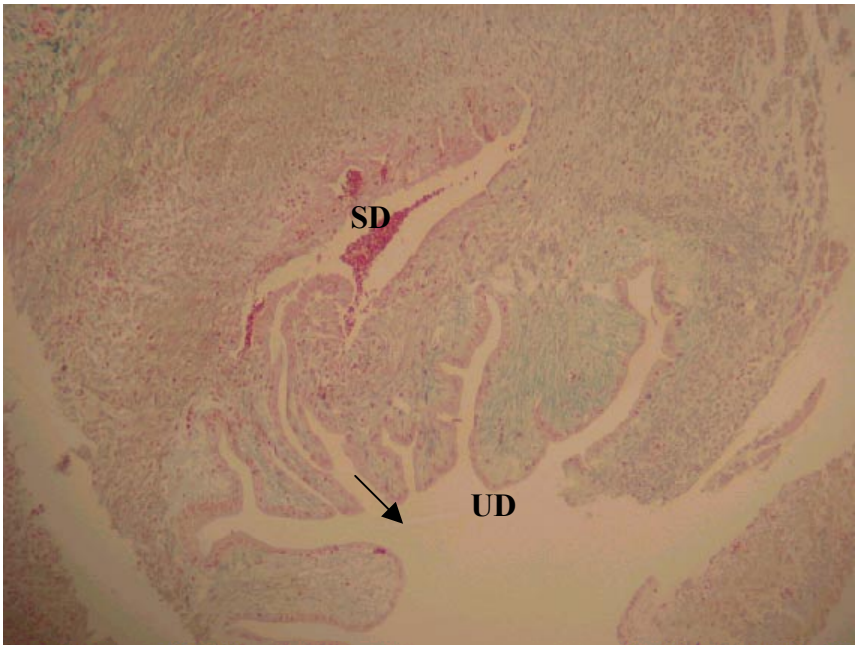

Figure 4.17 Transverse section through the common sperm duct (SD) and the urinary duct (UD) showing joining of the two ducts, MT × 100.

Several germ cell types were noted in *L. victorianus* testes. Type A spermatogonia had round-to-oval shaped nuclei; two nucleoli associated with the nuclear envelope and heterochromatin granules (Fig. 4.15). Type B spermatogonia had one

eccentrically placed oval nucleolus and heterochromatin granules spread along the nuclear envelope (Fig. 4.15). Primary spermatocytes were smaller than the Type B spermatogonia, and had one to three nucleoli in the eccentric nucleus or close to the nuclear wall. In these cells, dense basophilic chromatin strands from the nuclear wall filled the nucleus. Condensation of the nucleus started with primary spermatocytes. Secondary spermatocytes were characterised by an extensive network of highly basophilic chromatin in the nucleus. The spermatids were smaller than spermatocytes (Table 4.4) with a prominent and condensed nucleus. Spermatozoa were the smallest germ cells observed and the most basophilic staining dark blue, in H&E, and were the only cells in the lumen of the lobules (Fig. 4.18). In "ripe and running" testes, the spermatozoa filled the entire lumen with a few resting spermatogonia visible close to the lobule boundary (Fig. 4.19). The nuclear cytoplasm ratio increased as the germ cells matured, such that by the secondary spermatocyte stage, the cytoplasm outline was not properly discernable. The lobules of the lateral region completed spermatogenesis, ahead of those in the medial aspect, close to the main sperm duct. Active cell division characterised by mitosis of spermatogonia and meiosis of secondary spermatocytes was evident at the recovery stage. Germ cell division was followed by spermiogenesis and rupture of the cyst to release spermatozoa in the lumen of the lobules (Fig. 4.18). As spermatogenesis progressed, the number of spermatogonia decreased and were eventually completely depleted.

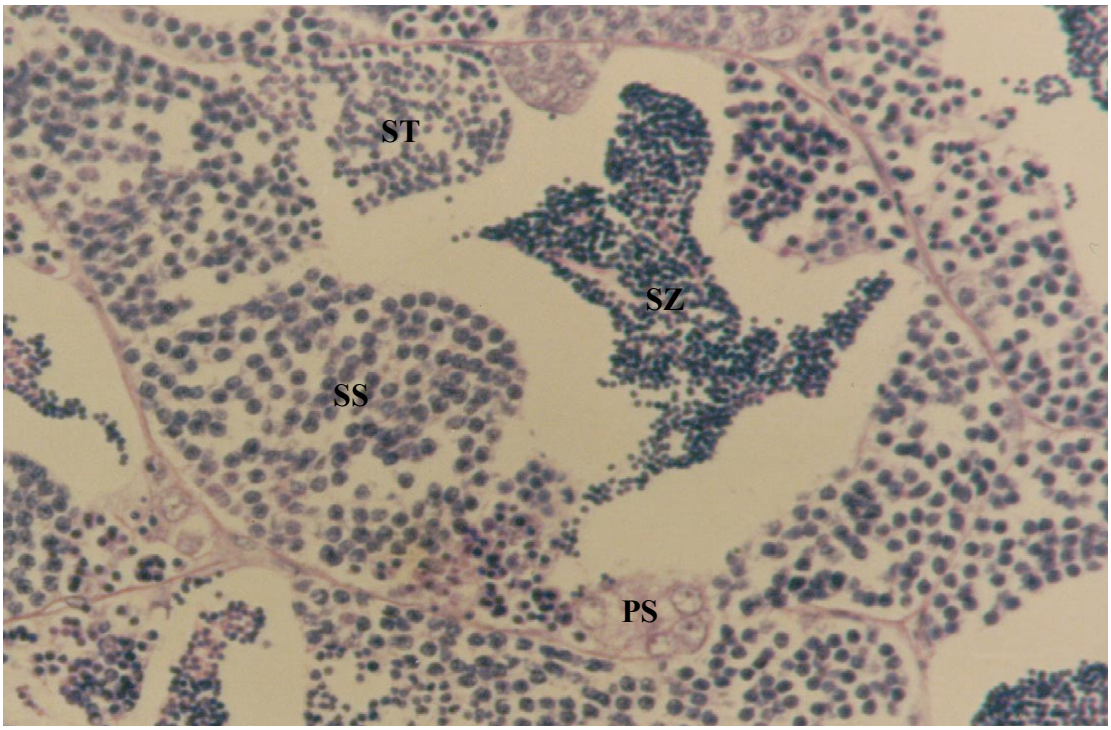

Figure 4.18 Transverse section through the testis showing spermatogenesis. Primary spermatocytes (PS), secondary spermatocytes (SS) and spermatids (ST) develop in cysts that ruptured to release spermatozoa (SZ) into the lumen of the lobule. H&E ×1000.

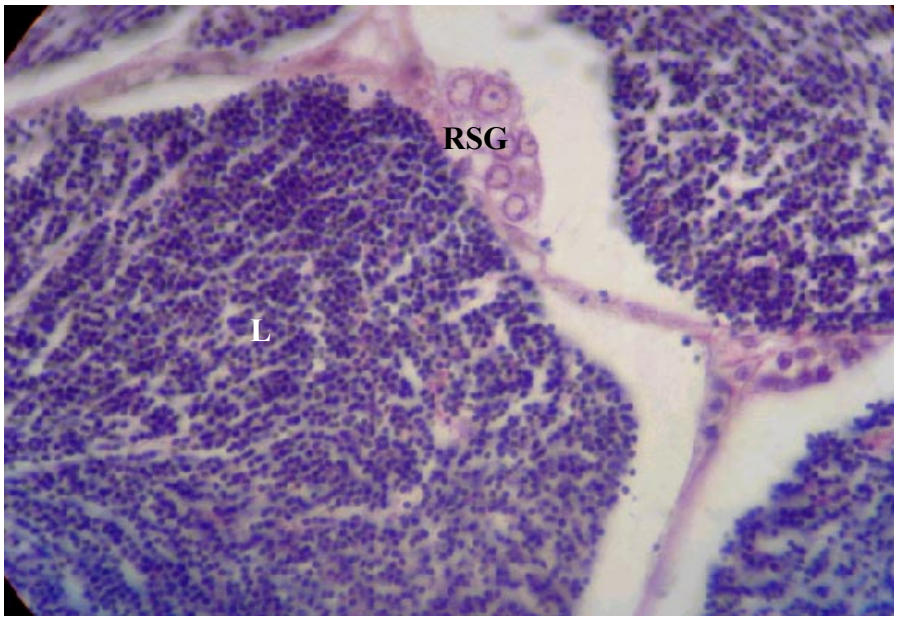

Figure 4.19 Transverse section through a “running” testis showing a lumen (L) completely filled with spermatozoa and resting spermatogonia (RSG), H&E ×1000.

Three types of somatic cells were found in the "spent" testes; Sertoli cells and granulocytes were intralobular while interstitial cells of Leydig were found in groups close to blood vessels in the connective tissue around the lobules. Sertoli cells were observed at the periphery and close to the spermatogonia (Fig. 4.20). The size of the lumen of the lobules varied with the state of the testes ranging in size from 52.9±6.6µm in "spent" to 197±12.6µm in ripe and running "testes".

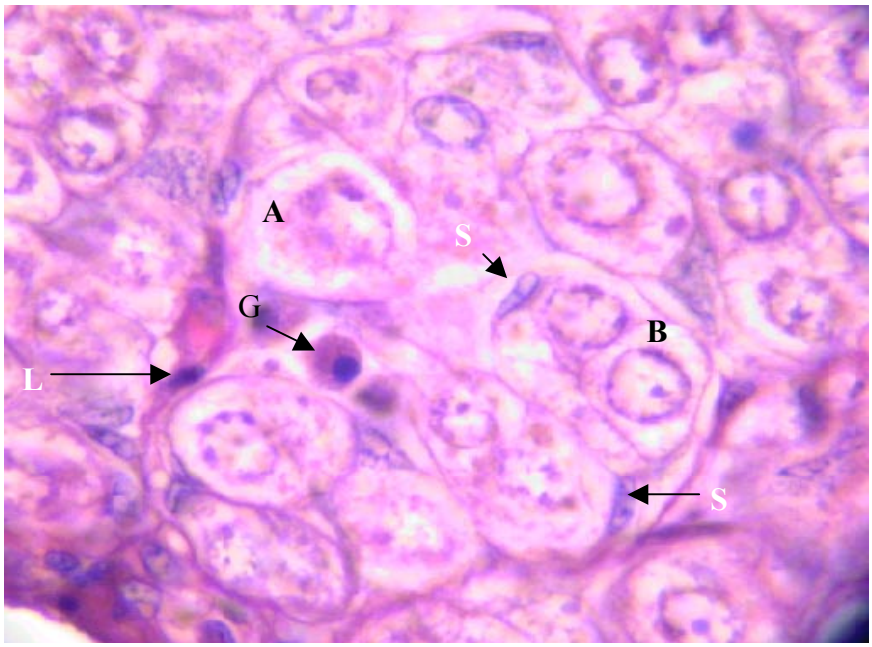

Figure 4.20 Transverse section through a "spent" showing Sertoli cells (S), Leydig cells (L), granulocyte (G) and spermatogonia Type A and B. H&E ×1000.

Transmission Electron Microscopy

Somatic cells, germ cells and connective tissue were observed in the testes. The somatic cells noted, included Leydig cells, Sertoli cells, fibroblasts, erythrocytes, endothelial cells and granulocytes. Leydig cells were oval, contained an irregular nucleus and occurred in the interstitium close to blood vessels (Fig. 4.21a). Sertoli cells had electron-lucent nucleus and cytoplasmic extensions that were joined by tight

junctions to form the cysts. Fibroblasts were long, fusiform in shape and were adjacent to the basement membrane. They contained a heterogeneous nucleus and microfibrils in the cytoplasm (Fig. 4.21b). Each endothelial cell contained a lobed nucleus and lined blood vessels in which the nucleated erythrocytes were found (Fig.4.21a). Lipid globules were observed in the cysts in "late-maturing stage" of the testes (Fig.4.21b). Granulocytes were characterised by a polymorphic heterogeneous nucleus and a cytoplasm with vacuoles and numerous electron dense granules.

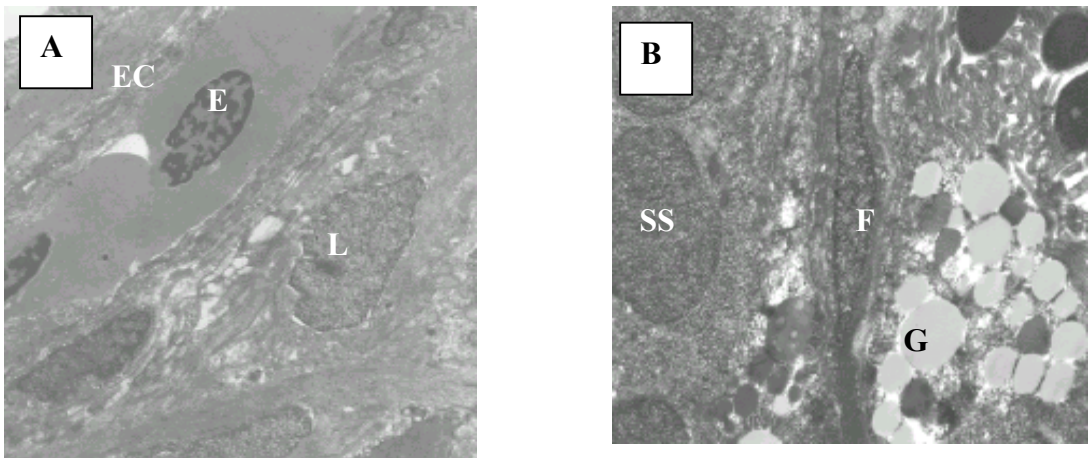

Figure 4.21 Electron micrographs of the testis showing; A, erythrocytes (E), endothelial cell (EC) and Leydig cell (L) in the interstitium. X 5000; B, a fibroblast (F) in tunica albuginea, secondary spermatocyte (SS) and lipid globules (G) in cysts. × 8000.

Germ cells

Primary spermatocytes, secondary spermatocytes and spermatids were found in the spermatocysts with only spermatozoa in the lumen of the testes. Germ cells in the cysts were noted to be at the same stage of development, completely surrounded by cytoplasmic extensions of Sertoli cells, and did not come into contact with the basal lamina of the lobule. Primary spermatocytes were ovoid cells characterised by

irregular light nuclei with central nucleolus and a rim of cytoplasm. Secondary spermatocytes were spherical with a high nucleus cell ratio. Their nuclei were electron-denser than those of the primary spermatocytes. They developed in the cysts and were the most abundant cells in "late-maturing" testes (Fig. 4.22). Meiotic division of the secondary spermatocytes produced spermatids. The young spermatids had a more electron dense nuclei than secondary spermatocytes and were surrounded by interconnected cytoplasm (Fig 4.23) that contained residual bodies. The nucleus: cell ratio varied from 0.59 in young spermatids to 0.8 in those about to complete transformation into spermatozoa.

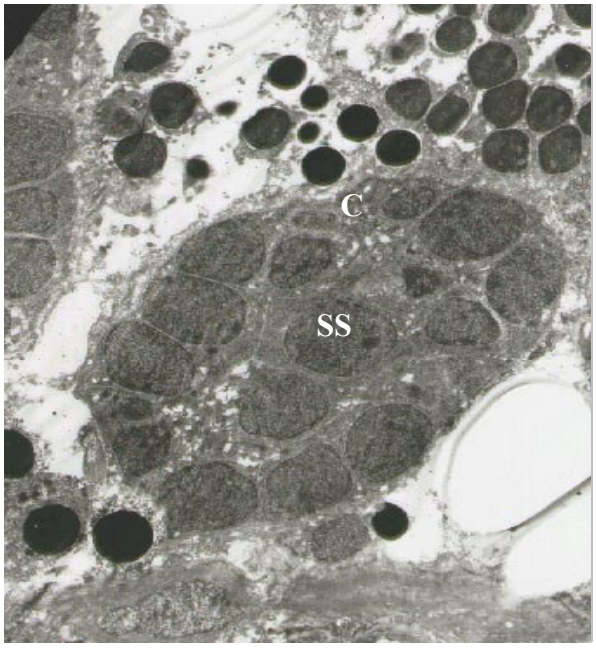

Figure 4.22 Transverse section of the testis showing synchronised development of spermatocytes (SS) within a cyst (C). ×2500.

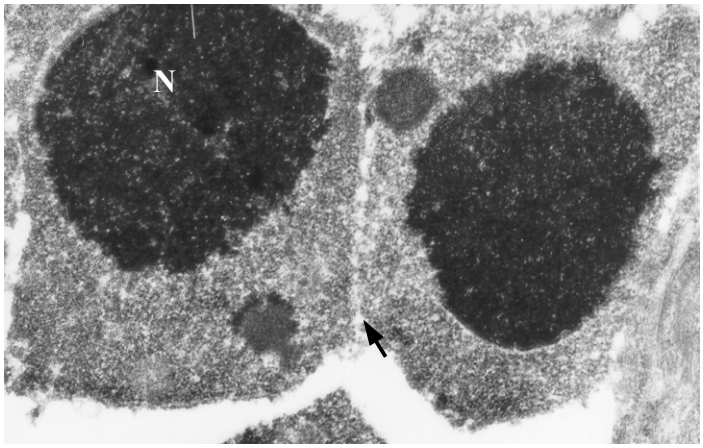

Figure 4.23 Transverse section through a young spermatid showing eccentric and condensed nucleus (N) cytoplasmic bridges (arrow) × 10000.

Transformation of spermatids to spermatozoa (Spermiogenesis) was characterised by increased electron density of the nucleus and discontinuation of the cytoplasmic bridges (Fig 4.24). The nucleus became more compact and located at one pole of the cell while the centriole in the implantation fossa and mitochondria were on the opposite pole. Spermiogenesis termination was marked by elimination of the residual body and rupture of the cyst to release spermatozoa in the lumen of the lobules (Fig. 4.24).

Spermatozoa

The spermatozoon of *L. victorianus* is uniflagellated. The head is spherical, measuring 1.54±0.11µm in diameter, and devoid of an acrosome (Fig. 4.25). A nucleus with electron dense granular chromatin occupied the head region with only a narrow rim of cytoplasm situated around it (Nucleus:cell ratio = 0.91±0.05). The nucleus was indented posteriorly to form a pit or implantation fossa in which the centriolar complex was located. The centrioles were oriented at an acute angle to each other forming a triangle with the nucleus. The proximal centrioles were 0.184±0.02 µm

long and 0.090±0.013 µm wide, while the distal centrioles were 0.294±0.048µm long and .094±0.012 µm wide. The two centrioles were held to each other, and to the nucleus, by fibrils. The distal centriole inserted laterally into the nucleus. An axoneme with the typical pattern of two central microtubules, surrounded by a ring of 9 doublets, originated from the basal body of the distal centriole and pervaded the mid piece.

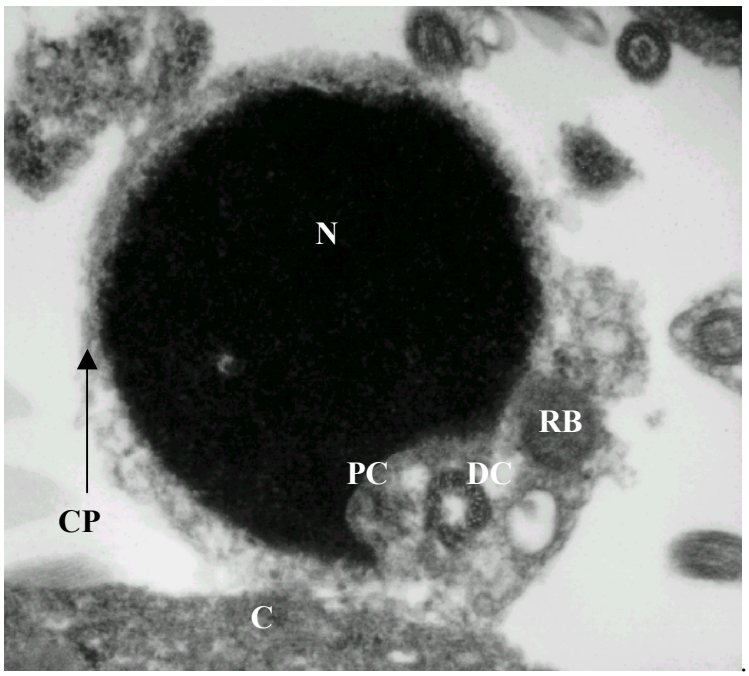

Figure 4. 24 Transverse through a young spermatozoon as it leaves the ruptured cyst (C) into the lumen. A rim of cytoplasm (CP) surrounds a highly electron dense nucleus (N). Distal centriole (DC) and proximal centrioles (PC) are contained in a nuclear fossa. The residue body (RB) is about to be eliminated. ×30000.

The midpiece was cylindrical and rich in cytoplasmic material. It contained six mitochondria and glycogen granules arranged around the postnuclear canal that was formed by invagination of the plasmalemma. The postnuclear canal passed through the midpiece but was not connected to it, except at the basal attachment. Glycogen granules were visible in the rim of the cytoplasm surrounding the nucleus (Fig. 4.25).

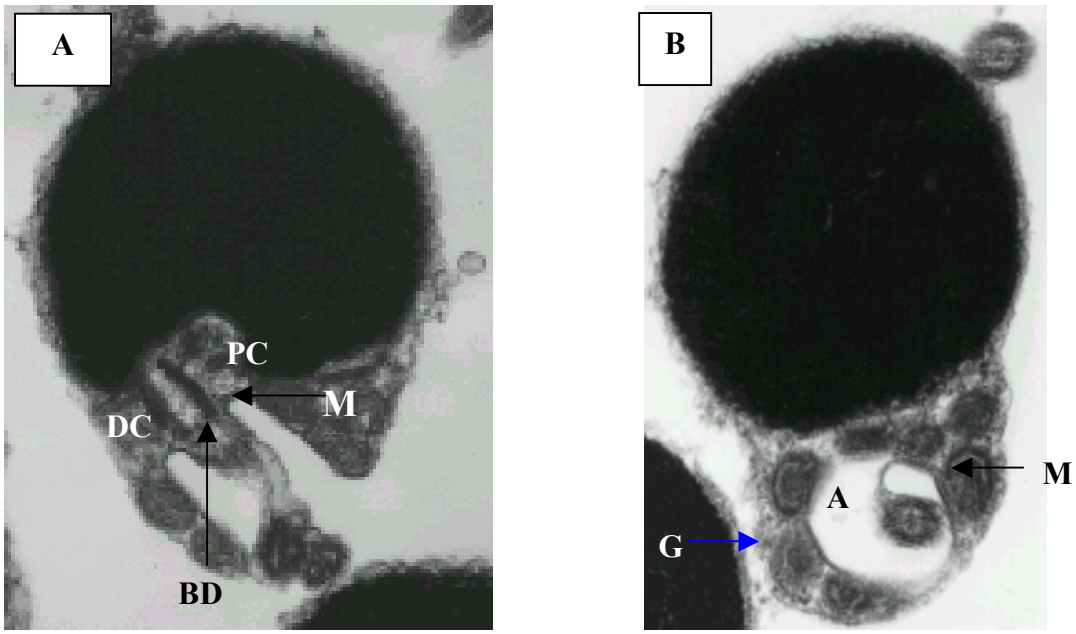

Figure. 4.25 The spermatozoon of *L. victorianus* showing; A, basal body (BD), microfibrils (M), proximal centrioles (PC) and distal centrioles (DC) inserting at acute angle to each other ×20000; B, Six mitochondrial and glycogen granules (G) in the mid piece cytoplasm around the axoneme (A). ×30000.

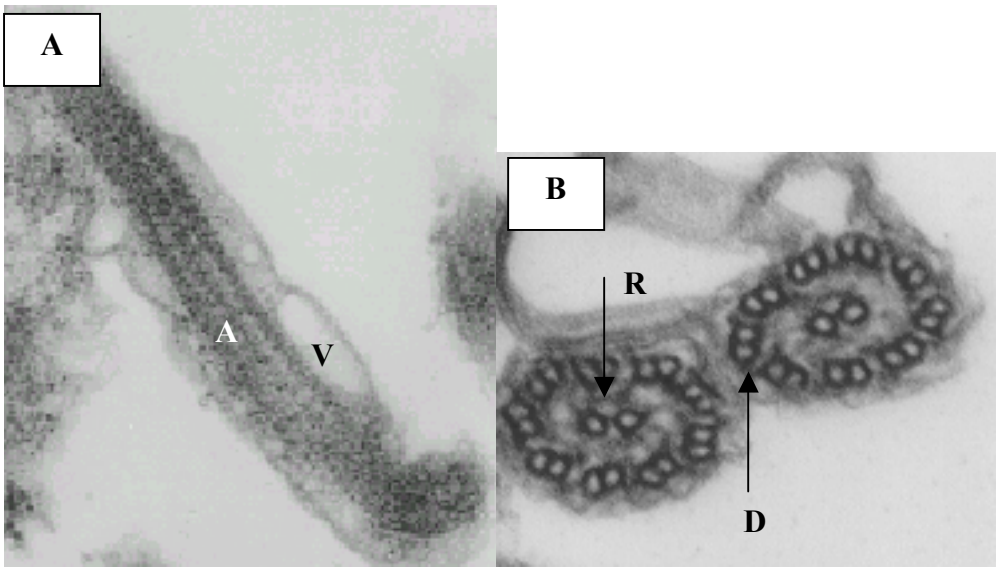

Figure 4.26 The tail of the sperm cell of *Labeo victorianus*; A, Longitudinal section showing axoneme (A) flanked by vesicles (V) ×20000; B, transverse section through the axoneme showing the 9+2 microtubule arrangement with Dynein arms (D) and radial spokes (R). ×60000.

A flagellum with the typical 9+2 microtubule arrangement was contained in the postnuclear canal and was flanked by vesicles (Fig. 4.26). Dynein arms extended from one doublet to another and radial spokes connected the sheath of the core doublet within the peripheral microtubules.

Table 4.4 Mean size (± standard deviation) of germ cells

Cell type	Cell diameter (µm)	Nucleus diameter (µm)	Number (n)
Type A Spermatogonia	14.1±4.1	8.4±2.0	69
Type B Spermatogonia	11.7±3.2	7.9±2.3	44
Primary spermatocytes	7.6±2.2	5.5±2.4	22
Secondary spermatocytes	4.2±0.2	2.9±0.3	81
Spermatids	2.2±0.3	1.7±0.2	65
Spermatozoa	1.64±0.5	1.51±0.1	69

Sperm count and motility

Mean sperm count for testes in the "developing" stage was 0.953±0.61 x 10^6 μl^{-1} for Kagera River population and 1.27±1.55 x $10^6 \mu l^{-1}$ for the Sio River population. In the "ripe and running" stage, the mean sperm count was 3.21±0.43 x $10^7 \mu l^{-1}$ and 3.16±0.44 x $10^7 \mu l^{-1}$ for the Kagera and Sio Rivers, respectively. In both stages there was no significant difference in sperm concentration between the populations for the "developing" stage (Mann-Whitney U test, U = 672, $p>0.05$ n = 38) and the "ripe and running" stage (Mann-Whitney U test, U = 903, $p>0.05$ n = 44).

Mean progressive sperm motility was 16±8 seconds in freshwater, 37±11 seconds in 0.9%NaCl solution buffered to a pH of 8.2 with 10 mmoll^- TRIS base, and 25mmoll^- glycerine, and 3±2 minutes in 0.5% NaCl solution with a pH 7.2. Mean total duration of motility was 29±14 minutes in 0.5% NaCl solution (pH 7.2) but was less than 45 seconds in the other diluents. No sperm motility was observed in undiluted milt.

Discussion

Microscopic investigation of *L. victorianus* ovaries indicated a typically cystovarian structure (Hibiya, 1982). Oocytes developed in cohorts up to the tertiary yolk vesicle oocyte stage followed by ovulation or atresia. In the "ripe-running stage" (Table 4.1), a new cohort of oocytes, developing up to the pre-perinuclear stage, were visible in ovaries dominated by tertiary yolk oocytes. From this observation *L. victorianus* could not be classified as quiescent (Cadwalladr,1965b) as there was no period of inactivity or rest between oogenetic cycles. Oogonia developing from the ovigerous lamellae of "ripe" ovaries were not affected by post-ovulatory changes, with oocytes from the preceding cohort only becoming atretic.

Oocytes from fish sampled two months after peak spawning periods were at the primary and/or secondary oocyte stage. The low prevalence of secondary oocytes was possibly due to rapid oocyte development to the tertiary oocyte stage. Ovaries of fish caught four months post-spawning included the new cohort of oocytes that had reached the tertiary yolk vesicle stage. Based on these observations, it is proposed that development from oogonia to tertiary yolk vesicle oocyte takes four months in *L. victorianus*. Future studies involving repeated ovarian biopsy are, however, required to confirm this observation.

In *L. victorianus,* Type III atresia of pre-vitellogenic oocytes was not characterised by invasion of follicles and phagocytosis (Hibiya, 1982). In contrast, atresia followed a degenerative pattern as in *Cheimerius nufar* (Coetzee, 1983). According to Hibiya (1982) the contents of vitellogenic oocytes liquefy and diminish during atresia, a description that conformed to Type I atresia noted in tertiary oocytes, and also

reported in the mullet, *Mullus surmuletus* (N'da and Déniel, 1993). Tyler and Sumpter (1996) observed that it was difficult to distinguish between an atretic follicle that failed to be ovulated in the previous spawning season, despite reaching full size, and a developing oocyte that becomes atretic before it reaches full size. In this study, atresia was observed in both successful, and failed spawning attempts. In successfully spawned ovaries, fully developed, i.e. tertiary yolk vesicle oocytes, underwent Type I atresia, while Type II was most common at the secondary yolk vesicle stage of development. Type III atresia was observed in Pre-vitellogenic oocyte stages only. In failed spawning attempts Type I atresia was absent but Type II occurred in all vitellogenic oocytes, and Type III in the pre-vitellogenic stages. During oocyte growth, Type III atresia was rare in both *L.victorianus* populations and did not seem to play an important role in determining the number of oocytes that developed to maturity.

Phagocytosis was characterised by areas staining brown in H&E, similar to macrophage centres noted in the spleen of teleosts (Hibiya, 1982). It is reported that follicle cells have the ability to acquire phagocytic properties and have been observed to phagocytise atretic oocytes in some fishes (Habiya, 1982; Hoar, 1969). In *L. victorianus* phagocytosis was only noted in the postovulatory follicles, and therefore appears to be the dominant process by which postovulatory follicles were cleared from the post-spawned ovaries.

Disturbance of spawning was noted histologically by low occurrence of post-ovulatory follicles and Type II atresia that marked the end of oogenesis. This was evident in most Sio River samples. No phagocytosis was observed during this type of

atresia but degenerative changes characterised by the break-up of yolk globules followed by vacuolar degeneration. According to Hibiya (1982) and Bhagyashri and Saidapur (1996) there is resorption of vitellogenin during this process. It could, therefore, be hypothesised that fish inhabiting the Sio River cope physiologically with failed ovulation by resorbing and recycling oocyte nutrients. The slow progression of this form of "atresia", which lasts up to four months, could be to facilitate nutrient transfer from the old oocyte via the liver, and then back into the new cohort of developing oocytes. This process is possibly facilitated by the high degree of vascularisation noted in the ovaries. Studies on the processes involved in nutrient resorption and recycling between the body and the developing oocytes are, therefore, recommended.

Histological investigations during this study have indicated important differences in recrudescence patterns of the two *L. victorianus* populations. Spawning seems to proceed uninterrupted in the Kagera River populations followed by Type I atresia and phagocytosis of post-ovulatory follicles. In contrast, there was over 90% spawning attempt failure in the Sio River populations, as characterised by Type II atresia. The incidence of follicular atresia is reported to be high under sub-optimal conditions (Tyler and Sumpter, 1996). Unfortunately, those mechanisms that regulate follicular atresia in this process are not properly understood as various factors, including environmental conditions, influence follicular atresia in fishes (Bhagyashri and Saidapur, 1996).

This chapter has shown that *L. victorianus* testes can be classified according to both Grier (1981) and Billard (1986; 1990) as unrestricted or lobular, with a structural

mechanism to control the exudation of milt. Several descriptions of the duct system in teleost testes have been reported (Van der Horst, 1978; Grier, 1981; Lahnsteiner *et al.,* 1994). In this chapter, the duct description of Van der Horst (1978) was adopted because it conforms to lobular type testes and also considers the ontogenetic origins of the ducts. No secondary and tertiary sperm ducts, as reported in the *Liza dumerili* (Van der Horst, 1978), were present, as an anastomosing network of tubules in the testis opened directly into the main sperm duct. Arrangement where germinal compartments terminate at the periphery of the testes is considered a feature of higher fishes (Grier and Taylor, 1998). Release of spermatozoa into the duct when spermatogenesis is still on-going probably reduced backward pressure on the spermatogenic cell thus creating space for more spermatozoa from the spermatocyst. The sperm duct is suited for holding spermatozoa before exudation by possession of the simple cuboidal epithelium, which is conspicuously absent in some teleosts like *Liza dumerili* (Van der Horst, 1978).

Results from sperm counts indicate that sperm concentration in the ducts is initially low and peaks when the testes are in the "ripe and running stage" stage. Although histomorphological investigations indicated that female fish were total spawners, it is not known whether *L. victorianus* males spawn once in a spawning season or do it several times. However, the fact that the process of testicular regression was gradual, starting from the rostral to caudal region of the testes, is a cue that exudation of milt in *L. victorianus* is a gradual process during a particular season. This study has showed that *L victorianus* spermatozoa remain viable within the ducts but do not survive in the ambient water conditions once exuded. This finding is in conformity with results reported in *Poecilia reticulata, Dicentrarchus labrax, Sparus auratus, Esox lucius* and

Onchorhynchus mykiss (Billard, 1978), where water was not found to be the best medium for survival of spermatozoa. Spermatozoa viability in the ducts did not, however, last longer than a spawning season as indicated by the massive elimination at the end of a breeding season.

Type A and B Spermatogonia and primary spermatocytes were the most active cells, as indicated by their hetrochromatin nuclei. Condensation of the chromatin within the nucleus initiated in the primary spermatocytes, is known to involve biochemical changes that prepare the male gametes for survival in its external environment during spawning (Grier, 1981). Maturation of germ cells occurred in cysts as in other teleosts (Grier, 1981; Coetzee, 1983) and has been associated with a high degree of gamete synchronization (Van der Horst, 1978).

Ultrastructure sections indicated that spermatogenesis in *L. victorianus* conformed to the cystic type, where spermiogenesis is completed within the spermatocysts and not the semi-cystic (Mattei *et al*., 1993). A comparison of *L. victorianus* spermatozoon morphometry with data obtained from other cyprinids (Table 4.5), indicate that the size of the head is approximately the same size as *Ctenopharyngodon idella Hypophthalmichthys molitrix*, *Rhodeus ocellatus* and *Aristichthys nobilis* but smaller than that of *Leuciscus cephalus*, *Leuciscus souffia Rutilus rubilio*, *Chondrostoma toxostom,* and *Barbus barbus plebejus*. The orientation of centrioles in *L. victorinaus* was also similar to that of *B. plebejus* (Baccetti *et al*., 1984) and all the species studied by Emel'yanova and Makeeva (1985). Analysis of spermatozoa morphometrical data within the Cyprinidae (Table 4.5) indicates a wide variation in the lengths of the flagellum (range = 31 – 60 μm) and the number of mitochondria in

the mid-piece (range = 2 – 10). The length of *L. victorianus* flagellum found in the present study was not exactly equal to any of the species summarised in Table 4.5 but was within the range of 31 – 60 µm while the number of mitochondria relates closely to that of *Rutilus rubilio*. The vesicles that covered the flagellum of *L. victorianus* spermatozoon despite being reported in most cyprinids cannot be considered a common feature within the family since they are absent in some species (Baccetti, *et al*., 1984; Emel'yanova & Makeeva, 1985).

Table 4.5 A summary of spermatozoon characters from 16 cyprinid species

Species	Mean head diameter (µm)	Nucleus diameter (µm)	Number of mitochondria	Presence of vesicles	Length of flagellum (µm)
Alburnus alburnus alborella [1]	2	1.5	2	+	50
Aristichthys nobilis [2]	1.6	-	4 - 5	+	34
Barbus barbus plebejus[1]	1.8	1.7 x 1.5	2	+	60*
Carassius auratus[1]	2*	2 x 1.5	10	+	58
Chrondrostoma toxostoma [1]	1.8	1.75	3-4	+	42
Ctenopharyngodon idella [2]	1.6	-	4 - 5	+	34
Cyprinus carpio[2]	1.8 – 1.9	-	7 - 9	+	40
Hemiculter eignmanni[2]	1.8	-	7 - 9	+	33
Hypopthalmichthys molitrix[2]	1.6	-	4 - 5	+	35
Labeo victorianus [3]	1.6	1.5	6	+	38
Leuciscus cephalus[1]	1.8	1.7	2 - 3	+	50
Leuciscus souffia[1]	1.8	1.3 x 1.6	4	-	56
Opsariichthys uncirostris [2]	1.7 – 1.8	-	2 - 4	+	-
Pseudorasbora parva[2]	1.8 – 1.9	-	2 - 3	+	36
Rhodeus ocellatus[2]	1.6	-	1	-	31
Rutilus rubilio [1]	2	1.5	5-6	+	36

* Stated as nm but assumed to be µm
[1]Baccetti *et al*. (1984), [2]Emel'yanova & Makeeva (1985), [3]present study

In systematic studies of a wide range of animals, frequent use is made of the morphological characteristics of sperm cells (Cross *et al*., 1999). Information from this study can, therefore, be used to define the relationship of *L. victorianus* with other species within the family Cyprinidae. Use can also be made of the morphological

characterisation of *L. victorianus* spermatozoa during sperm preservation and fertilisation since knowledge of the normal structure is vital for recognition of deformations in assessment of sperm quality (Billard, 1978).

In *L. victorianus*, the germinal epithelium was a tripartite arrangement consisting of a basement membrane, spermatogonia and associated Sertoli cells. No follicle cells, as reported for *Onchorhynchus masou* (Hiroi and Yamamato, 1970), covered the spermatogonia. It has been known that fish lack permanent germinal epithelium (Grier, 1981) but according to Grier and Taylor (1998) an arrangement of the basement membrane, Sertoli and germ cells constitute a permanent germinal epithelium that is discontinuous during breeding and continuous in non-breeding testes. The presence of resting spermatogonia in *L. victorianus* supports the hypothesis of a permanent germinal epithelium in teleosts. Depletion of spermatogonia observed in this study is an indication of seasonality in the male reproductive biology of *L. victorianus* and has been observed in other cycling teleosts (Grier and Taylor, 1998), but contrasts with spermatogenesis in the wild carp *Cyprinus carpio* where released spermatozoa are rapidly replaced by a new wave of germ cells (Shikhshabekov, 1972).

Mechanisms by which teleost testes are cleared of unused spermatozoa after spawning are not well defined. Phagocytosis of spermatozoa by macrophages or Sertoli cells has been documented in several species including *Salvelinus fontinalis* (Henderson, 1962), *Parasilurus aristotelis* (Iliadou and Fishelson, 1995) and *Centropomus undecimalis* (Grier and Taylor, 1998). An entirely passive process, where residue spermatozoa are simply voided via the genital opening after the breeding season, has

also been suggested by Grier and Taylor (1998) in the common snook *Centropomus undecimalis*. In *L. victorianus*, there was no cytological evidence of phagocytosis in the lumen where spermatozoa were confined. Presence of granulocytes in the cysts could, however, be a cue for phagocytosis of germ cells in the spermatocysts. Melano-macrophage centres, reported by Grier and Taylor (1998) in *Centropomus undecimalis* testes, were common in ovarian post-ovulatory follicles stained with Haematoxylin and Eosin but were conspicuously absent in similarly stained sections of the testes.

The composition and function of lipid globules seen in the spermatocysts could not be established by this study. Stoumboudi and Abraham (1996) observed lipid droplets in Setoli cells of the *Barbus longiceps* × *Capoeta damascina* hybrid, but their function was not explained. It has, however, been established that gonadotropin stimulates the secretion of 11-ketotestosterone from Leydig cells, which in turn activates Sertoli cells to stimulate premitotic spermatogonia and the complete process of spermatogenesis in fish (Yaron, 1995). Possibly, the oil globules seen in *L. victorianus* testes are part of the synthesis of this steroid hormone.

Gonadal staging is common in fisheries science, but unfortunately most studies concentrate on ovarian recrudescence because macroscopic changes are easily discernable (King, 1995). Omission of testicular staging can lead to loss of valuable information, especially in species where the sexes have different patterns of gonadal development. The only work on the staging of African labeine testes was conducted by Booth and Weyl (2000) who placed more emphasis on oogenesis than spermatogenesis. Findings within this chapter indicated several differences in the

gonadal staging of testes within the African labeine lineage when compared to *L. cylindricus* (Booth and Weyl, 2000). It was noted that the "spent" testes did not show all stages of spermatogenesis and were not dominated by spermatocytes, but by Type A spermatogonia and old spermatozoa (Fig. 4.15. The descriptions of the "developing" and "ripe" stages given by Booth and Weyl (2000) could also be improved by noting that the spermatozoa filled the lobules partially in the "developing" stage and completely when "ripe" (Fig. 4.18 and 4.19). "Ripe" testes cannot be confidently described as showing all the stages of spermatogenesis (Booth and Weyl, 2000) since some lobules had only the spermatozoa and the resting spermatogonia (Fig. 4.19). Gross observations alone were not sufficient to stage the testes since the "spent" and "maturing" testes were almost identical in appearance, but histologically different.

Results from this study underscore the importance of histology in the elucidation of the reproductive tactics adopted by a fish species. Reproduction in fishes is known to revolve around trade-offs between immediate reproductive output and "holding back" some reproductive effort/resources for the future (Matthews, 1998). Investment in the immediate reproduction or "holding back" involves gonadal structural changes that can be discerned by histological investigations.

CHAPTER FIVE

Juvenile intersexuality in *Labeo victorianus*

Introduction

During the embryonic development of vertebrates, totipotent primordial germ cells develop from an extragonadal location and migrate to the gonadal ridge (Brambel, 1956; Mezhnin, 1979; Van Winkoop *at al*., 1992; Mann, 2001). In most vertebrates, the gonads have a cortex and medullary origin with sex determination dependent on the development of these portions. The cortex leads to the formation of the ovary, while the medulla forms the testes. In teleosts, however, the origin of either gonad corresponds to the cortex of other vertebrates (Hoar, 1969).

Available information on sex development within the family Cyprinidae indicate both differentiated and undifferentiated forms, with contradictory reports for some species. *Carassius auratus* was reported to develop an ovo-testis during gonadal development similar to the juvenile intersexuality stage in *Barbus tetrazona* and *Brachydanio rerio* (Takahashi, 1977). Other workers have however reported *C. auratus* and *Barbus conchonius* to be differentiated gonochorists (Parmentier and Timmermans, 1985). Similar unclear information exists on sex development in *Cyprinus carpio.* While Davies and Takashima (1980) reported undifferentiated gonochorism in *C. carpio*, Parmentier and Timmermans (1985) found the species to be a differentiated gonochorist, although they also reported intersexuality in three out of the 36 specimens they investigated.

A comprehensive knowledge of sexuality in fishes is considered vital for the understanding of behaviour patterns, breeding periodicity, growth rates, body colour and shape or sexual dimorphism (Yamazaki, 1983). Information on sex development has also

been applied in fish culture to either initiate sexual change or to improve growth of a particular sex (Yamazaki, 1983; Nakamura *et al*., 1989). No data on sex development and differentiation in any African *Labeo* species are available. In this Chapter, gonadal development and sex differentiation in *L. victorianus* under both natural and culture environments are investigated.

Material and methods

Sample collection

Samples were collected from the natural environment in the Sio River and from induced spawning experiments conducted at KARS (Chapter 2). Juvenile fish were obtained from the mouth of the Sio River by beach seining and gill netting. In order to capture small *L. victorianus*, a small meshed (7 mm stretched mesh size) pond seine net was used. Mean ambient water temperature at the mouth of the Sio River was 23.12±0.62^{o}C. *L. victorianus* was induced to spawn (Chapter 7) and larvae cultured in ponds at the KARS. Pond temperatures ranged from 20.4^{o}C to 25.8^{o}C with a mean of 23.8^{o}C during the rearing period.

Sample processing

Live samples (169 from the ponds and 178 from the Sio River at different stages of gondal development) were anaesthetised with 2-phenoxyethanol (Fluka-Chemie-Sigma Aldrich) and measured for fork length to the nearest millimetre. Where gonads were not macroscopically visible the whole fish was fixed. Details regarding fixation and microtomy are outlined in Chapter 2. Structural measurements were made on scanned sections from at least five fish per microscopic stage using Sigma Scan Pro Image Analysis Software. A Student t-test was used to compare lengths at which the various cell types dominated in the two groups of fish.

Results

Macroscopic description.

Gonads were visible macroscopically in fish from 2.5 months of age and at a mean length of 3.2±1.1 cm FL. Gonads were visible as small irregular transparent tissue patches covered by a peritoneum close to the swim bladder.

Histological description of gonadal development

Microscopic development of the gonads was categorised into four stages:

Stage I: Quiescent primordial germ cell stage

Primordial germ cells (PGC) were visible on the dorsolateral aspect of the body cavity by 119 degree-days post-hatch (Fig. 5.1). PGC were conspicuously large (Table 5.1) with a large nucleus and a single nucleolus. Each PGC was encompassed by one to two somatic cells. By day 714 degree-days post-hatch, the PGC had increased in number and a gonadal ridge was discernable. Primordial germ cells remained quiescent but the accompanying somatic cells underwent mitotic division. At this stage the PGC increased in size (Table 5.1) and remained singly distributed with somatic cells filling spaces between them. By this stage two types of somatic cells were visible. One was spindle shaped, while the other was ovoid. Most cells of the latter group were undergoing mitotic division. Juvenile fish from the induced spawning experiments attained this stage at mean fork length of 6.0±0.50 cm FL. It was not possible to obtain fish of this size class from the wild.

Table 5.1 Summary of fish, cell sizes and number of nucleoli during gametogenesis in *Labeo victorianus*.

Dominant cell type	Cell size (µm)	Nucleus size (µm)	Number of nucleoli	Mean fork length (cm) Pond	River	Student-t and P-values
Quiescent Primordial Germ cells	23.10±4.20	11.60±2.10	1	0.60±0.50	-	-
Mitotic primordial Germ Cells	16.20±1.80	8.20±0.90	1	0.84±0.46 n = 30	0.83±0.07	t=0.44 P=0.66
Gonocytes	13.8.22±0.20	7.46±0.23	1	3.07±0.23 n = 20	3.05±0.18	T=0.23 P=0.82
Oogonia	13.11±0.80	6.80±0.30	1	6.17±0.1 n= 26	6.23±0.3	t=-0.41 P=0.68
Chromatin nucleolar oocytes	41.05±14.00	6.27±7.30	1	6.33±0.03 n = 31	6.51±0.05	t=-1.57 P=0.12
Pre-perinucleolus oocytes	57.50±3.20	42.10±1.90	7.60±0.83	7.81±0.24 n = 25	8.10±2.60	T = -0.32 P=0.75
Spermatogonia/ Atresia	12.90±2.10	7.70±2.10	1-2	9.70±0.63 n = 34	10.30±1.40	t=-1.25 P=0.21

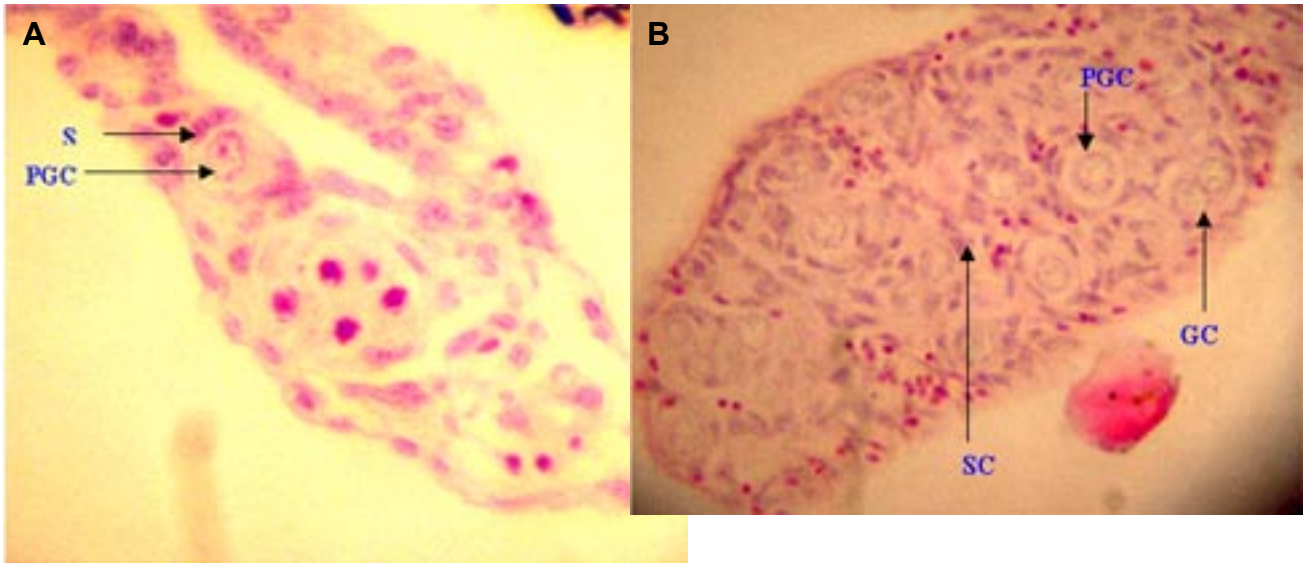

Figure 5.1 Longitudinal sections through the juvenile gonad at the various stages of sexual development in *Labeo victorianus*; A, a quiescent primordial germ cell (PGC) in the gonadal ridge surrounded by spindle shaped somatic cells (S); B, primordial germ cells PGC and gonocytes (GC) in gonadal stroma interspersed with somatic cells (SC).

Stage II: Mitotic primordial germ cell stage

Mitosis of PGC was noted after 1334 degree-days post-hatch. Each primordial germ cell remained surrounded by somatic cells. Division of the primordial germ cells gave rise to gonocytes. The gonocytes stained lightly in H&E and were in groups surrounded by somatic cells. Gonocytes were smaller than primordial germ cells with a dark staining nuclear wall, light staining nucleus and cytoplasm, and one eccentric nucleolus (Fig. 5.1). These cells continued to divide and appeared in cysts in a stroma interspersed with somatic cells.

Intersexuality stage

The first sign of gonadal differentiation was noticed after five months post-hatch. Condensation of chromatin in the nucleus followed by meiotic division of the gonocytes marked the initiation of sex differentiation (Fig. 5.2). All the gonads first developed oogonia, which underwent meiotic division and developed through chromatin nucleolar oocyte to the perinucleolus oocyte stage (Fig. 5.2). Transformation from gonocytes to oogonia was characterised by the spreading of intense basophilic chromatin strands into the nucleus and by an increase in the nucleus: cytoplasm ratio. The first oocyte cell (chromatin nucleolar oocyte) was larger than oogonia (Table 5.1) with a large centrally located nucleus and a light staining cytoplasm. A prominent thick nuclear wall surrounded lightly stained chromatin strands. Perinucleolus oocytes were the largest germ cells attained in gonads that later developed into testes. Several nucleoli in the nucleus and a dense basophilic cytoplasm characterised the perinucleolus oocytes. The gonad was well vascularised and enclosed in a connective tissue capsule. It was at this stage that the direction of further sexual development was defined. The onset and duration of this stage varied widely among individuals. In some individuals sexual development started at six months post-hatch and lasted two to four months. In stunted individuals, onset of

oogenesis was delayed and did not progress up to pre-vitellogenic oocytes before the onset of atresia. Histological sections from five stunted males from the pond showed that sex differentiation started after eight months and gonocytes did not develope beyond the chromatin nucleolar oocytes stage prior to the onset of atresia. (Fig. 5.2).

Stage IV: Sex differentiation stage

In gonads that were to develop into ovaries, oogenesis continued leading to formation of more perinucleolus oocytes. At this stage, ovarian capsules projected its vascularised connective strand or lamellae into the developing ovary. It was in the ovigerous lamellae that oogonial nests and somatic cells were found (Fig. 5.3). This stage was attained after nine months at a size of 10.30±1.40 cm FL and was the highest level of development attained in cultured fish when the study terminated. In the samples from the natural environment, the secondary oocyte growth phase was first observed in fish of mean length 15.2±0.6 cm FL.

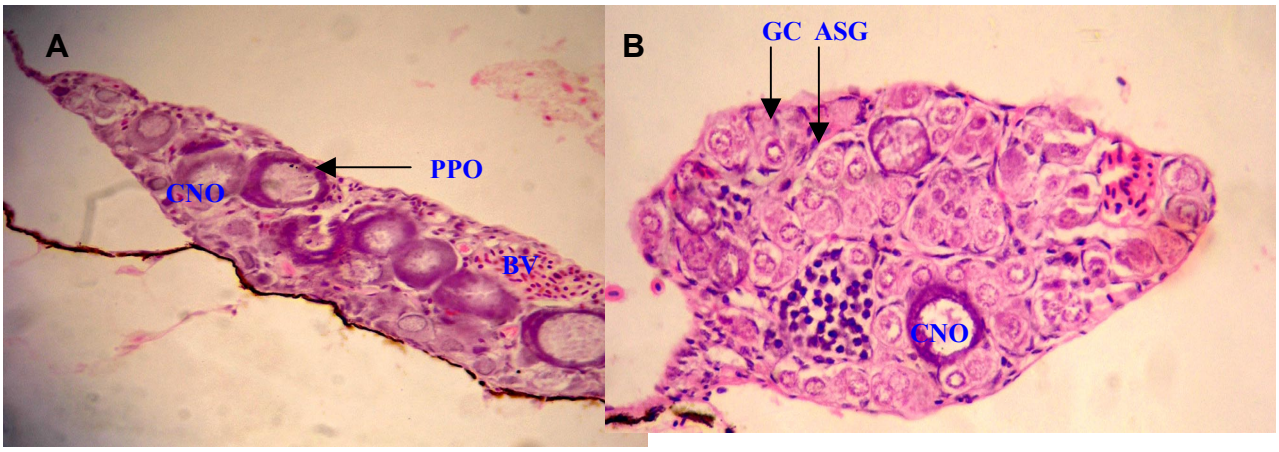

Figure 5.2 Longitudinal sections through the juvenile gonad showing; A, an initial ovary-like structure with chromatin nucleolar oocytes (CNO), pre-perinucleus oocyte, (PPO), and blood vessels (BV); B, in stunted individuals there was transient development in the female direction up to chromatin nucleolar oocyte (CNO) followed by atresia and fast onset of spermatogenesis marked by mitotic division of gonocyte (GC) and the production of Type A spermatogonia (ASG) x 100

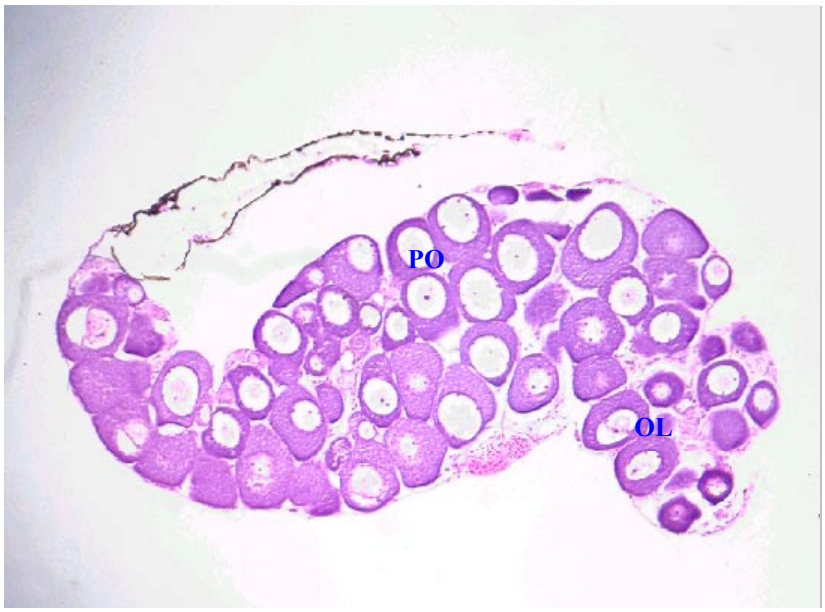

Figure 5.3 Longitudinal section through differentiated ovary of an eight months specimen with ovigerous lamellae (OL) and perinucleolus oocytes (PO). H&E ×100.

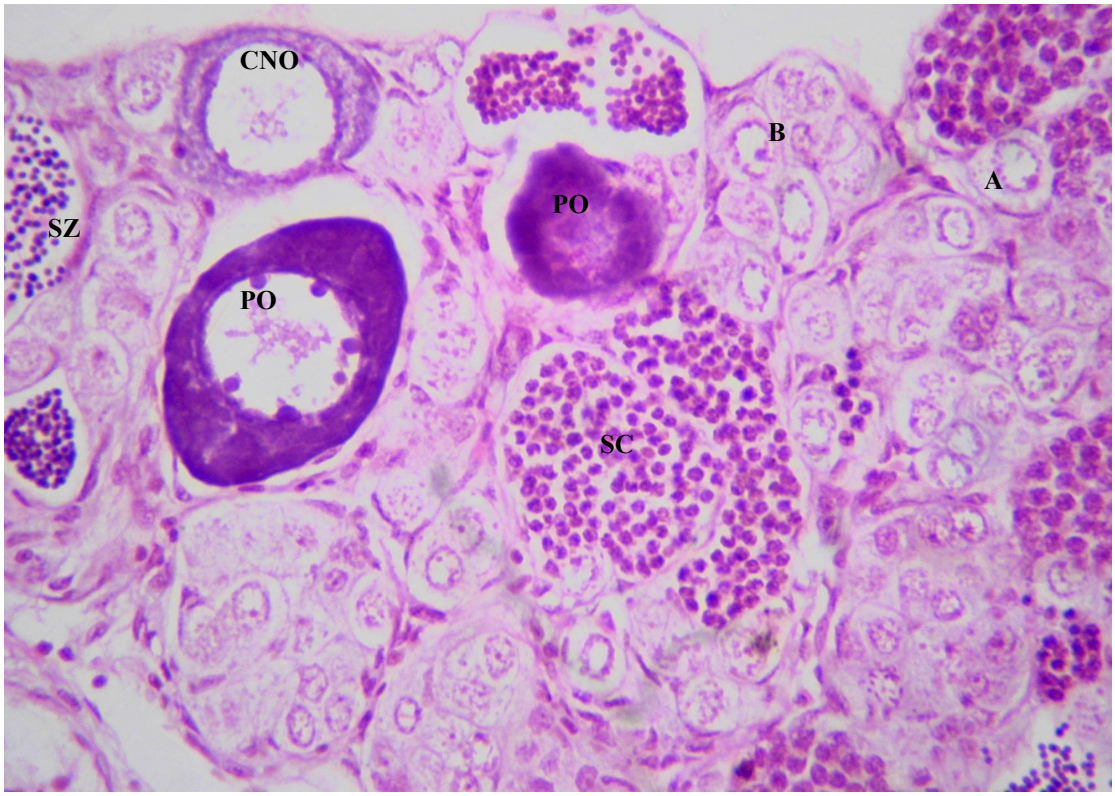

Figure 5.4 Transverse section through a juvenile gonad; Testicular differentiation was marked by atresia of perinucleolus oocytes (PO) and appearance of, Type A & B spermatogonia (A, B), spermatocytes (SC), and spermatozoa (SZ). H&E ×1000

In gonads destined to develop into testes, the perinucleolus oocytes underwent asynchronous atresia. Concomitant with the onset of oocyte atresia was the active division of gonocytes and the appearance of several spermatogonial nests, encompassed by the spindle shaped somatic cells (Fig. 5.4) This stage marked the onset of spermatogenesis. By this stage the ovary was covered by a connective tissue capsule or tunica albuginea consisting of dense irregular connective tissue, rich in collagen fibres, fibroblasts and smooth muscle. Lobules demarcated by invaginations of tunica albuginea were visible.

At the beginning of lobule formation, spermatogonial clusters formed a ring close to the lobular wall leaving an empty space at the centre (Fig. 5.4). The spaces were gradually filled with spermatozoa. The testes at this stage contained all the male germ cells (Fig. 5.4). Remnants of atretic pre-vitellogenic oocytes were still visible in some developing testes from wild juveniles at 15.2 cm FL. At this stage the spindle shaped somatic cells closely enveloped the spermatogonia, while the cuboidal cells were in the interstitial connective tissue at the periphery of the lobules. The pattern of sex development in *L. victorianus* is illustrated in Figure 5.5. There was no significant difference in fork lengths at which the various stages of germ cells dominated the gonads in the two groups of fish ($0.12 \leq P \leq 0.82$; Table 5.1).

Discussion

The quiescence of primordial germ cells found in this chapter indicates that mitotic division does not start immediately after reaching the presumptive gonadal area, as has been reported in *Oryzias latipes* (Satoh and Egami, 1972; Satoh, 1974; Hamaguchi, 1982). This finding does, however, confirm reports by other workers on the development of *Perca fluviatilis* (Mezhnin, 1979) and *C. carpio* (Van Winkoop *et al*., 1992). The

quiescent PGC reached the presumptive gonadal area before the formation of the gonadal ridge. This pattern of development has been observed in several other teleosts and reported to differ from other vertebrates where formation of the gonadal ridge precedes PGC migration (Parmentier and Timmermans, 1985).

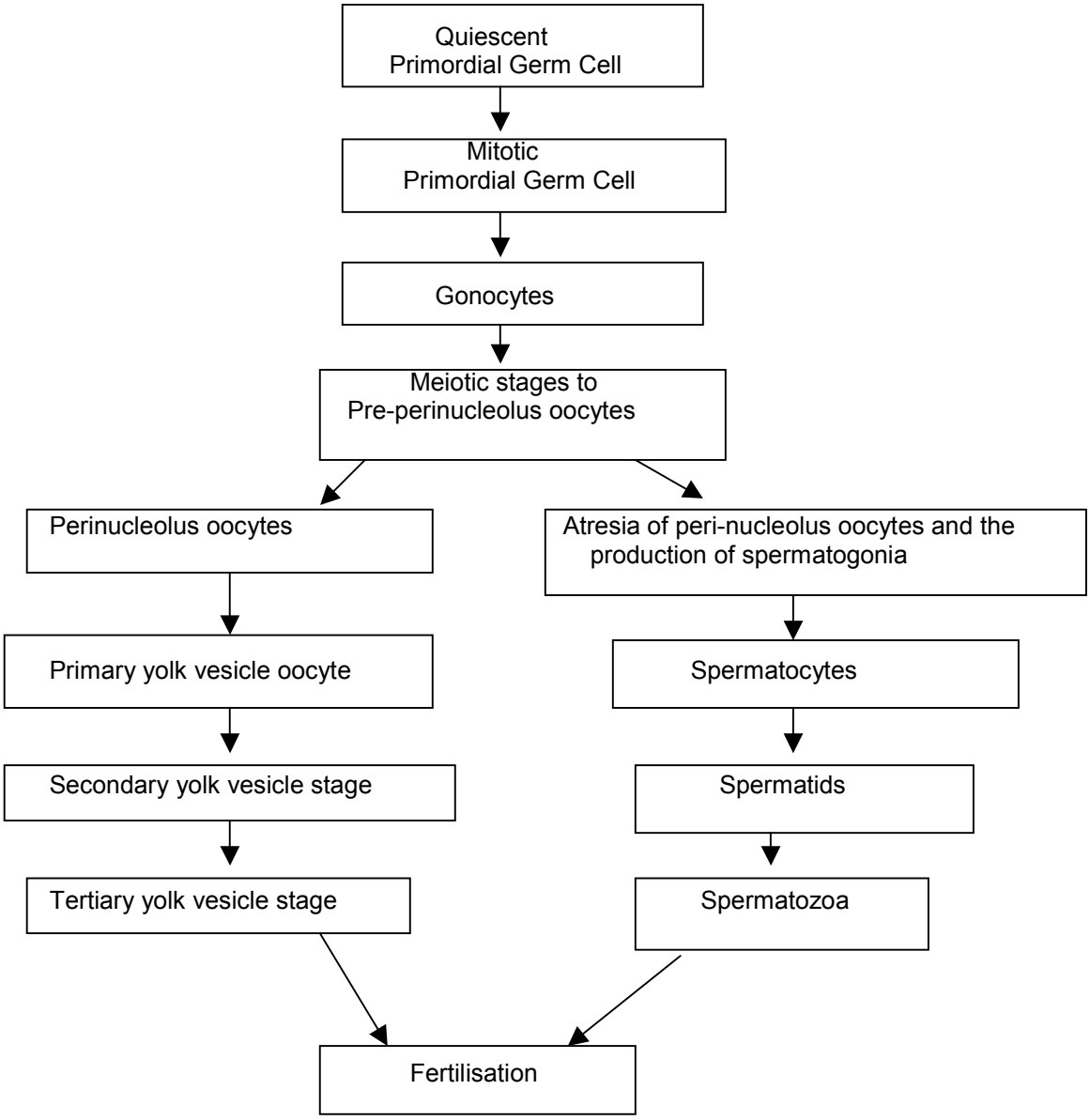

Figure. 5.5 Schematic representation of sex development in *Labeo victorianus*

Ultrastructure studies by van Winkoop *et al*. (1992) on the common carp *C. carpio* indicated that an increase in the size of the primordial germ cells in the quiescent period

was a preparatory phase, prior to rapid proliferation. In *L. victorianus*, germ cells develop in close association with somatic cells right from the primordial stage. The somatic cells actively multiplied and were abundant in the gonadal stroma before the onset of germ cell differentiation. At the start of oocyte atresia and at the onset of spermatogenesis, the spindle shaped cells closely enveloped the spermatogonial cysts and conformed to the Sertoli cells described in this species (Rutaisire *et al.*, in press) and corresponded to the steroid producing cells described by Nakamura and Nagahama (1993).

Various stages of germ cell development have been reported in teleosts. Foyle (1993) described primordial germ cells, definitive germ cells and spermatogonia or oogonia in the differentiated gonochorist *Oncorhynchus kisutch.* Colombo & Grandi (1996) classified *Anguilla anguilla* germ cells into stages such as; Primordial Germ Cells 1 and 2, Oocytes 1 to 4, Spermatogonia 1 and Spermatogonia A and B. Overall, the morphological description of germ cells in *O. kisutch* and *A. anguilla* did not differ from the ones observed in *L. victorianus*. The definitive Germ Cell and Primordial cells 1 corresponded to the quiescent primordial germ cells prior to division. The only difference was in the sequence of development. In *L. victorianus*, gonocytes initially gave rise to oogonia and then to Type A spermatogonia if the gonad differentiated into a testis.

The appearance of pre-meiotic germ cells and the increment of stromal cells in the developing gonads have been reported to mark the onset of sex differentiation in *Oncorhynchus masou* (Nakamura *et al*., 1974) *Salvelinus leucomaenis* (Nakamura, 1982), *Tilapia mossambica* (=*Oreochromis mossambicus*) (Nakamura *et al*., 1973). In *L. victorianus,* stromal tissue increase was not prominent during initial sex differentiation but rather during mitotic division of the primordial germ cells. At the initiation of sex

differentiation, stromal tissue was more concentrated towards the periphery of the gonad where it constituted the tunica albuginea.

It was observed that sex differentiation in *L. victorianus* initially follows an oogenic pathway. A pre-ovarian structure developed in all gonads during early stages of development, and in some cases, oogenesis was advanced up to the perinucleolus oocyte stage. The development of the testes seemed to be an inversion of the female gonad. This was exhibited by atresia of vitellogenic oocytes and the concomitant appearance of gonocytes that actively divided and transformed into spermatogonia. The source of gonocytes that gave rise to spermatogonia within a gonad that was already developing in the ovarian direction, could not be ascertained during this study. It has been reported that sex cell reserves in fish are continuously replenished from the germinal epithelium (Mezhnin, 1979). Shapiro (1987) proposed that a pool of biopotential germ cells remains quiescent during the initial development of sex and then differentiate into appropriate germ cells at the time of sex change.

Atresia of the pre-vitellogenic oocytes in *L. victorianus* was by cloudy swelling degeneration without melanomacrophage centres, as reported in *Epinephelus marginatus* (Marino *et al*., 2001). Results from this study revealed that spermatogenesis ensued from the start of oocyte atresia and the formation of spermatogonia. There was also no long quiescent period of spermatogonia as reported in *O. masou* (Hiroi and Yamamato, 1970). Rapid onset and progression of spermatogenesis was indicated by presence of spermatozoa in the lumen of gonads still with atretic oocytes.

It is documented that fishes exhibit a wide range of sexual ontogenies such as synchronous, protandrous and protogynous hermaphroditism, rudimentary

hermaphroditism (=late gonochorism) and gonochorism (Yamazaki, 1983; Buxton and Garratt, 1990). Developmental patterns characterised by intersexuality have been documented in several fish species including: *Petromyzon marinus* (Lowartz and Beamish, 2000), *Thalassoma lucasanum* (Warner, 1982), *Thalassoma duperrey* (Ross, 1984), *Liza saliens* (Mogil'naya and Moiseeva, 1985), *Chrysophrys aurata* (Francis and Pankhurst, 1988), *Pagrus major* (Matsuyama *et al.,* 1988), *E. marginatus* (Marino *et al*., 2001) and several Sparid species (Buxton and Garratt, 1990). Hoar (1969) suggested that intersexuality in teleosts may be attributed to the embryological development from a single primordium, whereas both Alekseev (1983) and Francis and Pankhurst (1988) proposed that juvenile sex change represents a secondary development from ancestral hermaphroditism towards gonochorism and, therefore, has no adaptive significance. Sadovy and Shapiro (1987) reviewed criteria for sexual categorisation of fishes as hermaphrodites or gonochorists. In their opinion, a species can only be considered hermaphroditic if a substantial proportion of individuals in a population function as both sexes, either simultaneously or sequentially.

Results in this chapter revealed the presence of gametes of both sexes in the same gonad during development, but the gonads were not yet functional as oogenesis in predestined males did not progress beyond the perinucleolus oocyte stage, *L. victorianus* can, therefore, not be considered to be hermaphroditic, but is an undifferentiated gonochorist (Yamamato, 1969; Yamazaki, 1983; Buxton and Garratt, 1990). Sex differentiation in gonochoristic teleosts has been categorised either as differentiated or undifferentiated (Mezhnin, 1979; Yamazaki, 1983). Species within the differentiated category express direct sexual development as either male or female. Such a developmental pattern has been documented in *P. fluviatilis* (Mezhnin, 1979), *O. kisutch* (Foyle, 1993) and *Salaria* (=*Blennius*) *pavo* (Patzner and Kaurin, 1997). In the undifferentiated form, the gonad first

develops into an ovary-like structure followed by one half becoming males and the other half continuing as females (Yamazaki, 1983). Based on this categorisation and the results obtained in this chapter, it can be inferred that *L. victorianus* is an undifferentiated gonochorist.

Occurrence of intersexuality in all the specimens collected from natural and culture environments indicated that undifferentiated gonochorism is the norm in this species. In *L. victorianus*, sex development and differentiation seemed to depend on size rather than age. In stunted fish from the pond, sex development lagged behind that of fast growing individuals within the same cohort. Dependency of sex expression on length was further indicated by the lack of significant differences in size between cultured and river fish during different stages of gonadal development, despite the fact that the age of the wild samples was not known. It is therefore proposed that fork length could be used as a potential standard measure of sex development. Similar patterns of sexual development dependent on size and not age was observed in *A. anguilla* where gonad differentiation differed in fish of the same age but of varied body size (Colombo *et al*., 1984; Colombo and Grandi, 1996).

This chapter has histologically demonstrated undifferentiated gonochorism in *L. victorianus* and provides the first report of such pattern of sex development in an African labeine cyprinid. Additional research is required to ascertain whether undifferentiated / late-gonochorism is the norm rather than the exception in the genus *Labeo*.

CHAPTER SIX

Morphological and genetic characterisation of two populations of *Labeo victorianus*

Introduction

The significant differences in reproductive biology parameters such as size-at-maturity and recrudescence patterns, noted in Chapters 3 and 4, could not be properly elucidated without ascertaining whether the two populations are phenotypically and/or genotypically homogenous. Unfortunately no genotypic information is available on the African labeines with all previous African labeine systematics having been based on meristic and morphological variations (Boulenger, 1909; Greenwood, 1966; Reid, 1985). Genetic information pertaining to the two populations is therefore required to establish whether the two populations are genetically closed and isolated from each other, or if there is some maintenance of gene flow. These data are requisite for both fisheries management and captive culture purposes. Genetic characterisation of distinctive populations within a species into "stocks" is important (Ward, 2000). Whereas conservation biologists are generally concerned with the assessment of the demographic structure and genetic diversity of the sub-populations in different parts of the lake, aquaculturalists interested in foodfish culture or captive breeding for restocking purposes. Both need information on genetic structure of discrete populations to maintain genetic diversity.

Methods used to measure genetic variation and reconstruction of phylogenetic relationships in fishes have included; variation in protein sequence/Allozyme variants (Agnèse *et al.*, 1997; Ragnon *et al*., 1998; Osinov and Lebedev, 2000), restriction fragment lengths polymorphisms (RFLP) (Agnèse *et al*., 1997; Yoshizaki *et al*., 1997;

Nielsen *et al*., 1998), randomly amplified polymorphic DNAs (RAPDs) (Chapman *et al*., 1999; Mamuris *et al*., 1999) and direct nucleotide sequencing (Okazaki *et al*., 2001; Takeyama *et al*., 2001).

Direct nucleotide sequencing methods have a high resolution and are therefore useful in phylogenetic studies (Avise *et al*., 1988; Sang *et al*., 1994; Chen *et al*., 1998) with both nuclear and/or mitochondrial DNA sources found to be valuable in investigating fish (Waters *et al*., 2000; Wilson *et al*., 2000). Because of its high rate of evolution and maternal inheritance, high substitution rate and apparent lack of recombination (Avise, 1986; Wenink *et al*., 1993), mitochondrial DNA (mtDNA) is widely used in molecular evolutionary studies (Ragnon and Guyomard, 1997; Krieg *et al*., 2000; Waters and Willis, 2001). Furthermore, mitochondrial DNA has been reported to be highly sensitive to successive bottlenecks in comparison to nuclear DNA (Krieg *et al*., 2000), and has been useful in studies of ancient and recent demographic changes such as expansion or decline (Goldsworthy *et al*., 2000; Fleischer *et al.,* 2001).

Due to high levels of sequence polymorphism, the displacement loop (d-loop) has been of particular importance in the study of closely related populations in various taxa including humans (Vigilant *et al*., 1991), birds (Wenink *et al*., 1993), gorillas (Garner *et al*., 1996) and fish (Chen *at al*., 1998; Wilson *et al.,* 2000; Waters and Wallis, 2001). Polymorphism in the d-loop region of mtDNA has been found not only to be useful in interspecific studies but has also been shown to be sensitive enough for intraspecific phylogenic/phylogeographical reconstruction of population relationships in fishes (Chen *et al*., 1998). In this chapter, polymorphism in the d-loop region of *L. victorianus* was used to

firstly measure genetic diversity, and secondly, to determine possible population subdivisions and the detection of past demographic changes.

Material and methods

Sampling Strategy

Samples used in this study were obtained from the Kagera and Sio River populations, which constitute the present range of *Labeo victorianus* in Uganda. Individuals from different localities along the rivers were randomly sampled using active fishing gear in order to minimise sampling of possibly closely related individuals and to maintain sample freshness. A total of 38 samples were obtained, 19 from each river.

Sampling technique

Tiny pieces of fresh muscle tissues were excised from under the skin taking care to avoid contamination, and then immediately transferred into a storage buffer.

Morphometric Analysis

Fifty-six specimens, 26 from the Kagera River and the 30 from the Sio River, were used for morphometric analysis. A truss dimension (Strauss and Bookstein, 1982) of each fish was constructed (Fig. 6.1; Table 6.1), using callipers, following landmarks adopted from Flemming *et al*. (1994) and Hauser *et al*. (1995). In addition, classical (non-truss) measurements were made on all the specimens. A total of twenty truss and nine non-truss morphometric variables (Tables 6.1 and 6.2) were measured on each specimen. All morphometric measurements were adjusted for variation in fish size (Reist, 1985) according to the formula; $\varepsilon = \log_{10}Y - \beta(\log_{10}X - \log_{10}X_{SL})$ where ε = standard measurement; Y = character length; β = slope of $\log_{10}Y$ against $\log_{10}X$ plot for each population, where X = standard length of the specimen and X_{LS} = mean standard length of

all the samples (Karakousis *et al*., 1993). Size adjusted measurements were submitted to a principal component analysis (PCA) to explore possible separation between groups into discrete clusters. The null hypothesis that the two rivers were separated was then tested using Discriminant Function Analysis. Landmarks indicated in Figure 6.1 and the dimensions measured are described in Tables 6.1 and 6.2.

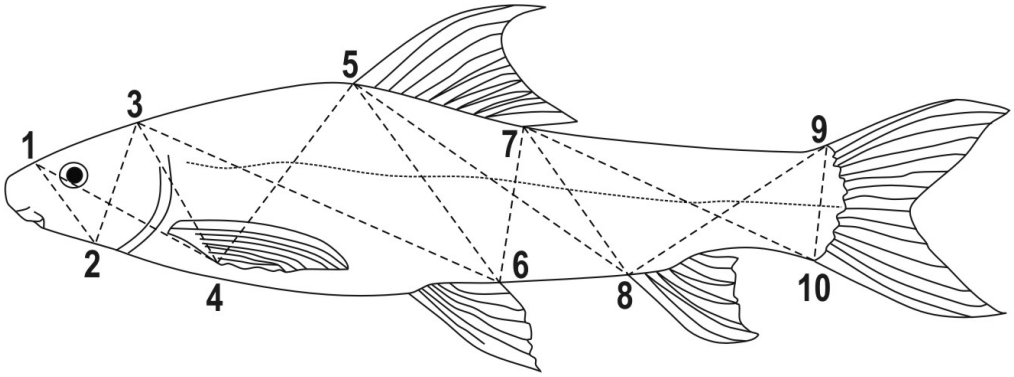

Figure 6.1 Sketch drawing of *Labeo victorianus* showing the location of landmarks and distances used for the truss system (adopted from Hauser *et al*., 1995).

Table 6.1 Descriptions of landmarks, corresponding to Figure 6.1, used in the morphometric analysis of *Labeo victorianus*.

Landmark	Description
1	Snout
2	Ventral junction of operculum
3	Supra orbital
4	Lower Pectoral Fin insertion
5	Anterior Dorsal Fin insertion
6	Posterior end of Pelvic Fin insertion
7	Posterior end of Dorsal Fin insertion
8	Anterior end Anal Fin insertion
9	Dorsal Caudal fin Insertion
10	Ventral Caudal fin insertion

Table 6.2 Truss and non-truss dimensions measured for the morphometric analysis of *Labeo victorianus*.

Truss	Non-truss
1. Snout to ventral operculum junction distance (SOJ)	1. Head depth (HD)
2. Snout to lower pectoral fin insertion (SPF)	2. Orbital distance (OD)
3. Snout to posterior end of supra orbital (SPO)	
4. Posterior end of supra orbital to lower pectoral fin insertion (PSOJ)	3. Intra-orbital distance (IOD)
5. Posterior end of supra orbital to ventral operculum junction (PSPF)	4. Inter-nostral distance (IND)
6. Posterior end of supra orbital to posterior end of pelvic fin origin (PSPP)	5. Mouth width (MW)
7. Posterior end of supra orbital to anterior dorsal fin insertion (PSAD)	6. Pectoral fin length (PEC)
8. Anterior dorsal fin to lower pectoral fin insertion (ADPF)	7. Pelvic fin length (PLV)
9. Anterior dorsal fin to posterior end of pelvic fin origin (ADPP)	8. Caudal fin length (CFL)
10. Anterior dorsal fin to anterior anal fin insertion (ADAF)	9. Anal fin length (AFL)
11. Anterior dorsal fin to posterior dorsal fin insertion (ADPD)	
12 Posterior dorsal fin insertion to posterior pelvic fin insertion (PDPP)	
13. Posterior dorsal fin insertion to anterior anal fin insertion (PDAF)	
14. Posterior dorsal fin insertion to dorsal caudal fin insertion (PDDC)	
15. Posterior dorsal fin insertion to ventral caudal fin insertion (PDVC)	
16. Lower pectoral fin origin to posterior pelvic insertion (PFPP)	
17. Posterior pelvic insertion to anterior anal fin insertion (PPAF)	
18. Anterior anal fin insertion to ventral caudal fin insertion (AFVC)	
19. Dorsal caudal fin insertion to ventral caudal fin insertion (DCVC)	
20. Anterior anal fin insertion to dorsal caudal fin insertion (AFDC)	

Storage of samples for DNA extraction

Samples were immediately stored in cryovial tubes containing 25% dimethylsulfoxide (DMSO) saturated with NaCl solution (Amos and Hoezel, 1991). DMSO prevents enzymatic decay of tissues and degradation of DNA. In the field, samples were kept at room temperature. In the laboratory, samples were immediately transferred to a freezer maintained at -80ºC pending DNA extraction. All equipment such as forceps and scalpels were either used once or were cleaned with hydrogen peroxide (H_20_2) and sterilised with 97% ethanol between uses in order to prevent cross contamination of samples. All samples were handled with latex examination gloves to minimise chances of cross contamination and contamination with human DNA.

Total genomic DNA was extracted from the samples using the QIAGEN Dneasy tissue kit (QIAGEN) following the manufacturers' protocol.

Mitochondrial DNA amplification using Polymerase Chain Reaction (PCR)

Design of primers

New primers for amplification of target regions are usually designed based on homologous sequences of closely related species that have already been sequenced (Palsbøll and Actander, 1998). By aligning homologous segments of sequences of several related species, conserved sequence blocks are identified from which primers can be designed to flank the region of interest. A similar approach was used to design primers that amplified the 5' end of the d-loop of *L. victorianus* based on data from the gene bank (Figs. 6.2 and 6.3).

The control region of the following fish species were retrieved from the gene bank, aligned and the conserved blocks at the beginning and upstream of the control region identified; *Rutilus rubilio* (accession number AJ388400), *Leuciscus multicellus* (accession number AJ388399), *Labeo bicolor* (accession number AJ388414), *Gobio gobio* (accession number AJ388393), *Carassius auratus* (accession number AJ388413), *Chondrostoma toxostoma* (accession number AJ388395) and *Barbus meridionalis* (accession number AJ388417). Nucleotide bases between positions 20 to 37 (Fig. 6.3) were used to design the forward primer LaviF (5'-CAC CCCC TGG CTC CCA AA-3') while nucleotide bases between position 794 to 821 were used to design the reverse primer LaviR (5' CCT CCT TGG TTT AGG GGT TTG ACA AGG-3') using the program AMPLIFY VER 1.2

(Engels, 1993). Figures 6.2 and 6.3 illustrate the positions from which the primers were designed.

Position Species

1 11 21 31 41

AAAAGGAGAT TTTAACTCCC ACCCCTGGCT CCCAAAGCCA GAATTCTAAA *Rutilus rubilio*

AAAAGGAGAT TTTAACTCCC ACCCCTGGCT CCCAAAGCCA GAATTCTAAG *Leuciscus multicellus*

AAAGGGAGAT TTTAACTCCC ACCCCTGGCT CCCAAAGCCA GAATTCTAAA *Labeo bicolor*

AGAGGGAGAT TTTAACTCCC ACCCCTGGCT CCCAAAGCCA GAATTCTAAG *Gobio gobio*

AAAGAGAGAT TTTAACTCTC ACCCCTGGCT CCCAAAGCCA GAATTCTAAA *Carassius auratus*

AAAAGGAGAT TTTAACTCCC ACCCCTGGCT CCCAAAGCCA GAATTCTAAA *Chondrostoma toxostoma*

AAAGAGAGAT TTTAACTCTC ACCACTGGCT CCCAAAGCCA GAATTCTAAA *Barbus meridionalis*

(primer LaviF is of the consensus of the underlined sequence in the alignment)

Figure 6.2 Aligned control regions of the seven cyprinid fish species used to design forward primers LaviF.

Position Species

786 796 806 816 826

CCTGTTATCC TTGTCAAACC CCGAAACCAAG GAAGGTTCGA GAACG *Rutilus rubilio*

CCTGTTCTCC TTGTCAAACC CCGAAAGCAAG GAAGGTTCGA GAACG *Leuciscus multicellus*

CCTGTTATCC TTGTCAAACC CCGAAACCAAG GAAGGTTCGA GAACG *Gobio gobio*

CCTGTTATCC TTGTCAAACC CCGAAACCAAG GAGGACCCAA GAACG *Carassius auratus*

CCTGTTCTCC TTGTCAAACC CCGAAACCAAG GAAGGTTCGA GAACG *Chondrostoma toxostoma*

CCTGTTATCC NTGTCAAACC CCTAAACCAAG GAGGACTCAA GAACG *Barbus meridionalis*

CCTGTTTTCC TTGTCAAACC CCTAAACCAAG GAGGACTCAA GAACG *Labeo bicolor*

(primer LaviR is complementary to the underlined portion of the light strand)

Figure 6.3 Aligned control regions of the seven cyprinid fish species used to design reverse primers LaviF.

PCR amplification and sequencing of Labeo victorianus d-loop

Four hundred, forty-six base pair long segments of the 5'hypervariable segment of the control region was PCR amplified using the designed primers. Symmetrical PCR amplifications (Saiki *et al.,* 1988) were carried out in 50 µl reaction volumes containing 2-5 ng of the total genomic DNA, 50 pmol of each primer 50 pmol dNTPs, 10 mM Tris HCl, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.8 units of *Taq Polymerase* (Boehringer Mannheim GmbH). The cycling parameters used were as follows: 1 cycle of initial denaturation at 94ºC for 5 min followed by 34 cycles of denaturation at 94ºC for 1 min, annealing at 56ºC for 2 min and extension at 72ºC for 3 min. A final extension of 5 min at 72ºC was used. Blank extractions and blank PCR reactions were included as controls to detect possible contamination.

One primer was 5'-end biotinylated, and the double stranded PCR product separated into single strands using straptavidin-coated paramagnetic beads (DYNAL®). Single stranded DNA was dissolved in distilled water and used as a template for sequencing by the dideoxy chain-termination method (Sanger *et al.,* 1977) using a sequenase kit version 2.0 (Amersham Pharmacia Biotech, Inc.), [α-^{35}]-dATP (Amersham Pharmacia Biotech, Inc.) and a primer complementary to the template. When the primer LaviF was biotinylated, primer LaviR2 was used as a sequencing primer when LaviR was biotinylated, LaviF was used as a sequencing primer. The products were electrophoresed in a 6% denaturating polyacrylamide /7M urea gel. The gel was fixed, dried, exposed for 24 – 48 hours and read manually.

Sequence analyses

The sequences were aligned manually using the program SeqApp version 1.9 (Gilbert, 1993). Genetic distances between haplotypes, nucleotide diversity and the net sequence divergence (Nei, 1987 equation 10.5) were estimated from the data using the program POPSTR version 1.25 (Seigismund, unplished). This program carries out a permutational contingency analysis of population subdivision and computes a K_{ST} statistic for each comparison (Hudson *et al.,* 1992). This statistic is a measure of the proportion of the total nucleotide variation between populations that is due to genetic differentiation - the extent of genetic subdivision between populations. Samples in pairwise comparisons were considered to be significantly heterogeneous when the probability of obtaining the observed value of K_{ST} was less than 0.05 in 1000 Monte Carlo simulations (Hudson *et al.*, 1992).

The relationship between the haplotypes was estimated in two ways. Firstly, maximum likelihood-based genetic distances between haplotypes were calculated, using transition/transversion ratio estimated from the data, and used to construct a neighbour joining algorithm (Saitou and Nei, 1987) implemented in PAUP* ver. 4.0 (Swofford, 2000). One homologous sequence of *Cyprinus carpio* was retrieved from the GenBank (accession number AF508938) and used as an out-group.

The second method involved collapsing the genetic sequences into haplotypes and calculating their frequencies. The frequencies were then used to estimate haplotype out-group probabilities which correlated with the haplotype age. (Castelloe and Templeton, 1994). The probability of parsimony (Templeton *et al*., 1992) was then calculated from which a network

was estimated that shows the maximum number of steps connecting haplotypes parsimoniously. This was implemented in the programme TCS (Clement *et al*., 2000).

Three approaches were used to test for past demographic changes in *L. victorianus* population. Firstly, Tajima's (1989) D statistic was initially used to test for differences between nucleotide diversity π and θ was calculated using the programme ARLEQUIN, version 2.0 (Schneider *et al*., 2000). A significantly negative D statistic is interpreted as indicative of a past sudden population expansion (Tajima, 1996). Secondly, a mismatch distribution (Harpending, 1994) describing pairwise differences between individuals within a population was analysed using the programme DnaSP (Rozas and Rozas, 1999). A smooth unimodal distribution indicates a past sudden population decline/expansion, while a multimodal distribution is a characteristic of a long-term stationery population. Lastly, a raggedness index (*r*) (Harpending, 1994) was calculated using ARLEQUIN, version 2.0 (Schneider *et al*., 2000). The index distinguishes the unimodal distribution from a ragged distribution. Values of *r* were tested for significance using 1000 Monte Carlo simulations.

Results

Morphometrics

Of the accumulated variance in the truss principal component analysis, over 80% was loaded within the first principal component. Only Posterior dorsal fin insertion to posterior pelvic fin insertion (PDPP) was loaded in the second component (Table 6.4). Analysis of the non-truss measurements indicated that the first component accounted for more than 90% of the total variance. Principal components analysis of the variables separated the two populations into two groups (Fig. 6.4 and 6.5). Discriminant Function Analyses of both truss and non-truss

variables showed a clear distinction between samples from the two populations (Wilk's λ = -016, $F_{(20,35)} = 105.4$, $p < 0.00$ for non truss and Wilk's $\lambda = 0.0024$, $F_{(9, 46)} = 2120$, $p < 0.00$ for non-truss variables).

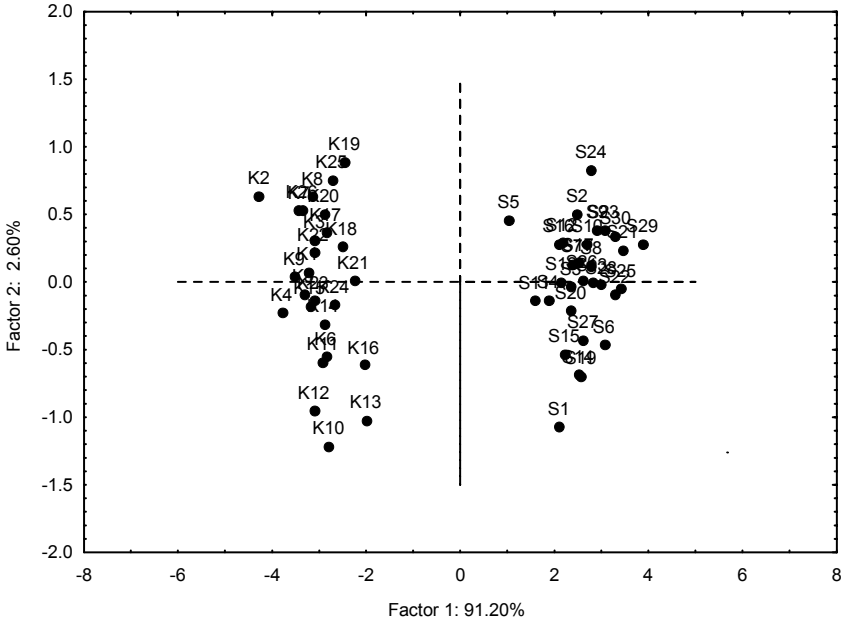

Figure 6.4. Plot of projection of the cases on the factor-plane (first and second) taken from principal analysis of eight non-truss variables measured on fifty-six *Labeo victorianus* specimens from the Kagera and Sio River populations.

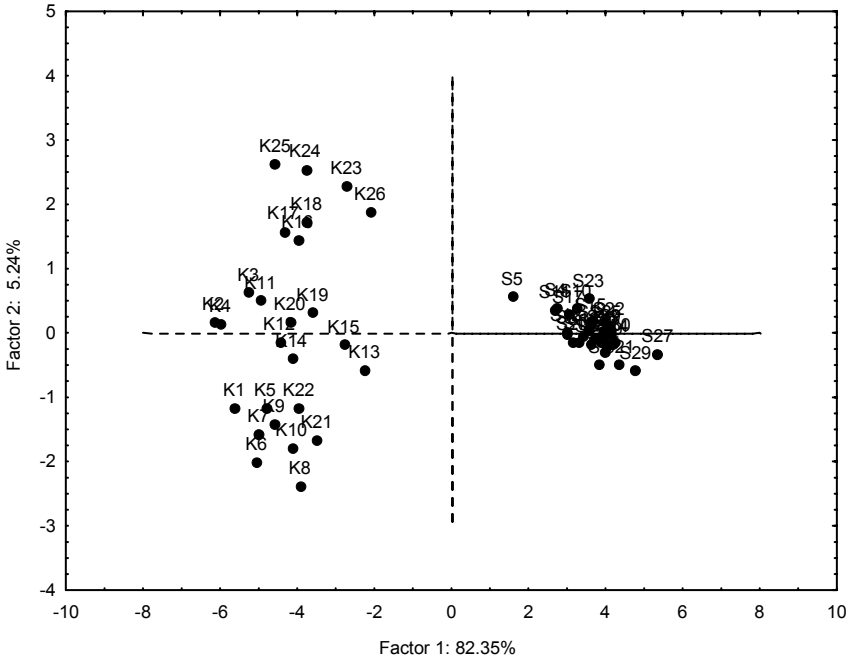

Figure 6.5. Plot of projection of the cases on the factor-plane (first and second) taken from principal analysis of twenty truss variables measured on fifty-six *Labeo victorianus* specimens from the Kagera and Sio River populations.

Table 6.3 Factor loadings extracted from principal component analysis of non-truss variables.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
HD	-0.966	0.018	-0.009	-0.097	-0.101	0.208	-0.051	-0.001
OD	0.974	-0.018	0.124	0.155	0.036	0.066	-0.012	0.056
IOD	-0.950	-0.189	-0.061	-0.012	0.212	0.013	-0.113	0.018
IND	0.973	0.006	0.177	0.076	0.0375	0.093	-0.040	-0.003
MW	-0.917	-0.239	0.295	-0.027	-0.093	-0.072	-0.016	0.003
PEC	-0.952	0.089	-0.066	0.246	-0.084	-0.033	-0.111	-0.033
PLV	-0.986	0.052	-0.047	0.048	-0.029	-0.008	0.067	0.125
CFL	-0.908	0.351	0.199	-0.051	0.101	-0.024	-0.008	-0.008
AFL	-0.966	-0.087	0.018	0.124	0.073	0.070	0.174	-0.054

Table 6.4 Factor loadings extracted from principal component analysis of truss variables

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
SOJ	-0.945	-0.127	-0.057	-0.096	-0.0219	0.010	0.107	0.048
SPF	-0.957	-0.039	0.219	0.009	-0.112	0.061	0.050	-0.0286
SPO	-0.939	-0.064	-0.102	-0.078	-0.030	0.019	-0.017	-0.300
PSOJ	-0.968	0.035	-0.013	-0.017	0.096	0.022	0.071	0.052
PSPF	-0.964	0.017	0.110	-0.0001	0.062	-0.095	0.082	0.008
PSPP	-0.891	0.097	0.340	0.065	-0.242	0.038	-0.060	-0.016
PSAD	-0.912	0.072	0.322	0.068	-0.084	-0.144	-0.009	0.040
ADPF	-0.949	-0.005	0.263	0.032	-0.071	-0.074	0.064	0.017
ADPP	-0.811	-0.074	-0.395	-0.166	-0.360	0.044	-0.087	0.084
ADAF	-0.973	-0.014	-0.131	-0.025	0.067	-0.116	-0.079	-0.005
ADPD	-0.694	0.061	-0.283	0.656	-0.041	0.030	0.040	-0.007
PDPP	0.115	0.984	-0.096	-0.076	-0.028	-0.021	0.009	-0.014
PDAF	-0.951	0.010	-0.193	-0.067	0.026	-0.173	-0.048	-0.003
PDDC	-0.952	-0.096	-0.159	-0.065	0.048	-0.119	0.042	0.028
PDVC	-0.980	-0.022	-0.101	-0.045	0.055	-0.027	-0.004	0.008
PFPP	-0.930	0.039	0.160	0.060	0.133	0.088	-0.250	0.0001
PPAF	-0.966	0.016	-0.025	0.006	0.152	-0.077	-0.088	0.045
AFVC	-0.935	0.077	0.001	-0.035	0.115	0.276	-0.054	0.056
DCVC	-0.958	0.033	-0.036	-0.066	0.057	0.110	0.102	-0.043
AFDC	-0.962	0.117	-0.017	-0.083	0.060	0.057	0.125	0.012

Mitochondrial DNA d-loop sequence analyses

Twenty-five segregating sites defining 23 different haplotypes were found among the 38 individuals sequenced (Fig. 6.7). Of these 13, were from the Kagera River population and 10 from the Sio River population. The within population number of segregating sites was higher in the Sio River (19) than in the Kagera River (14). Three haplotypes (K964, K967 and K1028) were shared between the two populations. Haplotype K965 occurred in more than one individual in the Kagera sample only while the rest were singletons. Of the shared haplotypes, K964 was the most abundant occurring in 21% of all the individuals analysed. Nucleotide diversity in the total sample (Kagera and Sio Rivers combined) was low (π = 0.0074 ± 0.0016; Nei, 1987). However, of the two populations, the Sio River population had a slightly higher nucleotide diversity (π = 0.00787 ± 0.00193, Nei, 1987) than the Kagera River population (π = 0.00687 ± 0.00258, Nei, 1987). The proportion of the total genetic variation in the total sample that could be attributed to genetic differentiation between the two populations, as represented by the K_{ST} statistic, was small (0.37%) and not statistically significant ($P > 0.05$).

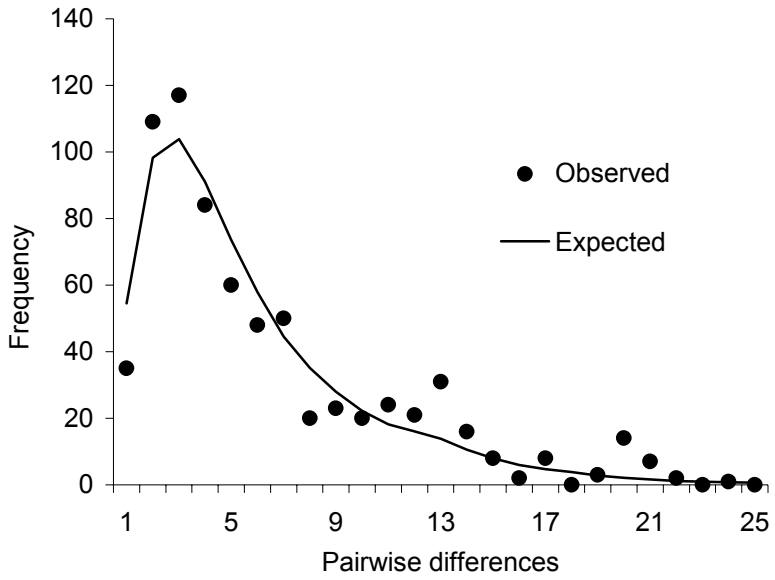

Figure 6.6 Frequency distribution of pairwise sequence difference between *Labeo victorianus* individuals.

		10 20				
		111 1111222222 23334				
		1457889055 8888000188 86793		Populations		
		9907349616 0246569356 90101	**Kagera**		**Sio**	**sum**
1	K963	-TC-GTATC- -TAATTCTCG ACCTT	1		-	1
2	K964	.........C C.........	5		3	8
3	K965	C........C C.........	3		-	3
4	K966	CC.......T C.T..CTA.. GT..C	1		-	1
5	K967	C......... C.........	1		1	2
6	K1002	.........C C......... GT..C	1		-	1
7	K1024	C........T C......... GT..C	1		-	1
8	K1025	...G.....C C..-......	1		-	1
9	K1028	.... C.........	1		5	6
10	K1029	.... C.....-... GT..C	1		-	1
11	K1036	C........T C.........	1		-	1
12	K1045	.CT.AC-.TT C.T..CTA.. GTA.C	1		-	1
13	K1048	.....C.... C.........	1		-	1
14	S746	....AC...T C.........	-		1	1
15	S751	.... C......... ...A.	-		1	1
16	S957	.... C.......GC	-		1	1
17	S962	.......C.C C.........	-		1	1
18	S1030	.... C......... GT..C	-		1	1
19	S1032	.C..AC-.TT TC........	-		1	1
20	S1033	C.......TT C.T..CTA.. GT..C	-		1	1
21	S1034	.C..AC-.TT C....CTA..	-		1	1
22	S1037	.C..AC-.TT C.........	-		1	1
23	S1038	.........T C...C.....	-		1	1
Number of bases		1221221222 2221222222 22222	**Sum**	**19**	**19**	**38**

Figure 6.7 Distribution of the 23 observed d-loop haplotypes from a sample of 38 *Labeo victorianus* individuals sampled from the Sio and Kagera Rivers in Uganda. The vertical numbers indicate the positions of the polymorphic sites relative to haplotype 1. A dash (-) represents a deletion introduced to optimise alignment.

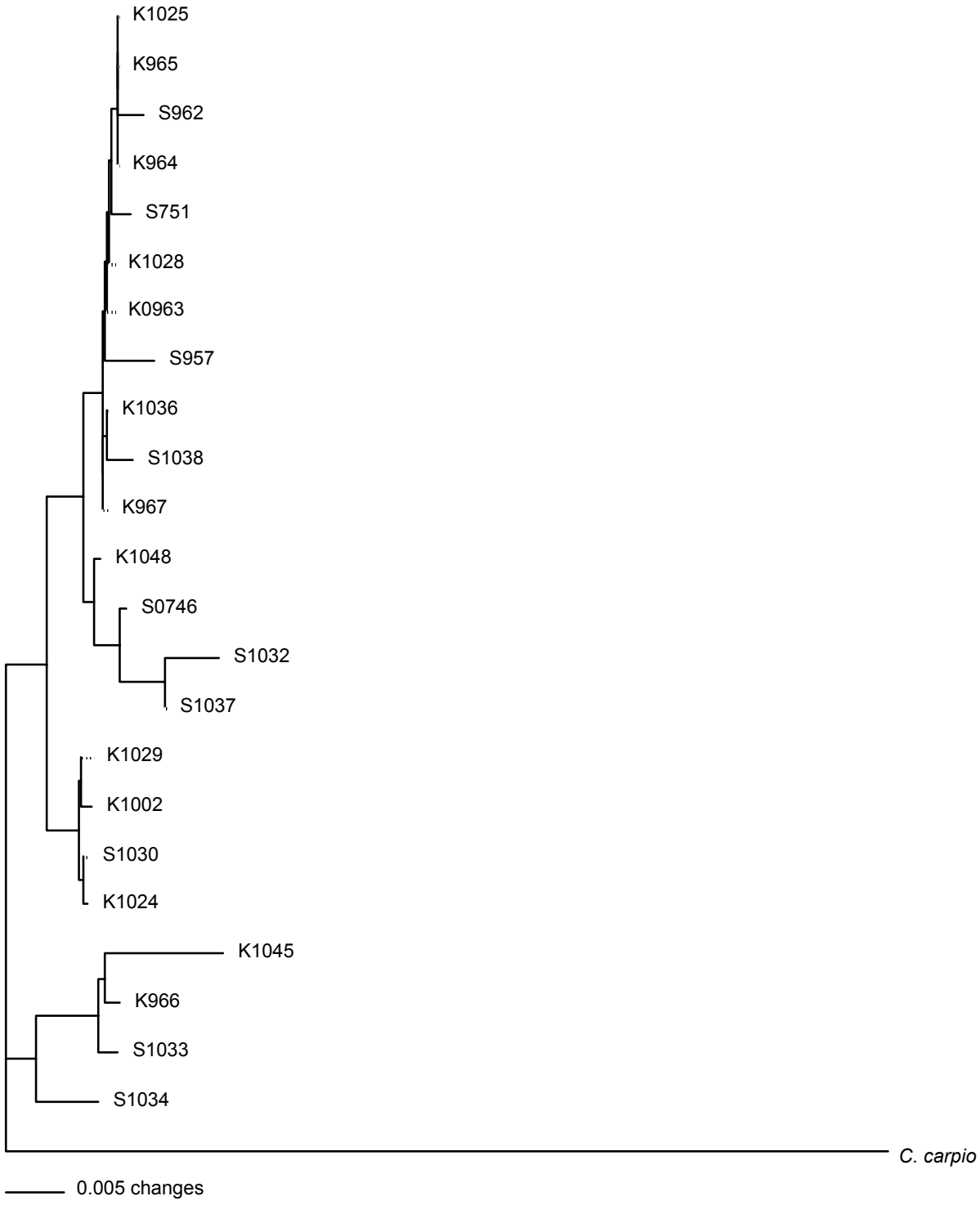

Figure 6.8 A neighbour joining haplotype tree, based on maximum likelihood distances. Letters on tips represent locality of origin, K, for the Kagera River and S, for the Sio River. A homologous sequence of *Cyprinus carpio* (accession number AF 508938) was used as an out-group.

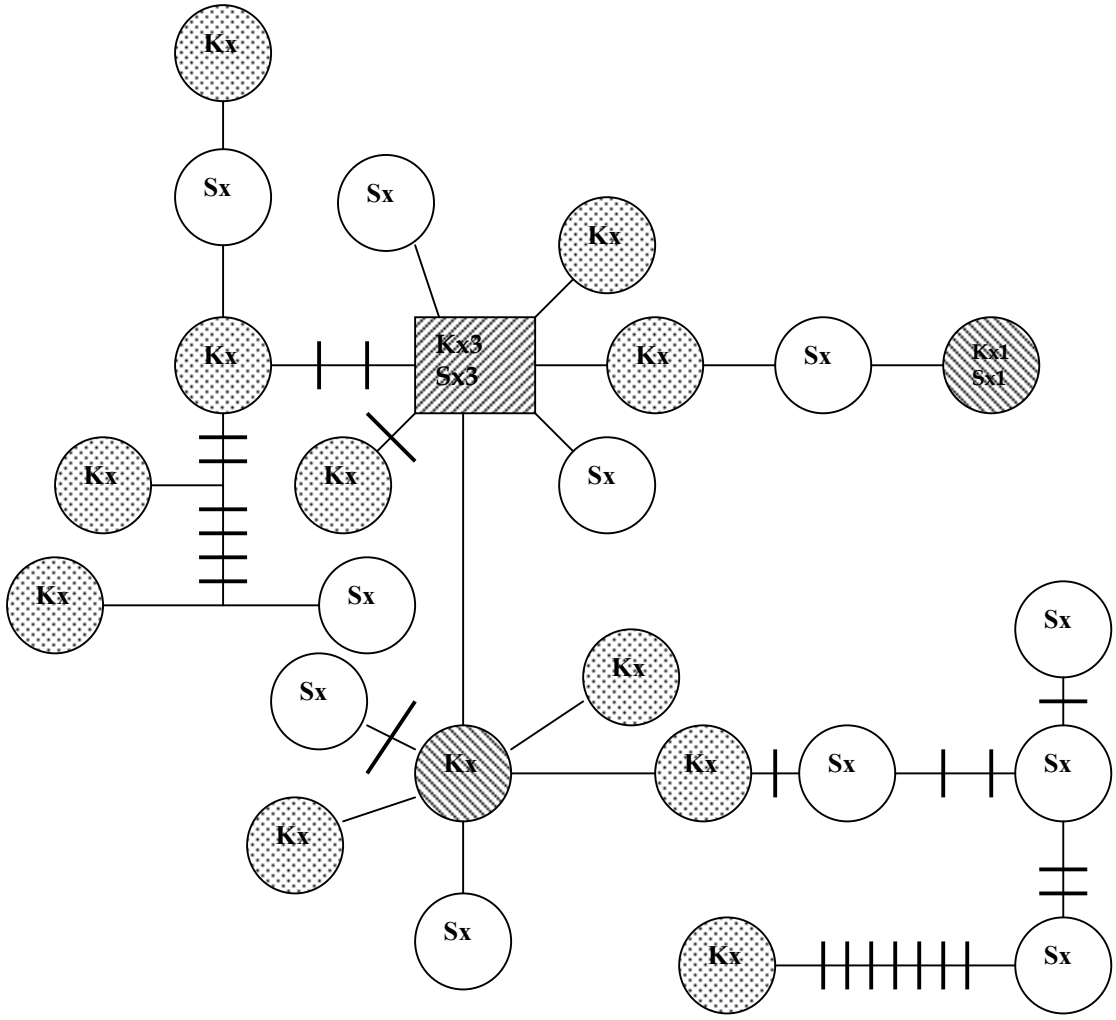

Figure 6.9 Haplotype network showing the phylogenetic relationships between the 23 observed haplotypes. Hatch marks along branches indicate the number of nucleotide differences in excess of one. A rectangle denotes inferred ancestral haplotype. Haplotypes unique to populations and shared are identified by differences in shading. Letters identify the area of origin (K = Kagera River; S = Sio River) of the haplotype while numbers represent occurrence.

The observed mismatch distribution of *L. victorianus* sequences was unimodal. The distribution did not differ from the simulated poisson expectation for a unimodal population decline model (Harpending, 1994) (Fig. 6.6). The raggedness index *r* (Harpending, 1994) was 0.018 with an associated p> 0.05. Analysis of the demographic history using Tajima's D (1989) was not significant (D= -1.095, p>0.01).

A neighbour joining tree showing the phylogenetic relationship between the 13 haplotypes of *Labeo victorianus* from the two populations (Fig. 6.8) revealed no geographical clusters. The minimum spanning network indicated that the different haplotypes were connected by short

branches (Fig. 6.9). There were no missing haplotypes for most of the network and clustering did not follow a geographical distribution. This finding was similar to the result obtained in Figure 6.8.

Discussion

Although multivariate character analysis indicated significant differences in morphometry, mtDNA sequencing noted a lack of genetic differentiation and a low level of nucleotide diversity between the two populations. Possible historical population decline was however noted (see below). It is clear from the K_{ST} statistic, the majority rule consensus tree and the minimum spanning network that there was neither subdivision nor reciprocal monophyly between the Kagera and Sio River haplotypes. This study, therefore, indicates that there has been a high degree of mixing and recent historical coalescences of the different haplotypes from the two populations. The fact that 21% of the individuals analysed shared a common haplotype is a further indication of a shared recent ancestry.

The incongruence between genetic and morphological data could therefore be attributed to environmental differences. Environment induced phenotypic divergence, also referred to as morphological plasticity, has been reported in other teleost fishes under both culture and natural conditions (Flemming *et al*., 1994). Currens *et al*., (1989) attributed this observation to "fitness or fatness" due to feeding regimes, while Flemming *et al*. (1994) linked it to underlying ontogenic mechanisms. Whatever the cause, it is suggested that potamological studies be undertaken on the Kagera and Sio River systems to provide additional information to explain the morphological divergence of the two *L. victorianus* populations that are genetically undifferentiated.

It is not clear whether the lack of population structuring is due to continuous gene flow through adult migration and/or larval dispersal between the two populations. *L. victorianus* falls in the non-guarder reproductive guild (Balon, 1975) and produces thousands of semi buoyant-eggs, which are dispersed by water currents (Chapters 3, 4 and 7). The extent of egg and larval dispersion is unknown and it has not been ascertained whether larval replenishment follows closed, single or limited distance source models of Carr and Reed (1992). However, the finding of only three shared haplotypes in the 38 analysed individuals suggests that the original panmictic population may have undergone recent isolation followed by random loss of many haplotypes. This would have resulted in the current situation of a few shared haplotypes and those unique to each population, but still closely related to each other. An alternative reason would be that the haplotypes have not had sufficient time to accumulate genetic differences from mutation and/or drift at the mtDNA level.

The mismatch distribution to Poisson expectation (Slatkin and Hudson, 1991), and the computed raggedness index (Harpending, 1994) revealed a pattern of historical decline. Although Tajima's D was not significant, it was negative confirming results of the mismatch test. From these results, a bottleneck event would therefore be the most plausible explanation for the low genetic diversity.

The observed low nucleotide diversity in the two populations is comparable with levels observed in whales (Lyrholm *et al*., 1996; Schaeff *et al*., 1997) as a result of low effective population size during bottleneck events. Similarly, as noted in American shad populations, low mtDNA diversity was also associated with bottleneck events and population declines (Brown *et al*., 1996; Waters *et al*., 2000). The observed low genetic diversity of less than 2% is a possible cue for a bottlenecked situation in the two populations. It would therefore appear

that the massive annual capture of migrating broodstock during each spawning season could have resulted in the stochastic elimination of some haplotypes; such as those large sized individuals leading to repeated population bottlenecks, since a single bottleneck event is unlikely to explain the low genetic diversity due to high preservation of pre-bottleneck heterozygosity (Nei *et al.*, 1975).

This Chapter provides evidence that the two *L. victorianus* populations are not differentiated at mitochondrial level and may be a single panmictic population. These findings should, however, be further verified since failure to detect population differentiation cannot preclude undetected subdivisions due to small sample size and inadequate temporal sampling. Random sampling of large numbers over a protracted temporal period is suggested for detection of population differentiation. This should be conducted together with migration and demographic studies to ascertain the extent of population mixing and possible age truncation that can result from the continuous removal of adult individuals through overfishing.

CHAPTER SEVEN

Induced Ovulation and Spawning of *Labeo victorianus*

Introduction

Previous attempts to induce spawning in *L. victorianus* have been unsuccessful (Fryer and Whitehead, 1959; unpublished KARS data) and were possibly due to inadequate knowledge concerning aspects of this species' reproductive biology. Information regarding sexual maturity, proximal environmental conditions associated with spawning, sperm ultrastructure spermatogenesis and oogenesis have been investigated in the preceding chapters. Unfortunately no data are available on the role of various reproductive hormones and induced spawning in this species.

It is well-known that reproductive processes in fishes are controlled by endogenous biological rhythms as well as by environmental cues (Munro, 1990). Endogenous control is mediated through actions of various hormones along the brain-hypothalamus-pituitary-gonad axis. Under natural conditions environmental stimuli are detected and relayed to the brain resulting in a release of hormones and neurotransmitters that regulate ovulation (Yaron, 1995; Peter and Yu, 1997). The most important reproductive hormone released from the pituitary is Gonadotropin releasing hormone (GnRH) that regulates gonadotropin hormone, GtH. (Peter and Yu, 1997). Twelve forms of GnRH are known today, all are highly conserved in length and in amino acid residues (King and Millar, 1995). Multiple forms of GnRH have been identified in most vertebrates including teleosts, and differ in spatial distribution throughout the brain (Sherwood *et al.*, 1994). The occurrence and physiological function of the various GnRH forms is reported to vary among teleosts (Peter and Yu,

1997). Gonadotropin release in teleosts is also influenced by a gonadotropin-inhibiting factor (GRIF) from the hypothalamus. This factor has been identified as dopamine and demonstrated to have inhibitory activity on the release of GtH (Peter *et al*., 1986; 1988). Dopamine directly inhibits the GnRH-induced GtH response from the gonadotrophs in a wide array of teleosts including cyprinids, salmonids, cichlids, siluroids and anguillids (Peter *et al*., 1991; Levavi-Sivan *et al*., 1995). Dopamine has, however, been reported to be absent in the sciaenid *Micropogonias undulutus* (Copeland and Thomas, 1989).

Unlike GnRH, which is known to exist in several forms, only two chemically different gonadotropins, GTH-I and GTH-II, have been characterized in teleosts (Kawauchi *et al*., 1989). GTH-I and GTH-II are generally conceived to be Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH), respectively. Follicle stimulating hormone or GtH-I is generally believed to be important for vitellogenin and early gonadal development, while LH or GTH-II stimulates events leading to final oocyte maturation, ovulation and spermiation (Yaron, 1995). Combinations of GnRH and dopamine antagonists have been used to induce ovulation in several cyprinids including *Carassius auratus* (Sokolowska *et al*., 1984), *L. rohita, Cirrhnus mrigala* (Halder *et al*., 1991) and *Cyprinus carpio* (Driori *et al*., 1994).

This chapter investigates two hormonal compounds – one with a GnRH and the other with a GnRH plus a dopamine antagonist – as stimulants for the induction of ovulation and the initiation of spawning *L. victorianus*, and elucidates locally feasible methods that could be used to spawn the fish outside its natural environment.

Materials and Methods

Collection of broodstock

Broodfish were caught using gill nets (27-75 mm stretched mesh) at the mouth of the Sio River (Fig. 2.1). The time for collection of ripe fish was predicted, based on cyclic variations in the gonadosomatic index and the prevalence of oocytes in advanced stages of development (Fig. 3.1). Ripe fish were obtained over two time periods of the year that were characterised by high gonadosomatic indices, and a dominance of tertiary yolk vesicle oocytes and fresh post-ovulatory follicles (Fig. 4.14).

The fish were induced to spawn under three different experimental conditions. Experiment 1 was conducted in tanks and involved the stripping of ripe fish. Experiment 2 was conducted in floating cages set within the Sio River and involved both stripping and natural fertilisation. Experiment 3 was conducted similar to Experiment 2 but the floating cages were set in ponds instead of the river. Two spawning inducing agents were tested for their efficacy in stimulating ovulation in *L. victorianus* under the three experimental conditions.

Detection of oocyte maturity

Prior to hormonal administration, fish were tested for readiness to spawn by siphoning a sample of oocytes from the ovary using a cannula tubing of 1.9mm in diameter. The samples were cleared in SERA fluid (ethanol: formaldehyde: acetic acid 6:3:1 *v*/*v*) and observed under a light microscope. Females were selected for induction when 80% of the sampled oocyte sample had eccentric nuclei.

Hormonal Administration

Fish were weighed, anaesthetized with 2-phenoxyethanol (Fluka-Chemie-Sigma Aldrich) prior to intramascular injection of the inducing agent between the dorsal fin and the lateral line. Dagin ([D-Arg^{6}, Pro^{9}-NEt])-sGnRH) combined with 20 mg.kg^{-1} of the water-soluble dopamine receptor antagonist metoclopramide (GnRH + MET) (purchased from Kibbutz Gan Shmuel Fish Breeding Centre, Israel) was administered at a dose of 10 µg.kg^{-1}. Aquaspawn, a simple, rapidly metabolised synthetic decapeptide GnRH (purchased from Exico, South Africa) was administered at 0.5ml.kg^{-1} as recommended by the manufacturer. Fish from a control group were injected with volumes of 0.6% saline solution (SS) equal to the volume of the hormones used. Water temperature was constantly monitored during the incubation period. Progression towards ovulation involved change of the oocyte from opaque yellowish-cream colour to becoming transparent. This process was divided into five phases that were used to assess ovulatory response to treatments. Oocytes were considered to be in Phase I if they were opaque, II if starting to clear, III if they showed low translucency, IV if highly translucent and V if transparent.

Experiment 1

Twenty females and thirty males were collected from the Sio River in October, corresponding to the second spawning peak (Table 3.1) and transported in an oxygenated 500 litre plastic container to the Sunfish Farm near the Kajjansi Aquaculture Research Station (Fig. 2.1). Water temperature was maintained at approximately 18^{o}C using ice blocks during transportation. On arrival at the farm, the fish were placed in rectangular concrete tanks supplied with gravity fed natural spring water. The tanks were covered with nets to prevent fish jumping out and to reduce

possible injury. After six hours of acclimatisation in the tanks the fish were grouped into six sets of two females and four males. Two sets were injected with either GnRH, GnRH + MET or SS. After injection, the fish were put in tanks according to theirs sets - females separate from males. The tanks were monitored for ovulation using gentle pressure applied to the belly of the females. Those fish whose oocytes had become transparent were stripped. Prior to stripping, a plastic container was cleaned, dried and weighed. Eggs from the ovulated females were stripped into the container and its weight recorded. The weight of the eggs was obtained as the difference between the weight of the container plus the eggs and the empty container. To determine the number of spawned eggs, a sample of eggs was taken out before the addition of milt, weighed and the eggs in the sample counted. Fertilisation was through the dry method as described by Rothbard (1981).

The fertilised eggs were non-adhesive and were therefore spread onto plastic mesh trays (with a mesh size of 0.6mm) held in wooden frames. After 120 minutes, fertilized eggs were discernable by a dark spot of the developing embryo in the transparent vitelline membrane. One hundred eggs were counted, using a tally counter in five sections of the tray and the fertilisation rate determined. The time at the onset and at the end of hatching was recorded. Hatching success is the percentage difference between unhatched and the total eggs at the start of incubation.

Experiment 2

This experiment was conducted during the first spawning season (Table 3.1). Broodfish were caught in reed enclosures at Sitengo (fig. 7.1) as they migrated upstream the Sio River.

Figure 7.1 A net cage in a reed enclosure set in a tributary of the Sio River during an induced spawning trial.

Fish were transported in a plastic bucket down stream to the Maddwa landing site and were tested for ripeness. Ripe fish were anaesthetized and hormonal injections were administered. In this experiment concrete tanks were replaced by floating net cages held in a reed enclosure that had been erected in tributaries of the river close to the main lake as illustrated in Fig. 7.1

Cage I measured 1×1×1.5m and contained two females used for the study of oocyte clearance. Cage II measured 2×4×1.5m and was subdivided into eight compartments, each measuring 1×1×1×1.5m with 0.5mm mesh netting. Each compartment contained one female and two males. This cage was set to allow for natural fertilization without stripping. Cage III measured 2×4×1.5m with two compartments containing fish for stripping. One compartment contained six females and the other twelve males.

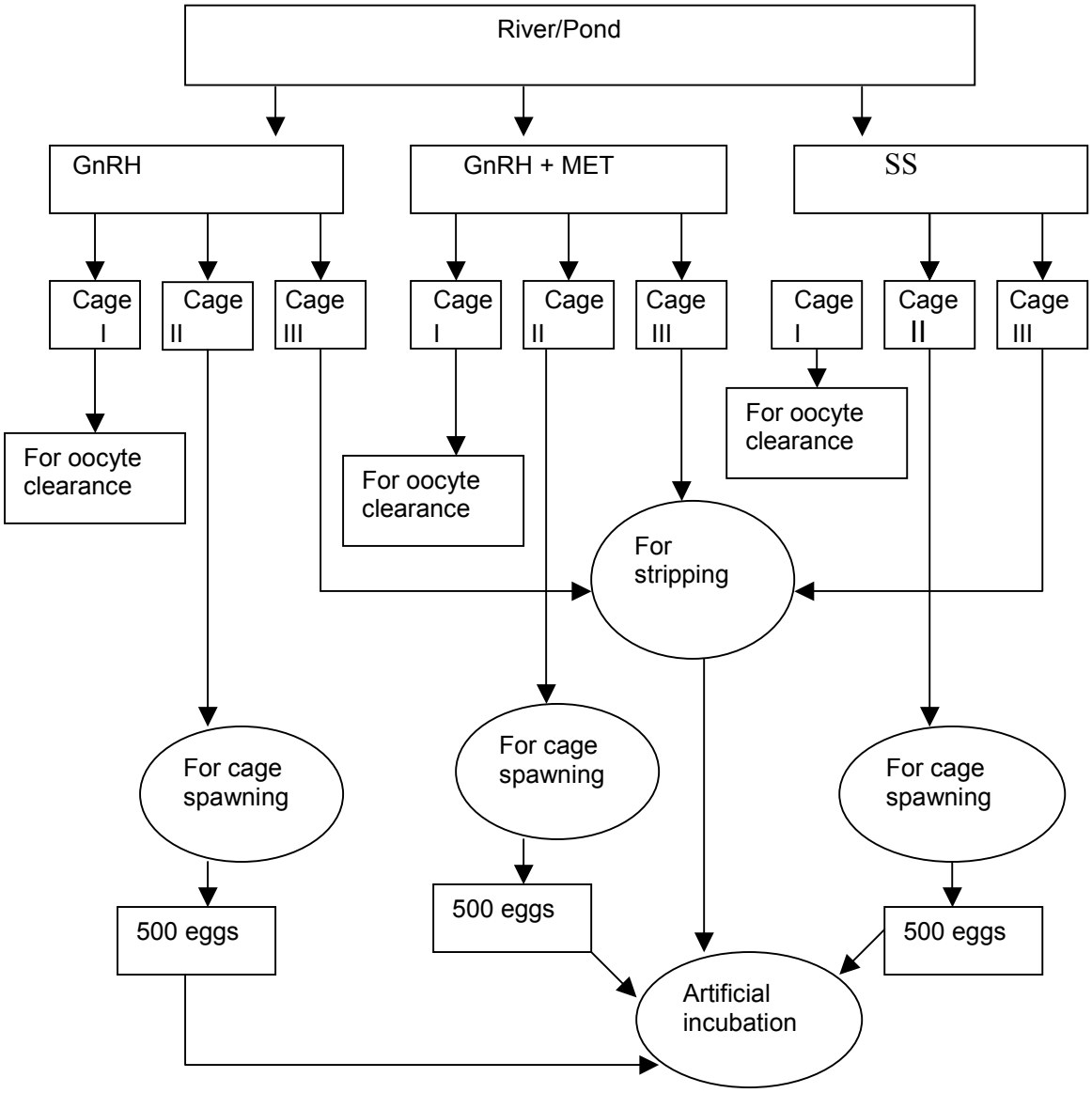

Figure 7.2 Illustration of the experimental design employed for Experiments 2 and 3.

Oocyte biopsies were obtained from the two females in Cage I of each treatment. The fish in Cage II and III were closely monitored for ovulation by lifting the cages out of the water and visually inspecting them. When ovulation was detected, fish in Cage III were stripped, while those in Cage II were left to fertilise in the cages.

Two hours after fertilisation, spawners were removed out of the cages. The number of eggs spawned was estimated by lifting the net out of the water to spread the eggs on the net. The total area occupied by the eggs was measured. Eggs in a quarter of the area was counted with a tally counter and used to estimate the total number of eggs spawned. Stripping was conducted as in Experiment 1 but the eggs were irrigated by river water from an overhead tank at 2 $l.min^{-1}$. Three hours post-spawning a total of 500 fertilised eggs from the each compartment in Cage II were collected and incubated in a plastic basin, supplied by the same tank as the stripped eggs and at the same flow rate.

Experiment 3

This experiment was conducted at the Kajjansi Aquaculture Research Station using brood fish that were collected from the Sio River and rested in ponds for eight days. The procedure for Experiment 2 (Fig. 7.2) was followed, except that the cages were suspended in the ponds. In all the three experiments hatching time was recorded from the start of spawning/fertilsation and not from the start of the experiment.

Statistical analysis

The time taken to induce ovulation, incubation period and duration of hatching were log_{10} transformed, while fertilisation and hatching rates were arcsine transformed and tested for homogeneity of variances using a Bartlett's test. Comparison of means was by a Student's t-test or a Mann-Whitney U test if the data did not fulfil parametric assumptions.

Results

Experiment 1 was unsuccessful as all the eggs and larvae died after approximately 10 hours. This experiment did, however, provide baseline information for Experiment 2 and 3. Comparisons between the induction times, fertilisation rates, and incubation and hatching durations could be drawn between Experiments 2 and 3. Results from the experiments are summarised in Table 7.1. At night, water temperature rose in the Sio River whereas in the ponds and tanks it decreased. A reversed trend was observed during the day (Fig. 7.3).

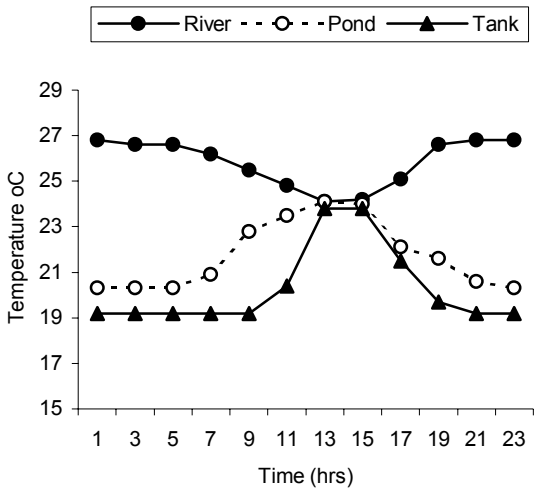

Figure 7.3 Diurnal temperature variations in the experiments during spawning latency, incubation and hatching.

Induction of spawning

The GnRH + MET treatment induced all fish to spawn in all replicates for all three experiments. However, in fish injected with GnRH and SS spawning did not occur. It was noted that the time taken to induce spawning was significantly shorter in the river than in ponds ($t = -84$, $df = 30$, $P < 0.01$). Eggs ovulated per unit body weight in the two environments were not significantly different ($t = 1.18$, $df = 30$, $P = 0.23$). Ovulated eggs were semi-buoyant and non-adhesive. On contact with water, the

vitelline membrane of the ovulated eggs swelled and increased from 1.2±0.1 mm to 5.8±0.3 mm in diameter. Swelling occurred whether the eggs were fertilised or not. GnRH only caused oocyte clearing up to Phase IV (Fig. 7.4).

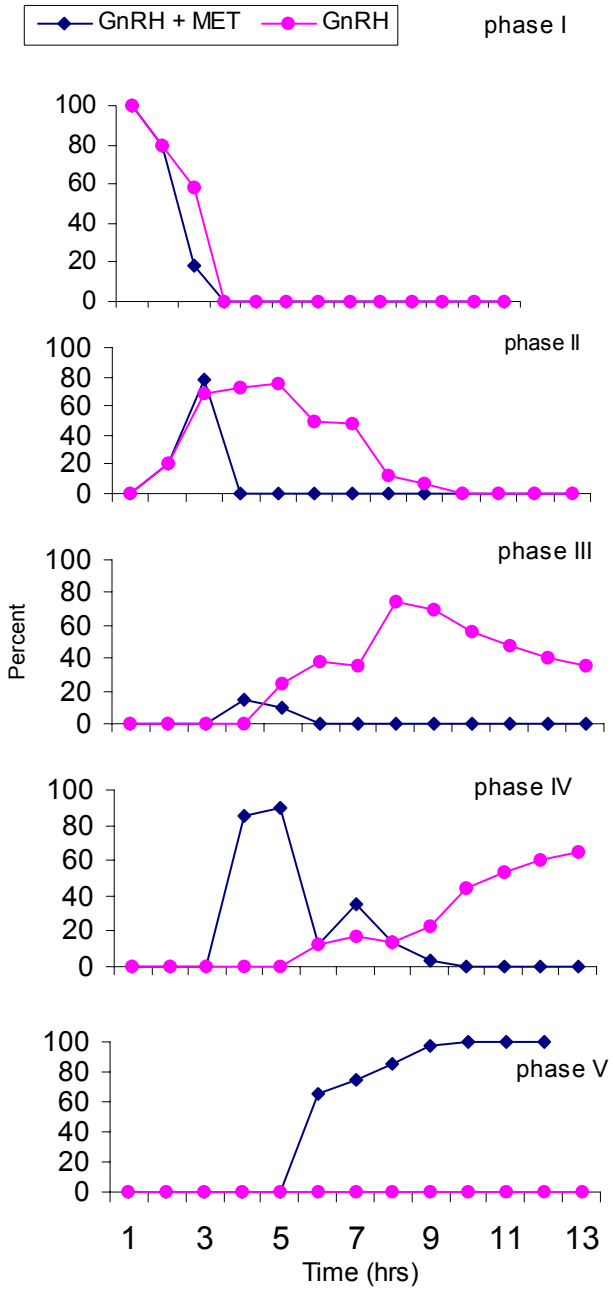

Figure 7.4 Progression of clearance in GnRH + MET and GnRH induced oocytes.

Table 7.1. Effect of different treatments on spawning of *Labeo victorianus* during three induced spawning experiments.

Treatment	Replicate	Time to ovulation (min)	Ovulation (%)	Eggs (10^3. kg^{-1})	Fertilisation (%)	Time to start of hatching (min)	Time to end of hatching (min)	Hatching (%)
				Experiment 1				
GnRH	II	-	-	-	-	-	-	-
GnRH	III	-	-	-	-	-	-	-
GnRH + MET	II	975	100	93.02	82.8	1625	10.10*	13.4
GnRH + MET	III	960	100	92.72	81.9	1664	8.45*	18.2
SS	II	-	0	-	-	-	-	-
SS	III	-	0	-	-	-	-	-
				Experiment 2				
GnRH	I	-	-	-	-	-	-	-
GnRH	II	-	-	-	-	-	-	-
GnRH + MET	I	518.6±8.8	100	100.96±10.1	94.7±2.6	1547±16.9	668±23.7	89.9±1.3
GnRH + MET	II	524±8.1	100	105.42±7.89	94.6±4.0	1554±22.5	677±19.3	90.0±1.4
SS	I	-	-	-	-	-	-	-
SS	II	-	-	-	-	-	-	-
				Experiment 3				
GnRH	I	-	-	-	-	-	-	-
GnRH	II	-	-	-	-	-	-	-
GnRH + MET	I	851±129	100	100.21±9.71	92.1±4.3	1623.6±33	1429±324	78.4±6
GnRH + MET	II	899±16	100	99.26±0.70	92.3±6.9	1625±17	1665±140	77.8±2.1
SS	I	-	-	-	-	-	-	-
SS	II	-	-	-	-	-	-	-

*All the eggs and larvae died.

Fertilisation

Fertilisation rate was high, above 69%, in all experiments with no significant difference in fertilisation rates between eggs spawned in the river cages (Experiment 2) and those spawned in pond cages (Experiment 3) ($t = 1.18$, $df = 30$, $P = 0.39$). Fertilisation rates did, however, differ significantly between stripped eggs and those fertilised naturally in the cages ($t = 8.08$, $df = 29$, $P < 0.01$).

Incubation and hatching

Incubation took a significantly shorter time to occur in the river than in the pond cages ($t = -11.22$, $df = 30$, $P < 0.01$). There was low hatchability in Experiment 1 (Table 7.1). In this experiment, all the larvae died on the day of hatching. Hatching was significantly higher in the river than in the ponds ($t = 19.6$, $df = 30$, $P < 0.01$); more than 77% in the two experimental conditions (Table 7.1). Attempts to hatch stripped eggs in Experiments 2 and 3 were unsuccessful, where the highest embryo mortality occurred between four and six hours post-fertilisation.

A hatching rate of 69.2% was obtained with the eggs collected six hours after fertilisation in the cages and incubated in plastic containers using the same water that the cages were floating in. The hatching process involved multidirectional larval movements at a rate of 78±5.1 min^{-1}, with embryos repeatedly hitting the vitelline membrane. The larvae measured 5.9±0.2 mm Total Length at hatching. Immediately after hatching, the newly hatched larvae lay in lateral recumbence on the bottom of the container. The larvae made vibrations as if to gain momentum followed by a thrust of vertical movements to the water surface and back to the bottom at a rate of 7±1.5 movements.min^{-1}. The movements ceased after 30 minutes. Hatching took

significantly more time in the ponds than in the river cages (Z adjusted = -4.824, $P < 0.01$).

Discussion

It is widely acknowledged that the inability to obtain an adequate seed supply is a major constraint to the initiation or expansion of aquaculture (Reay, 1984). The culture of some fish species depends on collection of wild larvae/juveniles, a method considered inefficient and unreliable (Kuo and Nash, 1975). For *L. victorianus* it would be hard to collect wild fry because of the difficulty in identifying spawning grounds and harvesting of the widely dispersed semi-buoyant eggs. Propagation of *L. victorianus* will, therefore, be dependent on successful induced spawning.

Due to the scarcity of *L. victorianus* broodstock, oocyte maturation and ovulation was attained in this chapter through the hypothalamic approach (Yaron, 1995). Unlike the hypophyseal approach, this technique does not involve killing donor fish. The 100% success achieved with GnRH + MET could be attributed to it being based on the linpe technique (Peter *et al*., 1988), which consists of a combination of gonadotropin-releasing hormone (GnRH) analogue plus a dopamine antagonist. Success of the linpe technique has been reported in several other cyprinids (Peter *et al*., 1988). Driori *et al*. (1994) reported high spawning ratios when the same technique was used in *C. carpio* and attributed the good spawning response to the rapid absorption of metoclopramide from the injection site. The failure of GnRH alone to cause oocyte clearance beyond phase IV could be explained by the lack of an effective dopamine antagonist and the fact that in many cyprinids, the endogenous inhibitory dopamine impact is so strong that it severely compromises the effectiveness of externally applied GnRH to increase

GtH release that would lead to ovulation and subsequent spawning (Peter *et al*., 1991; Driori *et al*., 1994).

The swelling of the egg vitelline membrane in *L. victorianus* has also been reported to occur in *L. mesops* and *L. cylindricus* (Msiska, 1990; Hiroyuki *et al*., 1999). Fryer and Whitehead (1959) suggested that the swelling is an adaptation to ephemeral flood conditions by providing a standard microenvironment to the developing embryo - effectively safeguarding it from a wide variety of environmental conditions. Production of semi-buoyant eggs also seems to be a characteristic of African labeines whose spawning has been studied; except *L. umbratus* where no precise information is available. Jackson and Coetzee (1982) reported that the eggs of *L. umbratus* are adhesive. This information could not, however, be confirmed as Mitchell (1984) observed that *L. umbratus* eggs sank rapidly after spawning.

The significant difference in GnRH + MET's latency, incubation time, hatching rate and duration of hatching between river and pond experiments was attributed to temperature differences in the two environments (Fig. 7.3). The same factor is thought to have caused prolonged hatching and low hatchability in the pond cages. Ambient water temperatures are known to affect latency of response to induced spawning treatments in *C. carpio* (Driori *et al*., 1994). The low hatchability of stripped and artificially fertilised eggs in Experiment 1 was originally attributed to the water conditions in tanks. This was, however, later disproved by incubation of stripped and artificially fertilized eggs using water from the river and ponds, in which naturally fertilised eggs were simultaneously being incubated. All the stripped and artificially fertilised eggs died before hatching, whereas those in the cages had a hatching rate of

more than 89%. Similar results were obtained during Experiment 3 where the water quality was the same as in tanks used in Experiment 1. It can, therefore, be concluded that induced spawning followed by natural fertilisation was the most successful method for *L. victorianus*.

The process of breaking through the vitelline membrane during hatching seemed to be energy intensive. It took 8 – 12 hours for some larvae to break through the membrane, while others got exhausted and died. The longest time to first hatching was in the concrete tanks and occurred after 27 hours and 44 minutes. In the other experiments it took less time for first hatching to occur. When compared with hatching of labeines in the tropics, these results contrast sharply with Fryer and Whitehead's (1959) findings in which the first hatching was reported to have occurred after 45 hours. Results in this chapter are similar to the incubation time for *L. mesops* eggs, which were reported to hatch after 28 hours (1680 minutes) (Anon, 1965).

This Chapter has established that *L. victorianus* can be successfully induced to spawn in captivity. The method used in this chapter does not require sophisticated equipment of modern hatcheries and is, therefore, affordable by potential breeders in the Lake Victoria basin. The Chapter has also indicated that once fertilisation has occurred in cages, eggs could be removed and successfully incubated in locally available receptacles. Incubation of eggs in a controlled environment would reduce predation on the developing embryos and larvae. The temperatures under which *L. victorianus* spawned in this chapter are within the normal range for most regions around the lake basin. Induction of spawning should be planned such that fertilisation, early embryonic development and hatching occur when diurnal water temperature is high.

Under controlled conditions, temperature at approximately 26^{o}C would be optimal for induced spawning and hatching. The high fecundity, high fertilization and hatching rates would, therefore, enhance the potential of *L.victorianus* as an aquaculture species.

CHAPTER EIGHT

General discussion and management considerations

Introduction

The management of Lake Victoria has, among a suite of responsibilities, been confronted with the task of reversing declining fish population trends together with the restoration of collapsed fisheries that have been based on indigenous species. This thesis acknowledges the increasing human pressures on the lake's fish resources and therefore considers various propositions for conserving the lake's ichthyofauna. These include the abolition of fishing during spawning periods (Cadwalladr, 1969), and indigenous fish captive propagation (Ribbink, 1987).

Prevention of fishing at certain times within defined areas has for over fifty years been one of the regulations enforced by fisheries management in Uganda. Unfortunately it has been largely ineffective due to implementation difficulties and because it has received little support by fishers and a general lack of political will. In Uganda, the generation of comprehensive biological data has often been considered expensive and too long a route to urgently needed management advice. This attitude is largely responsible for failure to heed scientific advice and seeking of "quick fix" solutions. Failure of previous attempts to induce *L. victorianus* and other indigenous species to breed can also be attributed to lack of adequate knowledge of their reproductive biology. Results from this thesis demonstrate the importance of fundamental research in contributing to solving management issues. After a critical analysis of the various factors influencing the sustainable utilisation of *L. victorianus*

discussed in Chapter 1, and *in lieu* of results from subsequent chapters, management strategies outlined in Figure 8.1 are proposed.

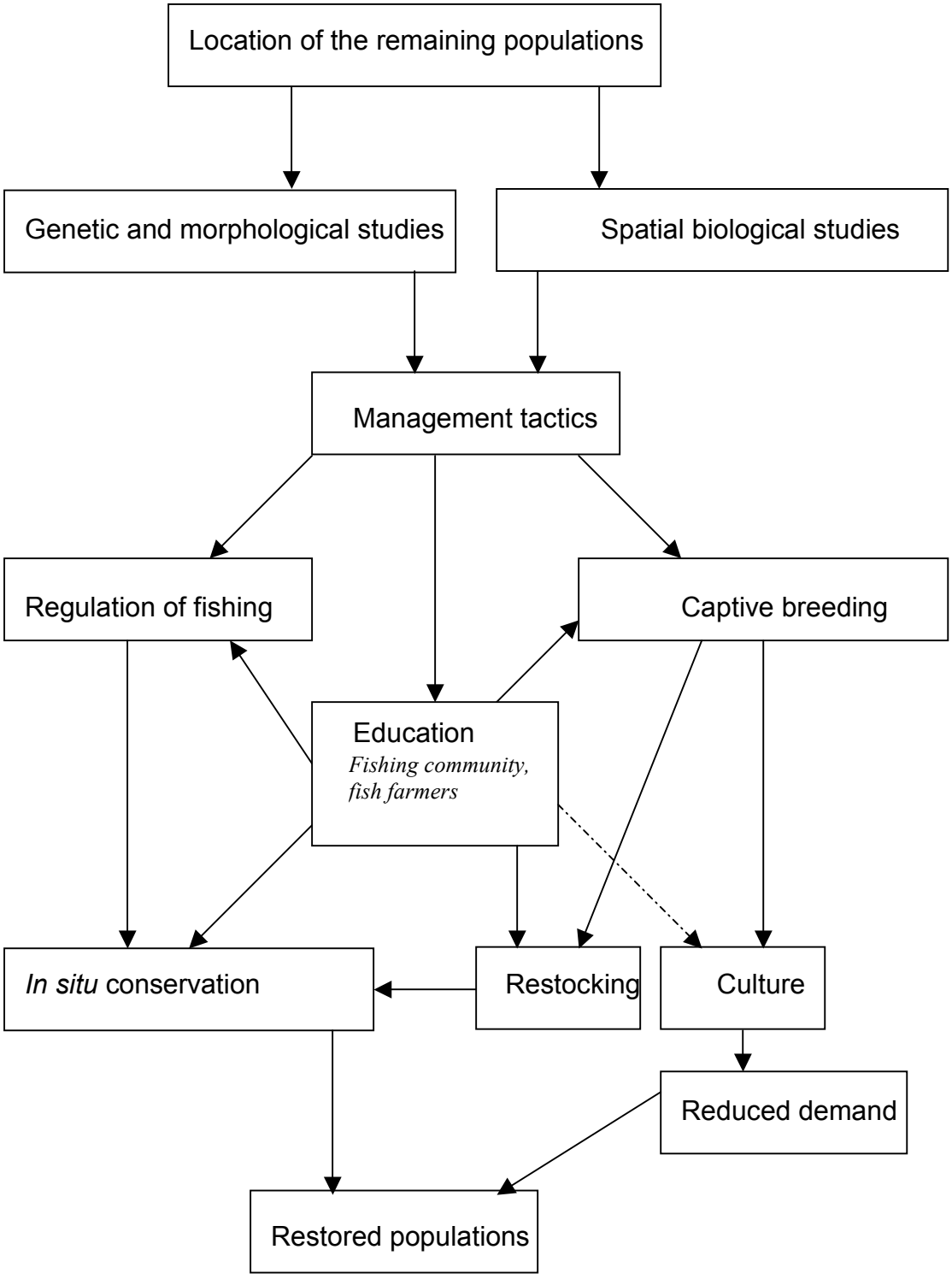

Figure 8.1 Flowchart of the proposed management strategy for *Labeo victorianus*.

The proposed strategy emphasises the importance of fundamental research, while simultaneously considering human requirements and perceptions in the formulation of resource management plans. Technical aspects of the proposed strategy, together with their management applications, are discussed below.

Location of the remaining populations and their movements

The absence of recorded specimens of *L. victorianus* from the Ugandan side of the lake basin in recent international collections (Table 2.1) - with the exception of two geographically distant populations - is of concern. This suggests that there has been some form of range contraction over the past 50 years. While specimens were obtainable from several locations, especially in the Kagera River (Table 2.1), little to no data are available on the population densities, genetic variability and migration patterns. According to Whitehead (1959), *L. victorianus* is a medium-pattern anadromous fish that enters the river in compact shoals and moves ten to fifteen miles upstream to spawn. This thesis, however, found fish that spawn within one kilometre from the lake (Chapter 3). The extent of upstream migrations is, therefore, unclear. The level of mixing within the Kagera system also needs to be ascertained.

Genetic, morphological and spatial biological studies

The causal mechanism for the observed spatial differences in the reproductive patterns could either be genetic or through some form of plastic life-history strategy. One fundamental question was, therefore, whether the two population constituted a homogeneous population or have they been genetically subdivided? Although this study was mainly concerned with reproductive biology, the lack of information on the

phylogeny of the two distant populations necessitated undertaking genetic studies as none of the casual mechanisms could be excluded without factual evidence. The lack of genetic differentiation at the mitochondrial d-loop control region between the two populations, as presented in Chapter 6, precluded the genetic sub-division hypothesis. This unfortunately does not necessarily exempt genetic influence on the observed disparities because ecological and genetic properties of a species are intimately linked (Adams, 1980). The incongruence of genetic and morphological studies observed in Chapter 6 could, be considered a case of varied phenotypic expression of a common genotype.

A species' life-history characteristics are a direct result of inherited attributes, and expressed in their immediate environment (Mann, 1989). According to Thorpe (1994), life-history characteristics are expressions of genetic blueprints for development that are realised differently under particular sets of environmental conditions. As the environmental conditions experienced by a population change, the individuals may display phenotypic plasticity in their physiology such as growth rate, or survival that mitigate the effects of the environment (Wootton, 1998). Disparities in life-history parameters, such size-at-maturity (Chapter 3), varied reproductive physiological pattern in gonadal recrudescence (Chapter 4) found between the Kagera and Sio River populations appear to provide some evidence in favour of this argument.

Fishes have an inherent ability to alter their life-history style by either adopting an altricial or a precocial state to "track" their immediate environment (Bruton, 1989) depending on prevailing abiotic or biotic conditions (Adams, 1980; Noakes and

Balon, 1982 and Bruton, 1989). Populations living within stable environments are more inclined to precocial life history style and will exhibit traits such as delayed maturity, slow growth and high longevity. In contrast, fish inhabiting unstable habitats, or harsh environments that are unpredictable and fluctuate regularly, tend to express an altricial life history style. Life history characteristics include early maturation, fast growth and a short life span. According to Stearns and Crandall (1984), an organism encountering an unavoidable stress that results in slower growth will, through the process of natural selection, have its age and size at maturity altered to maintain reproductive fitness as high as possible. Size-at-maturity is a critical stage in the life history of a fish. Resources utilised for growth and survival have to be partitioned to include reproductive demands (Wootton, 1998). According to Noakes and Balon (1982), if fish were to delay onset of sexual maturation, more of their available resources would be channelled into somatic growth. Therefore, these fish would be larger, with a higher fecundity at the time of their maturation.

Results from this study suggest a possible innate ability of *L. victorianus* to respond adaptively to proximate environmental conditions. The Kagera and Sio Rivers present contrasting environmental systems to which *L. victorianus* appears to have adapted accordingly. The Kagera River is a longer, larger and deeper river, whereas the Sio River is shallower and narrower (Chapter 2). Information gathered from the fishers, together with observation made during this thesis, indicate that the Sio River population are under more intense fishing pressure than the Kagera River population. Steep banks and the flow of large water volume in the Kagera River also makes fishing of *L. victorianus* using traditional gear difficult. The Sio River has conditions suitable for seining, gill netting and deep-trapping. The situation in Kagera River can,

therefore, be considered harsher to the fishers, and through the commensurate reduction of fishing effort, provides a stabler environment for fish. The converse holds for the Sio River. Results from Chapters 3 and 4 indicate that *L victorianus* in the Sio River exhibit a more altricial/r-selected life history style – in effect an opportunistic strategy. This could, therefore, be a phenotypic plastic response or a compensatory response, due to change of the gene pool through over-fishing.

According to Garrod and Horwood (1984), a species that is "successful" is one that is able to withstand some degree of adversity. This could be achieved through mechanisms dependent on the number of young born, or pertaining to their survival. Fish species either produce small numbers of eggs adapted for high survival, such as the cichlids, or large numbers of small eggs with low survival. In the latter example, the high numbers of eggs absorbs the risk of recruitment failure. *L. victorianus* appears to exhibit varying degrees of the two mechanisms. Both the Kagera and Sio River populations tend to maximise recruitment, as exemplified through the high production of yolked ova during a spawning season (Chapter 3) and the number of spawned eggs (Chapter 7).

Unfortunately, little information is available on the survival of *L. victorianus* eggs in the natural environment. The production of large number of semi-buoyant eggs Chapter 7), and the observation on natural spawning by Fryer and Whitehead (1959), therefore places the species into the non-guarder reproductive guild of Balon (1975). The absence of parental care will lead to high mortality, prompting synchronisation of spawning with onset of rainfall to ensure the availability of food for larvae. However, the capture of individuals that had spawned before onset of rainfall in the Sio River

(Chapter 3), together with similar observations in *Labeo capensis* (Cambray, 1985), suggest a deviation from the rainfall synchronised spawning pattern in African labeines as generalised by Skelton *et al*. (1991). It has not been ascertained whether such deviations confer any survival advantage to the offspring. What is clear within the Sio River is that there is less fishing activity especially trapping across the river during non-spawning periods. Possibly, spawning before the onset of rainfall provides a relatively stable microhabitat for the offspring. Further research into non-rainfall triggered spawning patterns, or facultative potamodromy, in African labeines is recommended. Observations of Type II atresia of retained vitellogenic oocytes in Chapter 4 was another biological disparity between the two populations that could possibly be attributed to the maximisation of lifetime reproductive output within the unpredictable environment (Matthews, 1998).

Results of this thesis reveal that *L. victorianus* has an adaptive life-history, which partially explains its persistence within a lake that has experienced massive fish extinctions. But how long can the species survive within an environment that is becoming increasingly hostile? Available data illustrate a crash in catches (Chapter 1) and a low genetic diversity within remaining populations (Chapter 6). Wootton (1998), after analysing the effects of fishing on life-history patterns using an idealised stock-recruitment curve, noted that populations will not recover if fishing mortality reduces the population below the minimum critical density even if fishing is halted. In the face of these threats, a prudent step would be to take advantage of the species inherent reproductive potential before further population declines and genetic erosion is noted.

Management tactics

Results from Chapter 6 illustrate extremely low levels of nucleotide diversity, together with the stochastic loss of haplotypes. Further analysis of nucleotide sequences indicated historical demographic decline thus providing the genetic basis for bottleneck situation in *L. victorianus* as previously postulated by Ribbink (1987) for small populations of Lake Victoria's native fishes. Frankel and Soulé (1981) discuss quantitative and qualitative handicaps imposed on populations by bottleneck situations. They argue that qualitatively specific alleles will be lost or retained. If lost then a mutation event is unlikely to replace them if the population is small. From a quantitative perspective, the variance of quantitative traits is reduced. On the whole, bottlenecks will have a greater qualitative than quantitative impacts. The consequence of reduced genetic diversity in a bottleneck situation is a populations' inability to cope with environmental changes or withstand disease conditions due to loss of vital alleles. Such a situation alone can lead to the demise of the population (Frankel and Soulé 1981; Ribbink, 1987).

The foregoing facts, together with the findings in Chapter 6, cast a grave prognosis to the continued existence of healthy *L. victorianus* populations in either the Kagera or the Sio Rivers. There is, therefore, an urgent need for management to intervene and not only restore population numbers but also genetic variability. Approaches to the proposed interventions are illustrated in Figure 8.1.

Regulation of fishing pressure

Although efforts to conserve native fish stocks by law enforcement have not been very successful in the lake, they should be reinvigorated with new ideas. A two-

pronged approach involving conservation of the remaining population in their natural environment and captive breeding is, therefore, likely to ameliorate the present precarious situation. Management should, therefore, base any intervention measures on relevant research information and institute medium- to long-term monitoring programmes. Populations within bottleneck situations are known to recover if given the opportunity to do so in their natural environment, but this is under the condition that the original causal agents are removed (Ribbink, 1986). Population recovery can be rapid for highly fecund species, such as *L. victorianus*. It must, however, be noted that a reduction of fishing pressure can only be effective if it is preceded by community education and provision of alternative sources from aquaculture.

Captive breeding

Captive propagation is a component of global strategy for conservation of genetic diversity (IUCN, 1987), and has already been suggested as one of the alternatives for management of Lake Victoria'a endemic species (Balon and Bruton 1986). However, Meffe (1987) cautions that such programmes must be preceded by filling of knowledge gaps in genetic information of the species, gaining experience in breeding, understanding fitness, life histories and biogeographical implications, and the recognition of role of education in conservation. According to Ribbink and Twentyman-Jones (1989), for captive propagation to be effective natural taxa must be characterised genetically and phenotypically followed by monitoring of changes that occur in captivity. Lack of such information could have contributed to failure to institute captive programme for Lake Victoria fishes despite having been recommended more than two decades years ago. Apart from assessing the problems, and subsequently making recommendations, there has been no generation of technical

information on which management could base the implemention of remedial measures.

Within a captive breeding programme, success or failure is ultimately determined by the ability to multiply the species under artificial conditions (Frankel and Soulé, 1981; Ribbink, 1987). In most fishes, including the labeines, final gamete maturation and ovulation does not spontaneously occur under captive conditions (Glubokov *et al*, 1991; Tan-Fermin and Emata, 1993). Results from the thesis provide the first comprehensive information that can form a basis for launching a captive breeding programme for *L. victorianus*, and possibly serve as a model for other native fishes inhabiting Lake Victoria.

Restocking and* in situ *conservation

Besides a reduction in fishing pressure, natural stocks can be enhanced through the collection of broodfish during their upstream migrations and inducing them to spawn. Reared juveniles would then be used to bolster wild populations. A similar approach was reported to have restored wild populations of anadromous fishes in the United States (Moring, 1986). However, captive breeding for the reintroduction of animals into the wild is a controversial subject (See review by Ribbink, 1987). Guidelines have, however, been drafted to allay fears expressed by opponents (Conway, 1980; Goodman, 1980, Frankel and Soulé, 1981, Foose, 1983; Ralls and Ballou, 1983; Schonewald-Cox, 1983; Templeton and Read, 1983).

According to Foose (1983), captive populations must be genetically managed so as to preserve as much of the heritable diversity that has evolved and exists in the wild

gene pool. This strategy would, therefore, necessitate establishing reference points of genetically characterised remaining wild populations prior to their propagation, followed up through monitoring. According to Foose (1983), a strategy for genetic management of a captive population should consider the acquisition of an adequate number of founders, the rapid expansion in population numbers from founders to the carrying capacity, the periodic regulation of genetic material exchange between populations to minimise inbreeding depression and possible genetic drift, and maximisation of an effective population size. These components would easily be addressed by a prudent *L. victorianus* captive propagation strategy.

It was ascertained during the preceding chapters that several hundred specimens could still be obtained from the remaining natural populations of *L. victorianus* for captive propagation purposes. Results of induced spawning in Chapter 7 indicate that *L. victorianus* populations can be expanded rapidly. The concern for regulation of exchange of genetic material between and within populations can be addressed by genetically characterising all the populations in the Lake basin (Table 2.1). Characterisation would provide a baseline for breeding between populations, while taking care to avoid the selection of particular attributes and hybridisation. It is known that effective population size is determined by the number, sex ratio and the quantity of offspring produced by an individual in its lifetime (Foose, 1983). The natural spawning behaviour of *L. victorianus* needs to be observed and effective sex ratios determined. Results from Chapter 3 indicated no sex bias during breeding months although it could not be ascertained whether all the individuals that migrated upstream participated in spawning activities. This uncertainty is confounded by reports of polyandry within the linage (Jackson and Coetzee, 1982; Mitchell, 1984 and Ried,

1985) together with findings in this thesis of gonadal structures that can permit intermittent release of spermatozoa in males and failed ovulations in females (Chapter 4). It is therefore possible that only a fraction of the total sexually mature individuals reproduce in a particular season in some natural environments.

Whereas increasing effective population size (N*e*) is important to control the loss of genetic polymorphism, Chasser (1983) proposes that an increase in N*e* may not be substantial enough to significantly curb the loss of genetic variation by genetic drift. Instead, captive propagation of species in numerous isolated populations is proposed. It can then be argued that although each population approaches fixation for a single allele at each locus, each population may become fixed for different alleles. Thus, if all the remaining populations of *L. victorianus* (Table 2.1) were to be examined, probably all or most of the original alleles may be recovered. Some of the advantages of this breeding strategy have been outlined as regaining of heterozygosity through cross mating among individuals from different populations, the maintenance of polymorphism, and compensation of loss of fitness due to inbreeding by the out-crossing process. It is, therefore, recommended that such management programmes be jointly undertaken by all the lake basin states. Although captive breeding knowledge generated from this thesis would be applicable to all the populations, genetic characterisation and some aspects of reproductive biology studies would have to be conducted for all the other populations in Table 2.1.

One other concern expressed towards the captive breeding is that of a population explosion beyond the habitat carrying capacity as a result of a successful propagation programme. Whereas this is a serious concern in other vertebrates, fishing pressure

would probably not allow a population explosion of *L. victorianus*. Fishing should, however, be monitored and possibly regulated to allow for the requisite level of reproduction and the maintenance of genetic diversity.

Management considerations for culture of *Labeo victorianus*

Knowledge of a species reproductive biology such as the structure of gametes, as presented in Chapter 4, can be used for accurate assessment of maturation and sperm preservation. Methods used to artificially spawn the fish (Chapter 7) can be improved and packaged for private commercial breeders who are stake-holders in Uganda's Plan for Modernisation of Agriculture (PMA). Increased multiplication of *L. victorianus* should be conducted simultaneously with education of the riparian communities about the potential of *L. victorianus* as an aquaculture species. The main biological attributes of labeines as aquaculture candidates is that they are non-carnivorous and their culture would not necessarily entail the collection of wild fish – two major limitations in Third-World aquaculture (Naylor *et al*., 2000). Strangely, labeines have been equated to cows (Reid, 1985) because of their habits of feeding on aufwuchs, zooplankton and detritus and insect larvae (el Moghraby and Rahman, 1984; Reid, 1985). Besides the production of a high biological value animal food, culture of the species would narrow the gap between supply and demand, and consequently reduce fishing pressure on wild stocks. It is, however, not known whether the inherent phenotypic plasticity noted in this species will lead to stuntedness in a cultured environment. In this situation, knowledge of sex differentiation (Chapter 3) would be important in control of reproduction through monosex culture.

In conclusion, this thesis has demonstrated that vital information for initiation of management policies can be generated within a reasonably short time period, of approximately three years, with modest financial resources. Results from reproductive biology and genetic studies underscore the importance of research-oriented approach in the conservation of Lake Victoria's native fish stocks. Possible options for increasing fish populations are explored based on an indigenous cyprinid *L. victorianus*. Finally, there is a need for management to get out of theoretical arguments and take rational steps towards conservation of the lake's ichthyofauna.

REFERENCES

Acheng, A.P. 1990. The impact of the introduced Nile perch *Lates niloticus* (L.) on the fisheries of Lake Victoria. *Journal of Fish Biology* **37**A: 17-23.

Adams, P.B. 1980. Life History patterns in marine fishes and their consequences for fisheries management. *Fish Bulletin* **78**: 1-12.

Agnèse, J.F., Teugels,G.G., Galbusera, P., Guyomard, R. and Valckaerts, F. 1997. Morphometeric and genetic characterisation of sympatric populations of *Clarias gariepinus* and *C. anguillaris* from Senegal. *Journal of Fish Biology* **50**: 1143-1157.

Alekseev, F.E. 1983. Hermaphroditism in porgies (Perciformes: Sparidae). II. Sexual structure of the populations, mechanism of its formation and evolution in scups, *Pagrus pagrus, P. orphus, P. ehrenbergi and P. auriga. Journal of Ichthyology* **23**: 61-73.

Amos, W. and Hoezel, A.R. 1991. Long term preservation of whale skin for DNA analysis. *Report on the International Whaling commission* **13**: 99-104.

Anon, 1965. Annual report of the Department of Agriculture and Fisheries 1964. Fisheries Research, Part II. Government Printer, Zomba, Malawi.

Anon, 1997. Fish registrations within the museum database of the Vertebrate Section of the Royal Museum for Central Africa. MRAC, Tervuren, Belgium.

Anon, 1999. Fish collection database of the Natural History Museum, London Natural History Museum, London (formerly British Museum of Natural History).

Anon, 2000. Fish collection database of the J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa.

Avise, 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. *Philosophical Transactions of the Royal Society of London*. **312:** 325-342.

Avise, J.C., Ball, R. A. and Arnold, J. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. *Molecular Biology and Evolution* **5**: 331-344.

Baccetti, B., Burrini, A.G., Callaini, G., Gibertini, G., Mazzini, M. and Zerunian, S. 1984. Fish germinal cells.I. Comparative spermatology of seven cyprinid species. *Gamete Research* **10**: 373-396.

Bagenal, T.B. 1969. Relationship between egg size and fry survival in brown trout salmon, *Salmo trutta* L. *Journal of Fish Biology* **1**: 349-353.

Balon, E.K. 1975. Reproductive guilds of fishes: A proposal and definition. *Journal of the Fisheries Research Board Canada* **32**: 821-826.

Balon, E.K. and Bruton, M.N. 1986. Introduction of alien species or why scientific advice is not heeded. *Environmental Biology of Fishes* **16**: 225-230.

Barbini, G.P. and McCleave, J.D. 1997. Fecundity of the American *eel Anguilla rostrata* at 45ºN in Maine, U.S.A. *Journal of Fish Biology* **51**: 840-847.

Barrel, C.D.N. and Witte, F. 1986. Cichlid species flock of Lake Victoria on the verge of extinction. *Annales du Musée Royal de l'Afrique Centrale Science Zoologique* **251**: 171-173.

Baynes, S.M. and Scott, A.P. 1987. Cryopreservation of rainbow trout spermatozoa: the influence of sperm quality, egg quality and extender composition on post-thaw fertility. *Aquaculture* **66**: 53-67.

Bhagyashri, A. and Saidapur, S.K. 1996. Atretic follicles and corpora lutea in the ovaries of fishes: structure-function correlations and significance. pp.147-168. *In*: D. J.S. Minshi and H.M. Dutta (eds.) *Fish morphology horizons of new research*. Science Publishers, Inc. Enfield.

Billard, R. 1986. Spermatogenesis and spermatology of some teleost fish species. *Reproduction Nutrition Development* **26**: 877-920.

Billard, R. 1978. Changes in structure and fertilising ability of marine and freshwater fish spermatozoa diluted in media of various salinities. *Aquaculture* **14**: 187-198.

Billard, R. 1990. Spermatogenesis in teleost fish. pp. 183-212 *In*: G. Lamming (ed.) *Marshall's physiology of reproduction in males*. Churchill Livingston, Edinburgh.

Bishop, W.W. and Trendall, A. F. 1967. Erosion surfaces, tectonics and volcanic activity in Uganda. *Quarterly Journal of the Geological Society of London* **122**: 385-420.

Booth, A.J. and Weyl, O.L.F. 2000. Histological validation of gonadal macroscopic staging criteria for *Labeo cylindricus* (Pisces: Cyprinidae) *African Zoology* **35**: 223-231.

Boulenger, G.A. 1909. *Catalogue of the freshwater fishes of Africa* pp. 322-323. Longmans. London.

Brambell, F.W.R. 1956. Ovarian changes. pp. 397-542. *In*: A.S. Parkes, (ed.) *Marshall's physiology of reproduction*. Longmans, Green and Co. London.

Bromage, N. 1995. Broodstock management and seed quality: general considerations. pp 1-2. *In*: N.R. Bromage and R.J. Roberts, (eds.) Broodstock management and egg and larval quality. Blackwell Sciences, London

Bromage, N., Hardiman, P., Jones, J., Springate, J. and Bye, V. 1990. Fecundity, egg size and total volume differences in 12 stocks of rainbow trout, *Oncorhynchus mykiss* Richardson. *Aquaculture and Fisheries Management* **21**: 269-284.

Brown, B.L., Epifanio, J.M., Smouse, P.E. and Kobak, C.J. 1996. Temporal stability of mtDNA haplotype frequencies in American shad stocks: to pool or not to pool across years? *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 227-2283.

Bruton, M.N. 1989. The ecological significance of alternative life-history styles. pp. 503-553. *In*: M.N. Bruton (ed.) Alternative Life-History Styles of Animals, Kluwer Academic Publishers, London

Bruton, M.N. 1990. The conservation of the fishes of Lake Victoria, Africa: an ecological perspective. *Environmental Biology of Fishes* **27**: 161-175.

Bruton, M.N. 1995. Have fishes had their chips? The dilemma of the threatened fishes. *Environmental Biology of Fishes* **43**: 1-27.

Buxton, C.D. and Garratt, P. A. 1990. Alternative reproductive styles in seabreams (Pisces: Sparidae) *Environmental Biology of Fishes* **28**: 113-124.

Bye, J.V. 1984. The role of environmental factors in the timing of reproductive cycles. pp. 187-205. *In*: G.W. Potts and R.J. Wootton (eds.) Fish reproduction strategies and tactics. Academic press, London

Cadwalladr, D.A. 1964. Studies on *Labeo victorianus* Blgr., of Lake Victoria. Report of the East African.Freshwater Fisheries Organisation 1963: 50-58.

Cadwalladr, D.A. 1965a. The decline in the *Labeo victorianus* Blgr (Pisces: Cyprinidae) fishery of Lake Victoria and an associated deterioration in some indigenous fishing methods in the Nzoia River, Kenya. *East African Agricultural and Forestry Journal* **30**: 249-256.

Cadwalladr, D.A. 1965b. Notes on the breeding biology and ecology of *Labeo victorianus* Boulenger (Pisces: Cyprinidae) of Lake Victoria. *Revue de Zoologie et de Botanique Africains* **72**: 109- 134.

Cadwalladr, D.A. 1969. A discussion of possible management methods to revive the *Labeo victorianus* fishery of Lake Victoria with special reference to the Nzoia River, Kenya. *Uganda Fisheries Department Occasional paper* 2: 1-6.

Cambray, J.A. 1982. The life-history strategy of a minnow, *Barbus anoplus* in a man-made lake in South Africa. MSc. Thesis, Rhodes University, South Africa. pp. 77-78.

Cambray, J.A. 1985. Observations on spawning of *Labeo capensis* and *Clarias gariepinus* in the regulated lower Orange river. *South African Journal of Science* **81**: 318-321.

Carr, M.H. and Reed, D. 1992. Conceptual issues relevant to marine harvest refuges: examples from temperate reef fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **50**: 2019-2028.

Castelloe, J. and Templeton, A. R. 1994. Root probabilities for intra-specific gene trees under neutral coalescent theory. *Molecular Phylogenetics and Evolution* **3:** 102-113

Chamberlain, G.W. 1993. Aquaculture trends and feed projections. *World Aquaculture Report* **24**: 20.

Chapman, L.J., Chapman, C.A., Brazeau, D.A., McLaughlin, B. and Jordan, M. 1999. Papyrus swamps, hypoxia, and faunal diversification: variation among populations of *Barbus neumayeri*. *Journal of Fish Biology* **54**: 310-327

Chasser, R.K. 1983. Isolation by distance: relationship to the management of genetic resources. pp. 66-77. *In*: C.M. Schonewald-Cox S.M. Chambers B. MacBryde and L. Thomas (eds.) Genetics and conservation: a reference for managing Wild Animal and Plant Populations. Benjamin/Cummings.

Chen, I.S., Hsu, C.H., Hui, C.F., Shao, K.T. Miller, P.J. and Fang, L.S. 1998. Sequence length and variation in the mitochondrial control region of two freshwater gobiid fishes belonging to *Rhinogobius* (Teleostei:Gobioidei). *Journal of Fish Biology* **53**:179-191.

Clearwater, S.J. and Pankhurst, N.W. 1994. Reproductive biology and endocrinology of female red gurnard, *Chelidonichthys kumu* (Lesson and Garnot) (Family Trigilidae), from the Hauraki gulf, New Zealand. *Austrarian Journal of Marine and Freshwwater Research*. **45**: 131-139.

Clement, M., Posada, D. and Crandall, K.A. 2000. TCS: a computer programme to estimate gene genealogies. *Molecular Ecology* **9**: 1657-1659.

Coetzee, P.S. 1983. Seasonal histological and microscopic changes in the gonads of *Cheimerius nufar* (Ehrenberg, 1820) (Sparidae: Pisces). *South African Journal of Zoology*. **18**: 76 – 88.

Colombo, G. and Grandi, G. 1996. Histological study of the development and sex differentiation of the gonad in the European eel. *Journal of Fish Biology* **48**: 493-512.

Colombo, G., Grandi, G. and Rossi, R. 1984. Gonadal differentiation and body growth in *Anguilla anguilla* L. *Journal of Fish Biology* **24**: 215-228.

Conway, W.G. 1980. An overview of captive propagation. pp. 199-208 *In*: M.E. Soulé and B.A. Wilcox (ed.) *Conservation Biology: an Evolutionary-Ecological perspective*. Sinauer Association, Sounderland.

Copeland, P.A. and Thomas, P. 1989. Control of gonadotropin release in the Atlantic croaker (*Micropogonias undulates*): evidence of lack of dopaminergic inhibition. *General and Comparative Endocrinology* **74**: 474-483.

Cross, R. Botha, T. and Pinchuck, S. 2001. Manual for preparation of biological material for electron microscopy. Electron Microscopy Unit, Rhodes University, Grahamstown, South Africa.

Currens, K.P., Sharpe, C.S., Hjort, R., Schreck, C.B. and Li, H.W. 1989. Effect of different feeding regimes on the morphometrics of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*). *Copeia* **3**: 689-695.

Davies, P.R. and Takashima, F. 1980. Sex differentiation in the common carp, *Cyprinus carpio*. *Journal of the Tokyo University of Fisheries* **66**: 191-199.

Dawson, A.S. and Grimm, A.S. 1980. Quantitative seasonal changes in the protein, lipid and energy content of the carcass, ovaries and liver of adult female plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* **16**: 493-504.

Driori, S., Ofir, M., Levavi-Sivan, B. and Yaron, Z. 1994. Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. *Aquaculture* **119**: 393-407.

el Moghraby, A.I. and Rahman, A.A. 1984. Food and feeding habits of *Labeo niloticus* (Pisces: Cyprinidae) in Jabel Aulia Reservoir, Sudan. *Hydrobiologia* **110**: 327-332.

Emel'yanova, N.G and Makeeva, A.P. 1985. Ultrastructure of the spermatozoids of some cyprinid fishes. *Journal of Ichthyology* **25**: 66-75.

Engels, W.R. 1993. Contributing software to internet: the Amplify programme. *Trends in Biochemical Sciences* **18**: 448- 450.

Fleischer, R.C., Perry, E.A., Muralidharan, K., Steven, E.E. and Wemmer, C.M. 2001. Phylogeography of the Asian elephant (*Elephas maximus*) based on mitochondrial DNA. *Evolution* **55**: 1882-1892.

Fleming, I.A., Johnsson, B. and Gross, M.R. 1994. Phenotypic divergence of sea-ranched, farmed and wild salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 2808-2824.

Food and Agricultural Organisation (FAO). 1995. Report on Lake Victoria Environmental Management Project tasks. pp. 11- 17.

Foose, T.J. 1983. The relevance of captive populations to the conservation of biotic diversity. pp. 374-401 *In*: C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde and L. Thomas (eds.) *Genetics and conservation: a reference for managing Wild Animal and Plant Populations*, Benjamin/Cummings Publ. London.

Foucher, R.P. and Beamish, R.J. 1980. Production of non-viable oocytes by pacific hake, *Merluccius productus*. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 41-48.

Fouda, M.M., Hanna, M.Y. and Fouda, F.M. 1993. Reproductive biology of a red sea goby, *Silhouettea aegyptia*, and a Mediterranean goby, *Pomatoschistus marmoratus*, in Lake Timsah, Suez canal. *Journal of Fish Biology* **43**:139-151.

Foyle, T.P. 1993. A histological description of gonadal development and sex differentiation in the coho salmon (*Oncorhynchus kisutch*) for both untreated and estradiol immersed fry. *Journal of Fish Biology* **42**: 699-712.

Francis, M.P. and Pankhurst, N.W. 1988. Juvenile sex inversion in the New Zealand snapper *Chrysophrys auratus* (Bloch and Schneider, 1801) (Sparidae). *Australian Journal of Marine and Freshwater Research* **39**: 625-631.

Frankel, O.H. and Soulé, M.E. 1981. *Conservation and evolution.* Cambridge University Press, London. pp. 31-161.

Franklin, I.R. 1980. Evolutionary changes in small populations. pp. 135-139. *In*: M.E. Soulé and B.A. Wilcox (ed.) *Conservation biology: an evolutionary-ecological perspective*. Sanauer Associates, Saunders.

Froese, R. and D. Pauly. 2002. FishBase (eds.). World Wide Web electronic publication, www.fishbase.org . 08 April 2002

Fryer, G. and Whitehead. P.J.P. 1959. The breeding habits, embryology and larval development of *Labeo victorianus* Boulenger. (Pisces : Cyprinidae). *Revue de Zoologie et de Botanique Africain* **59**: 33-49.

Gaigher, I.G. 1983. Reproduction of *Labeo umbratus* (Pisces, Cyprinidae) in Wuras Dam, a shallow, turbid impoundment. *South Afican Journal of Zoology* **19**: 105-108.

Garner, K.J. and Ryder, O. 1996. Mitochondrial DNA diversity in gorillas. *Molecular Phylogenetics and Evolution* **6**: 39-48.

Garrod, D.J. and Horwood, J.W. 1984. Reproduction strategies and the response to exploitation. pp. 367-384. *In*: G.W. Potts and R.J. Wootton (eds.) *Fish reproduction strategies and tactics*. Academic press, London.

Garrod, D.J. 1961. The history of the fishing industry of Lake Victoria, East Africa, in relation to the expansion of marketing facilities. *East African Agricultural and Forestry Journal* **27**: 95-99.

Gee, J.M. 1964. Nile perch investigations. East African Freshwater Fisheries Research Organisation Annual Report 1962/63

Gilbert, D. 1993. SeqApp 1.9 Biosequences editor and analysis, biocomputing office. Biology department, Indiana University, Bloomington, IN.

Glubokov, A.I. Motloch, N.N. and Sedova, M.A. 1991. Effect of a synthetic LHRH analogue and dopamine antagonists on the maturation of bream, *Abrama brama* L. *Aquaculture* **95**: 373-377.

Goldschmidt, T., Witte, F. and Wanink, J. 1993. Cascading effects of the introduced Nile perch on the detritivorous/phytoplanktivorous species in the sub-littoral areas of Lake Victoria. *Conservation Biology* **7**: 686-700.

Goldworthy, S., Francis, J., Boness, D. and Fleischer, R. 2000. Variation in the mitochondrial control region in the Juan Fernández Fur Seal. *The American genetic Association* **91**: 371-377.

Gonçalves. E.J. and Almada, V.C. 1997. Sex differences in resource utilisation by the peacock blenny. *Journal of Fish Biology* **51**: 624-633.

Goodman, D. 1980. Demographic intervention for closely managed populations. pp. 171-195 *In*: M.E. Soule and B.A. Wilcox (eds.) *Conservation biology: an evolutionary-Ecological perspective*. Sanauer Associates, Saunders.

Gophen, M., Ochumba, P.B.O. and Kaufman, L.S. 1995. Some aspects of perturbation in the structure and biodiversity of the ecosystem of Lake Victoria (East Africa). *Aquatic Living Resources* **8**: 27-41.

Graham, M. 1929. The Victoria Nyanza and its fisheries. A report on the fish survey of Lake Victoria 1927-1928 and appendices. Crown agents for the Colonies. London. pp. 255.

Greenwood, P.H. 1966. *The fishes of Uganda*. pp. 58 - 73.The Uganda Society, Kampala

Greenwood, P.H. 1972. New fish fossils from the Pleiocene of Wadi Natrun, Egypt. *Journal of Zoology London* **168**: 503-519.

Grier, H. J. 1981. Cellular organisation of the testis and spermatogenesis in fishes. *American Zoology* **21**: 345-357.

Grier, H.J. and Taylor, R.G. 1998. Testicular maturation and regression in the common snook. *Journal of Fish Biology* **53**: 521-542.

Gunderson, D.R. 1980. Using the r-K selection theory to predict natural mortality. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 2266-2271

Halder, S., Sen, S., Bhattacharya, S., Ray, A.K., Ghosh, A. and Jhingran, A.G. 1991. Induced spawning of Indian major carps and maturation of a perch and a catfish by murrel gonadotropin releasing hormone, pimozide and calcium. *Aquaculture* **97**: 373-382.

Hamaguchi, S. 1982. A light and electron microscope study on the migration of primordial germ cells in the teleost *Oryzias latipes*. *Cell Tissue Research* **227**: 139-151.

Hambalyn, E.L. 1960. The Nile perch project. *East African Freshwater Fisheries Research Organisation Annual rep*ort 1960, 26-31

Harpending, H.C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch. *Human Biolology* 66: 591-600.

Hauser, L., Carvalho, G.R. and Pitcher, T.J. 1995. Morphological and genetic differentiation of the African clupeid *Limnothrissa miodon* 34 years after its introduction to Lake Kivu. *Journal of Fish Biology* **47**: 127-144.

Hecky, R.E. 1993. The eutrophication of Lake Victoria. *Proceedings of International Association of Theoritical and Applied Limnology*. **25**: 39-48.

Henderson, N.E. 1962. The annual cycle in the testes of eastern brook trout. *Salvelinus fontinalis*. *Canadian Journal of Zoology* 40: 631-641.

Hibiya, T. 1982. *An atlas of fish histology: normal and pathological features*. Kodansha Scientific books, Tokyo, Japan. pp 104-110

Hiroi, O. and Yamamato, K. 1970. Studies on the maturation of Salmonid fishes –II. Changes in the testis of the masu salmon, *Oncorhynchus masou* during anadromous migration. *Bulletin of the Faculty of Fisheries, Hokkaido University* **20**: 252–263

Hiroyuki, C., Kamanga, G., Mususa, W. and Kataya, P.B. 1999. Technical report and instruction of Aquaculture in Malawi on seed production No. 1. Pages 9-19. Ministry of Forestry, Fisheries and Environmental affairs and Japan international Cooperation Agency.

Hoar, W.S. 1969. Reproduction. pp. 1-72. *In*: W.S. Hoar and D. J. Randall (eds.) *Fish physiology*. *Reproduction and growth, bioluminescence, pigments and poisons*. Academic press, London.

Hudson, R.R., Boos, D.D. and Kaplan, N. 1992 A statistical test for detecting geographical subdivision. *Molecular Biology and Evolution* **9**: 138-151

Hughes, N.F. 1992. Growth and reproduction of the Nile perch *Lates niloticus,* an introduced predator, in the Nyanza gulf, Lake Victoria East Africa. *Environmental Biology of Fishes* **33**: 299-305

Idler, D.R. and Bitners, I.1959. Biochemical studies on sockeye salmon during spawning migration. V. Cholesterol, fat, protein and water in the body of standard fish. *Journal of the Fisheries Research Board of Canada* **16**: 235-241.

Iliadou, K. and Fishelson, L. 1995. Histology and cytology of the testes of the catfish *Parasilurus aristotelis* (Siluroidae, Teleostei) from Greece. *Japan Journal of Ichthyology* **41**: 447–454.

International Union for Conservation of Nature (IUCN), 1987. Policy statement on

captive breeding. Survival commission, captive breeding specialist group, Glands, Switzerland. pp 4.

Jackson, P.B.N. and Coetzee, P.W. 1982. Spawning behaviour of *Labeo umbratus*, (Smith) (Pisces Cyrinidae). *South African Journal of Science* **78**: 293 – 295

Jhingran, V.G. and Pullin, R.S.V. 1985. A hatchery manual for the common, Chinese and Indian major carps. Asian Development Bank, Manila, Philippines. pp. 13-15

Johnson,T.C., Scholz, C.A., Talbot, M. R., Kelts, K., Ricketts, R.D., Ngobi, G., Beuning., K.R.M., Ssemmanda, I. and McGill, J.W. 1996. Late Pleistocene desiccation of Lake Victoria and rapid evolution of Cichlid fishes. *Science* **273**: 1091 - 1093.

Johnson, T.C., Kelts K. and Odada, E. 2000. The halocene history of Lake Victoria, *Ambio* **291**: 2-11.

Jolly, M.C. and Clonts, A.H. 1993. *Economics of aquaculture*. pp. 4-5. Food Products Press, New York.

Jonsson, N. and Jonsson, B. 1999. Trade-off between egg mass and egg number in brown trout. *Journal of Fish Biology* **55**: 767-783.

Karakousis, Y., Peios, C., Economidis, P. and Triantaphyllids, C. 1993. Multivariate analysis of the morphological variability among *Barbus peloponnesius* (Cyprinidae) populations from Greece and two populations of *B. meridionalis meridionalis* and *B. meridionalis petenyi. Cybium* **17**: 229-240.

Kawauchi, H., Suzuki, K., Itoh, H., Swanson, P., Naito, N., Nagahama, Y., Nozaki, M., Nakai, Y. and Itoh, S. 1989. The duality of teleost gonadotropins. *Fish Physiology and Biochemistry* **7**: 29-38.

Kaufaman, L. 1993. The changes of the World's great Lakes. *Conservation Biology* **7**: 447-449.

Kaufman, L. 1996. Preface. pp. 2-8. *In*: Seehausen, O. (ed.) *Lake Victoria rock cichlids, taxonomy, ecology and distribution*, Verduyn Cichlids.

Kudhongania, A.W. and Cordone, A.J. 1974. Benthos-spatial distribution and biomass estimates of the major demersal fishes in Lake Victoria. *African Journal of Tropical Hydrobiology and Fisheries* **3**: 15-31.

Khumalo, N. 2001. Diet of moggel *Labeo umbratus* in Katriver dam Eastern Cape province South Africa. Honours dissertation. Rhodes University 2001

King, M. 1995. *Fisheries biology, assessment and management*. pp. 154. Fishing News Books, Oxford, London.

King, J.A. and Millar, R.P. 1995. Evolutionary aspects of gonadotropin-releasing hormone and its receptor. *Cell Molecular Neurobiology* **15**: 5-23

Krieg, F., Triantafyllids, A. and Guyomard, R. 2000. Mitochondrial DNA variation in European populations of *Silurus glanis*. *Journal of Fish Biology* **56**: 713-724.

Kuo, C. and Nash, C.E. 1975. Recent progress on the control of ovarian development and induced spawning of the grey mullet *Mugil cephalus* L. *Aquaculture* **5**: 19-29.

Lahnsteiner, F., Berger, B., Weisman, T. and Patzner, R. 1995. Fine structure and motility of spermatozoa and composition of the seminal plasma in the perch. *Journal of Fish Biology* **47**: 492-508.

Lahnsteiner, F., Patzner, R.A. and Weisman, T. 1994. Testicular main ducts and spermatic ducts in some cyprinid fishes I; Morphology, fine structure and histochemistry. *Journal of Fish Biology* **44**: 937-951.

Levavi-Sivan, B., Ophir, M. and Yaron, Z. 1995. Possible sites of dopaminergic inhibition of gonadotropin release from the pituitary of a teleost fish, tilapia. *Molecular and Cellular Endocrinology* **109**: 87-95.

Ligtvoet, W. 1989. The Nile perch in Lake Victoria: A blessing or a disaster? *Annales du Musée Royal de l'Afrique Centrale Science Zoologique.* **257**: 151-156

Lowartz, S.M. and Beamish, F. W. H. 2000. Novel perspectives in sexual lability through gonadal biopsy in larval sea lampreys. *Journal of Fish Bio*logy **56**: 743-757

Lyrholm, T., Leimar, O. and Gyllensten, U. 1996. Low genetic diversity and biased substitution patterns in the mitochondrial DNA control region of sperm Whales: Implications for estimates of time since common ancestry. *Molecular Biology and Evolution* **13**: 1318-1326.

Luna, L.G. 1968. Manual of histological staining methods of the Armed Institute of Pathology. pp. 5 - 46. McGraw-Hill Book Company, New York.

MacFarlane, R.B., Norton, E.C. and Bowers, M.J. 1993. Lipid dynamics in relation to the annual reproductive cycle in yellow tail rockfish, *Sebastes flavidus*. *Canadian Journal of Fisheries and Aquatic Sciences* **50**: 391-401.

Mackinnon, J.C. 1972. Summer storage of energy and its use for winter metabolism and gonadal maturation in the American plaice, *Hippoglossoides platessoides*. *Journal of Fisheries Research Board of Canada* **29**: 1749-1759.

Mamuris, Z., Stamatis, C., Bani, M. and Triantaphyllidis, C. 1999. Taxonomic relationships between four species of the Mullidae family revealed by three genetic methods: allozymes, random amplified polymorpic DNA and mitochondrial *DNA Journal of Fish Biology* **55**: 572-587.

Mann, J.R. 2001. Imprinting in the germ cells. *Stem cells*. **19**: 287-297

Mann, R.H.K. 1989. Fish population dynamics in the River Frome, Dorset. *Regulated Rivers Research and Management* **4**: 165-177

Marino, G., Azzurro, E., Massari, A., Finoia, M.G. and Mandich, A. (2001). Reproduction in the dusky grouper from the southern Mediterranean. *Journal of Fish Biology* **58**: 909-927.

Mason, J.C. 1985. The fecundity of the walleye Pollock, *Theragra chalcogramma* (Pallas), spawning in Canadian waters. *Journal of Fish Biology* **27**: 335-346.

Matsuyama, M., Lara, R.T. and Matsuura, S. 1988. Juvenile bisexuality in the red sea bream, *Pagrus major*. *Environmental Biology of Fishes* 21: 27-36

Mattei, X. 1991. Spermatozoa ultrastructure and its systematic implications in fishes. *Canadian Journal of Zoology* **69**: 3038-3055.

Mattei, X., Siau, Y., Thiaw, O.T. and Thiam, D. 1993. Peculiarities in the organisation of testis of *Ophidion* sp. (Pisces: Teleostei). Evidence for two types of spermatogenesis in teleosts fish. *Journal of Fish Biology* **43**: 931-937.

Matthews, W.J. 1998. *Patterns in freshwater fish ecology*. Chapman & Hall. New York. pp. 419-454.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge. pp. 797.

Medford, B.A. and Mackay, W.C. 1978. Protein and lipid content of gonads, liver and muscle of northern pike, *Esox lucius* in relation to gonadal growth. *Journal of the Fisheries Research Board of Canada* **35**: 213-219.

Meffe, G.K. 1987. Conserving fish genomes: philosophies and practices. *Environmental Biology of Fishes* 1**8**: 3-9.

Mezhnin, F.I. 1979. Development of the sex cells in the early ontogeny of the common perch *Perca fluviatilis*. *Journal of Ichthyology*. **18**: 71-86

Miller, P.J. 1984. The tokology of gobioid fishes. pp. 119-153. *In*: G.W. Potts and R.J. Wootton (eds.) *Fish reproduction strategies and tactics*. Academic press, London

Mills, C.A. 1991. Reproduction and life history. *In*: I.J. Winfield and J.S. Nelson (eds.) Cyprinids Fishes: Systematics, Biology and exploration. pp. 483-488 Chapman & Hall, London.

Ministry of Agriculture Animal Industry and Fisheries, Entebbe, Uganda, 1995 Fisheries Department, Fish catch statistics 1995.

Ministry of Agriculture Animal Industry and Fisheries, Entebbe, Uganda, 1988 Fisheries Department, Fish catch statistics 1988.

Ministry of Agriculture Animal Industry and Fisheries, Entebbe, Uganda, 2000. Fisheries Department, statistics abstract 2000.

Mitchell, S.A. 1984. Further observation on the breeding behaviour of *Labeo umbratus* (Smith) (Pisces: Cyprinidae). *Journal of the Limnological Society of Southern Africa* : 28-34.

Mogil'naya, N.A. and Moiseeva, E.B. 1985. Gonad development of the black sea leaping gray mullet *Liza saliens* during early ontogeny. *Journal of Ichthyology* **25**: 76-84.

Moore, R. 1979. Natural sex inversion in the giant perch *Lates calcarifer*. *Australian Journal of Marine and Freshwater Research* **30**: 803-813.

Moring, J.R. 1986. Stocking of anadromous species to restore or enhance Fisheries. pp. 59-74. *In*: Stroud, R.H. (ed.) Fish culture in Fisheries Management, a proceeding of a symposium on the role of Fish Culture in Fisheries Management at Lake Ozark, Missouri, March 31 – April 3, 1985. Bethesda, Maryland

Msiska, O.V. 1990. Reproductive strategies of two cyprinid fishes in Lake Malawi and their relevance for Aquaculture development. *Aquaculture and Fisheries Mana*gement **21**: 67-75.

Mugidde, R. 1993. The increase in phytoplankton productivity and biomass in Lake Victoria, Uganda. *Proceedings of International Association of Theoritical and Applied Limnology* **25**: 846-849.

Munro, A.D. 1990. General introduction. pp. 1-11 *In*: A.D. Munro, A.R. Scott and T.J. Lam (eds.) *Reproductive seasonality in teleosts: environmental influences*. CRC Press, Boca Raton, Florida, USA.

N'da, K. and Déniel, C. 1993. Sexual cycle and seasonal changes in the ovary of the red mullet, *Mullus surmuletus*, from the southern coast of Brittany. *Journal of Fish Biology* **43**: 229-244.

Nakamura, M., Takahashi, H. and Hiroi, O. 1973. Gonadal differentiation in *Tilapia mossambica* with special regard to the time of estrogen treatment effective in inducing

complete feminisation of genetic males. *Bulletin of the Faculty of Fisheries, Hokkaido University* **24**: 1-3.

Nakamura, M., Takahashi, H. and Hiroi, O. 1974. Sex differentiation of the gonad in the masu salmon *Onchorhynchus masou*. *Scientific Report of Hokkaido Salmon Hatchery*. **28**: 1-8.

Nakamura, M. 1982. Gonadal sex differentiation in whitespotted charr, *Salvelinus leucomaenis*. *Japanese Journal of Ichthyology* **28**: 431-436

Nakamura, M., Hourigan, T.F., Yamauchi, K., Nagahama, Y. and Grau, G. 1989. Histological and ultrastructural evidence for the role of gonadal steroid hormones in sex change in the protogynous wrasse *Thalassoma duprrey*. *Environmental Biology of Fishes*. **24**: 117-136.

Nakamura, M. and Nagahama, Y. 1993. Ultrastructure study on the differentiation and development of steroid-producing cells during ovarian differentiation in the amago salmon *Onchorhynchus rhodurus. Aquaculture* **112**: 237-251.

Nassour, I. and Léger , C.L. 1989. Deposition and mobilisation of body fat during sexual maturation in female trout *Salmo gairdneri. Aquatic living Resources* **2**: 153-159.

National Environmental Management Authority (NEMA). 1998. Report on the state of the environment report for Uganda. Kampala, Uganda pp. 121 – 130.

Naylor, R.L., Goldburg, R.J, Primavera J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. 2000. Effect of aquaculture on world fish supplies. *Nature* **405**: 1017-1024.

Nei, M., Murayama,T. and Charkraborty, R. 1975. *The bottleneck effect and genetic variability in populations.* Evolution **29**: 1-10.

Nei, M. 1987. Molecular Evolutionary Genetics. pp. 256-257. Columbia University Press.

Nielsen, E.E., Hansen, M.M. and Mensberg, K.L.D. 1998. Improved primer sequences for the mitochondrial ND1, ND3/4 and ND5/6 segments in salmonid fishes: application to RFLP analysis of Atlantic salmon. *Journal of Fish Biology* **53**: 216-220.

Noakes, D.L.G. and Balon, E.K. 1982. Life histories of tilapias: an evolutionary perspective. pp. 61-82. *In*: R.S.V. Pullin and R.H. Lowe-McConnell (eds.) *The Biology and Culture of Tilapias*, ICLARM Conference Procedings 7, Manila.

Ochumba, P.B.O. 1994. Conservation plans for Lake Victoria? *Swara* **17**: 16-19.

Ochumba, P.B.O. and Kibara, D.I. 1989. Observations on blue-green algal blooms in the open waters of Lake Victoria, Kenya. *African Journal of Ecology* **27**: 23-34.

Ogari, J. 1985. Distribution, food and feeding habits of *Lates niloticus* in the Nyanza gulf of Lake Victoria (Kenya). Committee for inland fisheries of Africa (CIFA). Report on the third session of the sub-committee for development and management of fisheries of Lake

Victoria, Jinja, Uganda 4-5 October, 1984. Food and Agricultural Organisation, Fisheries Report 335, 68-80.

Ogari, J. 1992. Trends in fisheries development, prospects and limitations. Report, National Seminar on the development and Management of the Kenya fisheries of Lake Victoria., Bujumbura (Burundi), Dec 1992. pp. 59-73.

Ogutu-Ohwayo, R. 1990. The decline of fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile Tilapia, *Oreochromis niloticus*. *Environmental Biology of fishes* **27**: 81-96.

Okaranon, J.O. 1994. Current composition, distribution and relative abundance of the fish stocks of Lake Victoria, Uganda. *African Journal of Tropical Hydrobiology and Fisheries* **52**: 89-100.

Okazaki, M., Naruse, K., Shima, A. and Arai, R. 2001. Phylogenetic relationships of bitterlings based on mitochondrial 12S ribosomal DNA sequences. *Journal of Fish Biology* **58**: 89-106

Okedi, J.Y.O. 1970. Further observation on the ecology of the Nile perch *Lates niloticus* (L) in Lake Victoria and Lake Kyoga. *East African Freshwater Fisheries Research Organisation Annual Report.* 1970. pp. 42-55.

Okuda, N. 2001. The costs of reproduction to males and females of a paternal mouth brooding cardinalfish, *Apogon notatus. Journal of Fish Biology* **58**: 776-787.

Osinov, A.G. and Lebedev, V.S. 2000. Genetic divergence and phylogeny of the Salmoninae based on allozyme data. *Journal of Fish Biology* **57**: 354-381.

Palsbøll, P.J. and Actander, P. 1998. Primers for mitochondrial DNA: The importance of species-specific primers. *In*: A. Karp, P.J. Isaac and D.S. Ingram (eds.) *Molecular tools for screening of Biodiversity; Plants and Animals* Cambridge Unversity press, London.

Parmentier, H.K. and Timmermans, L.P.M. 1985. The development of germ cells and gonads during development of carp *Cyprinus carpio* L. A study with anti-carp sperm monoclonal antibodies. *Journal of Embryology and Experimental Morphology* **90**: 13- 32.

Patino, R. 1997. Manipulation of the reproductive systems of fishes by means of exogenous chemicals. *Progressive Fish Culturalist* **55**: 18-128.

Patzner, R.A. and Kaurin, G 1997. Sexual differentiation in *Salaria* (=*Blennius*) *pavo*. *Journal of Fish Biology* **50**: 887-894.

Peter, R.E., Chang, J.P., Nahorniak, C.S., Omeljaniuk, R.J., Sokolowska, M., Shih,S.H. and Billard, R. 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. *Recent Progress in Hormonal Research* **42**: 513-48.

Peter, R.E., Lin, H.R. and Van Der Kraak, G. 1988. Induced ovulation and spawning of cultured freshwater fish in China: Advances in application of GnRH analogues and Dopamine antagonists. A*quaculture* **74**: 1- 10.

Peter, R.E., Trudeau, V.L., Sloley, B.D., Peng, C. and C.S. Nahorniak. 1991. Actions of catecholamines, peptides and sex steroids in regulation of gonadotropin-II in the goldfish. p. 91. *In*: A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe, (eds.) Proceedings of the 4th Symposium on the Reproductive Physiolology of Fish.

Peter, R.E. and Yu, K.L. 1997. Neuroendocrine regulation of ovulation in fishes: basic and applied aspects. *Reviews in Fish Biology and Fisheries* **7**: 173-197.

Pillay, T.V.R. 1990. *Aquaculture principles and practices*. pp. 7-11. Fishing News Books, Oxford.

Pitman, W.R. 1979. Effects of female age and egg size on growth and mortality in rainbow trout. *Progressive Fish Culturalist* **41**: 202-205.

Ragnon, X., Teugels, G.G., Guyomard, R., Galbusera, P., Andriamanga, M., Volckaert, F. and Agnèse, J. F. 1998. Morphometric and allozyme variation in the African catfishes *Clarias gariepinus* and *C. anguillaris*. *Journal of Fish Biology* **53**: 192-207.

Ragnon, X. and Guyomard, R. 1997. Mitochondrial DNA differentiation among East and West African Nile tilapia populations. *Journal of Fish Biology* **51**: 204-207.

Ralls, K. and Ballou, J. 1983. Extinction: lessons from zoos. pp. 164-184. *In*: C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde and L. Thomas (eds.) Genetics and conservation: a reference for managing Wild Animal and Plant Populations. Benjamin/Cummings Publ. London.

Reay, P.J. 1984. Reproduction tactics: A non-event in Aquaculture? pp.291-309. *In*: G.W. Potts and R.J. Wootton (eds.) *Fish reproduction strategies and tactics*. Academic press, London.

Reid, G. McG. 1985. A revision of African species of *Labeo* (Pisces: Cyprinidae) and a redefinition of the genus. Thesis Zoological. J. Cramer Verlag Braunschweig **6**: 223-226.

Reid, W.V. 1997. Strategies for conserving biodiversity. *Environment* **39**: 16-43.

Reidmiller, S. 1994. Lake Victoria Fisheries: the Kenyan reality and environmental implications. *Environmental Biology of Fishes* **39**: 329-338.

Reist, J. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Canadian Journal of Zoology* **63**: 1429-1439.

Ribbink, A.J. 1986. Perspectives in species conservation. *Annales du Musée Royal de l'Afrique Centrale Science Zoologique* **251**: 163-170.

Ribbink, A.J. 1987. African lakes and their fishes: conservation scenarios and suggestions. *Environmental Biology of Fishes* **19**: 3-26.

Ribbink, A.J and Twentyman-Jones, V. 1989. Captive propagation as a conservation tool. *Annales du Musée Royal de l'Afrique Centrale Science Zoologique* **257**: 145-150.

Riciardi, A. and Rasmussen, J.B. 2000. Extinction rates of North American fresh water fauna. *Conservation Biology* **13**: 1220-1222.

Ross, R.M. 1984. Anatomical changes associated with sex reversal in the fish *Thalassoma duperrey* (Teleostei: Labridae*). Copeia* **1**: 245-248.

Rothbard, S. 1981. Induced reproduction in cultivated cyprinids-the common carp and the group of Chinese carps: I the technique of induction, spawning and hatching. *Bamidgeh* **33**: 103-121.

Rozas, J. and Rozas, R. 1999. DnaSP, version 3: an integrated programme for molecular population genetics and molecular evolution analysis. *Bioinformatics* **15**: 174-175.

Rutaisire, J. 1997. Captive breeding of mirror carp, *Cyprinus carpio* and the epidemiology of survivorship of fry at Kajjansi, Uganda. Master's thesis, Makerere University, Kampala.

Rutaisire, J., Muwazi, R.T. and Booth, A.J. 2003. The structure and cytology of the testes of *Labeo victorianus* (Pisces: Cyprinidae) *African Zoology* **38**: 119-126.

Sadovy, Y. and Shapiro, Y. 1987. Criteria for the diagnosis of hermaphroditism in fishes. *Copei*a **1**: 136-156.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**: 487-491.

Saitou, N. and Nei, M. 1987. The Neighbour joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

Sang, T.K., Chang, H.Y., Chen, C.T. and Hui, C.F. 1994. Population structure of the Japanese eel, *Anguilla japonica*. *Molecular Biology and Evolution* **11**: 250-260.

Sanger, F. Nicklen, S. and Coulsen, A.R. 1977. Sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences of USA* **74**: 5463-5467

Satoh, N. and Egami, N. 1972. Sex differentiation of germ cells in the teleost, *Oryzias latipes*, during normal embryonic development. *Journal of Embryology and Experimental Morphology* **28**: 385-395.

Satoh, N. 1974. Ultrastructural study of sex differentiation in the teleost *Oryzias latipes*. *Journal of Embryology and Experimental Morphology* **2**: 195-215.

Schaeff, C.M., Kraus, S.D., Brown, M.W., Perkins, J.S., Payne, R. and White, B.N. 1997. comparison of genetic variability of North and South Atlantic whales (*Eubalaena*) using DNA fingerprints. *Canadian Journal of Zoology* **75**: 1073-1080.

Schneider, S., Roessli, D. and Excoffier, L. 2000. Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

Schonewald-Cox, C.M. 1983. Conclusions: guidelines to management: a beginning attempt. pp. 135-139. *In*: C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde and L. Thomas (eds.) Genetics and conservation: a reference for managing Wild Animal and Plant Populations, Benjamin/Cummings Publ. London.

Shapiro, D.Y. 1987. Differentiation and evolution of sex change in fishes. *Bioscience* **37**: 490-497.

Shapiro,Y.D. 1984. Sexual reversal and sociodemographic processes in Coral Reef Fishes. pp.103-117. *In*: G.W. Potts and R.J. Wootton (eds.) Fish reproduction strategies and tactics. Academic press, London.

Sherwood, N.M., Parker, D.B., McRory, J.E. and Lescheid, D.W. 1994. Molecular evolution of growth hormone and gonadotropin-releasing hormone. pp 13: 3-66. *In*: W. S Hoar and D. J. Randall, (eds.) *Fish Physiology*. New York Academic Press.

Shikhshabekov, M.M. 1972. The annual cycle of gonads in wild carp (*Cyprinus carpio*) (L.) from Terek Delta. *Journal of Ichthyology* **12**: 855-859.

Siddiqui, A.Q., Chatterjee, A. and Khan, A.A. 1976. Reproductive biology of the carp, *Labeo bata* (Ham.) from the River Kali, India. *Aquaculture* **7**: 181-191.

Skeleton, P.H., Tweddle, D. and Jackson, P.B.N. 1991. Cyprinids of Africa. . pp. 211-239. *In*: I.J. Winfield and J.S. Nelson (eds.) *Cyprinids Fishes: Systematics, Biology and exploration*. Chapman & Hall, London.

Slatkin, M. and Hudson, R.R. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* **129**: 555-562.

Sokolowska, M., Peter, R.E., Nahorniak, C.S., Pan, C.H., Chang, J.P, Crim, L.W. and Weil, C. 1984. Induction of ovulation in goldfish, *Carassius auratus*, by pimozide and analogues of LH-RH. *Aquaculture* **36**: 71-83.

Soule, M.E. and Wilcox, B.A. 1980. The threshold of survival Conservation biology: maintaining fitness and evolutionary potential. pp. 151-169. *In:* M.E. Soule, and B.A. Wilcox, (eds.) *Conservation biology: an evolutionary-ecological perspective*. Sanauer Associates, Saunders.

Springate, J.R.C. and Bromage, N.R. 1985. Effects of egg size on early growth and survival in rainbow trout, *Salmo gairdneri*. *Aquaculture*. **47**: 163-172.

Stearns, S.C. and Crandall, E. 1984 Plasticity for Age and Size at sexual maturity: A life-history response to Unavoidable stress. pp. 13-33. *In*: G.W. Potts and R. J. Wootton (eds.) *Fish reproduction strategies and tactics*. Academic press, London.

Stoumboudi, T.M. and Abraham, M. 1996. The spermatogenetic process in *Barbus longiceps, Copoeta damascina* and their natural sterile hybrid (Teleostei: Cyprinidae). *Journal of Fish Biology* **49**: 458-468.

Strauss, R.E. and Bookstein, F.L. 1982. The truss: body form reconstruction in morphometrics. *Systematic Zoology* **31**: 113-135.

Swofford, D.L. 2000: PAUP*: Phylogenetic inference using parsimony (* and other methods), version 4.0. Sinauer Assoc., Sunderland,Massachusetts

Tajima, F. 1996. The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. *Genetics* **143**: 1457-1465.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.

Takahashi, H. 1977. Juvenile hermaphroditism in the zebra fish *Brachydanio rerio. Bulletin of the Faculty of Fisheries, Hokkaido University* **28**: 57-65.

Takeyama, H., Chow, S., Tsuzuki, H. and Matsunaga, T. 2001. Mitochondrial DNA sequence variation within and between tuna *Thunnus* species and its application to species identification . *Journal of Fish Biology* **58**: 1646-1657.

Talling, J.F. 1965. The photosynthesis activity of phytoplankton in East African lakes. *International Revue of Hydrobiology* **50**: 1-32.

Talling, J.F. 1966. The annual cycle of stratification and phytoplankton growth in Lake Victoria (East Africa). *International Revue of Hydrobiology* **51**: 545-621.

Tan-Fermin, J.D. and Emata, A.C. 1993. Induced spawning by LHRHa and pimozide in the Asian catfish *Clarias macrocephalus* (Gunther). *Journal of Applied Ichthyology* **9**: 89-96.

Taylor, R.G., Grier, H.J. and Whittington, J.A. 1998. Spawning rhythms of common snook in Florida. *Journal of Fish Biology* **53**: 502-520.

Templeton, A.R. and Read, B. 1983. The elimination of inbreeding depression in a captive herd of Speke's gazelle. pp. 241-262. *In*: C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde and L. Thomas (eds.) *Genetics and conservation: a reference for managing Wild Animal and Plant Populations*. Benjamin/Cummings Publ. London.

Templeton, A.R., Crandall, K.A. and Sing, C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.

Thorpe, J.E. 1994 Performance thresholds and life-history flexibility in salmonids. *Conservation Biology* **8**: 877-879.

Tyler, C.R. and Sumpter, J.P. 1996. Oocyte growth and development in teleosts. *Reviews in Fish Biology and Fisheries* **6**: 287-318.

Van der Horst, G. 1978. The structure of the testes of the mullet *Liza dumerili* (Teleostei; Mugilidae) with special reference to spermotogenesis. *Zoologica Africana* **13**: 233 – 243.

van der Merwe, W., Van Vuuren, J.H.J. and Vermaak, J.F. 1988. Cyclic histomorphological changes in the ovary of mudfish, *Labeo capensis*. *Aquaculture* **72**: 349-358.

van Oijen, M.J.P., Witte. F. and Witte-Maas, E.L.M. 1981. An Introduction to ecological and taxonomic investigations on the haplochromine from the Mwanza Gulf of Lake Victoria. *Netherlands Journal of Zoology* **31**: 149-174.

van Winkoop, A., Booms, G.H.R., Dulos, G. J. and Timmermans, L.P.M. 1992. Ultrastructural changes in primordial germ cells during early gonadal development of the common carp *Cyprinus carpio* L.: Teleostei). *Cell Tissue Research*. **267**: 337-346.

van Zyl, B.J., Hay, C.J. and Steyn, G.J. 1995. Some aspects of the reproduction biology of *Labeo capensis* (Smith, 1941) (Pisces: Cyprinidae) in relation to exploitation and extreme environmental conditions in Hardap Dam, Namibia. *South African Journal of Aquatic Science* **21**: 88-98.

Vigilant, L., Stoneking, M., Harpending, H., Hawkes, K. and Wilson, A.C. 1991. African populations and the evolution of human mitochndrial DNA. *Science* **253**: 1503-1507.

Wallace, J.C. and Aasjord, D. 1984. An investigation of consequences of egg size for the culture of Arctic Charr, *Salvelinus alpinus* L. *Journal of Fish Biology* **24**: 427-435.

Ward , R.D. 2000. Genetics in fisheries management. *Hydrobiologia* **420**: 191-201.

Warner, R.R. 1982. Mating systems, sex change and sexual demography in the rainbow wrasse. *Thalossoma lucasanum. Copeia* **3**: 653-661.

Waters, J.M., Epifanio, J.M., Gunter, T. and Brown, B.L. 2000. Homing behaviour facilitates subtle genetic differentiation among river population of *Alosa sapidissima*: microsatellites and mtDNA. *Journal of Fish Biology* **56**: 622-636.

Waters, J.M. and Willis, G.P. 2001. Mitochondrial DNA phylogenetics of the *Galaxias vulgaris* complex from South Island, New Zealand: rapid radiation of a species flock. *Journal of Fish Biology* **58**: 1166-1180.

Weigand, M.D. 1996. Composition, accumulation and utilisation of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries* **6**: 259-286.

Wenink, P.W., Baker, A.J and Tilanus, M.G.J. 1993. Hypervariable-control-region sequences reveal global population structuring in a long-distance migrant shorebird, the Dunlin (*Calidris alpina*). *Proceedings of Natational Academy of Science USA* **90**: 94-98.

West, G. 1992. Methods of assessing ovarian development in fishes: a review. *Australian Journal of Marine and Freshwwater Research* **41**: 199-222.

Weyl, O.L.F. and Booth, A.J. 1999. On the life history of a cyprinid fish, *Labeo cylindricus*. *Environmental Biology of Fishes* **55**: 215-225.

Whitehead, P.J.P. 1959. The anadromous fishes of Lake Victoria. *Revue de Zoologie et de Botanique Africains* **59**: 329-363.

Wilson, E.O. 1985. The biological diversity crisis. *Bioscience* **35**: 700-706.

Wilson, P.J., Woods, C.M., Maina, J.N. and White, B.N. 2000. Genetic structure of Lake Magadi tilapia populations. *Journal of Fish Biology* **56**: 590-603.

Witte, F., Msuku, B.S., Wanink, J.H., Seehausen, O., Katunzi, E.F.B., Goundswaard, P.C. and Goldschmidt, T. 2000. Recovery of cichlid species in Lake Victoria : an examination of factors leading to differential extinction. Reviews in Fish Biology and Fisheries **10**: 233 - 241

Witte, F., Goldschmidt, T., Goudswaard, K., Ligtvoet, M.J.P., van Oijen, M. and Wanink, J. 1992a. Species extinction and concomitant ecological changes in Lake Victoria. *Netherland Journal of Zoology* **42**: 214-232.

Witte, F., Goldshmidt, T., Wanink, J., van Oijen, M., Goudswaard, K., Witte-Maas, E. and Bouton, N. 1992b. The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. *Environmental Biology of Fishes* **34**: 1-28.

Wootton, R.J. 1984. Introduction: Strategies and tactics in fish reproduction Fishes. pp.1-12. *In*: G.W. Potts and R.J. Wootton (ed.) *Fish reproduction strategies and tactics*. Academic press, London.

Wootton, R.J. 1998. *Ecology of Teleost fishes*. pp. 141-282. Kluwer Academic publishers, London.

Yamamato, T. 1969. Sex differentiation. pp.117-175. *In*: W.S. Hoar and D. J. Randall (eds.) *Fish physiology, reproduction and growth, bioluminescence, pigments and poisons*. Academic press, London.

Yamazaki, F. 1983. Sex control and manipulation in fish. *Aquaculture* **33**: 328-354.

Yaron, Z. 1995. Endocrine control of gametogenesis and spawning induction in the carp. *Aquaculture* **129**: 49-73

Yoshizaki, G., Yamaguchi, K., Oota, T., Strüssmann, C.A. and Takashima, F. 1997. Cloning and characterization of pejerrey mitochondrial DNA and its application for RFLP analysis. *Journal of Fish Biology* **51**: 193-203.