Aspects of the ecology and biology of the Lowveld largescale yellowfish (*Labeobarbus marequensis*, Smith, 1843) in the Luvuvhu River, Limpopo River System, South Africa.

by

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THESIS

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Abstract

Aspects of the ecology and biology of the Lowveld largescale yellowfish (*Labeobarbus marequensis*) were studied in the Luvuvhu River over a period of three years.

In this study the origin of the species, its phylogenetic relation to the other South African yellowfish species, its distribution and gross morphology are discussed and the lack of knowledge regarding aspects of the species is pointed out. The study area is identified and its geology, hydrology, climate and water quality as well as the historic distribution of the species is discussed.

Adapted "truss" techniques were applied to measure and calculate the morphometric features related to feeding and habitat preference of the species. From this data the habitat preferences and requirements were inferred after which it was compared to data obtained during field surveys. The study of the breeding biology and ecology of the species included investigations of gonad and egg structure and development as well as seasonal surveys of selected breeding sites. The diet of the species was established through stomach content analyses and related to the digestive tract morphology. Data obtained from the Xikundu fishway was used to establish the migratory behaviour.

Results show that nine distinct stanzas or growth phases, each with its own morphometric characteristics, were identified. The body form, and some morphological aspects, of the species make it suitable to cope with flowing water. Ontogenetic changes in body form and the identified morphological aspects were observed and related to the habitat preferences of the stanzas. A distinct ontogenetic shift in preferred habitat was illustrated. The species was shown to be fractional spawner with two spawning events per annum. A major extended spawning event occurred during spring or early summer and coincided with a temperature increase and in particular with an increase in flow. Breeding occurred at sites with fast flowing water over cobble or boulder beds and it was observed that the presence of nursery areas related to breeding biotopes was extremely important. Although the diet of the species was dominated by plant and algal matter, juvenile stanzas ingested large amounts of animal material. It was found that the spatial movements of the species could be characterised as migrations and that breeding and dispersal migrations occurred.

Opsomming

Aspekte van die ekologie en biologie van die Laeveldse grootskubgeelvis (*Labeobarbus marequensis*) is oor ’n tydperk van drie jaar in die Luvuvhurivier bestudeer.

As deel van die studie is die oorsprong, filogenetiese verwantskappe met ander Suid Afrikaanse geelvisspesies, verspreiding en die uitwendige kenmerke van die spesies bespreek. Die gebrek aan data in sekere aspekte word ook uitgewys. Die studiegebied word geidentifiseer en die gebied se geologie, hidrologie, klimaat, water kwaliteit asook historiese verspreiding van die spesies in die gebied bespreek.

Aangepasde meetings tegnieke is gebruik om die morfometries kenmerke wat verband hou met voeding en habitat te meet en te bereken waaruit die habitat voorkeure dan afgelei is. Tydens veldopnames is hierdie afleidings getoets. Die biologie en ekologie van die broei is bestudeer deur na gonade en eier ontwikkeling te kyk en ook seisonale opnames by moontlike broei habitatte te doen. Maaginhoudanalise is gebruik om dieet samestelling te bepaal en is met die morfologie van die dermkanaal vergelyk. Data van opnames wat by die Xikundu visleer gedoen is, is gebruik om vas te stel of die spesies migreer al dan nie.

Uitslae het gewys dat nege “stanzas” of groeifases, elk met sy besondere morfometriese kenmerke, in die spesies bestaan. Die liggaamstruktuur en ander morfologiese aspekte is kenmerkend van ’n spesie wat in vloeiende water voorkom. Die bestaan van ontogenetiese veranderinge in liggaamstruktuur en verwante aspekte is uitgewys en met habitat voorkeure verbind. Daar is gevind dat *L. marequensis* ’n veelvuldige en uitgebreide broei het en twee keer per jaar. Die hoof broei geleentheid het gedurende lente of vroeg somer plaasgevind en het saamgeval met ’n toename in temperatuur en vloei. Broei het in vinnig vloeiende water oor growwe substraat plaasgevind en aangrensende “versorgareas” is as belangrik beskou. Alhoewel die dieet oorheers is deur plant en alg materiaal het die kleinste “stanzas” groot hoeveelhede diere materiaal ingeneem. Omdat die beweging van die spesie aan vereistes voldoen is dit as migrasie geklassifiseer, en dan spesifiek as broei en verspreidings migrasie.

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Chapter 1: Introduction.

1.1 The rationale of the study.

Given the appropriate water quality, the distribution of fish in a river is primarily controlled by the flow regime and associated factors. These factors include timing of flow, velocity and depth. According to the microhabitat approach to riverine ecology the longitudinal changes in the community composition could be a function of variables which include mean depth, mean velocity, water quality or the other characteristics, such as substrate and cover that exhibit gradational changes (Fouché and Gaigher, 2001).

Although certain physical macro-habitat characteristics, such as temperature, define the tolerance limits for different species, fish do not respond directly to these characteristics, but rather to microhabitat characteristics (Wood and Bain, 1995). Size, shape, swimming performance, feeding strategy, predation and competition all combine to define the suitability of a species to a microhabitat as well as the limits of its tolerance. The microhabitat utilised also differs for the different life history stages and is often a reflection of the evolutionary history. Morphological differences, both at inter- and intra-specific levels, can therefore be construed as an adaptation to living in a particular type of microhabitat and can vary considerably. For example; species that occur in swift flowing, rocky-bottomed mountain streams require specific adaptations to resist turbulence. These adaptations are enlarged pectoral spines (*Amphilius* sp.) or suckers formed by a modified mouth (*Chiloglanis* sp.). Both genera further have an elongated, dorsally flattened body with a humped longitudinal profile and enlarged pectoral fins. All these adaptations serve as downward-raking hydroplanes that make these fish obligate rheophiles (Skelton, 2001).

Over and above studying microhabitat adaptation, attempts have been made, as early as 1980 (Gatz, 1981), to associate the morphology of a species with its niche. In these studies the niches were equated to the biological role of morphological features and niches and as a result permanent or semi-permanent morphological changes, or adaptations, occurred in species and probably in the life stages of a species.

Whereas the niche could, and does, lead to permanent morphological adaptations, certain temporary or seasonal changes, which are illustrated below, can be observed. The dynamics of condition, and to a large extent the fat storage content, are not only closely related to the linear growth of a fish (Nikolsky, 1963) but also reflect seasonal changes which in turn can be related to the age and sexual maturity of the fish. The traditional mode of expressing the condition of a fish is the use of a mathematical expression proposed by Fulton in Nikolsky (1963). It has been observed that an increase in the condition factor often precedes a spawning event as the fish starts storing energy for the event in the form of fat that is accumulated around the intestines (Shul'man, 1974). Thus, by determining the condition factor over a period of time, the spawning event of a species can be predicted.

Changes in microhabitat can lead to changes in fish assemblage composition. Some fish are more susceptible to adverse or negative changes and because of their inability to make drastic adaptational changes could vanish from the specific habitat when such changes occur. These species are described as "sensitive" or "intolerant" (Angliss, 1999; Kleynhans, 1991). Other species that are not affected by the changes or who have the ability to adapt could be regarded as less sensitive or tolerant and would persist in the changed environment.

Numerous attempts to develop indices of biotic integrity that employ the sensitivity or tolerance of fish and fish assemblages have been undertaken (Hocutt, 1981; Karr, 1981; Fausch *et al.*, 1984; Kleynhans, 1999; Gaigher and Fouché, 2000). A derivation, the Fish Assemblage Integrity Index (FAII), developed by Kleynhans (1999), is currently applied in South African rivers as an integral part of the National River Health Programme (RHP). These indices, and specifically the FAII, are strongly based on the intolerance of species. Kleynhans (op cit.) states that the factors used in the compilation of the intolerance ratings of fish are predominantly based on incidental observations and professional judgment and that experimental information (and therefore empirical data) is largely lacking. The same arguments would apply if a sensitivity, rather than intolerance rating, were used. Angliss (op cit.) adapted the sensitivity index produced by Kleynhans (1991) for the Limpopo Province by using the best possible information and personal experience. It is important to note that the decision making process of both Angliss (1999) and Kleynhans (1991 and 1999) would have been simplified if quantitative data, such as measured velocities, as well as experimental data had been available.

The argument concerning experimental or quantitative data, or the lack thereof, used above becomes even more relevant when Instream Flow Requirements or Ecological Reserves are determined. In these studies the habitat, which includes flow, velocity and water depth that fish require during various life stages, are important. The response of fish, or the stress experienced by fish, if conditions are not favourable is used to determine a stress index known as the Fish Response Assessment Index (FRAI) which is used to determine the minimum requirements for fish assemblages in different river reaches (Kleynhans, 2007).

Relatively little is known about the ecology, habitat preference, niche differentiation and the associated morphological adaptations of most of the indigenous fish of southern Africa. A literature survey of the situation in South Africa revealed that this lack of knowledge also applies to the South African cyprinids and to *Labeobarbus marequensis* in particular (Appendix I).

The Lowveld largescale yellowfish, *L. marequensis,* is a "successful" species in the selected geographical study area that consists of the Luvuvhu River and tributaries, because of its reported abundance and the fact that it apparently occurs in a wide range of habitats. Gaigher (1973) referred to it as an “unspecialized” species with a wide distribution that occurs in the Limpopo River system in pools and rapids of perennial streams at all altitudes. The species has been collected in most of the tributaries of the Luvuvhu River. Records of the South African Institute of Aquatic Biodiversity (SAIAB), collections done by Polling *et al.* (1983), data provided by the Limpopo Province directorate of Environmental Affairs and personal collections confirm these findings (Fouché, 2002; Fouché and Gaigher, 2001; Fouché *et al.*, 2005a; State of Rivers Report, 2001). Hecht (1982) found that because of its slow growth rate the species is considered to be of little commercial importance in aquaculture but he suggested that it be monitored and conserved for anglers in the future.

During recent years an initiative to promote the nine large “yellowfish” species (*L. marequensis, L. aeneus, L. kimberleyensis, L. polylepis, L. capensis, L. natalensis, Barbus serra, B. andrewi and B. matozzi*) as angling species in southern Africa has been launched by the Federation of Southern African Flyfishers (FOSAF). One of their aims is to use these magnificent fish to draw international tourists to the country.

It is important to realise that the outdoor sportsmen from the USA and Europe are often both hunters and fishermen, as opposed to their counterparts in South Africa who is usually one of the two. It was with this philosophy that the Yellowfish Working Group was established at a meeting in 1997. At the annual meetings since this first venture, much progress has been made to get the fly-fishing fraternity, conservation bodies, non-governmental-organizations and researchers around the same table. Research strategies, conservation priorities and fishing needs have led to many constructive discussions and projects. James (2002), who also enthusiastically promotes these fish as a “quarry” for fly fishers, however points out that most populations of yellowfish are declining due to reasons such as *inter alia* habitat degradation. Although not traditionally a "target" of the fly-fishing fraternity in South Africa, its dimensions (maximum size and length) and its general appearance could make it a worthwhile target species (http//: wwwfishingafrica.co.za).

Among the Venda speaking people the lowveld largescale yellowfish is known as “thanzwi” or “pupela” and it forms an important protein component of the diet of rural people. It is fished using different methods and for various reasons which include recreation and for food. Between 5 and 10% of the total mass of fish caught in the Mutshindudi River (van der Waal, 2000) are lowveld largescale yellowfish.

To develop the possibility of utilizing this fish as a possible fly-fishing candidate would however imply that the species should be aggressively promoted and conserved (James, 2002). In an effort to do this it is important to gather detailed (and quantified) information of its breeding ecology, feeding needs, niche differentiation, habitat preferences and other general habitat requirements. Although many experts have some ideas about these issues, very little detailed and scientifically substantiated information has been published in scientific journals. The tabularized summary in Appendix I show the related information published over the past 40 years. A closer inspection of these publications reveal that as far as habitat preference and trophic niche differentiation is concerned most of the deductions are based on personal observations and very little on empirical scientific data. As an example it is interesting to note that no publications dedicated to the habitat preferences or feeding habits could be found during the literature search. From the summary it is evident that no knowledge is available on juveniles. The areas where the lack of knowledge exists include the breeding ecology, the habitat preference, the feeding needs and the morphological adaptations.

With regard to the breeding ecology three definite periods, each with their own problems and the specific knowledge “shortfalls” can be identified. These periods occur prior to spawning, at the onset of spawning and during spawning. In these periods the knowledge shortfalls are the following:

a) In the period prior to spawning the habitat needs, gonadal development, changes in condition of the breeding fish and seasonal migration patterns should be investigated.
b) At the start of spawning little is known about changes in the environmental factors that might act as cues that trigger spawning.
c) During the spawning more knowledge should be gathered concerning the habitat needs for spawning, the microhabitat factors needed for breeding and the number of times they spawn per season.

Other aspects that need to be investigated include detailed knowledge of habitat preferences and needs through the developmental stages, the feeding needs of the different size classes and the morphological adaptations and changes in the morphometry of the different size classes.

All the abovementioned aspects could be transformed into research questions:

- Are the microhabitat preferences of the various life stages of *L. marequensis* similar?
- Do the various life stages of *L. marequensis* display morphological adaptations that can be related to habitat preference and trophic niche differentiation?

- If it does utilise different niches through body form and alimentary tract modifications, what is the nature of these niches?
- Does its niche change with an increase in size?

In reaction to these questions this study firstly hypothesised that the morphological adaptations of the various life stages of *L. marequensis* reflect their habitat preferences and niche differentiation. Furthermore it hypothesized that changes in the macro-and microhabitat characteristics, which can be of physical or chemical origin, initiate or trigger reproductive behaviour in the fish and that at the onset of the reproductive process these fish migrate to suitable microhabitats that will allow the spawning and developmental processes to take place.

In order to investigate this hypothesis the aims and objectives of this study were:

i) To establish the community structure or assemblage composition of fish at the sites where *L. marequensis* occurs.

ii) To determine the morphological characteristics of the various size classes of *L. marequensis* and to infer the habitat preferences and trophic niche differentiation from this data.

iii) To determine the habitat preference of the various size classes or life stages of *L. marequensis* through direct observations of the physico-chemical microhabitat characteristics and through inferences from morphometric characters.

iv) To determine the trophic niche differentiation of the various size classes or life stages of *L. marequensis* through direct observations of stomach contents and changes in intestinal morphology and dimensions.

v) To determine the environmental factors that trigger spawning behaviour in *L. marequensis.*

vi) To determine the preferred microhabitat and conditions required for spawning and the early developmental stages of *L. marequensis.*

vii) To determine the biological changes that precedes spawning and spawning behaviour.

viii) To determine whether *L. marequensis* migrates or whether the observed movement only occurs on a localized scale.

1.2 The structure of the thesis.

Chapter 1: Introduction.

This chapter presents the rationale for the study by identifying and formulating the relevant research questions based on an investigating of the existing knowledge of the species in question. From these questions a hypothesis is formulated and following on this the aims and objectives are set.

Chapter 2: The species: a survey of existing knowledge.

At the start of this chapter the origin of *L. marequensis* and its phylogenetic relation to the other South African yellowfish species is introduced. The distribution of the species and the general factors leading to this distribution is then discussed. The gross morphological characteristics are shown and include aspects such as live colouration and the variable mouth form. In conclusion the lack of knowledge regarded its habitat preference, feeding and growth is pointed out.

Chapter 3: The Study area and the historic distribution of the *Labeobarbus marequensis* in the area.

Aspects such as the general geology, hydrology, climate and water quality of the study area, the Luvuvhu River catchment, are discussed at the onset of this chapter. This is followed by an appraisal of the ecological status of the river, based on the outcome of River Health Programme (RHP) surveys, and a discussion of the fish diversity and the status of *L. marequensis* within the catchment.

Chapter 4: The morphology and morphometry of *Labeobarbus marequensis*.

This chapter is structured to address the second aim of the study namely to "determine the morphological characteristics of the various size classes of *L. marequensis* and to infer the habitat preferences and trophic niche differentiation from this data". In the chapter truss morphometric techniques are applied to measure and calculate the morphological features related to the feeding habits, food size and habitat preference of the species. Statistical verification methods include multivariate analyses of covariance and the establishment of significant differences between morphological features. In addition the length: mass relationship is analysed to establish if growth phases or stanzas could be identified. Ontogenetic changes and differences between the stanzas with regard to body form and morphometry is investigated to infer the habitat preferences of the stanzas.

Chapter 5: The population structure, ecology and habitat preference of *Labeobarbus marequensis* in the Luvuvhu River catchment.

This chapter addresses two aims of the study. These are i) to establish the community structure or assemblage composition of the fish at the sites where the species occur and ii) to determine the habitat preference of the various size classes or life stages of the species through direct observations of the physico-chemical microhabitat characteristics. The methods in the chapter include site selection and the identification, mapping and sampling of the microhabitat or biotope.

Chapter 6: The reproductive biology and ecology of the *Labeobarbus marequensis* in the Luvuvhu River catchment.

This chapter investigates the breeding biology of the species using traditional methods that *inter alia* include studies of the gonadal development, sizing of egg and egg counts. In addition the breeding ecology is studied through a seasonal investigation of selected breeding sites in the Luvuvhu River system.

Chapter 7: The feeding biology of *Labeobarbus marequensis* in the Luvuvhu River.

This chapter addresses the fourth aim of the study, namely "to determine the trophic niche differentiation of the various size classes or life stages of *L. marequensis".* The methods applied include stomach content analyses and an investigation of the intestinal morphology and dimensions.

Chapter 8: The movement and migration of *Labeobarbus marequensis* as observed in the Luvuvhu River.

In this chapter the aim is to investigate the movement of the species in order to establish if different forms of movement take place. The seasonality of the movement and diel patterns as well as the possible cues is investigated. In addition the physiological preparation prior to migrations is established. This research reported on in this chapter formed part of an ongoing project that was undertaken at the Xikundu fishway in the lower Luvuvhu River.

Chapter 9: Conclusion.

In this chapter the results of the project are reviewed and summarized. It also particularly establishes whether the aims and objectives set in chapter one has been achieved. The possible contributions of the findings and the shortcomings of the project are discussed.

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APPENDIX I: A summary of the available literature on *L. marequensis* at the onset of this study.

I) BREEDING

Sexual maturity	
Females	Males
1. Skelton ,2001 2. Gaigher,1969a	3. Weeks *et al.*,1996 4. Skelton , 2001 5. Gaigher, 1969a 6. Vlok, 1992
Breeding ecology	
Habitat requirements	Feeding requirements during breeding
1. Crass, 1964 2. Gaigher 1973 3. Bell-Cross & Minshull, 1988	

II) FEEDING

Feeding methods & adaptation to feeding	
Adults	Juveniles
1. Lauzanne & Gaigher, 1988	
Food	
Adults	Juveniles
1. Crass, 1964 2. Bell-Cross & Minshull, 1988 3. Jubb, 1961 4. Skelton, 2001	

III) MORPHOLOGY

Morphology and morphological adaptations	
Adults	Juveniles
1. Jubb, 1961 & 1967 2. Le Roux and Steyn, 1978 3. Crass, 1964	
Morphometrics	
Adults	Juveniles

IV) GENERAL HABITAT REQUIREMENTS

Adults	Juveniles
1. Weeks *et al.*, 1996 2. Gaigher, 1973 & 1998 3. Bell-Cross and Minshull, 1988 4. Jubb, 1967 5. Russell, 1997 6. Skelton, 2001	

V) DISTRIBUTION

1. Skelton *et al.*, 1995
2. Polling *et al.*, 1983
3. Hecht and Scholtz, 1981
4. Jubb,1961 , 1967
5. Crass, 1964
6. Bell-Cross and Minshull, 1988
7. Jackson, 1961
8. Skelton, 2001

VII) MIGRATION AND MOVEMENT

Adults	Juveniles
1. Jubb, 1961, 1967 2. Meyer, 1974 3. Bell-Cross and Minshull, 1988 4. Skelton , 2001 5. Fouché *et al.*, 2005b	

Chapter 2: The species *Labeobarbus marequensis*: a survey of existing knowledge.

It is generally accepted that the family Cyprinidae evolved in Asia during the Tertiary Era and then dispersed to Africa, Europe and North America with ancestral forms of the genera of this family reaching the southern tip of the African continent (Jubb, 1967). Being stenohyaline, or true freshwater forms, the dispersion could only be through the freshwater links associated with the evolution of the continent. Paleogeographic maps, in which the drainage basins at the end of the Tertiary Era are reconstructed, suggest that the area representing present-day South Africa was drained by distinct inland systems namely the Limpopo, Damara and Basutoland basins as well as by the shorter east and south flowing rivers in the coastal belt (Roberts, 1975).

The Limpopo basin was the only system with a link to the inland Okavango-Ngami sedentary basin. The fact that the current fish fauna of the Limpopo River shows an affinity with both the upper and lower Zambezi River fauna lends support to this suggested temporary link. The Damara basin, which consisted of the Orange River and tributaries, as well as the Basutoland basin, which contained the current Vaal River and its tributaries which later formed the present day Orange-Vaal River basin. The headwaters of the current South Coastal drainage basin are in close proximity with the tributaries of the Orange and shared species between the basins is evidence that at some stage links existed between the systems. The present day Limpopo River drains “a great deal” of the highveld of the current Gauteng and North-West provinces as well as the south-western portion of Zimbabwe. Its fish fauna not only contain remnants of the Zambezi fish fauna but is not homogenous throughout the river (Jubb, op cit.) with species closely related to the Orange-Vaal system in its southerly tributaries.

Until recently the species commonly regarded as the South African “yellowfish” all resorted under the huge genus *Barbus*, but taxonomists have for some time recognized that some of these species are distinct from the European “barbels” (Skelton, 2002). Oellerman and Skelton (1989) showed southern African “yellowfish” to be hexaploid, with around 150 chromosomes in comparison with the European barbel-group species that are tetraploid with around 100 chromosomes. Although the hexaploid lineage was first considered within the subgenus *Labeobarbus,* they were elevated to full generic status by Skelton (2001).

Currently the South African "yellowfish" consist of two genera namely *Barbus* and *Labeobarbus* represented by three species and six species respectively (Table 2.1). Whereas the representatives of hexaploid *Labeobarbus* have longitudinal or parallel striated scales and a spinous dorsal fin ray, the representatives of the genus *Barbus* are tetraploid with radialy striated scales and a serrated dorsal spine (Skelton, 2001).

Table 2.1: The Scientific and common names of the South African "yellowfish" (Adapted from Skelton 2002).

Species	**Author and date**	**Recommended common name**
Labeobarbus aeneus	Burchell 1822	Vaal-Orange smallmouth yellowfish
Labeobarbus capensis	Smith 1841	Clanwilliam yellowfish
Labeobarbus kimberleyensis	Gilchrist and Thompson 1913	Vaal-Orange largemouth yellowfish
Labeobarbus marequensis	Smith 1841	Lowveld large-scale yellowfish
Labeobarbus natalensis	Castelnau 1861	KwaZullu-Natal yellow fish
Labeobarbus polylepis	Boulenger 1907	Bushveld smallscale yellowfish
Barbus andrewi	Barnard 1937	Berg-Breede Whitefish
Barbus serra	Peters 1864	Clanwilliam sawfin
Barbus rapax authority	Guimaraes 1884	Bushveld papermouth

The Lowveld large-scale yellowfish, *Labeobarbus marequensis* (A. Smith, 1841), more commonly known as the large-scale yellowfish, is the common large-scaled yellowfish of the Limpopo and middle Zambezi systems (Jubb, 1967) and is widely distributed from the middle and lower Zambezi System in the north to the Phongolo system in the south (Figure 2.1). In South Africa, it commonly co-occurs with another true yellowfish, the Bushveld small-scale yellowfish *Labeobarbus polylepis* (Boulenger, 1907) and less frequently with a "yellowfish-type" species, the Bushveld papermouth, *Barbus rapax authority,* in southern Limpopo tributaries (Skelton, 2001).

According to Skelton *et al*. (1995) the distribution of organisms is governed by a complex combination of factors which *inter alia* include climate and topography. In freshwater fish the hydrographic and geomorphological history plays an equally important role. *L. marequensis* and *B. rapax authority* are part of the Zambezian fauna that occur in the

Zambezi River and its historically associated drainage basins. Contrastingly, *L. polylepis* forms part of the Southern Temperate fauna like the rest of the South African "yellowfish" species, all of which are endemic to southern Africa.

There is considerable morphological variation across this species' range which could be a major reason why so many previous workers have described the variants as separate species. Table 2.3 shows these synonyms and the areas or rivers where type specimens were collected. In South Africa, *L. marequensis* is most commonly found in the east flowing rivers (Le Roux and Steyn, 1978). Although Crass (1964) reported the Phongolo River as the most southern distribution, specimens were also collected in the Bloemveld Dam, in the Mfolozi system, near Vryheid in 1972 (Coke, pers. com.)[1]. These specimens are most likely alien and may be invasive as well.

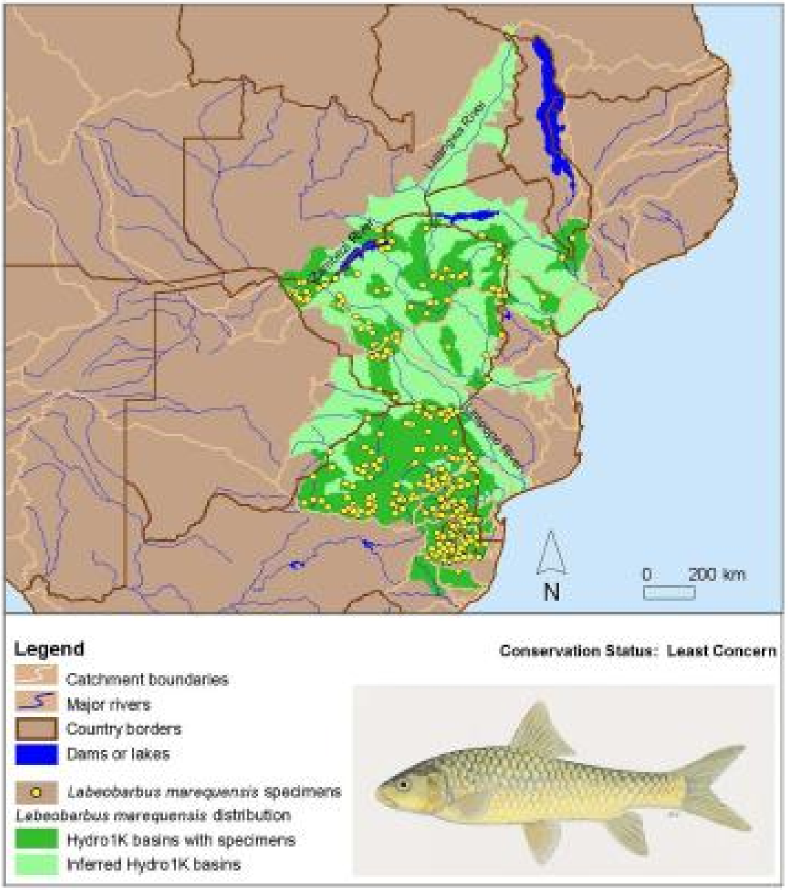

Figure 2.1: Distribution of *Labeobarbus marequensis* in southern Africa based on voucher records (Adapted from Scott *et al.*, 2004and Fouche, 2008).

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[1] Coke, M.D. Independent Freshwater Ecologist and Ichthyologist , Kwazulu-Natal, South Africa

Gaigher (1973) classified the species as eurytopic and eurythermal. The term eurytopic indicates that it occurs in a wide variety of habitats that range from pools and rapids. Being eurythermal indicates that the species can tolerate a wide range of temperatures which would enable them to survive in mountain streams and lowland rivers. The findings of Gaigher (1969a, 1969b and 1973) concerning the habitat preference of the species are summarized in Table 2.2**.** Crass (1964) reported the presence of the species in the Phongolo River up to an altitude of 1220m. Although Hecht and Scholtz (1981) did not collect the species at this altitude in the Steelpoort River, they reported its presence throughout the river at lower altitudes. In the Groot Letaba River, Chutter and Heath (1993) reported the species present in the escarpment as well as in middleveld and lowveld sections.

The conservation status of *L. marequensis* is classified as "Least Concern" as it is still relatively abundant and widespread throughout its natural distribution range (IUCN 2007).

Table 2.2: The influence of altitude on the distribution of *Labeobarbus marequensis* (Adapted from Gaigher 1969a, 1969b and 1973).

	Percentage frequency of occurrence		
	Perennial		Seasonal
m ASL	Rapids	Pools	Pools
0 - 305	100	55	0
306 - 610	95	50	10
611 - 915	78	70	30
916 – 1220	75	48	0
1221 and higher	38	30	0

The general body structure of the species reflects, like the other cyprinids, its strong swimming ability (Figure 2.2). The large scale-size of the species and the consequent scale counts of 27 to 33 in the lateral line and twelve around the caudal peduncle, is possibly the most outstanding used in identification of the species. *L. marequensis* and *L. codringtonii* are the only two yellowfish species in the genus with large scales (Skelton, 2001) that occur in southern Africa. In both species, the dorsal fin is situated in front of the pelvic fins, but in the case of *L. marequensis* the dorsal fin height is less than the length of the head. This dorsal fin height to head length ratio and four spines and eight to ten branched rays in the dorsal fin can also distinguishes the species from *L. codringtonii*. In addition the dorsal fin of *L. marequensis* is one aspect that shows a large degree of variation with Jubb (1967) as well as Bell-Cross and Minshull (1988) reporting an east-west and a north-south decrease in height. The dorsal fin height can even vary within a single population (Skelton, 2001).

The mouth is terminally positioned, with two pairs of barbels. The mouth form and the lips, as shown in figure 2.3, are extremely variable (Jubb 1961, 1967; Bell-Cross and Minshull 1988). Pienaar (1978) refers to the square mouth form with the chisel-shaped lower cutting jaw as *forma varicorhinus*, the thick and rubbery *forma gunningi* and the intermediate form

Table 2.3: A list of the "species" or synonyms *Labeobarbus marequensis* and localities historically described by various authors (Adapted from Fouché, 2008). (* spelling of words as by the original author).

Synonym	**Locality**	**Reference**
Barbus (Cheilobarbus) marequensis	Interior of South Africa	Smith 1841
Barbus brucii	Olifant River,Transvaal, South Africa	Boulenger 1907
Varicorhinus brucii,	Klein Olifant River, Transvaal, South Africa	Boulenger 1907
Barbus cookei,	Crocodile River, Transvaal, South Africa	Gilchrist & Thompson 1913
Barbus dwaarsensis	Dwaars River*, Transvaal, South Africa	Gilchrist & Thompson 1913
Barbus fairbairnii	Gorge below Victoria Falls, Zambezi River, Zambia/ Zimbabwe	Boulenger 1908
Barbus (Dangila) inermis	Lower Zambezi River, Mozambique	Peters 1852
Barbus gunningi	Thabina and Pienaars rivers and Six-mile Spruit, Transvaal, South Africa	Gilchrist & Thompson 1913
Varicorhinus nasutus	Gorge below Victoria Falls, Zambezi River. Zambia/Zimbabwe	Gilchrist & Thompson 1911
Barbus rhodesianus	Near Mazoë, Zimbabwe	Boulenger 1902
Barbus sabiensis	Sabie River, Malelane, Transvaal, South Africa	Gilchrist & Thompson 1913
Barbus sector	Groot Olifants River., Transvaal, South Africa	Boulenger 1907
Barbus swierstrae	Thabina, Dwars , Magalies , and Pienaars rivers, Transvaal, South Africa	Gilchrist & Thompson 1913
Barbus victoriae	Gorge below Victoria Falls, Zambezi River	Boulenger 1908
Labeobarbus zambezensis	Zambezi River at Tette, Mozambique	Peters 1852

Figure 2.2: The body structure of a sub-adult *Labeobarbus marequensis* (Fouché, 2008).

as *forma typica*. The variance in lip and mouth form and placement is further complicated by the ability of the species to change its lip form during its early life stages. According to Pienaar (1978), the significance of lip form is not clearly understood but has no diagnostic value.

Jackson (1961) was of the opinion that lip form is related to habitat and in particular feeding in various substrates where for example the chisel type mouth form allows a scraping action during feeding on food attached to a rocky substrate.

The live colouration of the fish also varies and adults in clear water are golden yellow (Bell-Cross and Minshull 1988; Skelton 2001) but pale olive in turbid water. Pienaar (1978) indicates that the adults can be silvery in more turbid waters but usually have a darker dorsal side and white belly. Juveniles are silvery with a characteristic dark spot on the caudal peduncle (Jubb 1967) and a distinct pigmentation pattern (Figure 2.4). Another distinctive characteristic is that tubercles develop on the top and side of head as well as on the anal fin when breeding occurs (Skelton 2001).

According to Pienaar (op cit.) and Bell-Cross and Minshull (op cit.), the Lowveld large-scale yellowfish is partial to deep, rocky pools where the current is swift and strong but is also found in sandy stretches and reed-fringed pools in both perennial and seasonal streams.

Figure 2.3: The variation in mouth form displayed by *Labeobarbus marequensis* with *forma varicorhinus* **shown in the upper two photographs and** *forma gunningi* **in the two lower photographs (Fouché, 2008).**

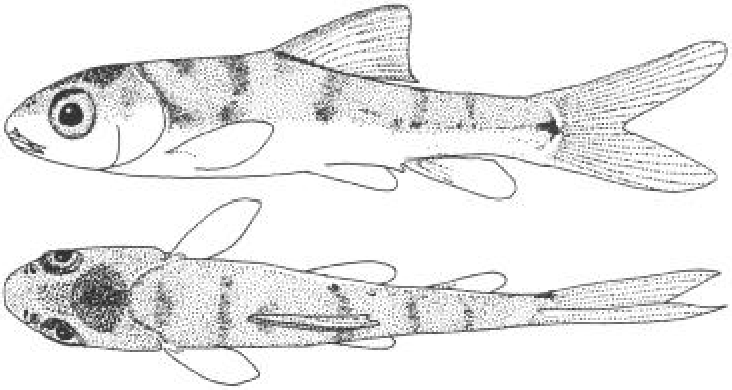

Figure 2.4: *Labeobarbus marequensis* juveniles showing the juvenile pigment pattern (Fouché, 2008).

Fouché *et al*. (2005), when comparing the Mutale and the Luvuvhu rivers, found that the species occurred in a third of the fast-flowing habitats surveyed in former and almost all these habitats in the latter river. In the Groot Letaba River, Chutter and Heath (1993) reported that their preferred habitat that consisted of pools, rapids and man-made impoundments. These observations are supported by the more detailed habitat-preference work done by Russell (1997) in the Kruger National Park who found that large-scale yellowfish prefer the swift-water seams between the rapids and stream margins, particularly where they have gravel and/or cobble beds. Although they favour sites with fringing aquatic vegetation they do not frequently occur in this vegetation. Gaigher (1973) *inter alia* referred to the species as "unspecialized with a wide distribution and dependent on rapids for breeding purposes". The fact that they are absent from the lower Limpopo because it is devoid of rapids, illustrates the breeding dependence.

Although the species is generally considered to be "unspecialized" (Gaigher, op cit.) and "non-sensitive" (Kleynhans, 1991), it is regarded as sensitive to reduction in flow rates and increased levels of siltation, especially at breeding sites (Fouché and Gaigher, 2001).

Because of its distribution and reported flow dependency, the species is often selected as an indicator species of rheophilic or semi-rheophilic habitats in the application of biomonitoring indices. There is however a lack of detailed empirical data of the physical parameters that form part of the habitat needs of the various life stages. The reported size of 280mm (Skelton, 2001) at which females mature also raises a concern. If the size limitations of catches indicate that fish larger than 200mm may be kept this would impact on the breeding females. Before this is implemented it should be established if females only reach maturity at that size; whether there are differences at various altitudes and under different conditions.

Migrations, most likely associated with spawning, reportedly occur during spring and summer (Crass 1964; Pienaar 1978; Skelton 2001). Bell-Cross and Minshull (1988) reported that adults migrated up swollen rivers to spawn in rapids between October and April in Zimbabwe. Other migrations, such as the recolonization of areas by juveniles, also take place but the seasonality of these movements are not well documented.

The distinct difference in the size at breeding reported is reason for concern and needs to be investigated. According to Crass (op cit.) males mature at a fork-length of approximately 70 mm while females mature at a length of 280mm (Skelton, op cit.).

Russell (1997) indicated that very little is known about the growth rate of the species. Göldner (1969) found that in Loskop Dam the fish attained a fork length of 110mm at the end of the first year and 150 – 160 mm at the end of the second year. As is the case with many fish species females grow faster, attain a greater length and on average become older (Gaigher, 1969a). The male to female ratio of 25:75 observed by Göldner (op cit.) underpins these findings. Gaigher (1969a) established that the size obtained by the fish could be related to their distribution and specifically altitude. He found that in areas higher than 610mASL, the dominant fork length was 160mm while at an altitude lower than 610mASL specimens of lengths up to 300mm were common. A similar situation was reported by Groenewald (1960) who found that 69, 8% of the specimens he collected were less than 170mm and only 4,2 % longer than 280mm. Russell (op cit.) found that in the Olifants River within the KNP the modes with the highest frequency within the length frequency distribution were with at 20–30, 101-120, 161-170 and 240 – 250mm respectively.

Various authors (Crass, 1964; Pienaar, 1978; Gaigher, 1979; Bell-Cross and Minshull, 1988 and Skelton 2001) regard the species as omnivorous and have indicated that the diet consisted of algae, plant detritus, larval and adult stages of aquatic insects, snails and even small fish. Fouché *et al*. (2005) found that algae, as was reported by Gaigher (1969a) and Skelton (op cit.), formed a more important component in the diet of the size groups smaller than 60 mm. Fouché and Gaigher (2001) found that the large-scale yellowfish they collected had a relative gut length that was 2,24 times longer than the fork length, an aspect that is typical of herbivores.

Although some very important aspects of the ecology and biology of species are addressed in the in the preceding text, it is clear that to a large extent detailed knowledge of the species is still lacking (Appendix 1). This gap in the knowledge in particular exists with regard to *inter alia* the adaptive morphology habitat preference and trophic status of the various age groups, or size classes, of the species. In the chapters that follow an attempt is made to address this lack of knowledge.

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Chapter 3: The study area and the historic distribution of the *Labeobarbus marequensis* within the study area.

3.1 The Luvuvhu River System.

The study area comprises the Luvuvhu River catchment. The Luvuvhu catchment forms part of the larger Limpopo River system with the Luvuvhu River a major tributary The catchment area of the Luvuvhu River is 5941 km^2 (State of the Rivers Report, 2001) and is elongated in shape extending for 250 km from approximately 20km east of Louis Trichardt in a north-east direction to Pafuri in the Kruger National Park (KNP). The catchment is bisected along its longer east-west axis by the Soutpansberg mountain range and the majority of the tributaries originate on its southern slopes. The main tributaries on these slopes are the Latonyanda, Dzindi and Mutshindudi rivers. The only tributary on the northern slopes is the Mutale River that feeds into the main stem of the Luvuvhu River within the KNP (Figure 3.1).

The drainage pattern is strongly influenced by the geomorphology of the area and in particular by the Soutpansberg mountain range. Only a very small portion of the catchment along the watershed between the towns of Louis Trichardt and Thohoyandou, receives a mean annual precipitation (MAP) in excess of 1200mm. Most of the remainder of the catchment receives between 500 and 1000mm per year (Pullen in DWAF, 1997) and the headwaters of the Latonyanda, Barotta, Dzondo, Dzindi and Mutshindudi rivers lies within this area. These tributaries contribute 73% of the natural run-off that enters the river. In the very arid areas that exist in the extreme east of the catchment and around the Doringspruit in the west, rainfall is less than 500mm per annum and therefore the resulting tributaries are non-perennial.

The general gradient of the river upstream of the Albasini Dam and its main tributaries are very steep while the main river below the Albasini Dam to the Limpopo River is less steep with a decrease in altitude of 2,25m/km on average. The only exceptions in this lower part of the river is a very steep section that occurs immediately after the river has entered the Kruger National Park and the "flat" reach in the last section of the river before the confluence with the Limpopo River.

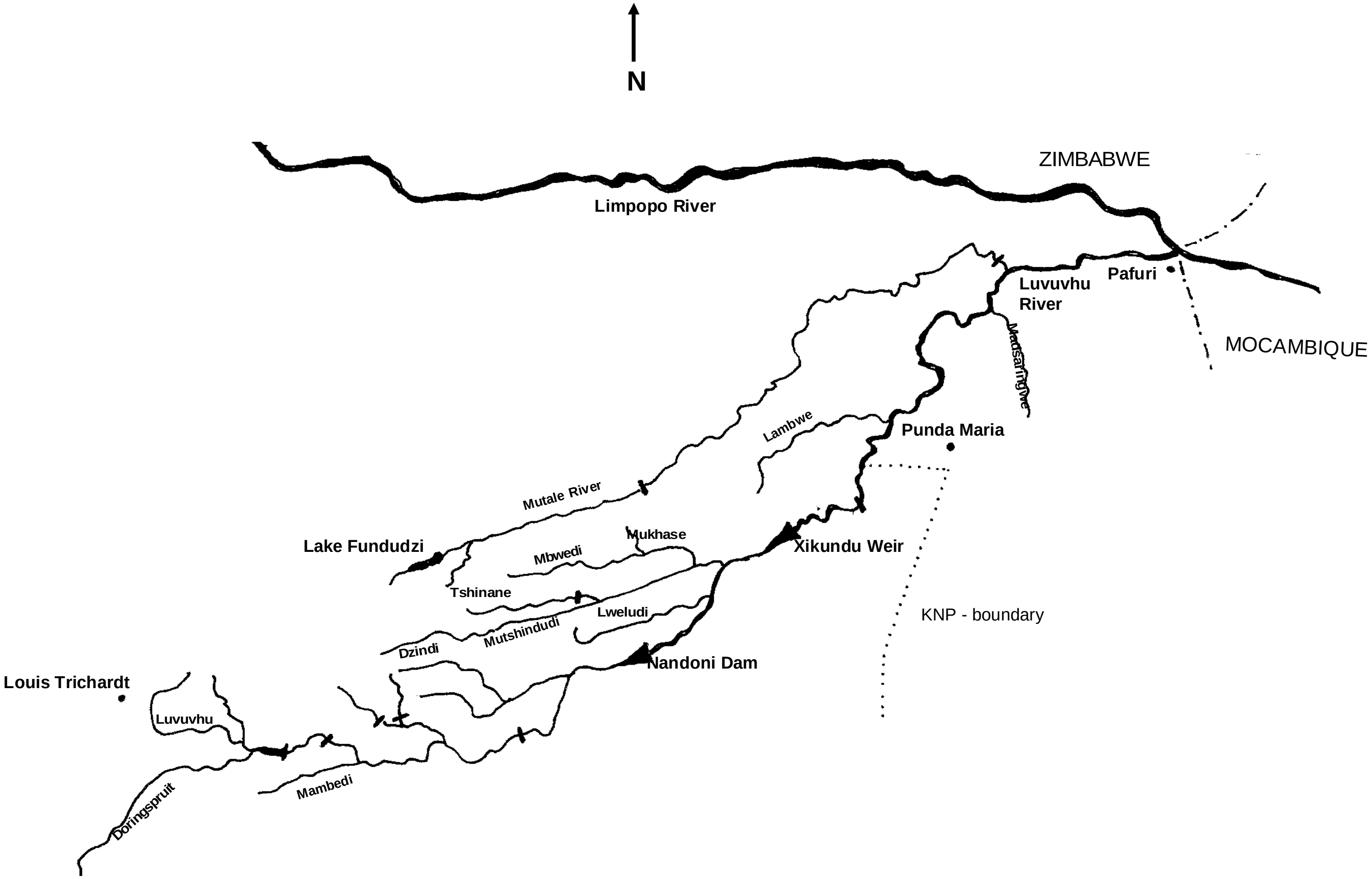

Figure 3.1: Sketch map of the Luvuvhu River and its major tributaries. (Adapted from DWAF, 1997).

The Luvuvhu River is a geomorphologically diverse system with a variety of alluvial and bedrock channel types occurring along its length. The upper Luvuvhu, Latonyanda, Dzindi, Mukhase, Mbwedi and Mutshindudi rivers are steep and narrow and dominated by cobble riffles and occasional pools with a few bedrock rapids. The lower reaches of the Mutshindudi and Luvuvhu Rivers, up to the Nandoni Dam are diverse and rapids, riffles and pools occur. Downstream of the Nandoni Dam, where the gradient declines dramatically, rapids are rare and the pools have sand and mud substrates.

The fact that the river and its tributaries flow through five level two eco-regions that form part of the Lowveld, Central Highlands and Limpopo Plain adds to the diversity of the river (Figure 3.2). The river and its tributaries are highly fragmented with six dams and ten gauging weirs. It should be noted that only the weir at Xikundu has a fishway.

O'Keeffe *et al.* (in DWAF, 1997) stated that in general the water quality could be regarded as "good" with conductivity that range from 13 – 16 mSm. They found the determinants of water quality to be dominated by total alkalinity as well as by the sodium, chloride and to some extent calcium concentrations and that the concentrations of all ions were fairly uniform along the length of the river. The nutrient concentrations were found by them to be similarly low with the same longitudinal tendency as for the ions. However in table 3.1 it is illustrated that increases in dissolved solids, sodium and alkalinity occurred downstream.

Table 3.1: Physico-chemic aspects recorded in the Luvuvhu River (Adapted from DWAF, 1990).

	Middle reach	Lower reach	Mutale River
pH (range)	5.9-7.8	6.0 – 8.2	5.1 – 8.4
TDS (maximum) mg/l	90	167	272
Sodium (maximum) mg/l	11.5	16.5	25.2
Total alkalinity (maximum) mg/l	61	83	113

Prior to 1910 human impact on the river was limited with no exotic afforestation, irrigation or dam construction. Afforestation development commenced in the upper basin area in 1911 and extended to the areas around Thohoyandou in 1950. The first dam built, the Albasini Dam,

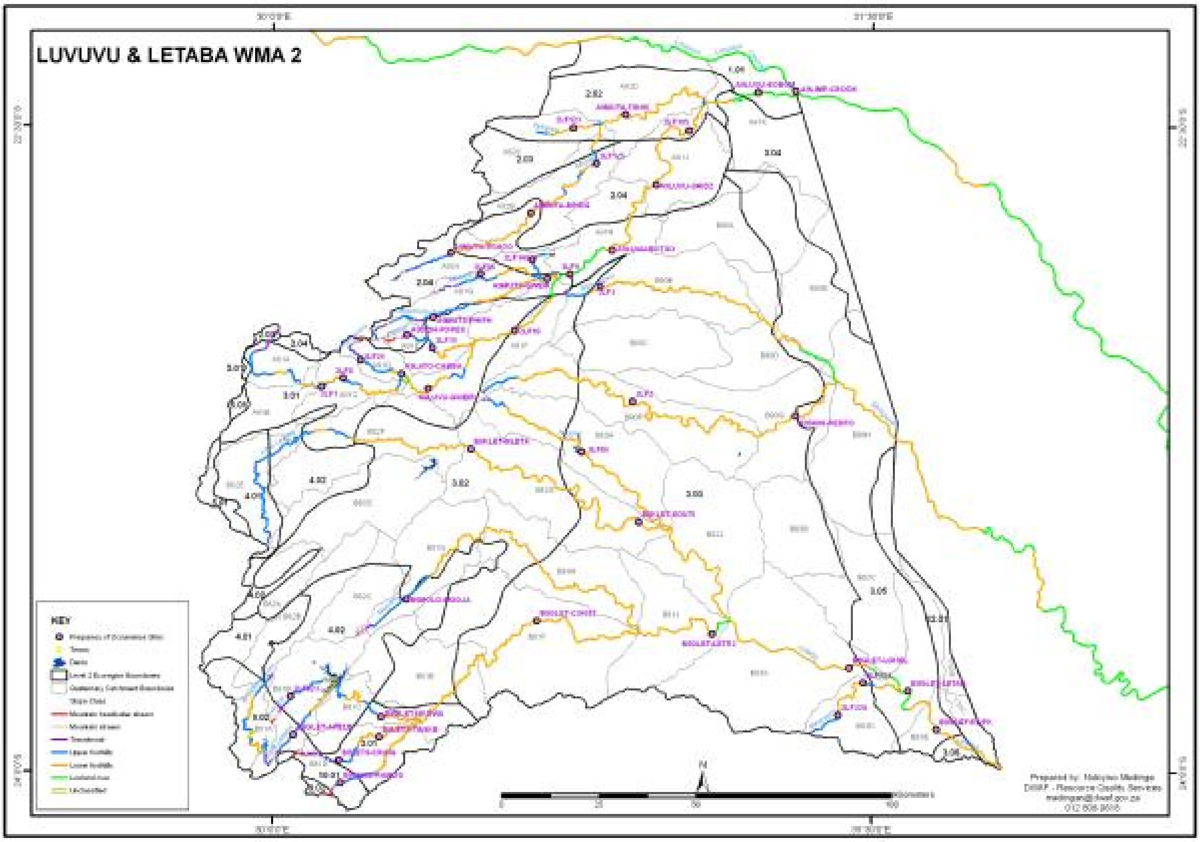

Figure 3.2: Level 2 Ecoregions in the Luvuvhu-Letaba Water Management Area (Adapted from Kleynhans *et al*., 2008)

was completed in 1952 and the amount of water used for irrigation, previously drawn from run-of-river weirs, increased dramatically (DWAF, 1990).

A change in run-off in the middle reach has been recorded for the ninety year period from 1900 to 1990, with run-off decreasing from ca. 389 million m^3 per annum to 308 million m^3 per annum in (DWAF, 1990). The same trend was observed in the Mutshindudi River with run-off decreasing from more than 128 million m^3 per annum to 104 million m^3 per annum. Historically the Luvuvhu River used to flow continuously but in recent times have ceased to flow for periods during the year. The first record of flow cessation was in 1946 (Moore *et al.*, 1991). These episodes are cause for concern because of the increase in salt and nutrient concentrations that could reach unacceptable levels for riverine biota. This situation is often observed in the KNP when there is no or little flow (State of the Rivers Report, 2001). Although the Luvuvhu River has always been a river with high silt loads, the possible increased levels of sedimentation, caused by badly planned farming practices, is however a reason for concern.

The flood of February 2000 was an historic event that deserves a special mention when the Luvuvhu River is discussed. This event not only reshaped the structure of the river but also affected the biota through extreme habitat changes (Fouché, 2002). This will be discussed later in this report.

3.2 The ecological status of the river.

When the data from the Luvuvhu-Letaba State of the Rivers Reports (State of the Rivers Report, 2001) which was an outcome of the National River Health Programme (RHP), is studied, the Fish Assemblage Integrity Index (FAII) scores can be regarded as a reflection of the status of the instream habitat. Table 3.2 shows that in the Luvuvhu River none of the twenty-four reaches surveyed were in a “natural” state. Being classified as natural would infer to a situation where no modification has occurred. On the other hand seven, or 29 percent, of the reaches were classified as “good”. This classification reflects that the biodiversity was largely intact. The Luvuvhu River was however in a better state than the Letaba River (State of the Rivers Report, 2001) where again no reach was classified as “natural” and only 17 percent was regarded as “good”. To expand on these results the FAII scores obtained at the majority of the sites are shown in Table 3.3.

In the same study (State of the Rivers Report, 2001) the state of the riparian vegetation, which directly impacts on the instream habitat, was also surveyed. Each site surveyed was classified into an Ecological Reserve Class, ranging from A (“unmodified”) to F (“critically modified”). The results obtained reflect a situation similar to the situation of the instream habitat with no sites in a natural state (class A) and only a few sites classified as “good” or ecological class B. The results obtained in the main stem of the Luvuvhu River and two of the larger tributaries are shown in figures 3.3 to 3.5.

Figure 3.2 shows the level 2 ecoregions through which the Luvuvhu River flows. The Latonyanda and Luvuvhu rivers originate in ecoregion 5.04, which consists of typical Sour Lowveld Bushveld and patches of Afromontane forest. Downstream of the Nandoni IFR site the main stem of the Luvuvhu River flows through ecoregion 5.03 and then back into ecoregion 5.04. Upon entry into the Kruger National Park, it first flows through ecoregion 2.01, then into 1.02 and finally 1.01. The Mutshindudi River is the only river that flows in one ecoregion, namely 2.01, for its whole length. Except for fact that it originates in ecoregion 2.01, the Mutale River follows the same pattern as the main stem of the Luvuvhu River. In the RHP study (State of the Rivers Report, 2001) the natural riparian vegetation observed and recorded at the sites corresponds with the vegetation expected in each of the ecoregions.

Table 3.2: The status of the reaches as reflected by the FAII scores in rivers surveyed as part of the RHP survey in 1999 (State of the Rivers Report, 2001).

	Percentage of reaches in the health categories.			
Rivers	**Natural**	**Good**	**Fair**	**Poor**
Luvuvhu (2001)	0	29	42	29
Letaba (2001)	0	17	22	61

Table 3.3: The river health categories of the individual sites surveyed in the Luvuvhu River. The category selection was based on the scores obtained with the Fish Assemblage Integrity Index (FAII) scores obtained in the 1999 RHP survey (Adapted from the State of Rivers Report, 2001).

River/Tributary	Ecoregion	Site Name	Coordinates	River health category
Dzindi	2.01	Below water-fall	S 22° 59.05' E 30° 20.05'	Poor
	5.04	Crocodile Ventures	S 23° 09.38' E 30° 28.41'	Fair
Latonyanda	5.04	Botha's Farm Bridge	S 22° 03.07' E 30° 14.07'	Poor
	5.04	Cabbage Farm IFR	S 22° 03.07' E 30° 19.07'	Poor
Sterkstroom	5.04	Joubert se plaas	S 23° 04.08' E 30° 04.03'	Good
Luvuvhu	5.04	Beja Bridge	S 23° 05.51' E 30° 04.08'	Poor
	5.04	Valdezia Weir	S 23° 05.10' E 30° 10.28'	Fair
	5.04	Roberts Farm	S 23° 06.18' E 30° 20.45'	Fair
	5.04	Gauging Weir A9h001	S 23° 06.26' E 30° 23.26'	Fair
	5.03	Hasani Crossing	S 23° 05.04' E 30° 28.16'	Fair
	5.03	Nandoni IFR site	S 22° 58.25' E 30° 36.10'	Good
	5.03	Tshifudi Bridge	S 22° 50.57' E 30° 45.09'	Good
	5.03	Botsoleni	S 22° 47.25' E 30° 50.91'	Good
	5.03	Mhinga pump station	S 22° 45.18' E 30° 53.35'	Good
	5.03	Lambani	S 22° 44.19' E 30° 52.93'	Good
	2.01	Dongodziva	S 22° 47.25' E 30° 50.91'	Good
	2.01	Shidzivani IFR SITE 1	S 23° 40.00' E 30° 57.05'	Good
	2.01	Madzaringwa	S 22° 29.90' E 31° 03.57'	Good
	1.02	Mutale Bend	S 22° 26.67' E 31° 04.56'	Good
	1.02	Mangala IFR SITE 2	S 22° 25.62' E 31° 10.47'	Good
	1.01	Bobomene camp	S 22° 25.05' E 31° 12.51'	Good
	1.01	Crooks corner	S 22° 25.0' E 31° 18.05'	Good
Mbwedi	2.01	Bridge at Mutshindudi confluence	S 22° 50.09' E 30° 39.43'	Fair
	2.01	Damani dam	S 22° 52.58' E 30° 31.1'	Poor
Mukhase	5.04	Mphaphuli cycad reserve	S 22° 48.62' E 30° 38.87'	Fair
Mutshindudi	2.01	Phiphidi	S 22° 56.06' E 30° 24.00'	Fair
	2.01	Hydroscheme	S 22° 56.21' E 30° 24.04'	Fair
	2.01	Tshivhulani	S 22° 54.54' E 30° 29.18'	Fair
	2.01	School turn	S 22° 53.17' E 30° 35.21'	Fair
	2.01	Malavhuwe bridge	S 22° 51.40' E 30° 38.37'	Fair
	2.01	New gauging weir	S 23° 09.38' E 30° 28.41'	Fair
Tshiombedi	5.04	Tshiombedi pools	S 22° 35.16' E 30° 48.32'	Poor
Saambandou	2.01	Bridge above Mutale confluence	S 22° 43.03' E 30° 39.03'	Good
Mutale	2.01	Tshirovha confluence	S 22° 48.55' E 30° 23.47'	Good
	5.04	Roadside	S 22° 48.51' E 30° 25.86'	Good
	5.04	School site	S 22° 47.34' E 30° 26.56'	Fair
	5.04	Below Saambandou	S 22° 42.04' E 30° 38.34'	Good
	2.01	Tshikundamalemma	S 22° 40.28' E 30° 42.09'	Good
	2.01	Guyuni	S 22° 35.16' E 30° 48.32'	Good
	1.02	Tshikondeni bridge	S 22° 28.44' E 30° 52.83'	Poor

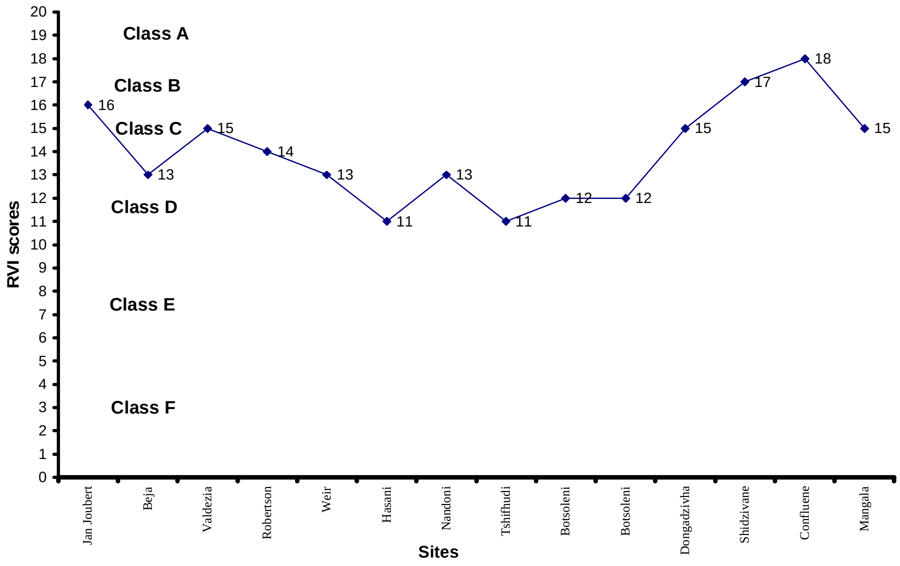

Figure 3.3: The ecological state of the riparian vegetation of the Luvuvhu River (Adapted from Fouché, 2000).

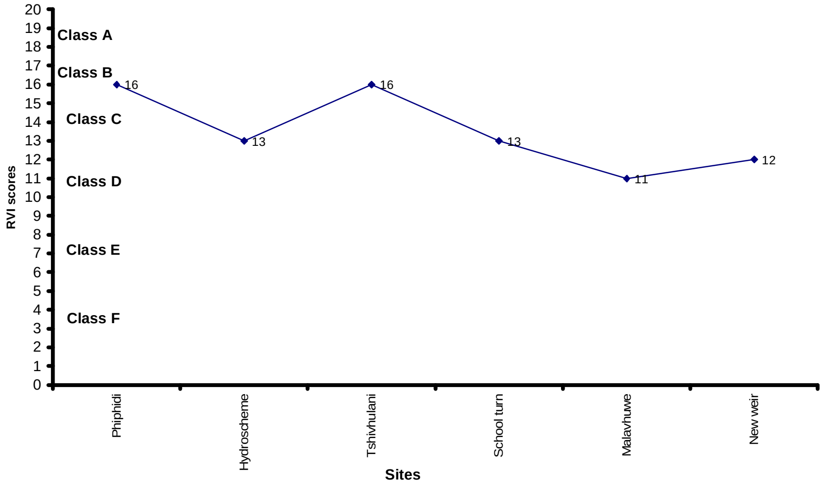

Figure 3.4: The ecological state of the riparian vegetation of the Mutshindudi River (Adapted from Fouché, 2000).

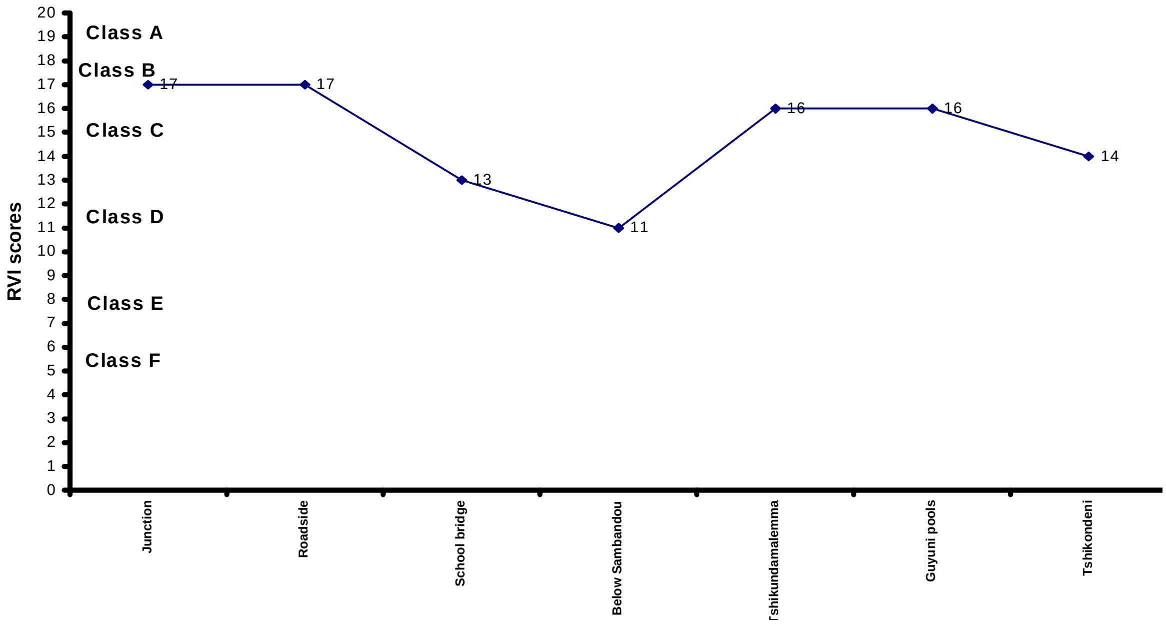

Figure 3.5: The ecological state of the riparian vegetation of the Mutale River (Adapted from Fouché, 2000).

Figures 3.3 to 3.5 show that with regard to their riparian vegetation the three rivers were within class B or C ("moderately modified") in the headwaters with a general downstream decline observed. As expected in the case of the main stem Luvuvhu River this decline was halted after entering the boundaries of the Kruger National Park.

3.2 Fish diversity and the status of *L. marequensis.*

Fouché and Gaigher (in Berger *et al*., 2003) listed forty four freshwater fish species, belonging to twelve families that occur in rivers related to the Soutpansberg mountain range that feed into the Limpopo River system (Table 3.4). Engelbrecht *et al.* (in DWAF, 1997) however pointed out that only thirty eight of these species could be expected in the Luvuvhu River and listed the species that were collected during surveys after 1990 (Table 3.5). Reports by Angliss (1999) and later work done by Fouché (2002) confirmed these species and indicated that *Barbus lineomaculatus* and *B. neefi* also occur in the Luvuvhu River system and should be added to the list.

Table 3.4: Fish diversity of the "Soutpansberg" (Adapted from Fouché and Gaigher in Berger *et al*., 2003).

Family	Species
Mormyridae	*Petrocephalus wesselsi, Marcusenius macrolepidotus*
Characidae	*Hydrocynus vittatus, Brycinus imberi, Micralestes acutidens*
Cyprinidae	*Labeobarbus marequensis, Barbus mattozzi, B. trimaculatus, B. paludinosus, B. afrohamiltoni,B. eutaenia, B. unitaeniatus,B. lineomaculatus, B. neefi, B. viviparus, B. annectens, B. toppini, B. radiatus, L. congoro, L. cylindricus, L. rosae, L. ruddi, L. molybdinus, Opsaridium peringueyi, Mesobola brevianalis*
Schilbeidae	*Schilbe intermedius*
Amphiliidae	*Amphilius uranoscopus*
Clariidae	*Clarias gariepinus, C. theodorae*
Mochokidae	*Synodontis zambezensis, Chiloglanis pretoriae, C. paratus* *C. engiops*
Cyprinodontidae	*Aplocheilichthys johnstoni*
Cichlidae	*Oreochromis mossambicus, Tilapia sparrmanii, T. rendalli* *Pseudocrenilabrus philander*
Gobiidae	*Glossogobius guiris, Awaous aeneofuscus*
Anguillidae	*Anguilla mossambica,, A. mormorata*
Aplocheilidae	*Notobranchius orthonotus, N. furzeri*

The "fish component" of the River Health Programme (RHP) survey of the Luvuvhu River represents what can be regarded as the most recent comprehensive survey of the river in its totality (State of the Rivers Report, 2001). During this survey all the available habitat at fourty sites (Tables 3.6 and 3.7) in the river was surveyed, using seine nets, electro-fishing and cast nets. With the exception of the site in the Sterkstroom, the Crooks corner site in the Luvuvhu River main stem, the Damani Dam site in the Mbwedi River and the site in the Mukhase River (Figure 3.6) specimens of *L. marequensis* were present at all the sites. Tables 3.6 and 3.7 show the fish diversity at the sites where *L. marequensis* occurred. Figure 3.7 shows a typical site where *L. marequensis* occur.

3.3 The effect of disturbance on the river.

During February 2000 the river was subjected to heavy flooding caused by exceptional high rainfall in the catchment. The high water levels led to high levels of impact and consequent changes to the river. In figures 3.8 and 3.9, which is of the "Gauging weir – A9H001 site" in the Luvuvhu River, the effect of these floods on both the riparian and instream habitat is illustrated. The arrows in the two figures point to a bedrock outcrop. This outcrop was unaffected by the energy of the flood and served as a fixed point marker.

Table 3.5: Scientific, English common names and abbreviations of the indigenous fish species expected in the Luvuvhu River (Adapted from Engelbrecht *et al*. in DWAF, 1997).

Scientific name	English common name	Abbre-viation	Expec-ted	**Collected after 1990**
Amphilius uranoscopus	Mountain catfish	AURA	+	+
Anguilla marmorata	Gaint mottled eel	AMOR	+	
Anguilla mossambica	Longfin eel	AMOS	+	+
Aplocheilichthys johnstoni	Johnston's topminnow	AJOH	+	
Awaous aeneofuscus	Freshwater goby	AAEN	+	
Barbus afrohamiltoni	Plump barb	BAFR	+	+
Barbus annectens	Broadstriped barb	BANN	+	+
Barbus eutaenia	Orangefin barb	BEUT	+	+
Barbus paludinosus	Straightfin barb	BPAU	+	+
Barbus radiatus	Beira barb	BRAD	+	+
Barbus toppini	East coast barb	BTOP	+	+
Barbus trimaculatus	Threespot barb	BTRI	+	+
Barbus unitaeniatus	Longbeard barb	BUNI	+	+
Barbus viviparus	Bowstripe barb	BVIV	+	+
Brycinus imberi	Imberi	BIMB	+	+
Chiloglanis engiops	Lowveld suckermouth	CENG	+	+
Chiloglanis paratus	Sawfin suckermouth	CPAR	+	+
Chiloglanis pretoriae	Shortspine suckermouth	CPRE	+	+
Clarias gariepinus	Sharptooth catfish	CGAR	+	+
Glossogobius giuris	Tank goby	GGUI	+	+
Hydrocynus vittatus	Tigerfish	HVIT	+	+
Labeobarbus marequensis	Lowveld largescale yellowfish	LMAR	+	+
Labeo congoro	Purple labeo	LCON	+	+
Labeo cylindricus	Redeye labeo	LCYL	+	+
Labeo molybdinus	Leaden labeo	LMOL	+	+
Labeo rosae	Rednose labeo	LROS	+	+
Labeo ruddi	Silver labeo	LRUD	+	
Mesobola brevianalis	River sardine	MBRE	+	+
Marcusenius macrolepidotus	Bulldog	MMAC	+	+
Micralestes acutidens	Silver robber	MACU	+	+
Opsaridium peringueyi	Southern barred minnow	OPER	+	+
Oreochromis mossambicus	Mozambique tilapia	OMOS	+	+
Petrocephalus wesselsi	Churchill	PWES	+	+
Pseudocrenilabrus philander	Southern mouth brooder	PPHI	+	+
Schilbe intermedius	Silver catfish	SINT	+	+
Synodontis zambezensis	Brown squeaker	SZAM	+	+
Tilapia rendalli	Redbreast tilapia	TREN	+	+
Tilapia sparrmanii	Banded tilapia	TSPAR	+	+

Figure 3.6: A typical “mountain steam” site in the Mutale River.

Figure 3.7: A typical lower foothill site in the Mutshindudi River.

Table 3.6: Fish diversity at the sites in the Dzindi, Latonayanda and Luvuvhu rivers observed in the 1999 RHP survey (Adapted from the State of Rivers Report, 2001) (For the abbreviations used for species names see table 3.5).

River	**Dzindi**		**Latonyanda**		**Luvuvhu**															
Site name	Below water-fall	Crocodile Ventures	Botha's Farm Bridge	Cabbage Farm IFR site	Beja Bridge	Valdezia Weir	Roberts Farm	Gauging Weir A9h001	Hasani Crossing	Nando-ni IFR site	Tshifudi Bridge	Botso-leni	Mhinga pump station	Lamba-ni	Dongo-dziva	Shidziva-ni IFR SITE 1	Madza-ringwa	Mutale Bend	Mangala IFR SITE 2	Bobo-mene camp
AMOS											2	1					1			
AURA	11		7	4	2		4						1	1						
BAFR																4	7	1		1
BANN												2								
BEUT		3		1		5	1													
BIMB									2							3	5	1	9	33
BLIN				1	1															
BNEE	38		6	1		3														
BPAU	21		1		100		1			2										
BRAD																8	11	10		
BTOP										2										
BTRI				1	6		2		39	1		12								
BUNI					8				1	2		1								
BVIV									4	8		36	12	42	59	13	17	17	1	
CGAR												8		3	1		2	3		5
CPAR											3	1					3	7	23	3
CPRE	7	58		11			117	40	103	11	99	164	200	28	75	22	2	18		10
CENG										4	2	3								
GGUI																	1		5	1
HVIT																			6	2
LCON																2				4
LCYL			1				1	6					3	3	7		6			26
LMAR	29	5	30	4	5	18	14	6	21	6	8	43	15	14	43	33	4	66	8	2
LROS																			12	11
LMOL				5	3		14	4	11	2	16	24	82	2	30	17	15	10		31
MACU								150			12	3	2		1	14	11	4	1	5
MBRE									3		2	1				8				
MMAC									1						1					2
OMOS					4	2			6	28				2		3		10	7	2
ONIL																			3	1
OPER	5																			
PWES									4					1			3			
PPHI					12	17			34	10		3								
SINT																	2			
TREN					10				27					5		16	1	6	6	3
TSPA					33	1		2	5			4								

Table 3.7: Fish diversity at the sites in the Mutshindudi , Sambandou and Mutale rivers observed in the 1999 RHP survey

(Adapted from the State of Rivers Report, 2001) (For the abbreviations used for species names see table 3.5).

River	**Mutshindudi**							**Saambandou**	**Mutale**						
Site name	Bridge above Mutsh. Confluence	Phiphidi	Hydro scheme	Tshivhu-lani	School turn	Malavu-he	New gauging weir	Bridge above Mutale confluence	Tshirovha confluence	Roadside	School site	Below Sambandou	Tshikun-damalema	Guyuni	Tshikondeni Bridge
AMOS	1		1				2						2	1	
AURA	3	16	21	3			1	2	4	5	7				
BAFR															
BANN															
BEUT	3	8	20					18		2		3	10		
BIMB															
BLIN			1					3							
BNEE			10	19				17	20	7	1	9	1		
BPAU			3							6		1			
BRAD						2		18				5	7		
BTOP															
BTRI					2	3	11	21		28	1	6			
BUNI							3								
BVIV	13					5	15	38				11	5	11	2
CGAR	1		1										3		
CPAR							6								
CPRE	36	11	83	71	88	18	43	4	5	6	50	47	98	15	78
CENG															
GGUI															
HVIT															
LCON															
LCYL							1	8	1	3	6	3	3	2	
LMAR	2	18	3	30	43	20	14	2	4	8	7	3	8	6	9
LROS	1													1	
LMOL	10		2	1	15	18	40	3			3	6	17	6	7
MACU	48				23	3	50	16		12					1
MBRE	12					1	49								
MMAC	1							20				1	8		
OMOS					4									23	
ONIL															
OPER	3				2	1			6	1					
PCAT	1					1	2	10				1	14		
PPHI							2							1	
SINT															
TREN	8														
TSPA								3		7		5			

As part of a project to determine the ecological status of the upper reaches of the Luvuvhu River (Fouché, 2002) the site was surveyed in November 1999 and again at three occasions in 2000 and 2001 (Table 3.8). These results show the effect of the flood on particularly the flow dependent species, ***Amphilius uranoscopus, L. marequensis, Labeo cylindricus*** and ***L. molybdinus***, that were absent from the sites in the June 2000 survey. It should be noted that ***L. marequensis*** was still absent from all the sites in the October 2000 survey and specimens were only collected once again in the January 2001 survey.

3.3 Conclusion.

Although not "natural" any more, approximately sixty percent the Luvuvhu River was found to be in "fair" to "good" condition during the 1999 RHP survey (State of the Rivers Report, 2001). It was in a better condition than its sister river, the Groot Letaba River, that also forms part of the same Water Management Area. As would be expected the "upstream" reaches were in a better condition than the downstream sites. The decline can be ascribed to the increase in anthropogenic impacts along the length of the river. The only exception in decline was in the Kruger National Park. The effect of disruptive impacts such as floods, and the subsequent "repair" was clearly illustrated at some of the sites. Despite all of the above, the river still has a high fish biodiversity with ***L. marequensis*** present at the majority of the sites.

Figure 3.8: The appearance of the instream and riparian habitat at the "Gauging weir A9H001" site in the Luvuvhu River during the 1999 RHP survey. The arrow indicates a bedrock outcrop that served as a landmark (Adapted from Fouché, 2002).

Figure 3.9: The appearance of the instream and riparian habitat at the "Gauging weir A9H001" site in the Luvuvhu River in March 2000 after the February 2000 flood. The landmark, also observed in figure 3.5 is indicated by the arrow (Adapted from Fouché, 2002).

Table 3.8: Changes in species assemblage observed at four sites in the Luvuvhu River before and after the February 2000 floods (Adapted from Fouché, 2002). (The abbreviations for the species correspond with table 3.5)

	1999 RHP Survey				June 2000 Survey				October 2000 Survey				January 2001 Survey			
	Crocodile Ventures	Gauging weir A9H001	Hasani crossing	Nandoni IFR	Crocodile Ventures	Gauging weir A9H001	Hasani crossing	Nandoni IFR	Crocodile Ventures	Gauging weir A9H001	Hasani crossing	Nandoni IFR	Crocodile Ventures	Gauging weir A9H001	Hasani crossing	Nandoni IFR
AMOS			1						1	1		1		1		
AURA	3			1	🟥	🟥	🟥	🟥	6	4			5	7	2	2
BLIN						1										
BTRI			9	1		1	1				1					
BUNI								3								
BVIV			3	3		1										
CGAR	1															1
CPAR												1				
CPRE	58	40	103	13	15	30	25	45	35	34	25	32	56	115	90	93
LCYL		5			🟥	🟥	🟥	🟥	7	4		6		6	2	
LMAR	5	4	19		🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	23	34	7	8
LMOL	10	4	11	2	🟥	🟥	🟥	🟥			2		4		1	2
MACU									1							
MBRE						1				2						
MMAC									1							
OMOS	1		1		2			1						1	1	2
PCAT													1			
PPHI			1													
TSPA			1													

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Chapter 4: The morphology and morphometry of the *Labeobarbus marequensis* in the Luvuvhu River.

4.1 Introduction

Broader habitat characteristics, such as temperature, define the tolerance limits, and consequently the distribution of fish species in the different reaches along the length of a river (Gaigher, 1973). Within a river reach, variation on a finer scale leads to the formation of different micro-habitats within habitats (Gatz, 1981) and differences in water velocity and substrate size *inter alia* forms part of this variation. The size, shape, swimming performance and feeding strategy of a species defines its suitability to a micro-habitat. The micro-habitat utilised also differs between the different life history stages and is often a reflection of the evolutionary history. While broader habitat characteristics do not directly lead to morphological adaptations, Wood and Bain (1995) were of the opinion that morphological differences could be related to micro-habitat characteristics. Morphological differences can therefore be construed as an adaptation to living in a particular type of micro-habitat and can vary considerably.

In addition to micro-habitat adaptation, the idea of associating the morphology of a species with its niche is an old and persistent one. Already in 1980 Bock (in Gatz, 1981) proposed equating the niche of an organism to the "sum of the biological roles of its morphological features and the selective forces that operate on those roles". According to Gatz (1981) the full potential of using morphology to define niches, and hence to investigate the relationship of species with ecological aspects, has seldom been tapped as most studies of this type suffer from at least one of three shortcomings. Gatz (op cit.) is of the opinion that in the first instance such studies have tended to concentrate on morphology related to either habitat or food, but not both. Gatz (op cit.) rates the fact that most studies have involved a limited number of morphological features as the second shortcoming and that only a selected group of congeners was studied in nearly all of the previously cited references rather than studying a complete assemblage of coexisting species as the third. Table 4.1 illustrates the array of morphological features that could be determined as well as their relation to habitat preference and food preference.

Table 4.1: Some of the morphological features of fish and their relation to habitat and food dimensions (Adapted from Gatz, 1981).

Category	Group	Feature	Code	Comments
Habitat preference and foraging site	Caudal fin	Aspect ratio	CFAR	High aspect ratio indicates continuous cruising
	Caudal peduncle	Flatness ratio	CPFI	Flat peduncle relates to high amplitude in movements
		Caudal span/body depth ratio	CSBD	As above the lower ratio relates to a high relative swimming speed
		Amount of red muscle	RED	More muscle in stronger swimmer
		Relative peduncle length	RPL	Longer peduncle in stronger swimmer
	Dorsal fin	Dorsal fin position	DORP	Determines steering abilities
	Eye	Eye position	EYEP	Dorsally in benthic species
	Body	Flatness index of body	FI	Laterally flattened in stiller water and vice versa
		Relative body depth	RBD	Deep body in still water
		Index of trunk shape	ITS	High value in cruising species
	Lateral line	Lateral line completeness	LLC	Incomplete in benthic and sluggish fishes
		Lateral line position	LLP	Indicates benthicity or vertical predator-prey relationship
	Mouth	Mouth orientation	MOUO	Indicates vertical position of prey
		Mouth position	MOUP	As above
	Pectoral fin	Area	PCA	Large in benthic species
		Aspect ratio	PCAR	
		Distance from center of gravity	PCCG	Anteriorly placed in maneuverable fish.
		Length	PCL	Long fins enhance low speed maneuvering
		Position	PCP	Relates to turning capacity
		Shape	PCS	Unclear
	Pelvic fin	Area	PVA	Large if fish is demersal
		Aspect ratio	PVAR	High aspect ratio for good maneuvering
		Distance from center of gravity	PVCG	Away from centre enhances maneuvering
		Length	PVL	Free swimmers have short fins
		Position	PVP	Relates to ability to turn and brake
		Shape	PVS	Falcate fins of current dwellers
Features related to food size and type		Number of barbels	BARB	More barbels in non-optic feeding
		Number of caeca	CAEC	More pyloric caeca with more protein in diet
		Eye size	EYE	Directly proportional to importance of sight in feeding
		Gill raker number	GILN	The more rakers the smaller the food particles
		Gill raker shape	GILS	Long thin rakers indicate small prey
		Gill raker fine structure	GRFS	Fine teeth on rakers for large prey.
		Gut length	GUTL	Gut length related to diet
		Jaw tooth presence	JAWP	Jaw teeth present if large prey is taken
		Jaw tooth shape	JAWS	Long pointed or canine like for large prey
		Hypertrophy of teeth	PHAR	If feeding is through suction
		Pharyngeal tooth shape	PHAS	
		Mouth protrusibility	MOPR	Highly protrusible for small prey
		Mouth height	MOUH	Directly proportional to prey size
		Mouth width	MOUW	Directly proportional to prey size
		Relative head length	RHL	Directly proportional to prey size
		Standard length	SL	Directly proportional to prey size

The results of previous studies by Fouché (in Fouché *et al*., 2005) and Gaigher (1969) indicated that the large specimens of *Labeobarbus marequensis* were usually present in deep slow flowing water and smaller specimens in fast flowing shallow water. This is also the opinion of Angliss (pers. com.)[2] It was therefore postulated that differences in the habitat preferences of the various size classes of *L. marequensis* might exist. Because of the lack of knowledge, illustrated in the summary in appendix I, it was decided to investigate this aspect.

It was hypothesized that morphological differences between various size classes or stanzas of *L. marequensis* existed and that these differences could be related to characteristics of the preferred biotopes and to biological aspects such as feeding.

The aim of this component of the study was to determine the morphological characteristics of the various size classes of *L. marequensis* and to infer the habitat preferences and trophic niche differentiation from this data. The accuracy of these inferences will be established in the components on feeding, breeding and habitat preferences of this study. As part of the investigation regarding the relationship between the size classes and habitat preference a secondary aim was to establish whether developmental stages or "stanzas" could be identified in the life cycle of the species.

4.2 Materials and methods.

To avoid the shortcomings described by Gatz (1981) as many as possible of the morphological aspects related to both habitat and food preferences were investigated. Aspects that were regarded to relate to interspecific differences were excluded. These included: eye position, aspects of the lateral line completeness, mouth position, barbels, gill rakers, caeca and teeth.

Morphometric data of attributes related to the remainder of the feeding and habitat preferences categories (Table 4.1) were measured and recorded on the relevant data form

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[2] Angliss, M.K. Senior Aquatic Scientist, Directorate Environmental Affairs, Limpopo Department of Economic Development, Environment and Tourism, South Africa.

(Data form 1 in appendix II). The attributes were measured using a combination of an adaptation of the truss measurement method described by Wood and Bain (1995) and physical measurements using calipers and string. For the truss measurements the specimen was placed on water-resistant paper and the selected "landmark locations", as is illustrated in figure 4.1, marked by pricking clearly visible holes in the paper with a dissection pin. The code number of each specimen, that was composed of the site name, date and fish number, as well as the fork length were recorded on the sheet. The "landmark locations" of the pectoral fin were marked by positioning the removed pectoral fin separately on the paper. After the prick marks had been made the distances were measured and recorded in the data sheet. These measurements and the consequent calculations are shown in table 4.2.

The truss marks used can be described as follows: **1** – the most posterior point of the maxillary, **2** – the most anterior point of the jaw, **3** - most posterior part of the neurocranium, **4** – the origin of the pelvic fin, **5** – the origin of the dorsal fin, **6** – the insertion of the dorsal fin, **7** and **8** the anterior attachment of the ventral membrane from the caudal fin, **9** - the tip of dorsal fin, **10** – the tip of pelvic fin, **11** the antero-dorsal origin of pectoral fin, **12** - the antero-ventral origin of pectoral fin, **13** – the postero-dorsal tip of pectoral fin, **14** - postero-dorsal tip of caudal fin, **15** – the angle formed by fork in caudal fin, **16** - the postero-ventral tip of caudal fin, **17** - the antero-dorsal origin of anal fin and **18** - a point directly dorsal of the antero-ventral attachment of the pectoral fin.

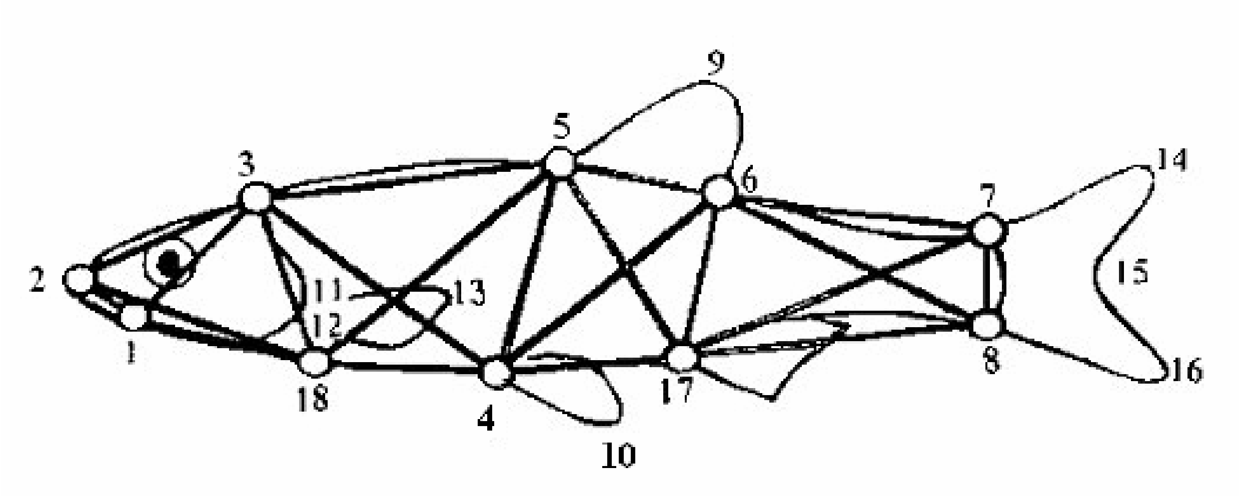

Figure 4.1: The location of the truss landmarks used for morphometric calculations (Adapted from Wood and Bain, 1995).

Table 4.2: Illustration of the morphometric measurements and consequent calculations carried out on *Labeobarbus marequensis.*

Feature	Code	Measurements between truss marks and calculations.
Fork length	FL	2 **à** 15
Body depth	BD	4 **à** 5
Relative body depth	RBD	BD/FL
Body width	BW	Caliper measurement
Relative body width	RBW	BW/FL
Caudal fin aspect ratio	CFAR	(7 **à** 14) / (7 **à** 15)
Relative caudal fin aspect ratio	RCFAR	CFAR / FL
Caudal fin area	CFA	[0,5 (7 **à** 15) X (14 **à** 15)] + [0,5(7 **à** 16) X(7 **à** 8)]
Relative caudal fin area	RCFA	CFA/FL
Caudal span	CS	String measurement
Relative caudal span	RCS	CS/FL
Caudal span/body depth ratio	CSBD	CS / BD
Caudal peduncle length	CL	17 **à** 8
Relative caudal peduncle length	RPL	CL / FL
Caudal peduncle width	RD	7 **à** 8
Relative caudal peduncle width	RCD	RD/FL
Dorsal fin position	DORP	3 **à** 5
Dorsal fin height	V	5 **à** 9
Dorsal fin area	DFA	0,5[(5 **à** 6) x (6 **à** 9)]
Relative dorsal fin area	RDFA	DFA:FL
Relative body depth	RBD	BD / FL
Pectoral fin area	PFA	0,5[(11 **à** 13) x (12 **à** 13)]
Pectoral fin length	PFL	11 **à** 13
Pectoral fin position	PFP	18 **à** 4
Relative pectoral fin area	RPFA	PFA/FL
Pelvic fin length	PVL	4 **à** 10
Pelvic fin area	PVA	0,5[(17 **à** 19) X (19 **à** 20)]
Relative pelvic fin area	RPVA	PVA/FL
Pelvic fin position	PVP	18 **à** 4
Eye size (diameter)	ED	Caliper measurement
Relative eye diameter	RED	ED/FL
Mouth width	MOW	Caliper measurement
Relative mouth width	RMOW	MOW/FL
Relative head length	RHL	(2 **à** 3) / FL

In order to establish whether differences could be related to the life history stages of the species, the fish measured were divided into fork length classes with a 10mm class interval and it was attempted to measure a minimum of 30 specimens in each length class. For each of the size classes the mean and standard deviation for each of the nineteen morphometric features listed in table 4.2 was calculated. Multivariate analyses of covariance, using ANOVA, were used to test for differences in morphology between the different size classes. In addition to test whether observed difference of calculated statistics were significant the ***z-value*** (standard score) between means was calculated at both 0.01 and 0.05 levels of significance using methods prescribed for uncorrelated data (Downie and Heath, 1970).

4.3 Results.

Because of large numbers of fish that were collected as part of the migratory studies (chapter 8) of the project, the majority of the fish measured were collected from the site at Xikundu weir in the Luvuvhu River. Very few specimens longer than 200mm were however collected in the fishway and specimens of these under-represented fork length classes collected as part of reproductive studies (chapter 6) were measured and included.

Table 4.3: A summary of the number of *Labeobarbus marequensis* specimens measured in the 10mm interval fork length size classes.

Class intervals (mm)	Number of specimens	Class intervals (mm)	Number of specimens
31 - 40	1	221 – 230	1
41 - 50	14	231 – 240	2
51 - 60	20	241 – 250	1
61 - 70	29	251 – 260	3
71 - 80	37	261 – 270	2
81 - 90	42	271 – 280	4
91 - 100	48	281 – 290	2
101 - 110	46	291 – 300	0
111 - 120	37	301 – 310	1
121 - 130	34	311 – 320	6
131 - 140	23	321 – 330	2
141 - 150	34	331 – 340	4
151 - 160	7	341 – 350	2
161 - 170	11	351 – 360	0
171 - 180	5	361 – 370	0
181 - 190	3	371 – 380	0
191 - 200	4	381 – 390	1
201 - 210	2	391 – 400	1
211 - 220	4	401 – 410	1

Table 4.3 shows that the morphometric features were measured in a total number of 434 specimens. It however also indicates that in only seven of the thirty eight fork length classes, thirty or more specimens were collected. Because of this lack of specimens in the larger size classes it was decided to apply a different approach to establish what size classes or stanzas existed.

4.3.1 The length-mass relationship of the species and the consequent stanzas.

The length and mass of specimens collected for habitat preference component of this study (Chapter 5) was added to the existing data base and statistically analyzed. Figure 4.2 shows the relationship between length and mass of a total of 581 specimens. As is the case in most fish this relationship is an indication of allometric growth ($M = cL^n$, where M is the mass, L the length and c and n are constants). Regression analyses of the length mass relationship indicates that a power or multiplicative trend line, with the formula $M = 0{,}023L^{3{,}0575}$, fits best with an R-squared value of 0,9876. This type of trend line is typical of the larger cyprinids (Hamman, 1974; Fouché, 1995) and shows inflections of the slope which corresponds with the mean fork lengths illustrated in the first column of Table 4.4. The slope at each observed inflection point was calculated by determining the length mass relationship at this point. The similarity in the calculated slopes were regarded as trends and used as a guide to select the size classes for comparison. The following stanzas were selected: Specimens that are less than 50mm in length, specimens that ranged from 51 to 80, 81 - 100mm, from 101 to 120, from 121 – 150, from 151 - 200mm, from 201 to 250mm, from 251 to 320 and specimens longer than 320mm. Although these stanzas do no correlate with the life stages (Skelton, 2001) of the species, the approach where stanzas were selected agrees with the approach of Gerbrandt (1998) where six predetermined size classes were selected.

4.3.2 The comparison of the morphometric data.

Tables 4.5 to 4.7 show the mean and standard deviation of the measured morphometric characters and calculated surface areas of the nine selected stanzas. The mean and standard deviation of the relative data are shown in tables 4.12 and 4.13. The results of the statistical

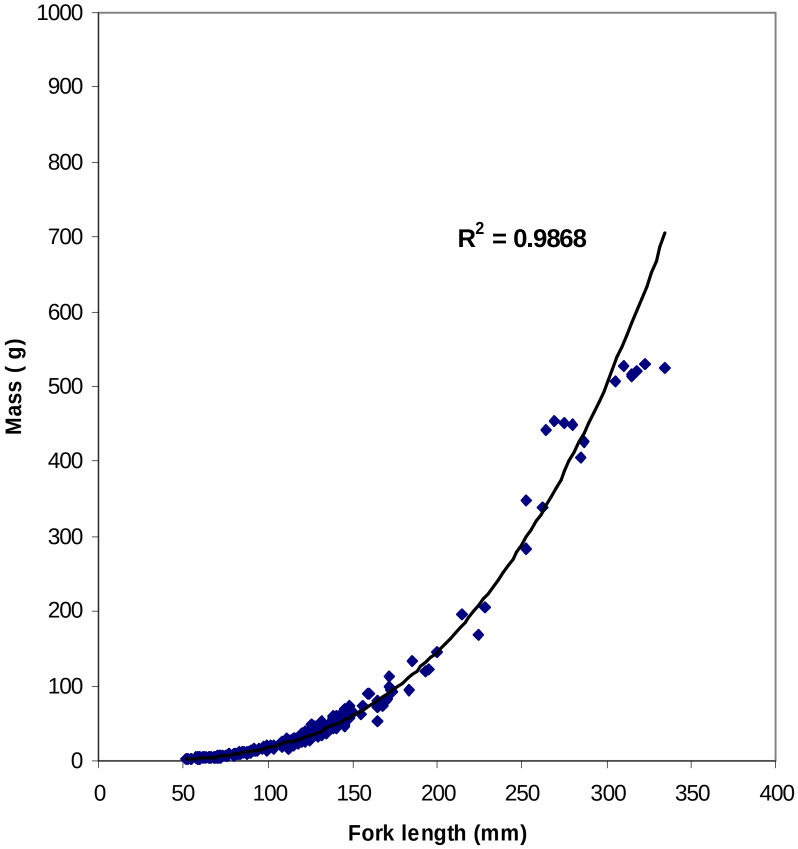

Figure 4.2: Regression analyses of the length: mass relationship of *Labeobarbus marequensis* collected from the Luvuvhu River.

Table 4.4: Calculation of the observed inclines at the inflection points observed in the fitted exponential trend line in the *Labeobarbus marequensis* length:mass relationship.

Mean fork length at inflection point. (mm)	Mean mass at inflection point (g)	Increase in X-axis value	Increase in Y-axis value.	Calculated slope of fitted trend line
44	0,8			
56	2.52	15	1,72	0.1147
80	7.98	24	5.46	0.2275
102	15.70	22	7.72	0.3509
131	46.75	29	31.05	1.0707
151	64.60	20	17.85	0.8925
175	106.32	24	41.72	1.7383
204	145.00	29	38.68	1.3338
228	240.00	24	95.00	3.9583
253	320.00	25	80.00	3.2000
275	405.00	22	85.00	3.8636
300	505.00	25	100.00	4.0000
325	530.00	25	30.00	1.2000

analyses of the measured and calculated data are shown in tables 4.8 to 4.12 and the results of the relative data are shown in table 4.15.

Where table 4.8 shows the analysis of variance within each stanza, tables 4.10 to 4.12 shows the analysis of variance between consecutive stanzas. Table 4.15 shows the difference between the means, based on the calculated z-value, of the relative data.

All the measured and calculated data, with exception of the aspect ratio of the caudal fin, (Tables 4.5 to 4.7) progressively increased in size from one stanza to the next. The substantial increases in body width (*ca* 140%), caudal length (*ca* 56%) as well as pectoral and pelvic fin lengths (*ca* 65 and 230% respectively) that were recorded should be noted (Table 4.7). With the exception of the lengths of the pectoral and pelvic fins, the dorsal fin height and the dorsal fin area in the class shorter than 50mm as well as the pelvic fin length in the 81 – 100mm size class, analyses of variance within the groups (Table 4.8) indicated that the variance within the fork length groups, were significant ($p \leq 0,05$). However when the variance between two consecutive stanzas were determined, body width with the exception of comparisons between stanzas 6:7 and 8:9 differed significantly (Table 4.10). This was also the case with pelvic and pectoral fin lengths where the only anomalies were in the comparison between stanzas 4:5, 6:7 and 8:9 (Table 4.11).

Although as stated above the aspect ratio of the caudal fin did not increase from one stanza to the next, but decreased slightly from the first to the second fork length class, then increased up to the seventh fork length class after which it decreased again. Analyses of variance show (Table 4.12) that the initial increase, at the comparison between stanzas 2 and 3 as well 3 and 4, were significant ($p \leq 0,05$) as was the decrease starting at the comparison between stanzas 7 and 8. Table 4.9 shows that the measured eye diameter of the stanzas did not significantly differ within the stanzas. This was also the case with mouth width up to a fork length of 150mm, but head length did however differ significantly. A comparison of the analyses of variance between consecutive stanzas showed that in the case of eye width, mouth width and head length (Table 4.10) only one instance where the difference between stanzas were not significant ($p > 0,05$) occurred in each trait. This was also the case with analysis of variance of the caudal peduncle length were the only instance where the difference was insignificant was between stanzas 2 and 3 (Table 4.12).

At this point the following aspect should be noted: “If the instances where no significant differences in traits between two consecutive stanzas were observed are accepted as artifacts that could for example have originated because of measurement errors, it can be accepted that an ontogenic increase in these traits did occur”. This decision refers to the analyses of data as reflected in table 4.9 to 4.13.

Table 4.5: Mean (+/- SD) of the measured morphological features related to feeding habits of the nine “stanzas” of *Labeobarbus marequensis* specimens collected in the Luvuvhu River (Standard deviations in parentheses).

Stanzas	N	Eye diameter (mm)	Mouth width (mm)	Head length (mm)
< 50	15	3.66 (0.339)	3.713 (0.395)	10.133 (1.846)
51 – 80	86	4.506 (0.525)	4.853 (0.714)	15.209 (2.653)
81 – 100	85	5.735 (1.141)	6.307 (0.576)	20.209 (1.977)
101 - 120	83	6.490 (0.721)	7.473 (0.697)	22.497 (2.483)
121 - 150	91	7.22 (0.885)	9.04 (1.075)	25.642 (3.171)
151 - 200	31	8.245 (0.824)	11.777 (1.931)	31.510 (3.766)
201 - 250	10	8.98 (1.230)	14.825 (1.676)	36.10 (8.6930
251 - 320	16	10.557 (0.844)	19.143 (3.090)	54.15 (8.26)
> 321	11	11.7I8 (0.938)	22.182 (5.956)	63.30 (12.037)

Table 4.6: Mean (+/- SD) of the calculated morphometric characters of the nine “stanzas” of *Labeobarbus marequensis* specimens collected in the Luvuvhu River (Standard deviations in parentheses).

Stanzas	N	Caudal fin aspect ratio	Caudal fin surface area	Pectoral fin surface area	Pelvic fin surface area
< 50	15	2.02 (0.33)	67.6 (14.21)	21.36 (29.35)	2.83 (1.42)
51 - 80	86	1.95 (0.43)	150.66 (35.32)	48.04(25.66)	31.94 (15.34)
81 - 100	85	2.28 (0.44)	249.88 (45.44)	98.35 (27.08)	58.02 (11.72)
101 - 120	83	2.1 (0.27)	362.12 (47.15)	123.06 (38.3)	72.64 (21.72)
121 - 150	91	2.3 (0.16)	543.86(101.79)	160.04 (93.7)	84.58 (55.28)
151 - 200	31	2.1 (0.29)	874.85 (188.4)	212.79 (93.5)	139.05 (100.79)
201 - 250	10	2.04 (0.24)	1514.3 (324.08)	279.05 (67.9)	154.77 (123.27)
251 - 320	16	2.01 (0.36)	2418.51 (598.2)	621.8 (22.69)	417.63 (202.51)
> 321	11	2.01 (0,35)	3443.27 (852.3)	908.7 (392.8)	436.24 (304.77)

Table 4.7: Mean (+/- SD) of the measured general body dimensions of the nine "stanzas" of *Labeobarbus marequensis* specimens collected in the Luvuvhu River (Standard deviations in parentheses).

Stanzas	n	Fork length FL	Body depth BD	Body width BW	Caudal peduncle span CS	Caudal peduncle length CL	Dorsal fin position DORP	Dorsal fin height V	Pectoral fin length PCL	Pelvic fin length PVL
< 50mm	15	46.733	13.933	5.793	13.367	9.200	10.800	3.933	6.933	2.800
		(4.53)	(1.71)	(0.61)	(2.04)	(1.93)	(2.54)	(1.03)	(1.28)	(0.68)
51 - 80	86	68.465	16.465	14.033	17.879	14.360	15.558	7.500	11.488	9.419
		(8.10)	(2.32)	(3.68)	(2.73)	(1.99)	(2.06)	(1.67)	(2.29)	(3.11)
81 – 100	85	90.365	21.612	20.877	24.329	18.930	19.859)	10.44	15.624	13.282
		(5.51	(2.22)	(2.55)	(3.19)	(1.79)	(1.56)	(1.88)	(1.81)	(1.39)
101 - 120	83	109.795	28.518	25.259	31.067	22.795	25.627	13.289	17.880	14.711
		(6.35)	(1.39)	(3.54)	(4.44)	(2.26)	(3.34)	(3.20)	(2.98)	(2.69)
121 - 150	91	135.407	37.912	29.327	39.953	22.692	34.187	17.615	19.824	15.154
		(9.11)	(5.70)	(6.72)	(6.50)	(3.20)	(4.38)	(3.86)	(5.99)	(5.57)
151 - 200	31	169.806	48.839	33.203	51.552	36.871	42.548	20.355	23.935	19.097
		(13.41)	(7.59)	(10.37)	(5.41)	(6.18)	(7.72)	(6.44)	(4.16)	(6.48)
201 - 250	10	222.400	67.300	40.400	69.320	45.300	57.600	30.300	26.400	19.800
		(14.14)	(9.26)	(8.32)	(6.74)	(4.62)	(13.26)	(7.29)	(5.76)	(7.37)
251 - 320	16	290.875	84.625	55.025	86.800	63.750	83.250	43.438	38.500	34.500
		(25.52)	(10.83)	(16.01)	(8.63)	(8.58)	(8.75)	(11.14)	(11.58)	(10.05)
> 321	11	353.818	100.818	62.745	103.782	73.456	97.454	52.727	45.182	34.272
		(29.26)	(14.01)	(17.98)	(10.82)	(9.38)	(13.21)	(8.16)	(11.64)	(13.58)

Table 4.8: Results of the analyses of variance within the groups of the measured and calculated morphometric characters of *Labeobarbus marequensis* collected in the Luvuvhu River ($p \leq 0,05$; critical F-value 1,9605).

Stanzas	Body depth	Body width	Caudal fin surface area	Caudal fin aspect ratio	Caudal peduncle span	Caudal peduncle length	Position of dorsal fin	Dorsal fin height	Pectoral fin length	Pectoral fin surface area	Pelvic fin length	Pelvic fin surface area
< 50	2.92	0.37	148.4	0.109	4.16	3.74	6.46	1.07	1.64	24.27	0.46	1.62
51 - 80	5.40	13.55	1248.0	0.184	7.44	3.95	4.23	2.77	7.78	658.79	9.68	235.38
81 - 100	4.87	6.47	2065.5	0.189	10.05	3.18	2.48	3.57	3.25	733.70	1.90	137.57
101 - 120	15.55	12.56	2222.7	0.072	19.69	5.12	11.19	10.21	8.86	1469.17	7.21	471.93
121 - 150	32.46	45.09	10362.4	4.48	42.26	10.26	19.20	14.88	35.88	8783.52	30.98	3056.16
151 - 200	57.61	107.56	35518.0	0.08	29.29	38.18	59.66	41.44	17.33	7381.45	41.96	6050.58
201 - 250	85.79	69.17	92467.5	0.06	45.34	21.34	175.82	53.12	33.16	4605.80	54.40	11237.51
251 - 320	130.13	256.19	357830.6	0.15	74.45	73.67	76.60	124.00	134.00	49589.28	101.07	41011.31
> 321	196.36	323.43	726568.5	0.124	117.16	88.07	174.47	66.62	135.56	154296.07	184.42	92886.67

Table 4.9: Results of the analyses of variance within the groups of the measured and calculated morphometric characters of *Labeobarbus marequensis* collected in the Luvuvhu River (p ≤ 0,05; critical F-value 1,9605).

Stanzas	N	Eye diameter	Mouth width	Head length
< 50	15	0.12	0.15	3.41
51 – 80	86	0.28	0.50	7.04
81 – 100	85	1.30	0.33	3.91
101 – 120	83	0.52	0.49	6.16
121 – 150	91	0.78	1.16	10.06
151 – 200	31	0.89	3.66	14.19
201 – 250	10	1.52	2.43	75.21
251 – 320	16	0.69	9.03	58.76
> 321	11	0.88	35.48	146.49

Table 4.10: Analyses of variance of the differences (p ≤ 0,05) in body depth, body width, eye width, mouth width and head length between the consecutive stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River.

Stanzas compared	F crit. (p = 0,05)	**Body depth** F	**Body width** F	**Eye width** F	**Mouth width** F	**Head length** F
1:2	3,937	16,20	74,20	42,237	37,08	50,43
2:3	3,897	233,10	202,40	78,31	219,58	196,41
3:4	3,898	198,0	84,79	22,90	141,20	43,40
4:5	3,896	157,0	24,29	35,02	126,99	52,89
5:6	3,920	71,25	5,72	5,94	96,69	71,82
6:7	4,091	40,20	3,97	0,15	24,13	5,64
7:8	4,300	14,28	7,07	14,67	17,99	32,75
8:9	4,279	11,15	1,37	12,12	2,50	3,81

Table 4.11: Analyses of variance of the differences (p ≤ 0,05) in pectoral fin length and area, pelvic fin length and area as well as dorsal fin height and position between the consecutive stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River.

Stanzas compared	F crit. (p = 0,05)	**Pectoral fin length** F	**Pectoral fin area** F	**Pelvic fin length** F	**Pelvic fin area** F	**Dorsal fin height** F	**Dorsal fin position** F
1:2	3,937	38,33	25,97	66,78	59,02	64,22	63,67
2:3	3,897	133,6	156,31	111,31	156,83	119,21	239,94
3:4	3,898	35,657	22,72	18,90	29,948	49,32	206,17
4:5	3,896	7,14	10,26	0,44	3,39	64,20	206,82
5:6	3,920	12,51	9,03	10,66	13,50	8,07	55,15
6:7	4,091	2,19	4,26	0,084	0,14	16,95	19,81
7:8	4,300	9,37	22,11	15,91	15,51	10,90	35,58
8:9	4,279	2,16	5,87	0,005	0,04	5,57	11,37

Table 4.12: Analyses of variance of the differences (p ≤ 0,05) in caudal fin surface area, caudal peduncle span, caudal fin aspect ratio and caudal peduncle length between the consecutive stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River.

Stanzas compared	F crit. (p = 0,05)	**Caudal fin aspect ratio** (calculated F value)	**Caudal fin area** (calculated F value)	**Caudal peduncle span** (calculated F value)	**Caudal peduncle length** (calculated F value)
1:2	3,937	0.257	83,325	37,30	86,74
2:3	3,897	6.281	255,58	205,09	252,96
3:4	3,898	5.778	248,29	129,38	151,73
4:5	3,896	0.519	221,19	108,78	264,47
5:6	3,920	0.314	152,14	79,72	69,12
6:7	4,091	0.142	65,53	72,35	15,66
7:8	4,300	43.37	19,49	29,59	38,76
8:9	4,279	57.39	13,537	20,53	7,72

Table 4.13: Mean (+/- SD) of the relative morphological features related to feeding habits of the nine "stanzas" of *Labeobarbus marequensis* specimens collected in the Luvuvhu River (Standard deviations in parentheses).

Stanzas	**N**	**Relative eye diameter ED:FL**	**Relative mouth width MW:FL**	**Relative head length HL:FL**
< 50	15	0.079 (0.01)	0.080 (0.01)	0.218 (0.04)
51 – 80	86	0.067 (0.01)	0.071 (0.01)	0.269 (0.19)
81 – 100	85	0.064 (0.01)	0.070 (0.01)	0.223 (0.02)
101 – 120	83	0.059 (0.006)	0.068 (0.005)	0.205 (0.02)
121 – 150	91	0.053 (0.008)	0.066 (0.009)	0.197 (0.08)
151 – 200	31	0.049 (0.006)	0.069 (0.009)	0.187 (0.02)
201 – 250	10	0.040 (0.005)	0.053 (0.03)	0.163 (0.04)
251 – 320	16	0.037 (0.003)	0.067 (0.01)	0.188 (0.02)
> 321	11	0.033 (0.003)	0.062 (0.01)	0.176 (0.03)

A comparison of the relative calculated features (Tables 4.13 and 4.14) showed that the means varied and did not show a trend of increase with the increase in fork length of the stanzas, as was the case with the majority of the actual measured data shown in tables 4.5 to 4.7. In the case of the relative eye diameter, the relative mouth width and the relative head length a general negative trend, i.e. a decrease, was observed. The trends in the other features (Table 4.14) were mixed and are graphically illustrated in figure 4.2 were it is shown that the relative caudal length and caudal width basically remained

Table 4.14: Mean (+/- SD) of the relative calculated morphological features related to habitat preference of the nine "stanzas" of *Labeobarbus marequensis* specimens collected in the Luvuvhu River (Standard deviations in parentheses).

Stanzas	N	Relative body depth BD:FL	Relative body width BW:FL	Relative caudal fin surface area CFA:FL	Relative caudal fin aspect ratio CFAR:FL	Relative caudal span CS:FL	Relative peduncle length RPL	Relative caudal width RCD	Relative dorsal fin area DFA:FL	Relative pectoral fin area PCA:FL	Relative pelvic fin area PVA:FL
< 50mm	15	0.301 (0.04)	0.124 (0.01)	1.430 (0.32)	4.387 (1.10)	0.287 (0.04)	0.195 (0.03)	0.122 (0.03)	0.465 (0.13)	0.300 (0.10)	0.057 (0.03)
51 - 80 mm	86	0.241 (0.03)	0.202 (0.04)	2.118 (0.32)	3.195 (0.877)	0.262 (0.04)	0.209 (0.01)	0.120 (0.01)	0.839 (0.21)	0.674 (0.32)	0.450 (0.20)
81 – 100	85	0.239 (0.02)	0.231 (0.02)	2.746 (0.39)	2.523 (0.489)	0.269 (0.03)	0.209 (0.02)	0.119 (0.02)	1.157 (0.29)	1.080 (0.27)	0.639 (0.11)
101 - 120	83	0.259 (0.03)	0.231 (0.03)	3.294 (0.34)	1.823 (90.25)	0.283 (0.04)	0.207 (0.02)	0.119 (0.01)	1.388 (0.53)	1.012 (0.50)	0.665 (0.20)
121 - 150	91	0.280 (0.04)	0.216 (0.05)	4.002 (0.60)	1.70 (1.532)	0.294 (0.04)	0.219 (0.02)	0.124 (0.02)	1.764 (0.62)	1.171 (0.67)	0.621 (0.39)
151 - 200	31	0.287 (0.03)	0.197 (0.07)	5.116 (0.75)	1.228 (0.216)	0.304 (0.03)	0.217 (0.03)	0.126 (0.01)	2.086 (0.91)	1.296 (0.54)	0.779 (0.45)
201 - 250	10	0.302 (0.03)	0.183 (0.05)	6.778 (1.02)	0.92 (0.129)	0,311 (0.03)	0.204 (0.02)	0.126 (0.01)	3.101 (0.82)	1.259 (0.33)	0.650 (0.48)
251 - 320	16	0.291 (0.03)	0.188 (0.05)	8.264 (1.68)	0.696 (0.138)	0.299 (0.03)	0.219 (0.02)	0.132 (0.01)	4.558 (1.68)	2.140 (0.80)	1.422 (0.66)
> 321	11	0.285 (0.03)	0.178 (0.06)	9.629 (1.59)	0.656 (0.107)	0.293 (0.02)	0.208 (0.02)	0.120 (0.01)	5.271 (1.41)	2.545 (0.95)	1.218 (0.84)

Table 4.15: Analyses of the difference between the means of the relative morphometric characters of consecutive stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River using the calculated z-values at a significance level of 5% (Null hypothesis: r – rejected, nr – not rejected).

Stanzas compared	**Relative body width**		**Relative caudal peduncle span**		**Relative caudal peduncle length**		**Relative caudal peduncle width**		**Relative pectoral fin area**		**Relative eye diameter**		**Relative mouth width**		**Relative head length**		**Position of the dorsal fin**	
	Z		z		Z		Z		z		z		Z		z		z	
1:2	16.984	R	-2.323	R	1.803	nr	-0.365	nr	55.557	r	-7.234	r	-5.135	R	0.503	nr	13.365	r
2:3	6.045	R	1.351	Nr	0	nr	-0.572	nr	11.026	r	-8.597	r	-3.320	R	-3.004	R	23.477	r
3:4	0	Nr	2.682	R	-0.780	nr	2.091	r	2.105	r	-6.101	r	-3.626	R	-3.101	R	29.255	r
4:5	-2.504	R	1.931	R	4.337	r	3.674	r	3.426	r	-8.008	r	0.598	nr	-4.179	R	11.791	r
5:6	-1.496	Nr	1.523	Nr	-0.357	nr	0.618	nr	0.554	nr	-6.921	r	-1.442	nr	-2.215	R	5.550	r
6:7	-0.756	Nr	0.715	Nr	-1.541	nr	2.349	r	4.616	r	-8.679	r	-0.714	nr	0.149	nr	15.710	r
7:8	0.272	Nr	-1.055	Nr	1.659	nr	-1.502	nr	4.329	r	-3.769	r	0.915	nr	0.825	nr	6.891	r
8:9	-0.490	Nr	-0.611	Nr	-1.229	nr	-67.571	r	-13.016	r	-39.975	r	-28.864	R	-32.365	R	-38.04	r

unchanged. The other relative features depicted in figure 4.3 did however change over time. Initial body width increase was first followed by a slight decrease after which it remained fairly constant. Relative caudal span and body depth initially decreased, then showed signs of increase and after which it finally remained constant.

Statistical analyses (Table 4.15) showed that in the case of the pectoral fin area, the eye diameter and position of the dorsal fin the difference between the means were significantly ($p \leq 0,05$) between all stanzas, while in the case of the relative body width the difference was only significant in the stanzas of fish smaller than 100mm in fork length. With regard to peduncle length and caudal width a similar pattern was observed. The means of the pectoral fin area, eye diameter and the position of the dorsal fin did however differ significantly between all stanzas. In the case of relative mouth width and head length, differences were significant in the majority of instances (Table 4.15).

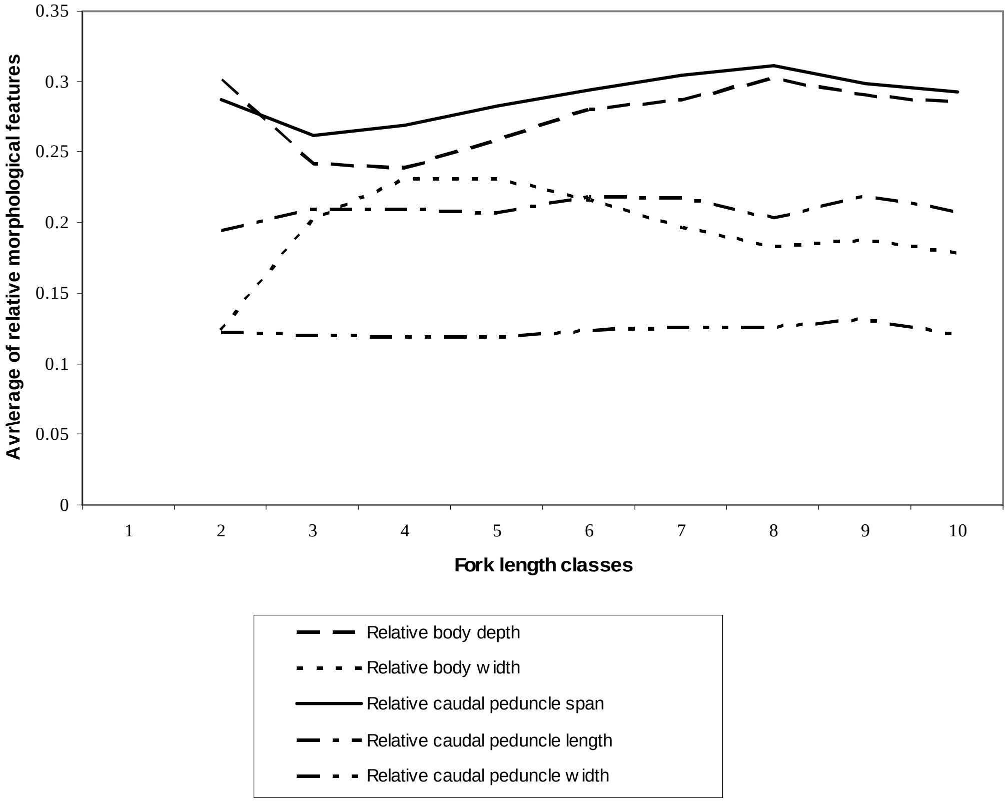

Figure 4.3: A graphical illustration of the changes in the means of five of the relative calculated morphological features of the nine stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River.

Because of the high values of the standard deviation with regard to the calculated surface areas of the fins (Table 4.6) it was decided to treat this data with care. The data on the actual surface does however indicate that a substantial increase in these surface areas did occur as the fish increased in fork length. This increase is echoed when the areas are expressed as a relative value of the fork length.

4.4 Discussion.

Since the density of water is only slightly less than that of living tissue, fish are almost perfectly supported and expend only a negligible amount of energy to overcome the forces of gravity. Consequently swimming is energy wise one of the most economic locomotory modes of the animal kingdom using only 0,39 kcal kg^{-1} km^{-1} as opposed to flying at the other end of the scale where an average of 1,45 kcal kg^{-1} km^{-1} is used (Hickman ***et al.***, 1984).

The energy costs of moving through water are however complicated by drag, which is the resistance that a medium offers. Directly around an object there is a thin layer of water that moves with the object but a distance away the water does not move at all. The amount of water between the two layers is known as the boundary layer and because the two layers are at different speeds, shearing forces between them develop in the boundary layer that slows down the object and this is the source of drag (Hildebrand, 1974). Two forms of drag, that are independent of one another, are recognized (Helfman *et al.*, 2000). The first is *viscous* or *frictional* drag which, as is discussed above, is caused by friction between the body and the medium and is not greatly affected by speed but rather by the shape and particularly by the smoothness of the body surface. The second form is *inertial* or *pressure* drag which is caused by the displacement of water as it flows around the fish. This form is affected by the speed of the fish and *per se* by the velocity of the water.

Laminar flow around the object occurs when there are no eddies and the flow is smooth and undisturbed. However if eddies are created, for example if the object is rough, the flow becomes turbulent and drag increases. An increase in speed leads to increase in turbulence which in turn increases drag (Hildebrand, op cit.). There are other causes of drag to consider.

Probably the most important occurs when an object moves through water and in the process displaces water. The volume of this water is at least equal to its own body volume plus a third of the volume of the boundary layer. This causes a backflow of water to form behind the object. If the backfill, which is the water that fills the backflow, is incomplete a separation of the boundary layer occurs. This creates suction and water follows in the wake. The second source concerns the positive pressure against the anterior and posterior parts of the object and negative pressure in the intermediate parts. The energy needed to overcome these pressure changes have to come from the object and the result is pressure drag (Hildebrand, 1974).

According to Hildebrand (op cit.) the general requirements of swimmers include the ability to a) reduce the resistance (drag) offered by water to motions of the moving body, b) propel themselves in a relatively dense medium, c) control their position in water and d) maintain orientation and steer the body.

Most fishes swim or propel themselves, by contracting a series of lateral muscles on one side of the body while relaxing the muscle on the other side. Muscle blocks, or myomeres, which can range in number from fourty to seventy, along the length of the body that are attached to one another by collagenous septa and ultimately to the backbone and the skin (Helfman *et al.*, 2000) are responsible for the precisely timed contractions. Miller and Harley (1992) described the result of the contractions as "waves of contraction that move in a posterior direction with an increasing amplitude and speed and pushes against the water at an angle that propels the fish forward" while Nikolsky (1963) indicated that in the "majority of fishes progressive movements are accomplished by means of bending the body as a wave which passes along the body".

Swimming muscles make up half of the body weight of fish and are divided into two groups (Pitcher and Hart, 1982). The first group, the red muscle with its high haemoglobin content and numerous mitochondria, are the "cruising motors" that enable continuous movement in aerobic conditions. White muscle, which function in anaerobic conditions and are regarded as the emergency accelerators, is the second group that the make up the bulk of body muscle.

From the above it can be deduced that vertebrate swimmers propel themselves with oscillating mechanisms. These oscillating movements can involve the whole body, which result in “eel-like” movement, or can involve only the posterior part of the body, namely the caudal peduncle and the caudal fin. The latter form occurs in fish that are less flexible than the eels (Hickman *et al.*, 1984). Regarding the second group, where the posterior body is involved, Hildebrand (1974) refers to two kinds of oscillating movements that are used. In one form an effective power stroke of the caudal peduncle is followed by a dissimilar and less effective or ineffective recovery stroke. The muscle contraction generated in the large muscle mass in the more anterior part of the body is transferred through tendons to the relatively non-muscular caudal peduncle and tail where thrust is generated. In the other kind each stroke is effective so the oscillations are symmetrical and there is no recovery stroke. In the latter form only the caudal fin sweeps back and forth from side to side.

The following factors are basic to the function of the caudal fin: i) the caudal fin should be broad and flat so that during the power stroke water will not easily flow around it but instead will resist the fins lateral motion, ii) the base of the tail (or supporting peduncle) should move from side to side carrying the caudal membrane with it iii) these motions should be timed so that the caudal fin angles back to the left as it sweeps from left to right and back to the right as it sweeps from right to left Hildebrand (op cit.). The last aspect above means that the caudal fin is thrusting obliquely against the water. The inertia of the water causes it to push with equal force in an opposite direction to the fin. When breaking this latter force into components a forward and a lateral component results. Where the forward component causes the fish to move forward, the lateral component causes the fish to pivot, with the anterior part of the body moving to the left and the posterior to right. This pivoting leads to the s-shaped movement pattern around the fish’s center of mass.

With regard to the other fins, both Nikolsky (1963) and Skelton (2001) regard the paired pelvic and pectoral fins to act as rudders and to assist in turning the fish on a horizontal plane. The unpaired dorsal and anal fins assist as stabilizing keels.

The shape of a fish gives an indication of its biology and habitat requirements (Skelton, 2001) and is the result of adaptation to a specific habitat. Active swimmers have a fusiform body that is the broadest in the middle and that tapers to both ends. This classic shape, that minimizes drag to a great extent, is round in cross section (Nikolsky, 1963) and has i) a circumference of 20 to 24% of the body length (Helfman *et al.*, 2000) or ii) a maximum width equal to 25% of its length (Hildebrand, 1974).

The aspect ratio or height to width ratio, of the caudal fin can also be indicative of the speed at which the fish swims. Hildebrand (op cit.) reported that where the aspect ratio of faster swimmers can exceed a ratio of 5 or more that of the slow swimmers would range between 1 and 2. Fish that are swift swimmers also have curved pointed fins and a deeply forked caudal fin (Helfman *et al*., op cit.). The latter aspect would also apply to the freshwater rheofiles, which are fish that live in fast flowing water. While the reduced surface of the forked caudal fin reduces the friction caused by large surfaces, the pointed tips minimize the vortices and thereby reduce inertial drag (Miller and Harley, 1992).

According to Nikolsky (op cit.) "good swimmers" in flowing water have a shorter and higher caudal stem than those in still water. The dorsal fins of rheofiles are short and are often placed relatively far back on the body. In contrast, the pool or slow water dwellers have long dorsal fins, caudal fins that are not forked and bodies that are deep and laterally flattened.

Fish in fast water are divided into four groups based on the nature of their habitat and the relevant adaptations (Hora 1930 in Nikolsky, 1963): a) Small species that occur in "still water" such as along the flanks and under waterfalls, b) good swimmers with a "strongly" cylindrical body, c) small bottom living fishes that live under stones and d) fish that can attach themselves to the substrate. Many river fish which inter alia include the African and Asian barbs belong to group b) above.

The Ostariophysi, the group of Teleost fish that *inter alia* include the cyprinids, have a fusiform body with an anterior dorsal fin with antero-ventral pectoral and postero-ventral pelvic fins (Jobling, 1996). Although it is postulated that deep-bodied fish, such as the cichlids that belong to the group Acanthopterygii have a superior turning ability, Schrank *et al.* (1999) showed that an ostariophysian body form or "bauplan" such as that of the goldfish was indeed superior to other forms for maneuvering.

The cyprinids have a body form that closest to what is commonly referred to as "sub-carangiform" body shape (Helfman *et al*, 2000). This body shape is generally characterized by a streamline body and broad V-shaped caudal fins. The propulsive elements of the sub-carangiform swimmers are concentrated in the posterior part of the body and they seemingly have the ability to accelerate rapidly. As is the case in most fish, sub-carangiforms can be regarded as a locomotor generalist rather than a specialist which would imply that the fish uses more than one swimming mode and could switch between modes, depending on what is needed.

Body size has an overriding influence on most aspects of fish biology and central to size are the concepts of scale and allometry (Schmidt-Nielsen, 1995). Changes in scale over ontogenetic time generally involve alterations in the dimensions, materials and design of structures. The way in which an increase in body size affects the swimming speed and ability of small and large members of a species is a good example of the effect of scaling. Allometry as a concept underscores a basic fact of growth and scaling, namely that changes in quantitative relationship between sizes and functions of growing body parts is seldomly linear. A doubling of the size of the fish will not necessarily lead to a doubling in its swimming speed as it is more complex and depends on the measure of the body size in question. The fact that the relative cost of swimming decreases with body size in most fish, both within and among species, is another example of an allometric relationship. Schmidt-Nielsen (1995) showed that it is more expensive for small fish to move 1 g of body mass a given distance than it is for a larger fish.

Growth processes, both those associated with general length and mass increase and those related to body proportions, help to explain many phenomena, which includes life history, behavioural, physiological and ecological aspects in the life of a fish. Helfman *et al.*, (2000) showed the existence of ontogenic differences within species, and in particular between the various size classes or life stages. The different stages often differ in habitat and ecology and must function both during definable stages as well as during transitional periods (Skelton, 2001). Adaptations appropriate to one stage may therefore create constraints for other stages. For example juveniles are often morphologically miniature adults but instead of feeding the same diet as the adults (for which their external morphology is suited) feed on something else. This puts them into competition with juveniles and adults of other species constructed to feed on that same diet for the rest of their lives and therefore have a competitive advantage.

Fish communities have been shown to be organised on the basis of habitat (Werner and Hall, 1976) and the relationship between morphology and habitat use is well documented (Keast and Webb, 1966; Gatz 1979a and b; Felley 1984, Wood and Bain, 1995). Although Nikolsky (1963) and Helfman *et al.* (2000) showed that on a large scale, such as within a river reach, habitat use by a species is generally regarded to be consistent over the range of a species, the work by Moyle and Baltz (1985) was one of the first that have shown that “micro-habitat use often varies among individuals of a species”. Gorman and Karr (1978) states the cyprinid species have been shown to partition habitat by “stratifying the water column into vertical zones”. They also pointed out that habitat segregation might be based on morphological adaptations. Felley (op cit.) found significant correlations between water depth and morphological traits. To some extent this was also shown to be the case in *L. marequensis* by Fouché *et al.* (2005) where the velocity-depth habitat characteristics relationship to fork length was examined. The pre-1990 morphometric research, as listed above, was limited to body structures, such as the length of fins, with little or no ability to quantify body shape. Their more recent work Wood and Bain (op cit.) included truss measurements, which made use of comprehensive measurements of body shape, and was reported to be more appropriate for differentiating both species and populations. In addition Wood and Bain (op cit.) was of the opinion that truss data may elucidate differences better among fishes which could lead to a better assessment of the relationship between habitat and morphology.

For the sake of the discussion that follows and in order to compare the morphometrics *L. marequensis* to aspects discussed in the foregoing text the concept flowing and in particular “fast flowing” is going to be equated with the concept “fast swimmer” which is used in the text referred to. The reasoning is based on the premise that in order to survive and live in a fast flowing habitat, the specimen should be able to swim as fast as or faster than the velocity of the current.

Based on its sub-carangiform body shape with its the "short" posteriorly placed dorsal fin and the V-shaped caudal fin it is apparent that *L. marequensis* has a general "bauplan" most suitable to cope with flowing water habitat rather non-flowing habitat. The caudal fin aspect ratio of the species, which ranges from just below 2.0 in the juveniles to 2.3 in the sub-adults, falls within what can be described as "median" (Helfman *et al.*, 2000) and indicates although the fish are not very fast swimmers they can not be rated as slow. Other aspects, such as the relative caudal peduncle length, furthermore indicate that the species is a strong swimmer. All the aspects however indicate that the species does not continuously cruise (Gatz, 1981).

However, at the onset of this chapter it was stated that the aim was determine whether the morphological characteristics of the various size classes of *L. marequensis* were different and if so the habitat preferences and trophic niche differentiation would be inferred from this data.

The fact that intraspecific variation does occur has been documented by various authors (Sheperd, 1991; Ehlinger 1990; Swain *et al*., 1991; Brőnmark and Miner, 1992). The results of this study shows that this variation also occurs within *L. marequensis* collected from the Luvuvhu River.

Ontogenetic changes in the caudal fin and related aspects that can be regarded as the propulsive force of *L. marequensis* were observed. As indicated in the results, the aspect ratio of the caudal fin, which is also a relative value, initially decreased, then increased after which it again decreased. Similar changes were observed in the relative caudal span (Figure 4.2). Although not part of the "propulsive machinery" this changes were observed in the relative body depth and to an extent also in the body width. The changes in body width in particular are of note as the cross section of fish obtains its most "circular" format from stanza 3 to stanza 6 (Table 4.14, Figure 4.2). As stated by Nikolsky (1963), Hildebrand (1974) and

Helfman *et al.* (2000) this is the classic form that minimizes drag to a great extent. Based on these changes in the relative morphological aspects it would seem that initially the species does not have the ability to swim fast, or cope with fast flowing water. It then develops the ability to swim faster and this adaptation is maintained for a few stanzas. The final adaptation then seems to refer back to "slower" habitats. It can therefore be inferred that while the juveniles (stanzas 1 and 2) are adapted to slow flowing habitats, stanzas 3, 4, 5 and 6 will dominate the fast flowing habitats. The largest specimens, stanzas 7, 8 and 9 will however again prefer slow flowing habitats Figure 4.4). It is however postulated that where the habitat occupied by the juveniles is shallow, those occupied by the larger specimens will be deep. These changes in morphology and the accompanying habitat preferences would constitute a "ontogenetic shift" as was described by Huckins (1997).

Figure 4.4: Example of an adult female caught in a deep pool illustrating the body form adapted to slow-flowing biotopes (Adapted from Fouché, 2008).

The relative caudal peduncle length did not show signs of change when the stanzas were compared. Longer relative caudal lengths are however regarded as a general aspect where "good swimmers in flowing water have a longer caudal stem than those in still water" (Nikolsky, 1963) This was illustrated by Paine *et al.* (1982) where the relative long caudal peduncle length of the darter *Etheostoma microperca* was related to its high frequency of occurrence in riffles.

With regard to the eye diameter, mouth width and head length differences in both the actual and relative sizes were observed. Palomares and Pauly (1998) found strong relationships between food ingestion, in the various body mass classes, and morphometric characters such the aspect ratio of the caudal fin and the body depth ratio. This clearly indicated that the change in feeding habits and diet of the various size classes could be related to the morphometry. It was shown that ontogenetic changes in both the aspect ratio of the caudal fin and the body depth also occurred in *L. marequensis* and it can be inferred that these changes can be related to changes in both the feeding habits and diet. Although this indicates that ontogenetic differences in the trophic adaptation of the species are imminent, no actual inference could be made to the actual diet make-up.

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APPENDIX II : Data form 1: Morphometrics of *L. marequensis* Specimen code (site/date/fishno) ……………………….

PROJECT ……………………………. DATE ………………….. TIME ………………….. RIVER ………………………….. Site no and name ……………

Category	Group	Feature	Code	Measurements	Points	Data
General aspects		Fork length	FL	Tip of snout to fork in caudal fin	**2 à 15**	
		Body depth	BD	Depth at widest part of body (excluding fins)	**4 à 5**	
		Body width	BW	Width at thickest part of body	**Caliper**	
Habitat preference and foraging site	Caudal fin	Caudal fin area	CFA	Surface area of fin	**0,5 (7 à 15) X [14 à 15]** **+** **0,5 (7 à 16) X [7 à 8]**	
	Caudal peduncle	Caudal span	CS	Circumference of caudal peduncle	**String measurement**	
		Caudal length	CL	Length of peduncle from post end of anal fin to anterior of caudal fin	**7 à 14**	
		Caudal span/body depth ratio	CSBD		*CS/BD*	
		Relative peduncle length	RPL		**CL/FL**	
	Dorsal fin	Dorsal fin position	DORP	Distance from operculum to anterior end of fin	**3 à 5**	
		Dorsal fin area	DFA		**0,5 (5 à 6) X [6 à 9]**	
	Body	Relative body depth	RBD		*BD/FL*	
	Pectoral fin	Pectoral fin area	PCA	Surface area of fin	**0,5 (11 à 13) X [12 à 13]**	
		Pectoral fin length	PCL	Base of fin to tip of longest ray	**11 à 13**	
		Pectoral fin position	PCP	Distance from operculum to anterior end of fin	**Operculum à 11**	
	Pelvic fin	Pelvic fin area	PVA	Surface area of fin	**0,5 (4 à 10) X [10 à 19]**	
Feeding		Pelvic fin length	PVL	Base of fin to tip of longest ray	**4 à 10**	
		Pelvic fin position	PVP	Distance from operculum to anterior end of fin	**18 à 4**	
	Eyes	Eye size (diameter)	EYE	Normal	**Caliper**	
	Mouth	Mouth width	MOW	At widest part with mouth fully open	**Caliper**	
	Head	Relative head length	RHL	Anterior tip of closed mouth to post edge of operculum	**2 à 3**	

Chapter 5: The population structure, ecology and habitat preference of *Labeobarbus marequensis* in the Luvuvhu River catchment.

5.1 Introduction.

A river, although complex, does not consist of a loose group of isolated ecosystems but is a single four dimensional entity with vertical, cross sectional, longitudinal and temporal components (Davies and Day, 1998). This view that rivers are continuous, longitudinal ecosystems is referred to as the River Continuum Concept (RCC) and was first introduced by Vannote *et al.* in 1980 (Davies and Day, op cit.). Based on the RCC, three sections, that is referred to as zones or reaches, can be distinguished in a river. Because of the descriptions used in the geomorphological hierarchy designed by Rowntree and Wadeson (1998) where the term *zone* is used for "an area in a catchment homogenous with respect to flood runoff and sediment production" while the term "reach" on the other hand refers to a uniform length of channel, it was decided that the term "zone" would be more appropriate. Each of the three zones has its own physical characteristics with the resultant differences in biota assemblages (Davies *et al.*, 1993). The zone referred to as the *"mountain stream"* is closest to the origin or headwaters and here the gradient is steep and the water clear, cool and fast flowing. The resultant substratum consists of bedrock, boulders and cobble. The channel is narrow with good canopy cover. Further downstream where the zone is referred to as the *"middle reach"* the gradient lessens and the water velocity decreases. However, more water has been added by tributaries and the water volume increases. The resultant substrate is boulders, cobbles, gravel and sand. The water is also more turbid and the channel is wider with less canopy cover. The *"lower reach"*, which is at the downstream end of the river, has the lowest gradient and therefore very slow flow. This results in a substrate that is dominated by gravel, sand and mud. There is however a very high water volume. The water is turbid with high sediment loads and the channel is so wide that canopy cover is restricted to the banks. In accordance with the decision to refer to use the term zone rather than reaches these three sections will be referred to as a *mountain stream zone*, a *middle zone* and a *lower zone* respectively in this study.

Within an aquatic environment, a mosaic pattern similar to that observed in a terrestrial environment is evident. In the case of the terrestrial environment the vegetation patches, which consists of patches of grass and clumps of trees form the parts of the mosaic and are spatially separated from one another. In the aquatic environment these patches result from an array of *inter alia* environmental components, which includes substrate, water depth, water velocity and cover. This mosaic leads to what is commonly refereed to as horizontal heterogeneity and which eventually adds to the physical complexity of community structure.

Each species can survive only in certain limited ranges of physical conditions that have been determined by its evolutionary past (Davies and Day, 1998). The variety of organisms in a river, and in particular the species that are found in a specific habitat or biotope, may thus be related to the physical conditions and consequently to the adaptation of the fish to these conditions. The preferential occupation or use of habitat is referred to by a number of terms in the literature. It would appear that the terms habitat utilization (Werner *et al.*, 1977), habitat partitioning (Werner *et al.*, op cit.), habitat preference (Gaigher, 1973; Felley and Hill, 1983) and habitat use (Wikramanayake and Moyle, 1989; Facey and Grossman, 1992; Wood and Bayne, 1995) are all used to describe a similar aspect.

Werner *et al.* (op cit.) mentioned that the patterns of habitat utilization and habitat partitioning are not known in all groups of organisms and where known the majority of knowledge is about terrestrial organisms. However a number of investigators have investigated habitat preference in freshwater fish and have *inter alia* shown that intraspecific differences exist in the spatial distribution of size classes (Werner *et al.*, 1976), that species could be segregated on the basis of microhabitat preference and that microhabitat utilization was so similar in each stream that it was possible to predict assemblages (Wikramanayake and Moyle, 1989) and that microhabitat use is velocity related (Facey and Grossman, 1992). As far as the cyprinids are concerned, Felley and Hill (1983) showed that seasonal and velocity related differences in microhabitat preference were evident.

With regard to the *L. marequensis* some work has been done on its habitat preference. According to a number of authors (Crass, 1964; Pienaar, 1978; Bruton *et al.*, 1982; Bell-

Cross and Minshull, 1988) the lowveld largescale yellowfish is “very partial” to deep, rocky pools where the current is swift and strong. They also report that it frequents sandy stretches of rivers as well as reed-fringed pools in both perennial and seasonal streams. Jubb (1967) pointed out that the species occur where rapids end in a pool. In addition Gaigher (1973) *inter alia* showed that the species favoured pools and rapids mainly in perennial streams and also referred to the species as “unspecialized with a wide distribution and dependent on rapids for breeding purposes”. The fact that they are absent from the lower Limpopo because it is devoid of rapids illustrates the breeding dependence. In the Groot Letaba River Chutter and Heath (1993) reported that the preferred habitat consisted of pools, rapids and man-made impoundments. None of the above work could be regarded as very specific with regard to the finer scale habitat preferences and in particular did not address the empirical aspects of the preferences.

Fouché *et al*. (2003), when comparing two sites in the Mutale and the Luvuvhu rivers, found that the species occurred in 33 % of the fast flowing (>0,3m/s) habitats surveyed in the Mutale River and in 96% of the same habitats surveyed in the Luvuvhu River.

Where the majority of work referred to in the preceding sections was based on expert observation, the work done by Russell (1997) in the Kruger National Park statistically underpinned some of the preferences. Russell (op cit.) *inter alia* showed that the habitat preference of the lowveld largescale yellowfish: a) lies midway between the rapids and marginal area but b) is not always associated with rapids but frequently with strongly flowing waters. He further pointed out that the species mostly occur in sites with a stony substratum and specifically where gravel and cobbles are dominant and that it is predominantly recorded in sites with fringing vegetation but is not collected among aquatic vegetation. This latter aspect probably indicates that their dependence on aquatic plants is unknown but it is most likely low.

Although the abovementioned presents a general view of what the habitat preferences of the species it was still regarded as important to establish the microhabitat requirements of the species. This gave rise to a number of questions such as: How heterogeneous should the habitat be in order to sustain the species? Is the habitat, and for that matter microhabitat, preferences of the various life stages of similar or are different requirements displayed by the different life stages? Are there specific determinant environmental components that can be related to the absence or presence of the species or its life stages on both habitat and biotope level?

This component of the project therefore aimed to establish: a) the general ecology of the sites where the species occur, b) the habitat preferences of the various life stages of the species, c) the correlation between the various habitat components and the species and d) the population structure of the species in the river.

For the assessment and evaluation of the habitat preference of the species it was regarded as important that habitat and its components be properly defined. According to Bain and Stevenson in Kleynhans *et al.* (2005) habitat is the "specific type of place within an ecosystem occupied by an organism, population or community that contains both living and nonliving components with specific biological, chemical, and physical characteristics including the basic life requirements of food, water, and cover or shelter". The term "habitat component" refers to "a single element (such as velocity, depth, or cover) of the habitat while habitat diversity refers to the number different habitat types within a given area.

The key to understanding "patterns of diversity among stream fishes lies within the diversity of the habitat and then specifically within the definition, understanding and measurement of relevant habitat components and characteristics" (Gorman and Karr, 1978). In order to assess and map habitat diversity, and to bring the data in line with current developments in South Africa, four velocity-depth classes (Kleynhans, 2007), also referred to as hydraulic habitat types (Jordanova *et al*., 2004) were distinguished. In this classification the cut-off point regarding depth was taken as 0,5 m with depths less than 0,5 m regarded as shallow and vice versa. On the same basis the cut-off point for velocity is taken as 0,3 ms^{-1} with velocities below 0,3 ms^{-1} regarded as slow and those above as fast. These resulting classes therefore are the following:

i) Slow-shallow habitats that include shallow pools and backwaters.

ii) Slow-deep habitats that include deep pools and backwaters.
iii) Fast-shallow habitats that include shallow runs, rapids and riffles.
iv) Fast-deep habitats which consist of deep runs, rapids and riffles.

This proposed velocity-depth classes was used in the study. Within each of these velocity-depth classes Kleynhans (2007) identified five broad cover types regarded as important to fish namely: overhanging vegetation, undercut banks and root wads, substrate, aquatic macrophytes and water column. These cover types were included in this study where they together with velocity were regarded as the "habitat components".

5.2 Materials and methods.

5.2.1 Site selection.

Sites, where *L. marequensis* were likely to thrive, were selected in the Luvuvhu River and its tributaries. The selection was based on historical data and care was taken that each site was representative of the specific river zone in which it occurred. It was also regarded as imperative that each site should have a well defined pool-riffle-pool sequence. The coordinates and altitude of each site were determined with a Garmin 76CSx GPS and recorded on the field form (Appendix III).

5.2.2 Habitat preference.

5.2.2.1 General habitat characteristics.

At each site the pH, dissolved oxygen, conductivity, total dissolved solids and temperature was determined *in situ* with handheld Eutech meters. In order to establish the water quality parameters of each river section, representative sites were selected in the main stem of the Luvuvhu River and tributaries. At these selected sites a water sample was collected for laboratory analyses.

5.2.2.2 Habitat description and mapping.

During the first site visit, each site was photographed and the possible habitats, based on the criteria in table 5.1, were identified and demarcated by judging the velocities and depths. The actual depths and velocities in each habitat were then measured. In cases where the depths and velocities in an identified habitat indicated the presence of more than one depth-velocity class, the habitat was subdivided into smaller units. Each of these units was then regarded as a microhabitat and was referred to as *"biotopes"* in this study. The decision to use this term in stead of microhabitat was based on the description of Rowntree and Wadeson (1998) that used the term biotope for "spatially distinct instream flow environments determined by temporally variable hydraulic and substrate characteristics" and that of Davies and Day (1998) who refer to a biotope as "an area of uniform environmental conditions" and who regard a habitat as a "combination of biotopes that make up the living space of an organism".

A sketch map to illustrate the habitat heterogeneity at the site was then drawn on which the biotopes were delineated and numbered.

Table 5.1: The Velocity-depth classes proposed by Kleynhans (2007).

Flow-Depth Class	Velocity (ms^{-1})	Depth (m)
Slow-deep (SD)	Less than 0,3	0,5 m and deeper
Slow-shallow (SS)	Less than 0,3	Less than 0,5
Fast-deep (FD)	0,3 and above	0,5 m and deeper
Fast-shallow (FS)	0,3 and above	Less than 0,5

5.2.2.3 The habitat components within the biotopes.

In each biotope the maximum length and width were measured from which the approximate surface area was later calculated. The water depth was then measured at four randomly selected points throughout the biotope. At the same points the velocity was determined (i) at 0.6 of the water column depth (Jones *et al.*, 1984; Facey and Grossman, 1992; Harding *et al.*, 1998) and (ii) directly above the substrate in meters per second using a Pasco Explorer 2000 velocity meter.

Evaluation of the substrate composition have take on many forms with Jones *et al.* (1984) as well as Wikramanayake and Moyle (1989) categorizing and scoring the substrates after which a coarseness index was calculated. Although no index was calculated, a similar method was followed in this study. To determine the substrate composition, the substrate type was determined at ten randomly selected points in each biotope. The substrate was classified as bedrock, boulder, cobble, pebble, gravel, sand or sediment according to the practical description listed in table 5.2. From the recorded data the percentage of each component was calculated and the dominant substrate class identified. A matrix that consisted of the dominant substrate type and the velocity depth class was constructed (Table 5.6) and this was used to classify the biotopes.

In each one of the recorded biotopes the overhanging vegetation and undercut banks, were identified, using the criteria shown in table 5.3 and their extent estimated and scored (Table 5.4). Because root wads and aquatic macrophytes are easy to identify no classification was needed but their abundance was scored in the same way.

Table 5.2: Substrate classification (adapted from Rowntree and Wadeson, 2000).

Substrate class	Size (mm)	Practical description (used in the field)
Bedrock	N/a	
Boulder	> 256	Larger than an adult head.
Cobble	64 – 256	Larger than an adult fist.
Gravel	2 – 64	From the size of a small pea à smaller than a fist.
Sand	0,06 – 2	Individual grains are visible.
Silt and clay	< 0,06	Powdery or soapy, grains not visible

Table 5.3: Classification of the estimated cover types (Kleynhans, 2007).

Cover type	Description
Overhanging vegetation	Vegetation that overhang the water surface by approximately 0.3m and that are not more than 0.1 m above the water surface.
Undercut banks	Banks that overhang the water surface by approximately 0.3 m and that are not more than 0.1 m above the water surface.

Table 5.4: Abundance scoring of cover types (adapted from Kleynhans, 2007).

Descriptor	Relative ecological value/ abundance score	Occurrence (% of area covered)
None	0	0
Rare	1	0 – 5
Sparse	2	6 – 25
Common/Moderate	3	25 – 75
Abundant	4	75 – 90
Very abundant	5	90 – 100

5.2.2.4 Rating of impacts.

Anthropogenic activities, which manifest as use of the surrounding land, in a specific river reach have an impact on the fish habitat integrity at the sites which are surveyed. The activities in a reach *inter alia* include weirs, impoundments, cultivated lands, grazing, plantations, industries, residences, roads, bridges and fishing. In addition activities such as water abstraction, flow- , bed- or channel modification at the site contribute to the impacts. In order to determine and compare the impacts at the sites, and in the reach, the impacts were assessed and classified using an adaptation (Fouché and Moolman, 2004) of the criteria proposed by Kleynhans (1999). The impact classes and their description are shown in table 5.5.

5.2.2.5 Comparison of the habitat diversity at the sites.

In order to compare the habitat diversity of the sites, each identified biotope was classified using a matrix (table 5.6) that combined velocity-depth classes with the dominant substrate.

Table 5.5: Descriptive classes for the assessment of impacts in the river reaches (Kleynhans, 2007).

Impact class	Description
None	No discernable modification. No impact on habitat quality, diversity, size and variability.
Small	The modification is limited to a few localities. Impact on habitat quality, diversity, size and variability is small.
Moderate	The modification is present at a small number of limited to a few localities. Impact on habitat quality, diversity, size and variability is limited.
Extensive	The modification is generally present with a clearly detrimental impact on habitat quality, diversity, size and variability. Large areas are however not affected.
Very extensive	The modification is frequently present and the habitat quality, diversity, size and variability in almost the whole of the area are affected. Only small areas are not influenced.
Critical	The modification is present overall with a high intensity. The habitat quality, diversity, size and variability in the whole of the area are affected detrimentally. Only small areas are not influenced.

Table 5.6: The matrix constructed to classify the biotopes.

	Dominant substrate					
Velocity-depth class	**Bedrock**	**Boulder**	**Cobble**	**Gravel**	**Sand**	**Silt**
FS	A	E	I	M	Q	U
FD	B	F	J	N	R	V
SS	C	G	K	O	S	W
SD	D	H	L	P	T	X

5.2.3. Sampling of fish.

There are various ways of linking fish to their habitats (Meng and Powell, 1999) and in *a priori* approach, which was followed in this study, habitat types were first identified after which fish are then sampled in each habitat type.

In fast-deep and fast-shallow biotopes the fish were electro-narcotized, using a 220V AC generator, and collected with scoop nets. In the slow-deep and slow–shallow biotopes, that were clear of snags, a small seine net (15m long X 1,5m deep with 10mm stretched mesh) was used. A pole-seine (2,5m long X 1,5m deep with 10mm stretched mesh) was used in the small pools, backwaters and in particular where sampling had to be done under and amongst vegetation. All the specimens collected were identified using the key from Skelton (2001). The following data from all the fish specimens collected were recorded on site: a) the number collected and b) the time of collection. The fork length of the *L. marequensis* specimens collected was determined and all the data were recorded in field form 1 (Appendix III).

5.2.4 Population studies.

In order to investigate the structural diversity of the population of *L. marequensis* in the Luvuvhu River all the fork length measurements taken over the whole period of the study were used to construct a length frequency distribution.

5.2.5 Laboratory analyses.

The water samples collected were placed on ice in a cool bag, transported to the laboratory and the following physico-chemical aspects determined the next day: chemical oxygen demand (COD), biological oxygen demand (BOD), total suspended solids (TSS), turbidity, ammonia, nitrates, sulfates and hardness. The nitrate, phosphate and calcium content as well as the alkalinity and hardness was determined with a Merck Reflectoquant photometer. The BOD and COD were determined with a Merck Spectroquant photometer. The turbidity was determined with a Novasina model 152 Nephelometer using double distilled water as a standard. The total suspended solids (TSS) was determined by calculating the weight increase after filtering a 200ml water sample through a filter paper of pre-determined mass.

5.2.6 Statistical analyses of the habitat preference of the species.

To investigate the habitat preference of the species and in particular the habitat preference of the size classes, two questions were formulated. The questions were: a) What local scale environmental variables are the best predictors of the presence of *L. marequensis*? b) Do the size classes of the species utilize different habitats and in particular biotopes?

Based on the above, the data analyses focused on the local scale i.e. the biotopes. Non-metric multi-dimensional scaling (MDS) was used to display the unconstrained relationships between fish sizes and the biotopes. An attempt was made to classify the biotope types using hierarchical agglomerative cluster analysis with group average linking in Primer v.6 (Clarke and Ainsworth, 1993). The robustness of the groups identified was tested by randomly permuting the similarity matrix that is used to construct the cluster classification. Relationship between fish and environmental variables were examined with redundancy analysis RDA, a constrained method of ordination using CANOCO V4.5, (Ter Braak and Šmilauer, 2002). A manual forward selection procedure was used to identify environmental variables that significantly explained species presence. This was done at biotope level and the relationships were displayed as tri-plots. The effects of environmental variables on the presence of *L. marequensis* were analysed with the BIO ENV procedure in Primer v.6 (Clarke and Ainsworth, op cit.).

Analyses of the length mass relationship of the species (see chapter 4) showed that, based on trends observed in the slopes, nine stanzas or growth phases could be identified in the life cycle of *L. marequensis*. It was therefore decided to establish whether the biotope utilization could be related to these stanzas. For the purpose of this investigation these stanzas were numbered from 1 – 9.

5.3 Results.

5.3.1 The sites selected.

Ten of the initial thirty five sites in the Luvuvhu River catchment, where L. *marequensi*s historically occurred, as reported on in chapter 3, were selected for the habitat preference study (Table 5.7 and Figure 5.1). They were selected for their habitat diversity and because they were regarded as representative of the river reaches in which they occur. Two additional sites, selected on the same criteria, were surveyed (table 5.8). The numbering of the sites is not related to the rivers but rather reflect their position within the river system as a whole.

Table 5.7: The ten initial sites in the Luvuvhu River catchment used in habitat studies.

River/Tributary	Site names	Site numbers	Altitude (m.a.s.l.)	Coordinates
Dzindi	Crocodile Ventures	3	549	S 22^{o} 59.05' E 30^{o} 20.05'
Luvuvhu	Gauging Weir (A9h001)	1	576	S 23^{o} 06.26' E 30^{o} 23.26 '
	Tshifudi bridge	11	447	S 22^{o} 50.57' E 30^{o} 45.09'
Mbwedi	Bridge above Mutshindudi/Mbwedi confluence	7	488	S 22^{o} 50.09' E 30^{o} 39.43'
Mutshindudi	Hydro bridge	4	636	S 22^{o} 56.21' E 30^{o} 29.18'
	Tshivhulani	5	550	S 22^{o} 54.54' E 30^{o} 20.05'
	New gauging weir	9	467	S 23^{o} 09.38' E 30^{o} 28.41'
	Malavuwe bridge	10	475	S 22^{o} 51.40' E 30^{o} 38.37'
Mutale	Tshirovha confluence	12	673	S 22^{o} 48.55' E 30^{o} 23.47'
Mukhase	Mphaphuli cycad reserve	8	523	S 22^{o} 48.62' E 30^{o} 38.87'

Table 5.8: The additional sites in the Luvuvhu River catchment used in habitat studies.

River/Tributary	Site names	Site numbers	Coordinates	Altitude (m.a.s.l.)
Luvuvhu	Tshino	2	S 23^{o} 06.26' E 30^{o} 23.26'	576
Tshinane	Bridge near jail	6	S 22^{o} 54 2.04’ E 30^{o} 31 37.26’	528

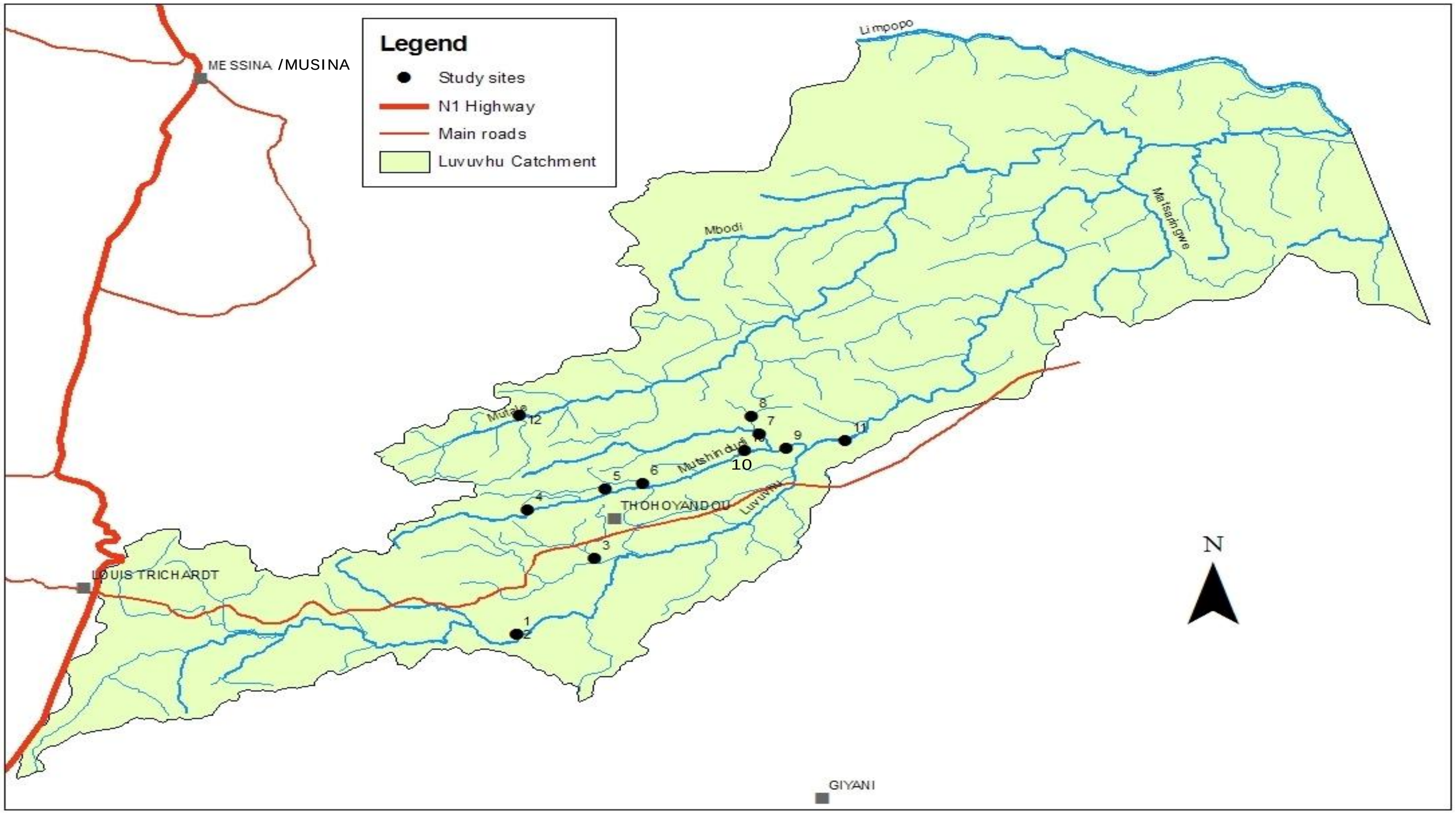

Figure 5.1: A map of the Luvuvhu River showing the sites used in the habitat preference study.

5.3.2 A general description of the surveyed sites and the impacts recorded.

The listing and the ratings of the impacts for each reach of the river in which the sites are situated form part of the site descriptions that follow below. The impact ratings at each site is however shown as averages in figure 5.31.

Site 1: “Gauging Weir A9h001 at Tshino”.

Site 1 is an historic site that was used in the River Health Programme and is situated at an old crossing in the main stem of the Luvuvhu River. Although there are a number of biotopes varying in depth and velocity (Figure 5.2), the substrate was found to be homogenous and dominated by cobbles. The site lacked marginal and aquatic vegetation cover. The only exception was biotope 1.1 where reeds were found at the fringes. Impacts in the river reach, that could negatively impact aquatic biota, included the weir upstream of the site, sand mining, commercial agriculture and grazing. Except for grazing, that were rated as “extensive”, all the other impacts were rated as “very extensive”.

Site 2: “Tshino 2”.

This site, which is *ca* 400m downstream of site 1, was selected because of the presence of a deep pool, which the former site lacked (Figure 5.3). Although only biotopes 2.1 and 2.4 had overhanging vegetation, the site had a more diverse substrate composition with some gravel and more boulders. The same impacts and ratings recorded at the previous site applied here.

Site 3: “Crocodile Ventures”

The substrate in the biotopes at this site in the Dzindi River (Figure 5.4) consists mostly of bedrock and boulders but sand and silt were found in biotopes 3.4a, 5 and 3.9. In biotopes 3.4a and 5 overhanging vegetation, rated as “sparse” and “moderate”, (Table 5.3) were present. Biotope 3.4 had a dense reed fringe and “abundant” root wads, which were remnants of an old growth of reeds, were present in biotope 3.3a. The site is located at the upper end of the “middle zone” of the river.

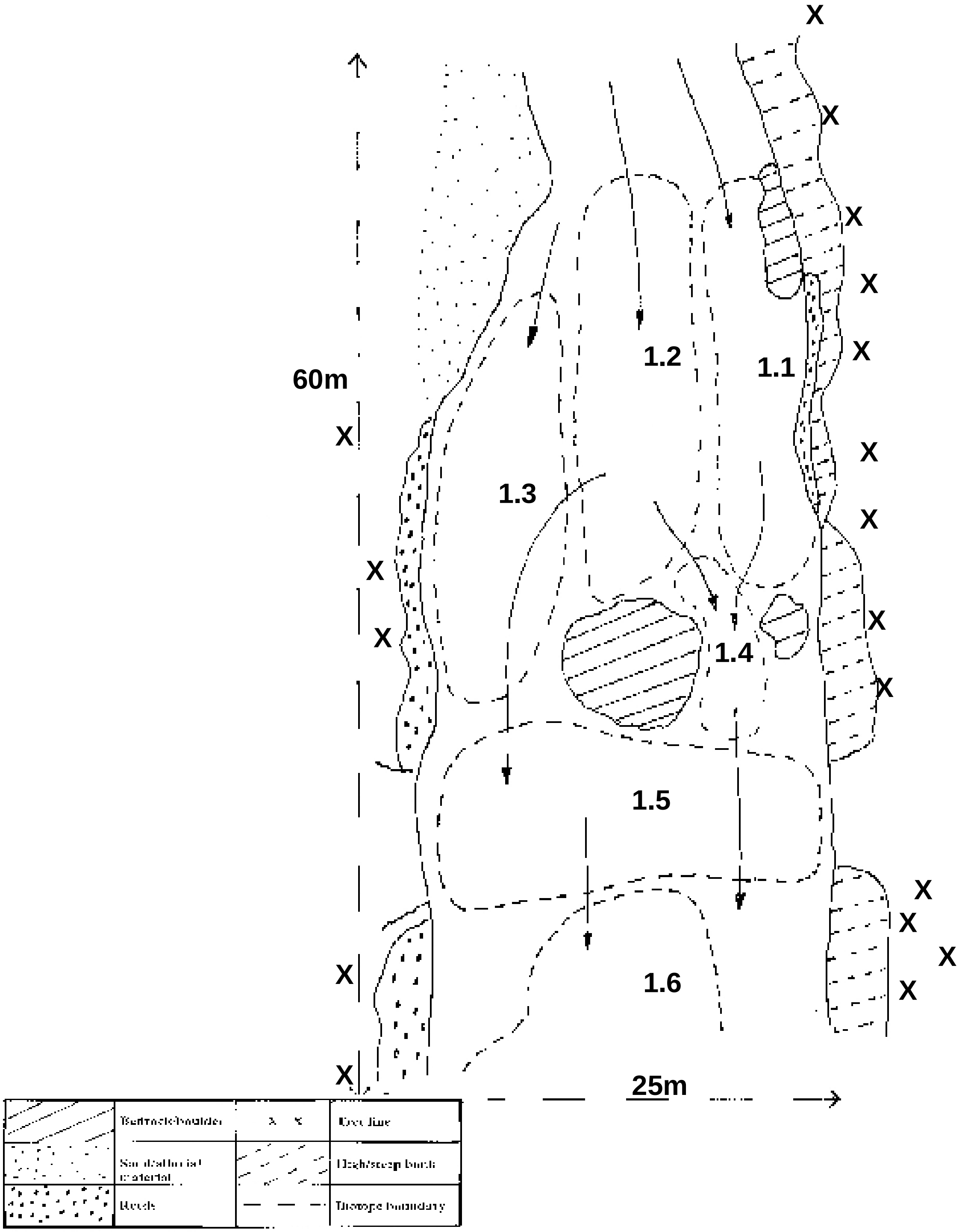

Figure 5.2: Sketch map of the site at the Gauging weir (Site 1) in the Luvuvhu River with areas numbered 1.1 – 1.6 showing the biotopes identified. (The arrows indicate the direction of flow).

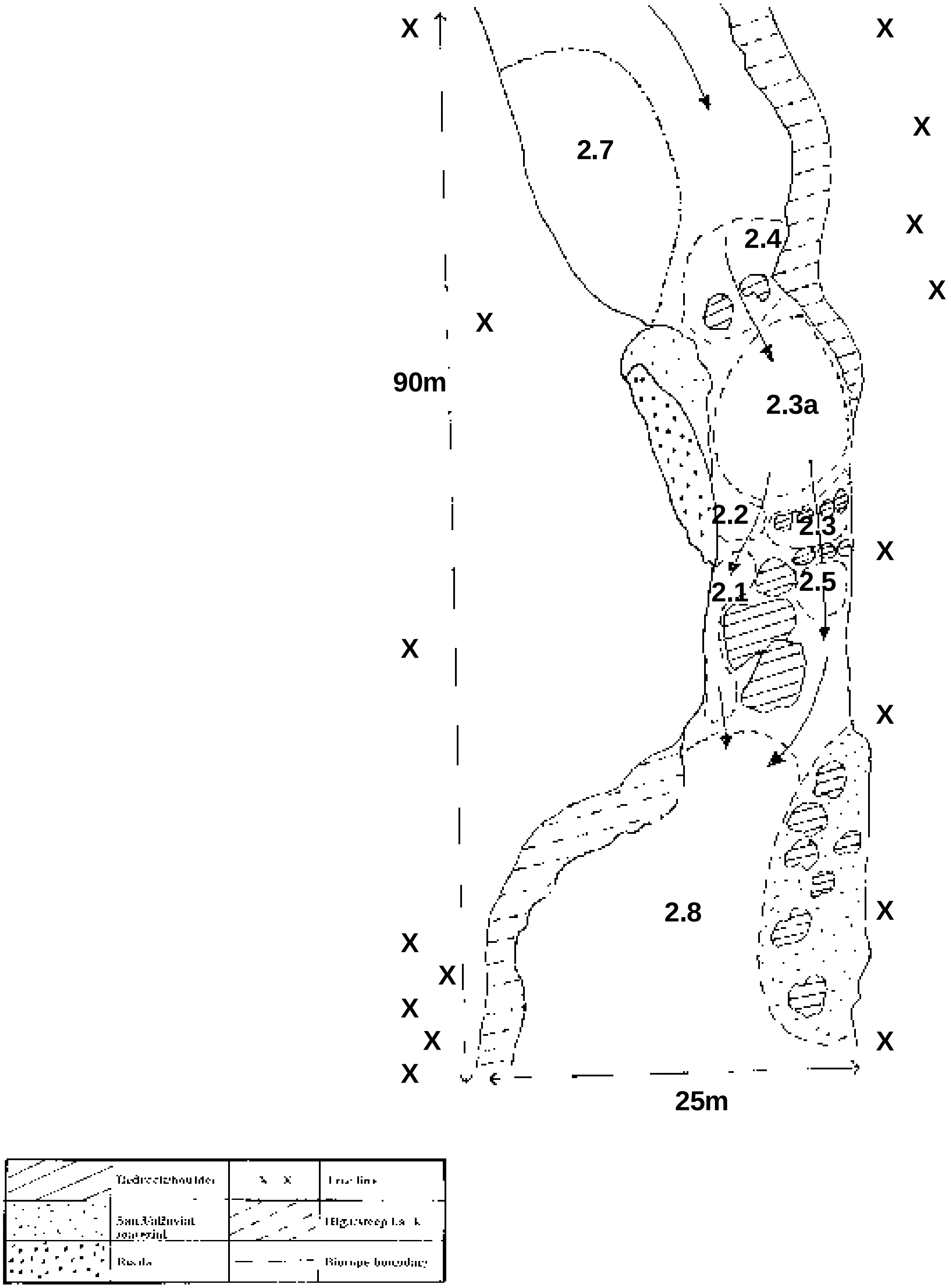

Figure 5.3: Sketch map of the site at Tshino (Site 2) in the Luvuvhu River with areas numbered 2.1 – 2.7 showing the biotopes identified. (The arrows indicate the direction of flow).

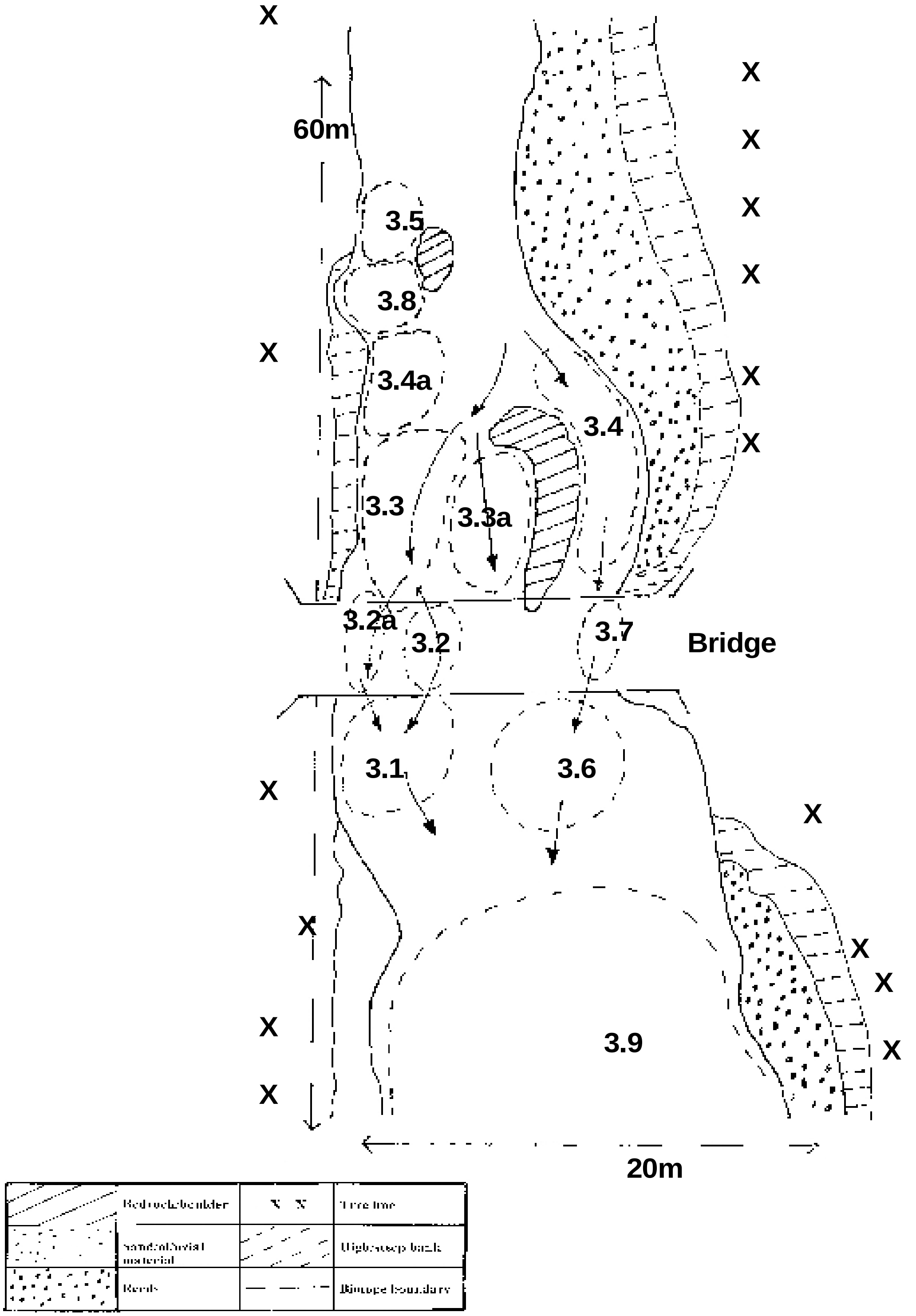

Figure 5.4: Sketch map of the site at Crocodile Ventures (Site 3) in the Dzindi River with areas numbered 3.1 – 3.9 showing the biotopes identified. (The arrows indicate the direction of flow).

Impacts in this river reach that could negatively impact aquatic biota consisted of subsistence farming, grazing and human settlements. Where the first two impacts were rated as “moderate”, the latter was rated as “extensive” because the presence of solid waste material in the aquatic habitat.

Site 4: “Phiphidi hydro bridge”.

This site in the upper reaches of the Mutshindudi River (Figure 5.5) is immediately downstream of a geographical barrier, the Phiphidi waterfall. The substrate composition of the biotopes, which is predominantly coarse alluvial material, is typical of a “mountain stream zone”, in which the site is located. The only exception is biotope 4.11, a deep pool, where the bottom substrate was covered with a thick layer of sediment and snags in the form of cut branches and other plant material. This was the only biotope where “abundant” root wads were observed. Except for the tree canopy on the left hand bank the only vegetation in the other biotopes were “rare” overhanging vegetation in biotope 4.15, “moderate” overhanging vegetation in biotope 4.2 and “rare” aquatic vegetation in biotope 4.16. Only “sparse” root wads were found in biotopes 4.12 and 4.15. The impacts in the river reach included plantations, commercial agriculture, settlements, grazing, roads and grazing which were all rated as “extensive". The effects of the impacts were particularly evident in the high rate of sedimentation observed in the biotope 4.11.

Site 5: “Tshivhulani”.

This site in the Mutshindudi River is a few kilometers downstream of site 4 and typical of a river zone at beginning of the “middle zone” where the water velocity starts slowing down (Figure 5.6). This is evident in the presence of finer alluvial material in the majority of the biotopes where gravel formed 70% of the substrate in biotope 5.3 and gravel and sand each formed 25% of the substrate in biotope 5.6. Although no aquatic macrophytes were observed, overhanging vegetation ranged from “very abundant” in biotope 5.5 to “moderate” in biotope 5.2 and “rare” in biotope 5.3. Undercut banks, rated as “abundant” in biotope 5.1, and root wads, rated as “abundant” in biotope 5.1 and “moderate” in 5.5 contributed to the heterogeneity of the site. Grazing and commercial farming, rated as “extensive” and “very extensive” respectively, are the most important impacts in this reach of the river.

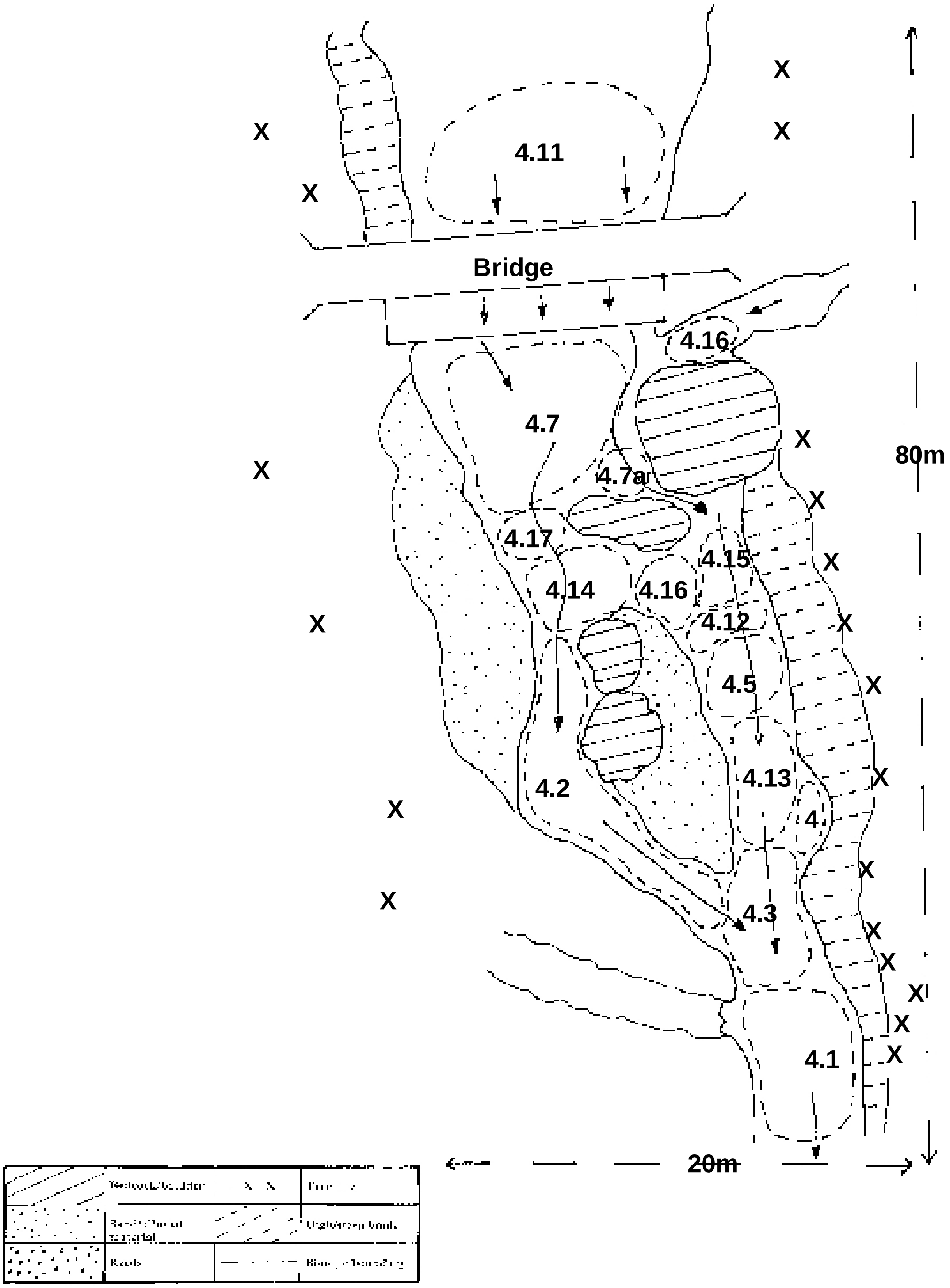

Figure 5.5: Sketch map of the site at Hydro bridge (Site 4) in the Mutshindudi River with areas numbered 4.1 – 4.16 showing the biotopes identified. The arrows indicate the direction of flow.

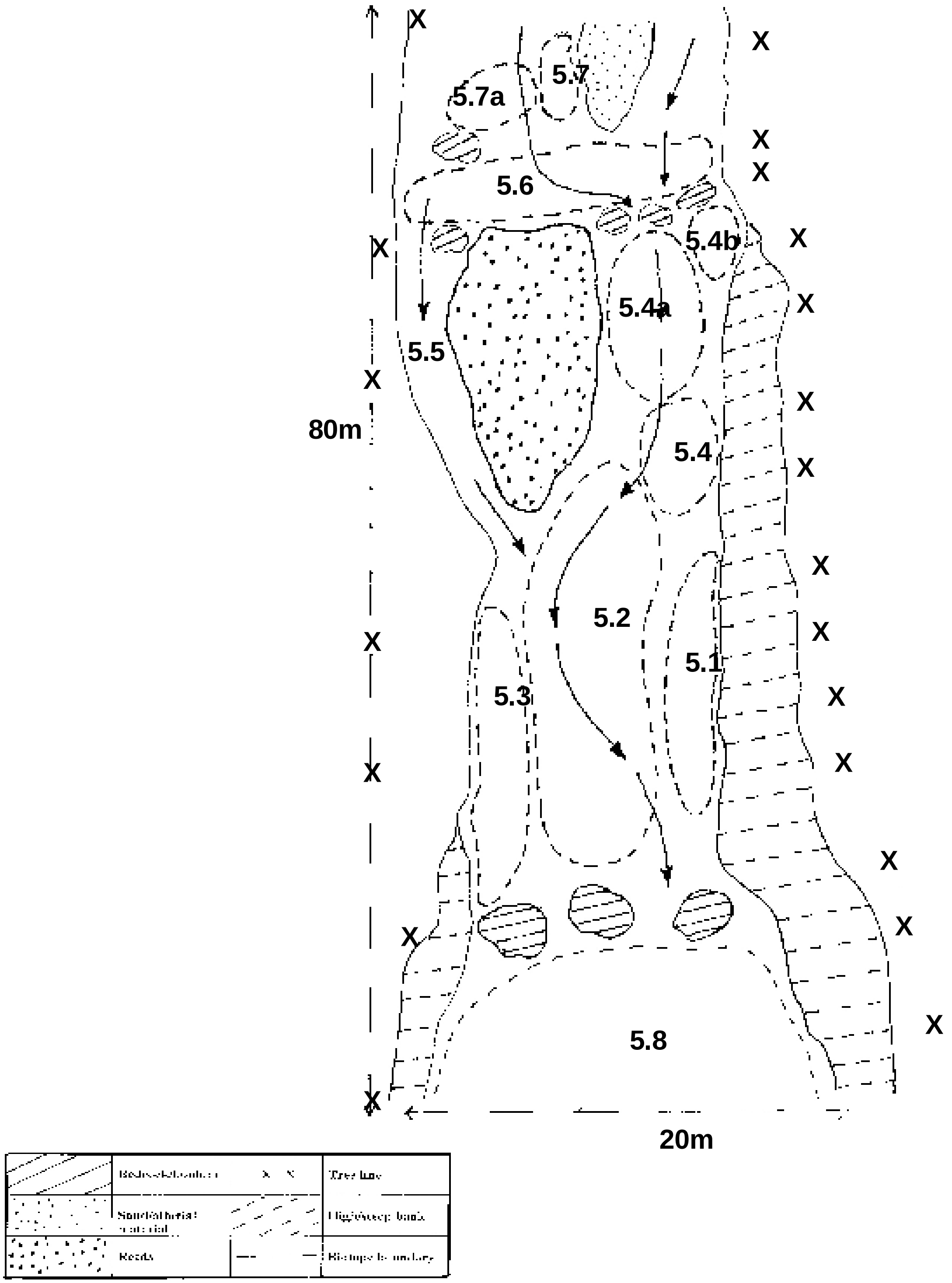

Figure 5.6: Sketch map of the site at Tshivhulani (Site 5) in the Mutshindudi River with areas numbered 5.1 – 5.8 showing the biotopes identified. The arrows indicate the direction of flow.

Site 6: “Bridge near jail”.

This site in the Tshinane River is at the lower end of the “mountain stream zone” of the river and is impacted by commercial farming and weirs, both rated as “very extensive”, as well as by residential areas where the impact was rated as “extensive” (Figure 5.7).

The substrate of the biotopes is dominated by boulders and cobbles but some finer alluvial material, namely gravel, sand and silt, were recorded in biotopes 6.3, 6.5 and 6.6. Other fish cover consisted of overhanging vegetation which was “moderate” and “sparse” in biotopes 6.3 and 6.6 respectively, undercut banks and root wads, both rated as “moderate” in biotopes 6.3 and 6.6.

Site 7: “Bridge above Mutshindudi/Mbwedi confluence”.

The substrate at this site in the Mbwedi River is dominated by cobbles with only a few boulders in the hydraulic control and some sand in biotopes 7.3, 7.3a and 7.5 (Figure 5.8). This is typical of a river reach at upper end of the “middle zone” of a river. Except for reeds fringes at biotopes 7.3, 7.5 and 7.6 the only other vegetation observed was the “sparse” overhanging vegetation in biotopes 7.5 and 7.6. In these two biotopes the root wads were rated as “moderate” and the undercut banks as “sparse”. The impacts consisted of roads, bridges, grazing and water abstraction at the site. The first two impacts were rated as “extensive” and the latter two as “moderate”.

Site 8: “Mphaphuli cycad reserve”.

This site in the Mukhase River is in a typical “mountain stream zone” where coarse alluvial and bedrock dominate the substrate (Figure 5.9). The only other alluvial material found in the biotopes was small amounts of sand that were observed in biotopes 8.3, 8.4, 8.8 and 8.10. The substrate of biotopes 8.9 and 8.9a, which together formed a deep pool, also contained sand but it was covered with leaves and other organic material of allochthonous origin. The latter was recorded as silt. In the deep pool (biotopes 8.9 and 8.9a) the cover for fish included undercut banks, root wads and overhanging vegetation all which were rated as “abundant”. Biotope 9a was the only biotope where “abundant” aquatic vegetation was recorded. Being within a protected area, all the possible impacts were rated as “small”.

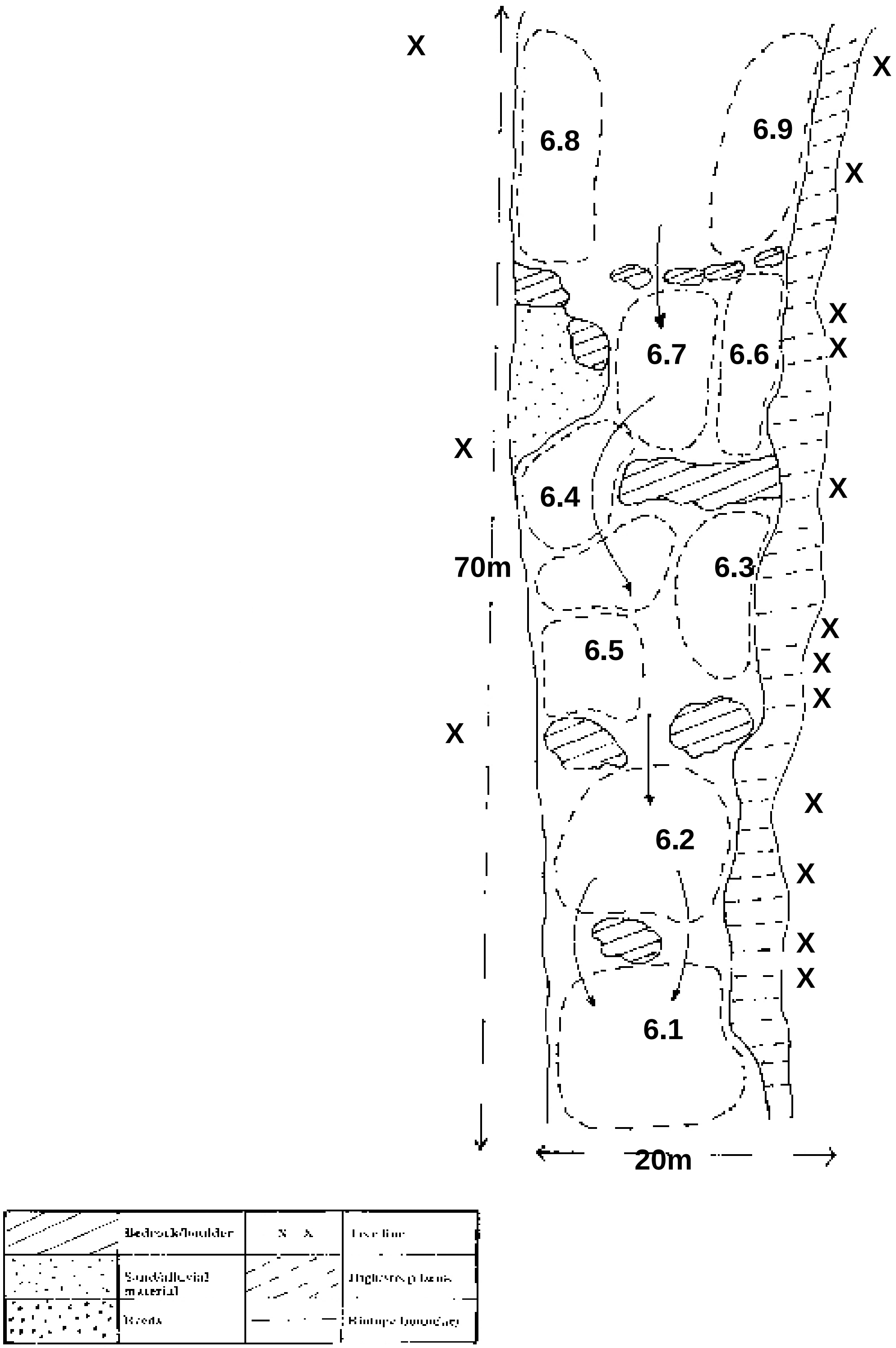

Figure 5.7: Sketch map of the site at Bridge near the jail (Site 6) in the Tshinane River with areas numbered 6.1 – 6.9 showing the biotopes identified. The arrows indicate the direction of flow.

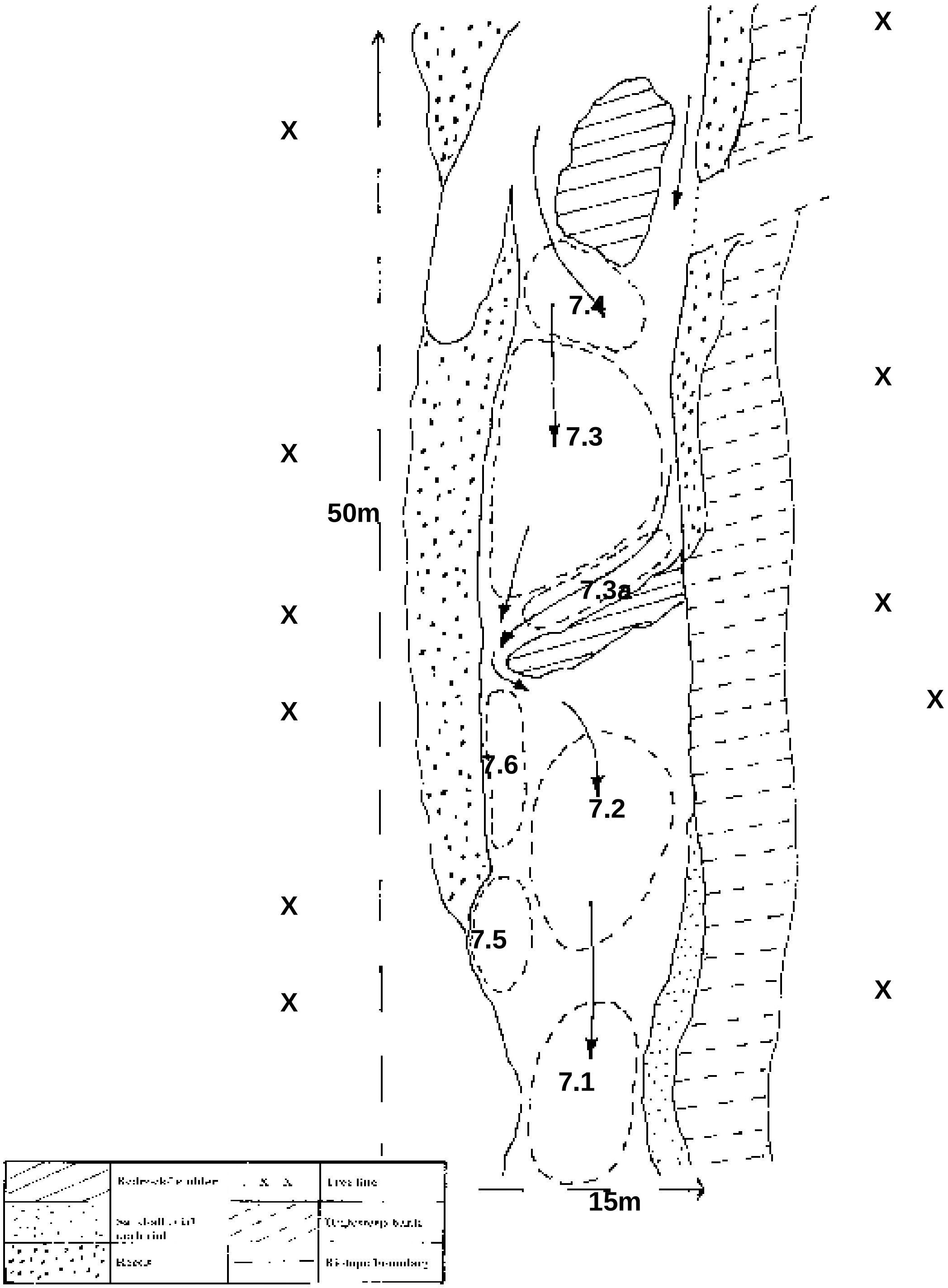

Figure 5.8: Sketch map of the site at the bridge above the confluence with the Mutshindudi River (Site 7) in the Mbwedi River with areas numbered 7.1 – 7.6 showing the biotopes identified. The arrows indicate the direction of flow.

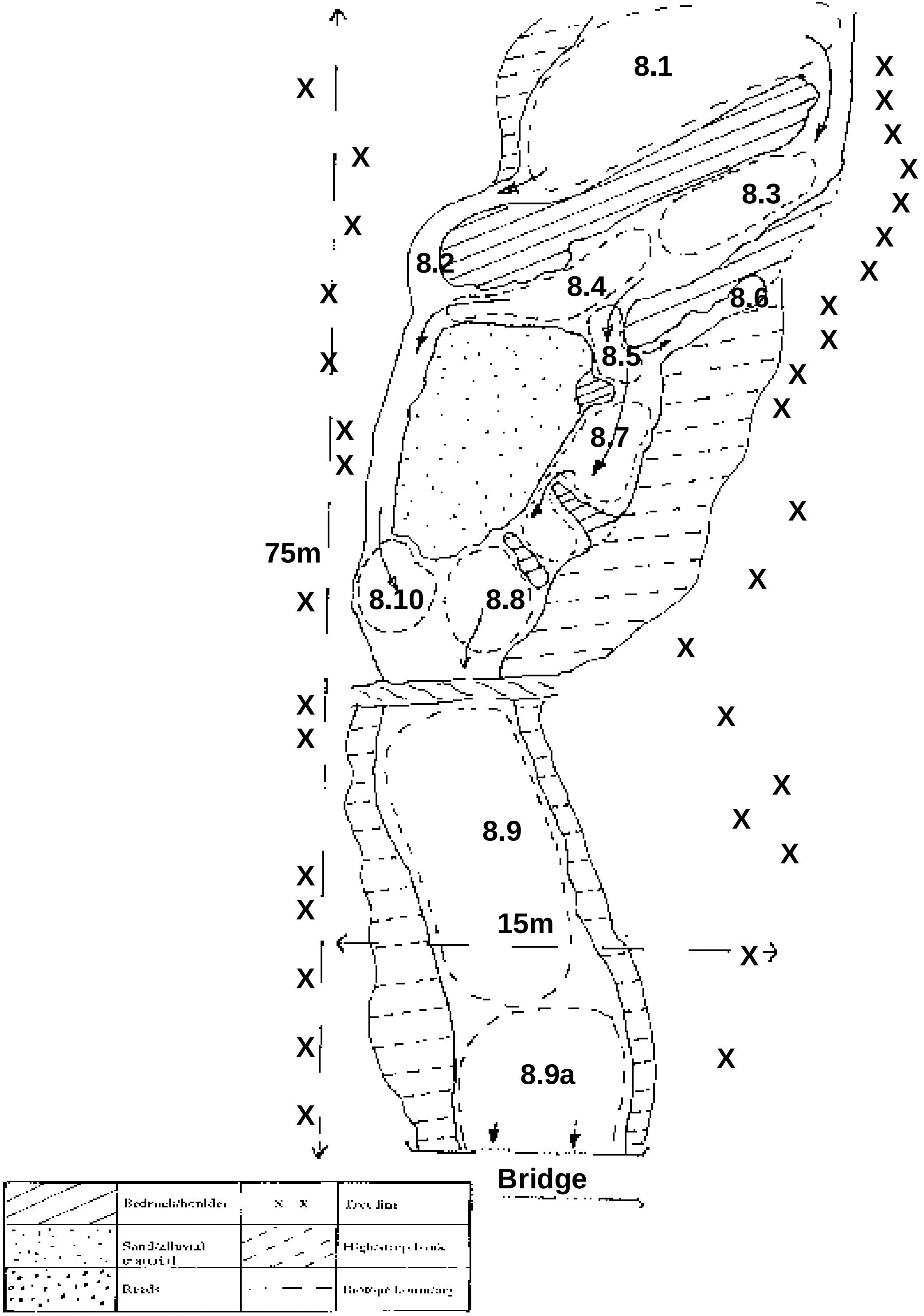

Figure 5.9: Sketch map of the site in the Mphaphuli Cycad Reserve (Site 8) in the Mukhase River with areas numbered 8.1 – 8.10 showing the biotopes identified. The arrows indicate the direction of flow.

Site 9: "New Gauging weir".

This site is in the Mutshindudi River, downstream of the Mbwedi River confluence. The river reach where the site is located, as is usually the case in a transition zone between "middle zone" and "lower zone", is characterized by long stretches of sandy bottom (Figure 5.10). The site however includes biotopes with coarser alluvial material, as is for example the case with biotope 9.5 where only boulders and cobbles occur, and biotopes 9.6, 9.8, 9.10 and 9.12 which are dominated by bedrock. Other cover for fish consisted of overhanging vegetation, rated as "sparse" in biotopes 9.2, 9.5 and 9.13, undercut banks, rated as "sparse" in biotopes 9.5 and 9.13 and lastly root wads rated as "sparse" in biotope 9.13. The double set of weirs and the water abstraction at the site as well as the erosion of the bank were the most important impacts recorded and were rated as "very extensive". The bank erosion is primarly caused by an access road that was constructed to service the weir.

Site 10: "Malavuwe Bridge".

This site is in the Mutshindudi River (Figure 5.11) upstream of the confluence with the main stem of the Luvuvhu River. The reach in which the site is located, is in the upper parts of the "lower zone". There are therefore long stretches in which the substrate is dominated by sand and mud and very few hydraulic controls that consist of coarse alluvial material are present. The site is located on one of these scarce hydraulic controls and with the exception of biotopes 10.1, 10.4 and 10.6 all the substrate consists of cobbles. Biotope 10.1 is a shallow pool, where the substrate consists mostly of silt with some sand while biotopes 10.4 and 10.6 are shallow sand dominated pools. Impacts in the reach consist of roads, bridges and crossings, grazing, subsistence agriculture and human settlements. All of these impacts were rated as "extensive". Except for "moderate" root wads, mostly associated with reeds, in biotopes 10.1, 10.4 and 10.5, "rare" undercut banks in biotope 10.5 and aquatic macrophytes rated as "rare" in 10.4 and 10.5, the only cover for fish was offered by the coarse alluvial material in the substrate.

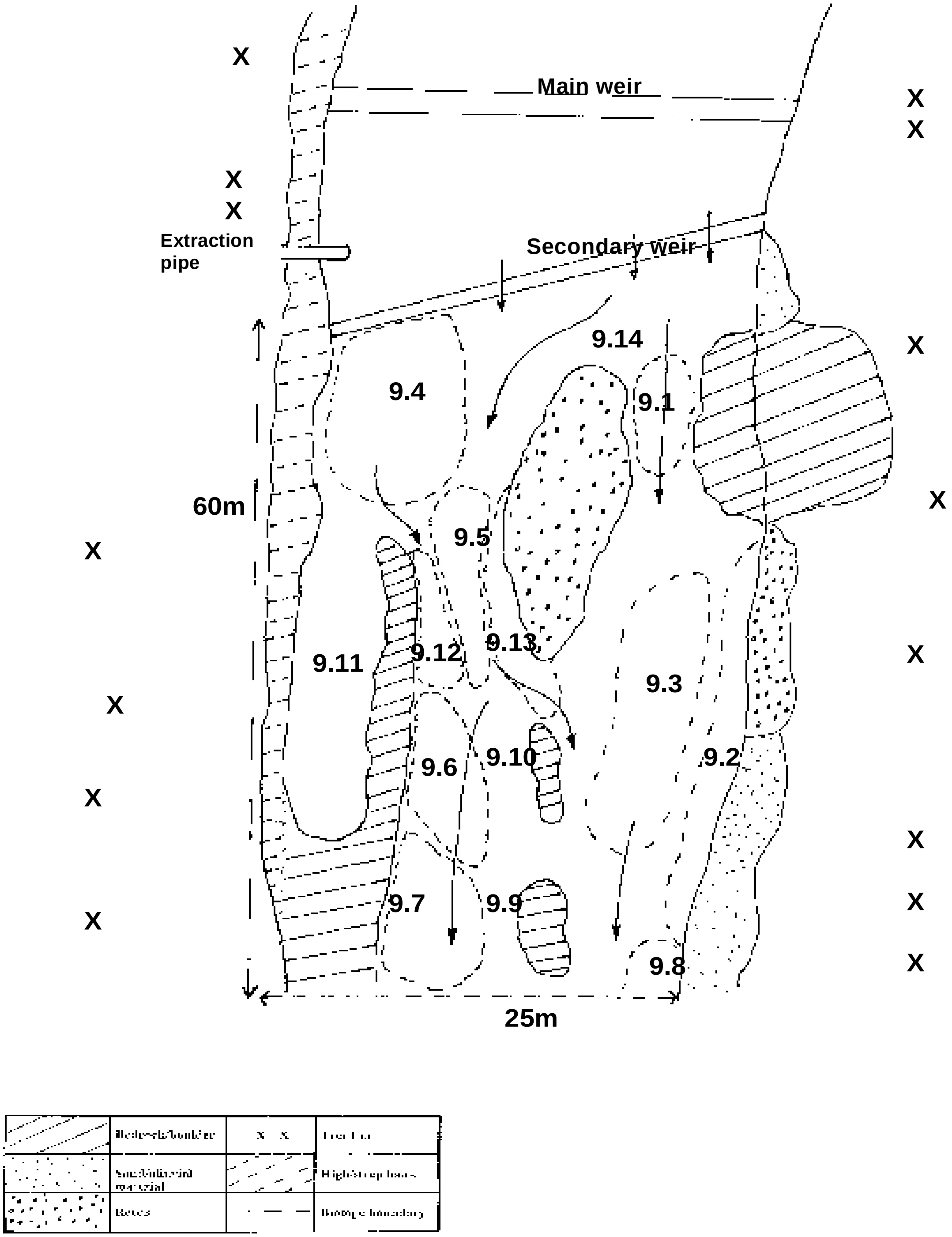

Figure 5.10: Sketch map of the site at the New Gauging weir (Site 9) in the Mutshindudi River with areas numbered 9.1 – 9.14 showing the biotopes identified. The arrows indicate the direction of flow.

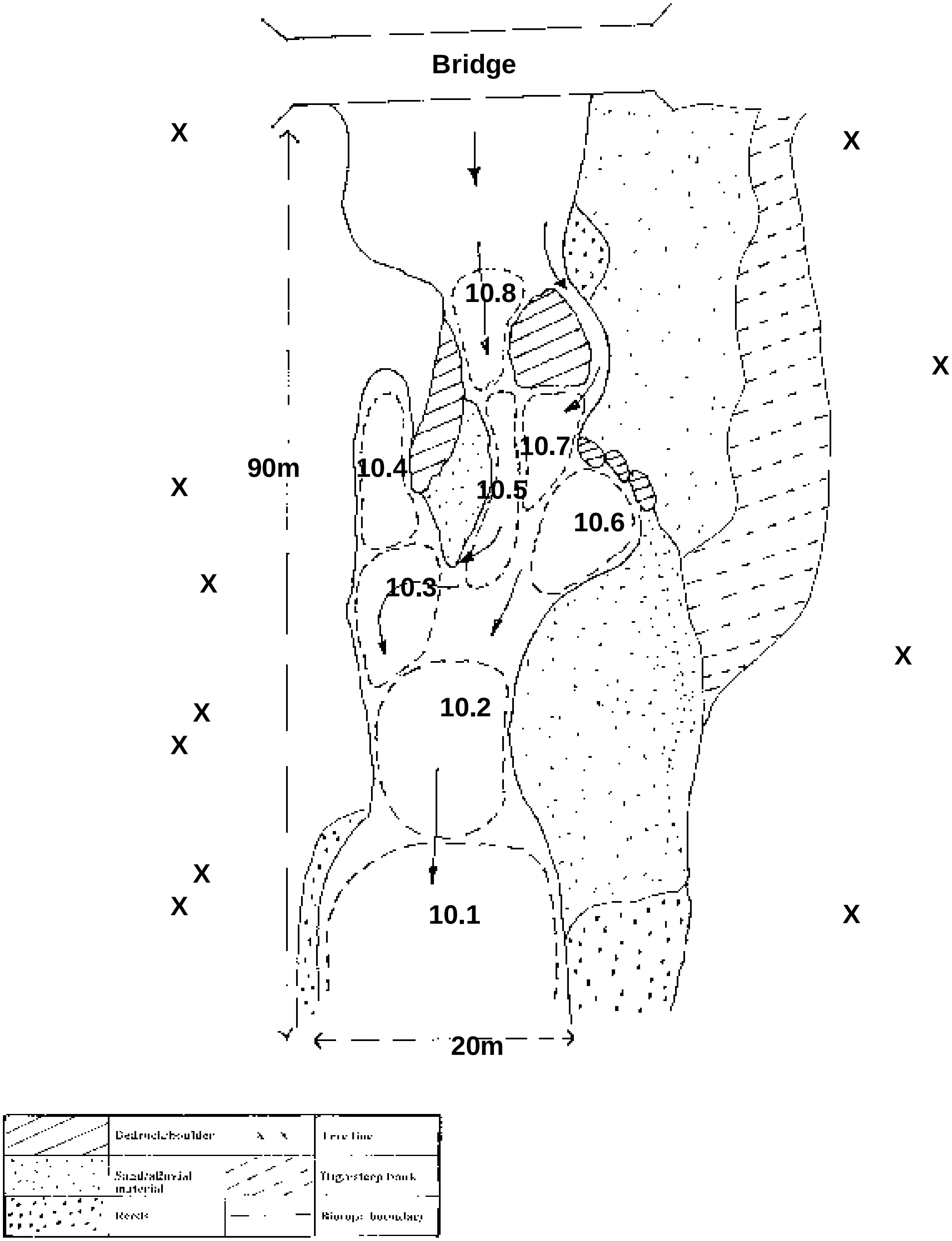

Figure 5.11: Sketch map of the site at Malavuwe bridge (Site 10) in the Mutshindudi River with areas numbered 10.1 – 10.8 showing the biotopes identified. The arrows indicate the direction of flow.

Site 11: “Tshifudi” bridge.

The reach in which this site is located is similar to the reach of the Mutshindudi River where site 10 is situated. The similarity refers to the fact that is a sand dominated river reach where hydraulic controls are scarce. As is the case at site 10, the impacts in this reach consist of roads, bridges and crossings, grazing, subsistence agriculture and human settlements and all were rated as “moderate”. To include as many biotopes as possible the actual site was divided into two sub-sites (Figures 5.12 and 5.13). Sub-site 11a where the substrate was mostly cobbles and gravel was regarded as the more homogenous of the two sub-sites. The exception was biotope 11.5b where some boulders were recorded and biotope 11.3 that is dominated by sand. Other fish cover at this site consisted of overhanging vegetation, rated as “very abundant” in biotope 11.2 and “abundant” in 11.3 and 11.5b, and “sparse” and “moderate” root wads in 11.2 and 11.5b respectively. Although cobbles form part of the substrate of the majority of the biotopes at sub-site 11b, the sub-site was more substrate diverse in that boulders were recorded in biotopes 11.6 and 11.7. In addition the other forms of fish cover were also more diverse. Overhanging vegetation and undercut banks rated as “abundant” were recorded in biotopes 11.8, 11.9 11.10 and 11.13 while root wads were rated as “abundant” in biotopes 10.8 and 10.9 and “moderate” in biotopes 11.10 and 11.13. The presence of the deep pool at the downstream end of sub-site 11b added to the heterogeneity and biotope diversity of the site.

Site 12: “Tshirovha-Mutale River confluence”.

This site in the Mutale River, at an altitude of 673m a.s.l., is the “highest” site included in the habitat studies and is a typical site associated with the “mountain stream zone”. The substrate in the biotopes is dominated by boulders with cobbles with sand only observed on the lateral sides of the deep pool (biotope 12.1) and biotope 12.2. As is typical in this part of a river the major part of the water surface is shaded by the leaf canopy. Overhanging vegetation was only present on the right hand bank of the pool (biotope 12.1) and in biotope 12.6a where it was rated as “moderate” and “sparse” respectively. Other forms of cover for fish consisted of undercut banks, rated as “moderate” in biotopes 12.1, 12.6a and 12.8, and root wads, rated as “moderate” in biotopes 12.1, 12.3, 12.6, 12.6a, 12.7 and as “abundant” in 12.8. The only biotope where aquatic macrophytes were observed was 12.6a where it was rated as “rare”.

The only impacts in this reach of the river, all rated as “small” included grazing, vegetation removal and one crossing upstream of the site.

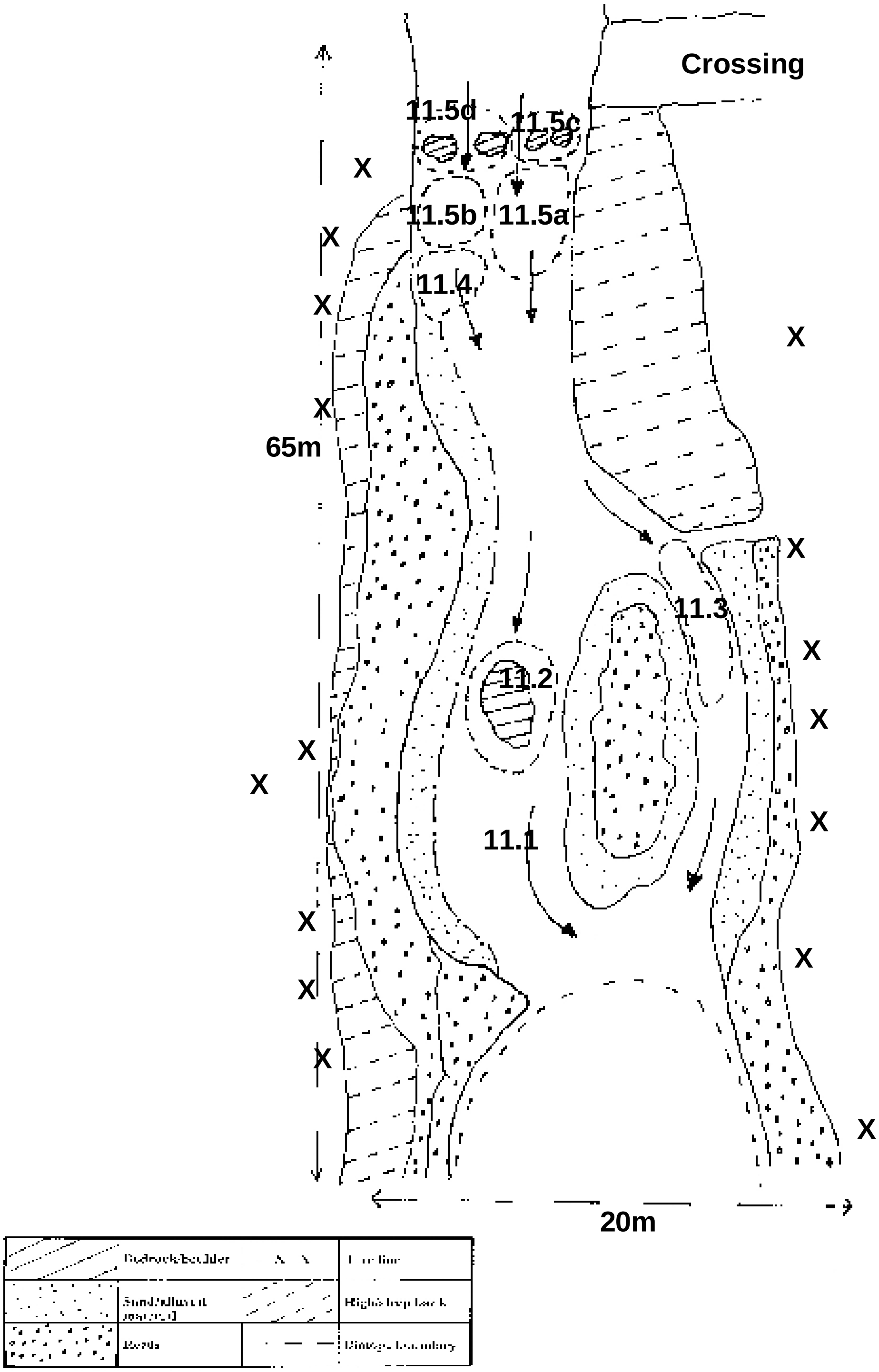

Figure 5.12: Sketch map of the sub-site at Tshifudi bridge (Site 11a) in the Luvuvhu River with areas numbered 11.1 – 11.5 showing the biotopes identified. The arrows indicate the direction of flow.

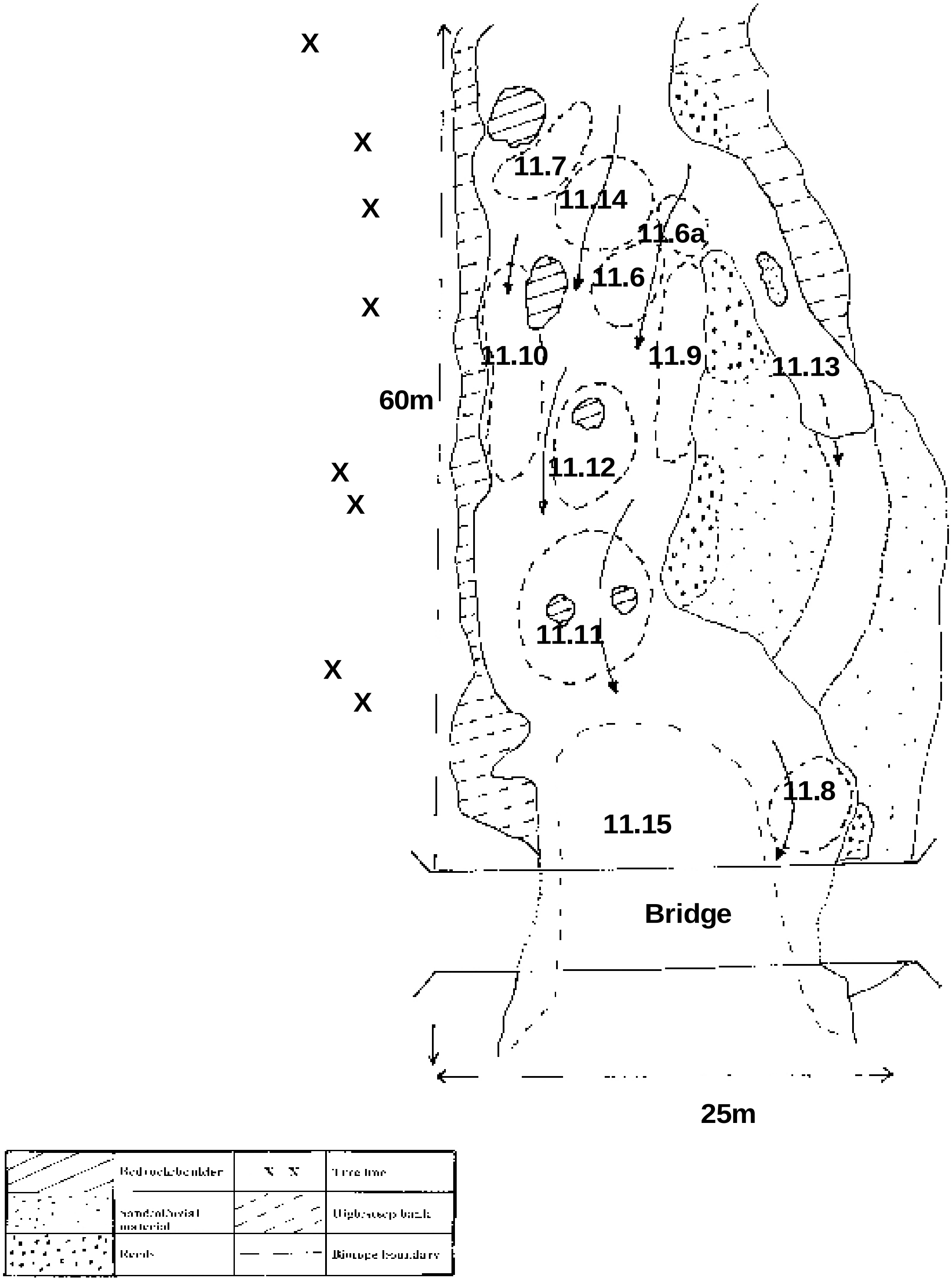

Figure 5.13: Sketch map of the sub-site at Tshifudi bridge (Site 11b) in the Luvuvhu River with areas numbered 11.7 – 11.15 showing the biotopes identified. The arrows indicate the direction of flow.

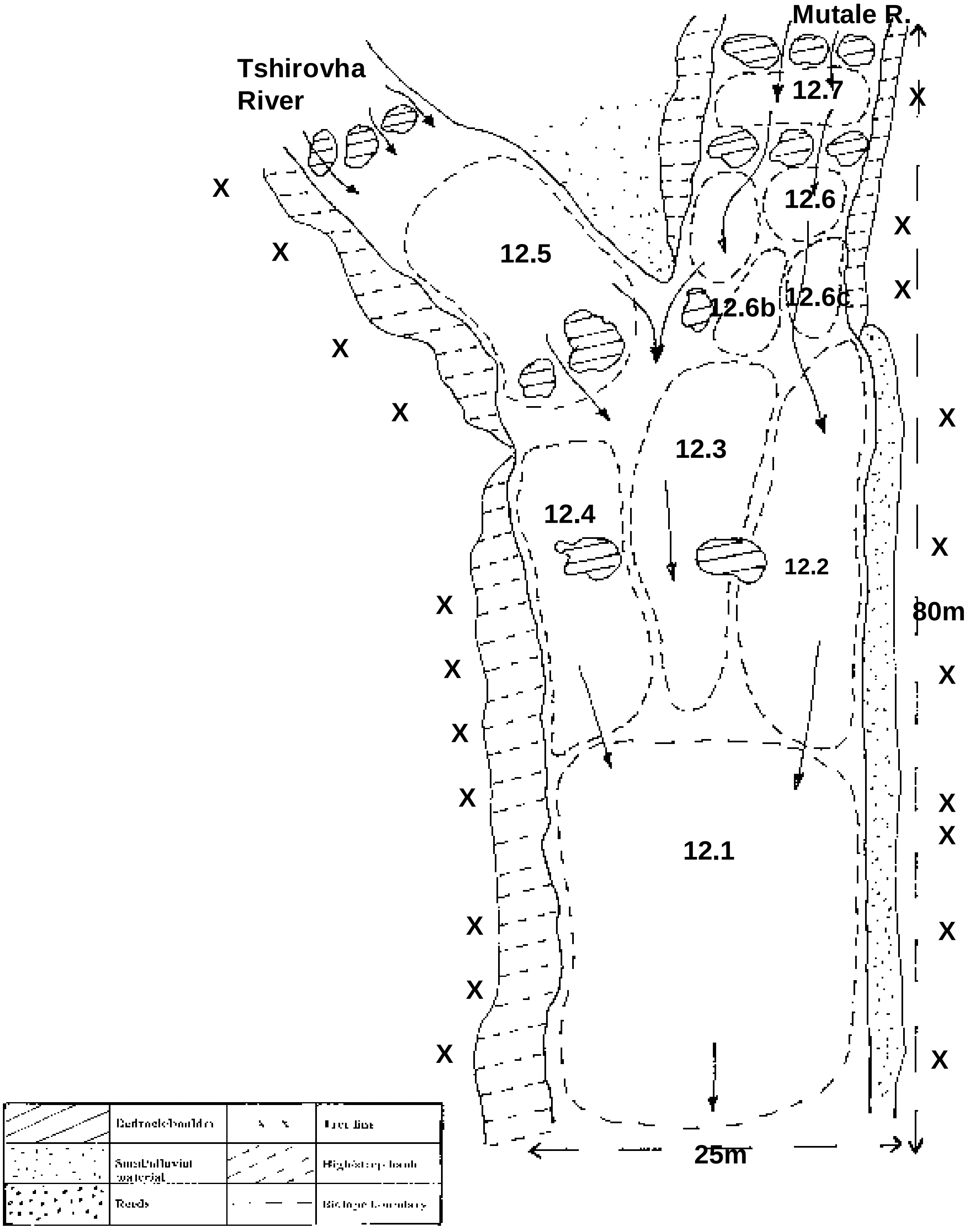

Figure 5.14: Sketch map of the site at the confluence of the Tshirovha and Mutale rivers (Site 12) with areas numbered 12.1 – 12.7 showing the biotopes identified . The arrows indicate the direction of flow.

5.3.3 A comparison of the biotope diversity at the sites.

The results obtained using the biotope classification matrix (Table 5.6) is shown in tables 5.9 to 5.14 and each table represents the diversity within each dominant substrate type. These tables show that cobbles is the most common substrate, followed by boulders. It further shows that although the sites are dominated by fast-flowing biotopes such as riffles, rapids and runs, which jointly constitute more than 51%, there are more than 23 % of the biotopes that were classified as "slow-shallow" and "slow-deep" respectively.

Table 5.9: The number of biotopes in the velocity-depth classes, expressed as a percentage, where the substrate is dominated by bedrock.

Site names and numbers	FS	FD	SS	SD
Gauging weir (1)	0	0	0	0
Tshino (2)	0	0	0	0
Crocodile Ventures (3)	25.00	0	8.33	0
Hydro bridge (4)	0	0	0	0
Tshivhulani (5)	9.09	0	0	0
Bridge near jail (6)	0	0	0	0
Bridge above Mutshindudi confluence (7)	0	0	0	0
Mphaphuli Cycad Reserve (8)	8.33	16.67	0	16.67
New gauging weir (9)	30.77	23.08	7.69	7.69
Malavuwe bridge (10)	0	0	0	0
Tshifudi bridge (11)	0	0	0	0
Tshirovha confluence (12)	0	0	0	0
Percentage of total number of biotopes sampled.	6.98	3.88	1.55	2.33

Table 5.10: The number of biotopes in the velocity-depth classes, expressed as a percentage, where the substrate is dominated by boulders.

Site numbers	FS	FD	SS	SD
Gauging weir (1)	0	0	0	0
Tshino (2)	0	0	0	0
Crocodile Ventures (3)	8.33	0	0	0
Hydro bridge (4)	33.33	6.67	0	0
Tshivhulani (5)	18.18	0	0	0
Bridge near jail (6)	11.11	33.33	0	11.11
Bridge above Mutshindudi confluence (7)	0	0	0	0
Mphaphuli Cycad Reserve (8)	8.33	0	0	0
New gauging weir (9)	15.38	0	0	0
Malavuwe bridge (10)	0	0	12.50	0
Tshifudi bridge (11)	0	0	0	47.37
Tshirovha confluence (12)	10.00	20.00	20.00	0
Percentage of total number of biotopes sampled.	10.08	4.65	2.33	7.75

Table 5.11: The number of biotopes in the velocity-depth classes, expressed as a percentage, where the substrate is dominated by cobbles.

Site numbers	FS	FD	SS	SD
Gauging weir (1)	66.67	33.33	0	0
Tshino (2)	50.00	12.50	25.00	0
Crocodile Ventures (3)	16.67	0	0	0
Hydro bridge (4)	6.67	0	20.00	26.67
Tshivhulani (5)	27.27	0	18.18	0
Bridge near jail (6)	11.11	0	11.11	0
Bridge above Mutshindudi confluence (7)	33.33	0	50.00	16.67
Mphaphuli Cycad Reserve (8)	0	0	25.00	8.33
New gauging weir (9)	0	0	0	0
Malavuwe bridge (10)	37.50	12.50	0	0
Tshifudi bridge (11)	10.53	10.53	0	0
Tshirovha confluence (12)	10.00	30.00		0.00
Percentage of total number of biotopes sampled.	17.83	6.98	10.85	4.65

Table 5.12: The number of biotopes in the velocity-depth classes, expressed as a percentage, where the substrate is dominated by gravel.

Site numbers	FS	FD	SS	SD
Gauging weir (1)	0	0	0	0
Tshino (2)	0	0	0	0
Crocodile Ventures (3)	0	0	0	0
Hydro bridge (4)	0	0	0	0
Tshivhulani (5)	0	0	9.09	9.09
Bridge near jail (6)	0	0	0	0
Bridge above Mutshindudi confluence (7)	0	0	0	0
Mphaphuli Cycad Reserve (8)	0	0	0	0
New gauging weir (9)	0	0	0	0
Malavuwe bridge (10)	0	0	0	0
Tshifudi bridge (11)	0	0	0	0
Tshirovha confluence (12)	0	0	0	0
Percentage of total number of biotopes sampled.	0	0	0.78	0.78

Table 5.13: The number of biotopes in the velocity-depth classes, expressed as a percentage, where the substrate is dominated by sand.

Site numbers	FS	FD	SS	SD
Gauging weir (1)	0	0	0	0
Tshino (2)	0	0	0	12.50
Crocodile Ventures (3)	0	0	16.67	0
Hydro bridge (4)	0	0	0	0
Tshivhulani (5)	0	0	0	9.09
Bridge near jail (6)	0	0	0	22.22
Bridge above Mutshindudi confluence (7)	0	0	0	0
Mphaphuli Cycad Reserve (8)	0	0	0	16.67
New gauging weir (9)	0	0	7.69	7.69
Malavuwe bridge (10)	0	0	0	0
Tshifudi bridge (11)	5.26	0	10.53	10.53
Tshirovha confluence (12)	0	0	0.00	10.00
Percentage of total number of biotopes sampled.	0.78	0	3.88	7.75

Table 5.14: The number of biotopes in the velocity-depth classes, expressed as a percentage, where the substrate is dominated by silt.

Site numbers	FS	FD	SS	SD
Gauging weir (1)	0	0	0	0
Tshino (2)	0	0	0	0
Crocodile Ventures (3)	0	0	16.67	8.33
Hydro bridge (4)	0	0	0	6.67
Tshivhulani (5)	0	0	0	0
Bridge near jail (6)	0	0	0	0
Bridge above Mutshindudi confluence (7)	0	0	0	0
Mphaphuli Cycad Reserve (8)	0	0	0	0
New gauging weir (9)	0	0	0	0
Malavuwe bridge (10)	0	0	25.00	12.50
Tshifudi bridge (11)	0	0	5.26	0
Tshirovha confluence (12)	0	0	0	0
Percentage of total number of biotopes sampled.	0	0	3.88	2.33

5.3.4 The surveys.

The site selection and mapping of the habitat was done in June 2006. During this survey the *in situ* physico-chemical aspects were recorded and water samples for turbidity and total suspended solid (TSS) analyses were collected at representative sites. The sites were then surveyed again in October 2006 and in January, June, August, September and October 2007. While water samples for chemical analyses were only collected in June 2007, the *in situ* physico-chemical aspects were determined during each survey. Additional water samples for turbidity and total dissolved solid (TDS) determination were collected in October 2006 and in January and June 2007. With the exception of the June 2006 survey, fish were sampled in the biotopes during all the surveys.

5.3.5 The physico-chemical aspects.

Table 5.15 shows the variation in the *in situ* physico-chemical aspects measured during all the surveys. The pH ranged considerably and although the water at the majority of the sites were in the lower “basic” range, it should be noted that the site at Tshivhulani (site 5) was acidic. The conductivity and TDS recorded at the sites were as expected with the sites in the upper catchment at the lower end of the scale. A pattern of a downstream increase in both TDS and electrical conductivity was observed in both the main stem of the Luvuvhu River and the Mutshindudi. Site 8 and site 12, both in the least impacted reaches of the two tributaries, are of particular importance as both have low TDS and conductivity values recorded. While the occasional low oxygen concentrations at sites 7 and 8 are reasons for concern the effect could be negated by the simultaneous degree of oxygen saturation recorded at these site. The low level of oxygen content with the accompanying low levels of oxygen saturation at site 9 are signs that the biota at these sites could be negatively affected. Table 5.16 shows the results of water chemistry analyses and table 5.17 shows the turbidity and total suspended solid contents recorded at the selected sites. The high chemical oxygen demand recorded at site 9 underpins the concerns above with regard to the oxygen content.

Table 5.15: The range, maximum and minimum, of the *in-situ* physico-chemical aspects recorded at the sites during the habitat surveys in the Luvuvhu River system during the period June 2006 to October 2007 (DO: Dissolved oxygen, TDS : total dissolved solids)

Site name	Site no	pH	Conductivity (μScm^{-1})	Temperature (^{o}C)	DO (mgl^{-1})	DO (%)	TDS (ppm)
Gauging weir	1	6.56 – 8.75	124.7 – 134.4	15.5 – 25.6	6.9 - 8.77	88.1 – 99.8	62.4 – 67.7
Tshino	2	6.56 – 8.75	124.7 – 134.4	15.5 – 25.6	6.9 - 8.77	88.1 – 99.8	62.4 – 67.7
Crocodile Ventures	3	6.6 – 7.14	124 – 148.6	13.9 – 22.7	6.82 - 9.1	78.5 – 95.0	62.3 – 74.4
Hydro bridge	4	6.3 - 7.93	45 – 64.0	14.4 - 17.3	7.87 – 8.3	78.9 - 98.8	22.4 – 34.8
Tshivhulani	5	6.54 – 6.9	100 – 111.4	14.4 - 22.5	8.61 – 11.45	92.0 - 106	50.3 – 55.7
Bridge near jail	6	6.3 - 7.79	90.4 – 120.0	15.5 - 18.8	5.74 - 7.5	61.6 - 79.0	45.4 – 68.6
Bridge above Mutshindudi confluence	7	7.9 - 8.35	80.4 - 113.6	13.8 - 23.2	4.38 – 6.5	60.0 - 52.8	48.7 - 76.0
Mphaphuli Cycad Reserve	8	7.7 - 8.69	35.7 – 42.0	13.0 - 19.5	4.96 – 9.0	54.5 – 97.0	17.8 – 22.4
New gauging weir	9	7.48 – 8.1	115 - 135.6	15.0 - 18.0	4.86 - 7.0	32.1 - 85.1	50.4 - 67.9
Malavuwe bridge	10	6.5 - 7.9	110.9	17.5	6.33 – 7.3	65.3 – 85.2	55.4 – 80.6
Tshifudi bridge	11	6.61 – 7.3	148.6 - 162	15.7 – 24.6	8.8 - 9.69	100.1 - 112	74.3 – 81.3
Tshirovha confluence	12	7.89 – 8.23	33.4 - 74.4	12.9 - 14.8	6.5 - 7.65	79.7 – 95.7	39.1 – 42.7

Table 5.16: The water chemistry recorded at eight sites during the habitat surveys of June 2007.

	Bridge near jail Site 7	**Crocodile ventures Site 3**	**Gauging weir Site 1**	**Tshifudi bridge Site 11**	**Hydro bridge Site 4**	**New gauging weir Site 9**	**Tshiro-vha confluence Site 12**	**Mpha-puli cycad reserve Site 8**
River/Tributary	**Mbwedi**	**Dzindi**	**Luvuvhu**	**Luvuvhu**	**Mutshin-dudi**	**Mutshin-dudi**	**Mutale**	**Mukhase**
NH_4 (mgl^{-1})	<0.2	0.2	0.2	0.2	<0.2	<0.2	<0.2	<0.2
NO_3-N (mgl^{-1})	0.4	0.6	0.6	0.6	0.3	0.6	0.4	0.3
SO_4 (mgl^{-1})	5	7	5	5	<5	7	5	6
Hardness (mgl^{-1})	32	20	10	10	16	44	20	8
COD	<10	<10	<10	<10	<10	36	<10	16
BOD	<10	<10	<10	<10	<10	<10	<10	<10

Table 5.17: The turbidity and Total Suspended Solid contents recorded at seven of the sites during the habitat surveys from January 2006 to October 2007.

	Bridge near jail (Site 7)	**Crocodile Ventures (Site 3)**	**Gauging weir (Tshino) (Site 1)**	**Tshifudi bridge (Site 11)**	**Tshirovha confluence (Site 12)**	**Mphaphuli cycad reserve (Site 8)**
River/Tributary	**Mbwedi**	**Dzindi**	**Luvuvhu**	**Luvuvhu**	**Mutale**	**Mukhase**
June 2006						
Turbidity (NTU)	53	22	19	35	3	2
TSS (mgl^{-1})	81	36	48.5	69.5	1.5	1.5
October 2006						
Turbidity (NTU)	58	76	36	30	5	3
TSS (mgl^{-1})	50	103.1	52.5	83.6	3	1
January 2007						
Turbidity (NTU)	38	9	9	11	2	1
TSS (mgl^{-1})	27	27	14	23.5	0.5	0.5
June 2007						
Turbidity (NTU)	11	9	9	8	2	1
TSS (mgl^{-1})						

5.3.6 The fish biodiversity at the sites.

A total of twenty four species were collected at the sites and at the most sites nine species were collected. The fish biodiversity observed at the individual sites is shown in table 5.18. The low diversity observed at site 12, the confluence of the Tshirovha and Mutale rivers is what was expected at the specific high altitude, while the high diversity observed at site 8 clearly reflects the low impact rating of a site within a conservation area.

Table 5.18: The fish biodiversity observed in the Luvuvhu River and tributaries during the habitat survey.

	Site numbers											
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Amphilius uranoscopus			X	X	X	X	X	X			X	X
Anguila mossambica									X		X	
Barbus euteania			X	X				X				
Barbus lineomaculatus					X			X				
Barbus neefi				X	X	X		X		X		X
Barbus trimaculatus			X					X		X		
Barbus viviparous		X					X	X	X	X	X	
Clarias gariepinus		X				X	X				X	
Chiloglanis paratus									X		X	
Chiloglanis pretoriae	X	X	X	X	X	X	X	X	X	X	X	X
Chiloglanis swierstrai											X	
Glossogobius guirius									X		X	
Labeobarbus marequensis	X	X	X	X	X	X	X	X	X	X	X	X
Labeo cylindricus	X	X	X	X	X	X	X	X	X	X	X	X
Labeo molybdinus					X	X			X			
Micralestes acutidens								X	X			
Mesobola brevianalis							X					
Marcusenius macrolepidotus			X			X		X		X		
Oreochromis mossambicus	X	X	X	X	X					X	X	
Opsaridium peringueyi				X	X			X				X
Pseudocrenilabrus philander	X	X	X		X	X	X	X			X	
Petrocephalus wesselsii			X									
Tilapia rendalii	X	X	X				X			X		
Tilapia sparmanni		X		X							X	
Total number of species	**7**	**9**	**11**	**9**	**10**	**9**	**9**	**12**	**9**	**9**	**13**	**6**

5.3.7 The population structure of *L. marequensis.*

The length frequency distribution graph obtained from the "pooled" fork length data of *L. marequensis* collected for all the components of this project (Figure 5.15) shows that the majority of length classes were collected in the river. It is however important to take note of the low frequency of the specimens longer than 180 mm.

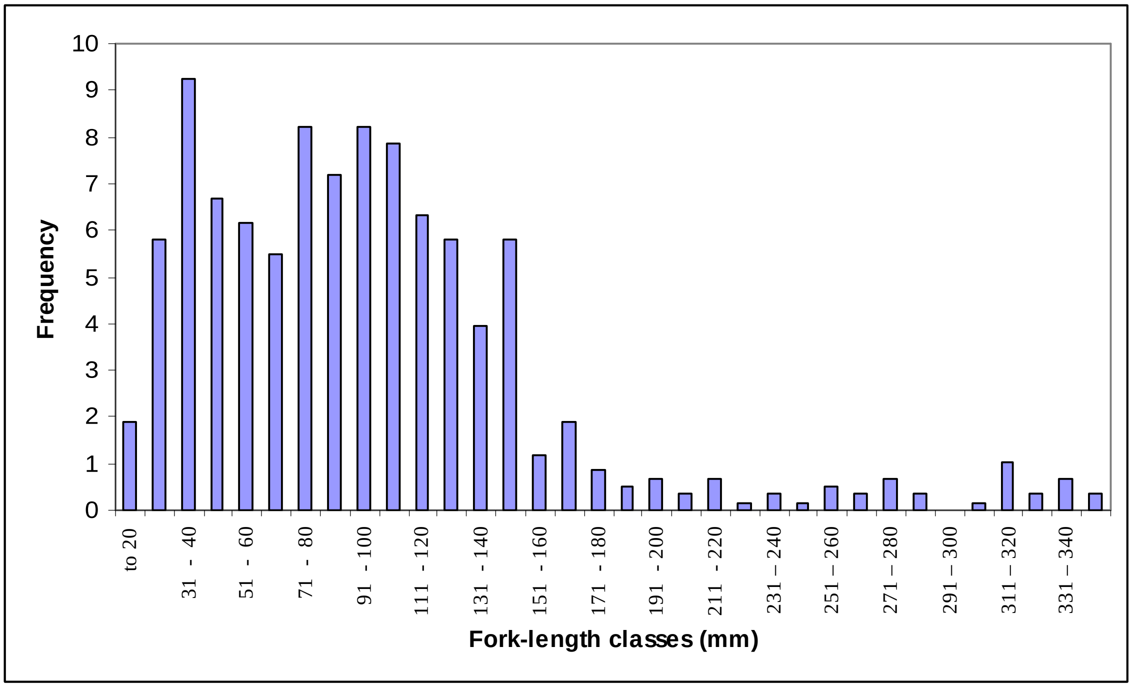

Figure 5.15: Population structure of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries.

5.3.8 The habitat preference of *L. marequensis*.

Table 5.19 shows the biotopes in which specimens of *L. marequensis* were collected and indicates the frequency of occurrence of the species within each biotope type. This analyses illustrates the preference of the species for fast flowing biotopes (A, B, E, F, I, J and Q) and for biotopes where coarser alluvial material (boulders and cobbles) dominate the substrate (E, F, I, J, K and L).

Table 5.20 shows the best correlations obtained from a Spearman rank correlation using the BIOENV method in Primer v.6. This table shows that the best correlation between the presence of the species was obtained with a selection of four variables namely maximum velocity, area, boulders and cobbles. It should however be noted that velocity, area and boulders featured in all of the variable selections.

Table 5.19: The number of biotopes observed and the calculated percentage frequency of occurrence (FROC) in the eighteen biotopes types present at the surveyed sites (LMAR = *Labeobarbus marequensis*).

	Biotope types																	
	A	**B**	**C**	**D**	**E**	**F**	**H**	**I**	**J**	**K**	**L**	**O**	**P**	**Q**	**S**	**T**	**W**	**X**
Number of biotopes present	8	5	3	3	7	1	1	27	4	13	2	1	1	1	5	7	3	3
Number of times the biotope type was surveyed.	19	4	6	3	10	1	1	67	9	28	2	4	3	4	16	11	10	6
LMAR Observations	7	1	2	0	8	1	0	37	2	15	1	1	2	4	9	7	4	3
Calculated % FROC	**36**	**25**	**33**	**0**	**80**	**100**	**0**	**55**	**22**	**53**	**50**	**25**	**66**	**100**	**56**	**64**	**40**	**50**

Table 5.20: Summary of the BIOENV analysis of the habitat components and the presence of *Labeobarbus marequensis*. Where 1 = max. velocity, 2 = max. depth, 3 = area, 4 = bedrock, 5 = boulders, 6 = cobbles, 7 = gravel, 8 = sand, 9 = silt, 10 = aquatic vegetation, 11 = overhanging vegetation, 12 = root wads and 13 = undercut banks.

Variable selection	Correlation
1, 3, 5, 6	0.333
1, 3, 6	0.326
1, 3, 5, 9	0.322
1, 2, 3, 5	0.311
1, 3, 5, 10, 12	0.306
1, 3, 5	0.304
1, 3, 5, 8, 10	0.301
1, 3, 5, 10, 13	0.301
1, 3, 5, 11	0.294

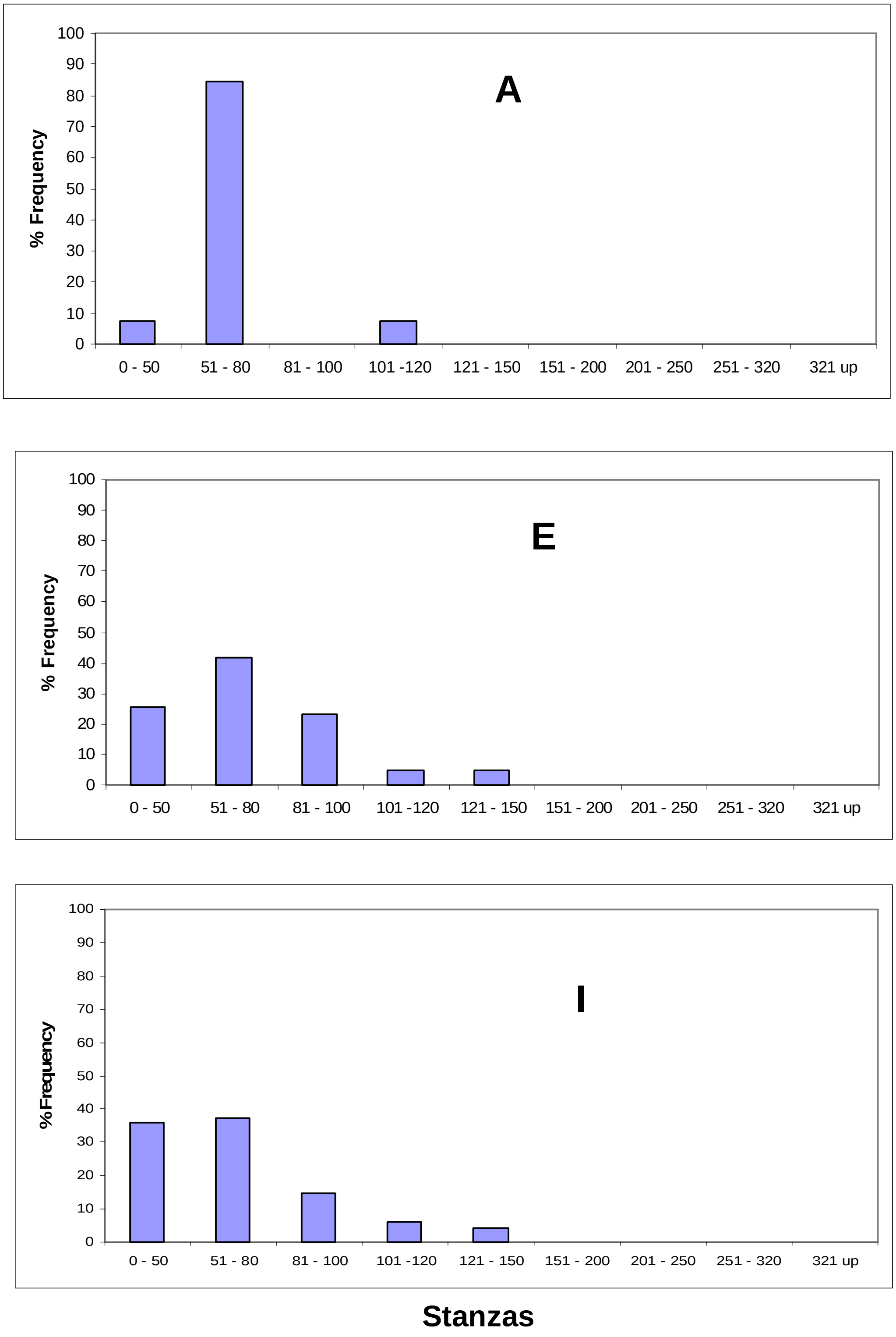

Figure 5.16: Frequency of occurrence of the stanzas of *Labeobarbus marequensis* in the fast-shallow biotopes (A = bedrock dominated, E = boulder dominated and I = cobble dominated). Stanza sizes (mm) are shown on the X –axis.

Figures 5.16 to 5.19 shows the frequency of occurrence of the various stanzas in each of the biotopes where *L. marequensis* was collected. The biotopes where no specimens were collected are excluded. In the figures the symbols A to X corresponded with the matrix of biotope types shown in table 5.6.

The results in figure 5.16 show that in all three the fast-shallow biotopes the majority of the specimens are between 0 and 80mm in length and that no specimens longer than 150mm were observed. The lengths of the specimens in these biotopes correspond to the first five stanzas identified in chapter 4 of this study. It should however be noted that first three stanzas (0 – 50, 51 – 80 and 81 – 100mm) had the highest percentage frequency of occurrence. In figure 5.17 a similar situation is observed with the exception that no specimens longer than 100mm were observed in any of the biotopes.

In the slow-shallow group of biotopes (Figure 5.18) the biotopes were dominated by smaller specimens, shorter than 120mm, and in particular those belonging to the first three stanzas. Although, as is typical in areas with low velocities, the substrate of the slow-shallow biotopes included the finer material such as gravel, sand and silt the high frequency of occurrence in the bedrock and cobble dominated biotopes should be noted. This indicates the role played by the larger particles in providing shelter against predation.

In the deeper pools, the slow-deep biotopes (Figure 5.19) dominated by sand and silt, larger fish, with fork lengths in excess of 150mm, were collected. The low frequency of the smaller fish (<120mm) in these two biotopes could be ascribed to the lack of shelter. This is underpinned by the higher frequency of occurrence in slow-deep biotopes with some shelter in the form of cobbles and gravel. In these biotopes (P and L) it is important to note that a relationship between substrate particle size and fish length seemed to exist with the slightly longer fish observed at a higher frequency of occurrence in the cobble dominated biotope L.

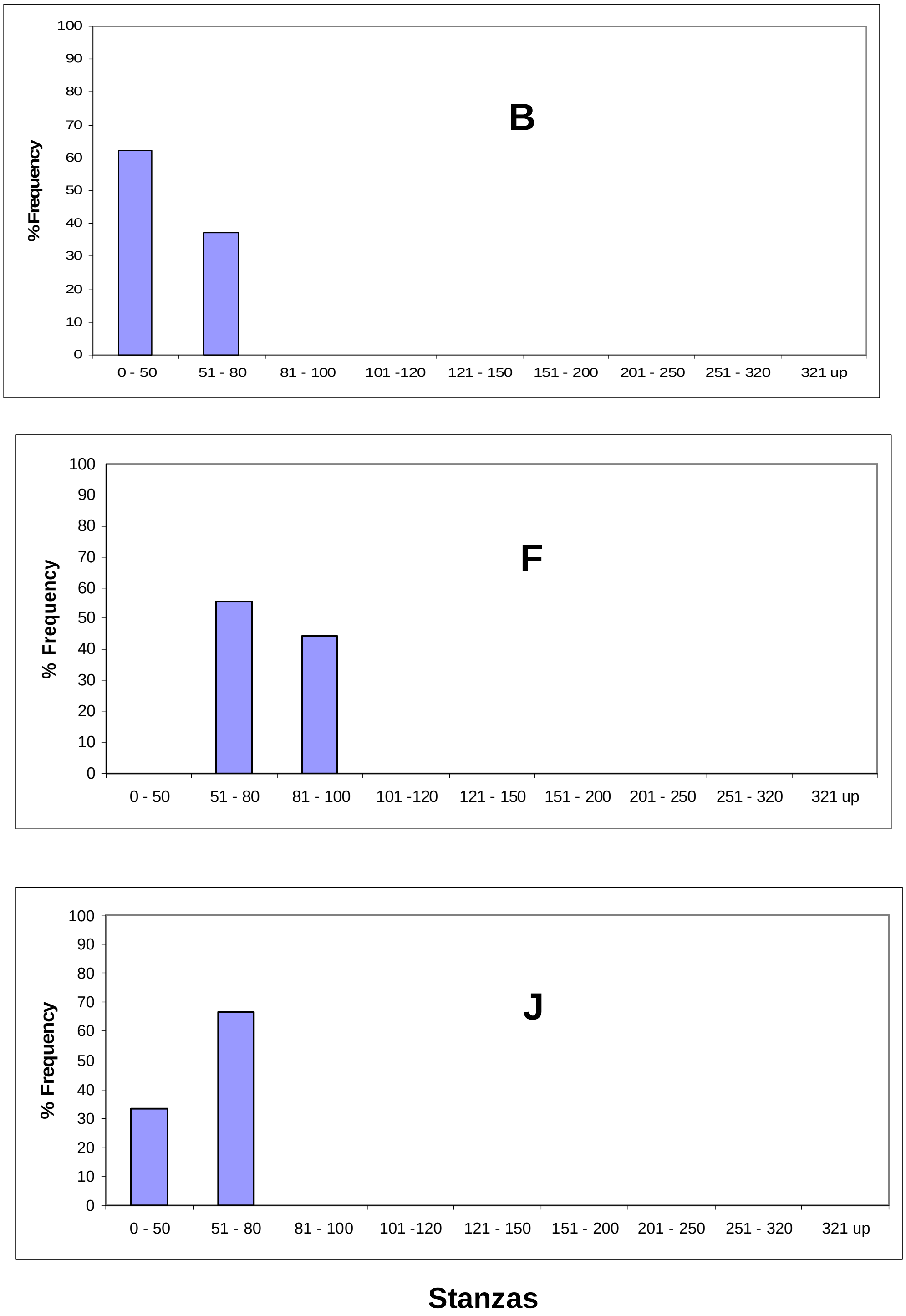

Figure 5.17: **Frequency of occurrence of the stanzas of *Labeobarbus marequensis* in the fast-deep biotopes (B = bedrock, F = boulder and J = cobble). Stanza sizes (mm) are shown on the X –axis.**

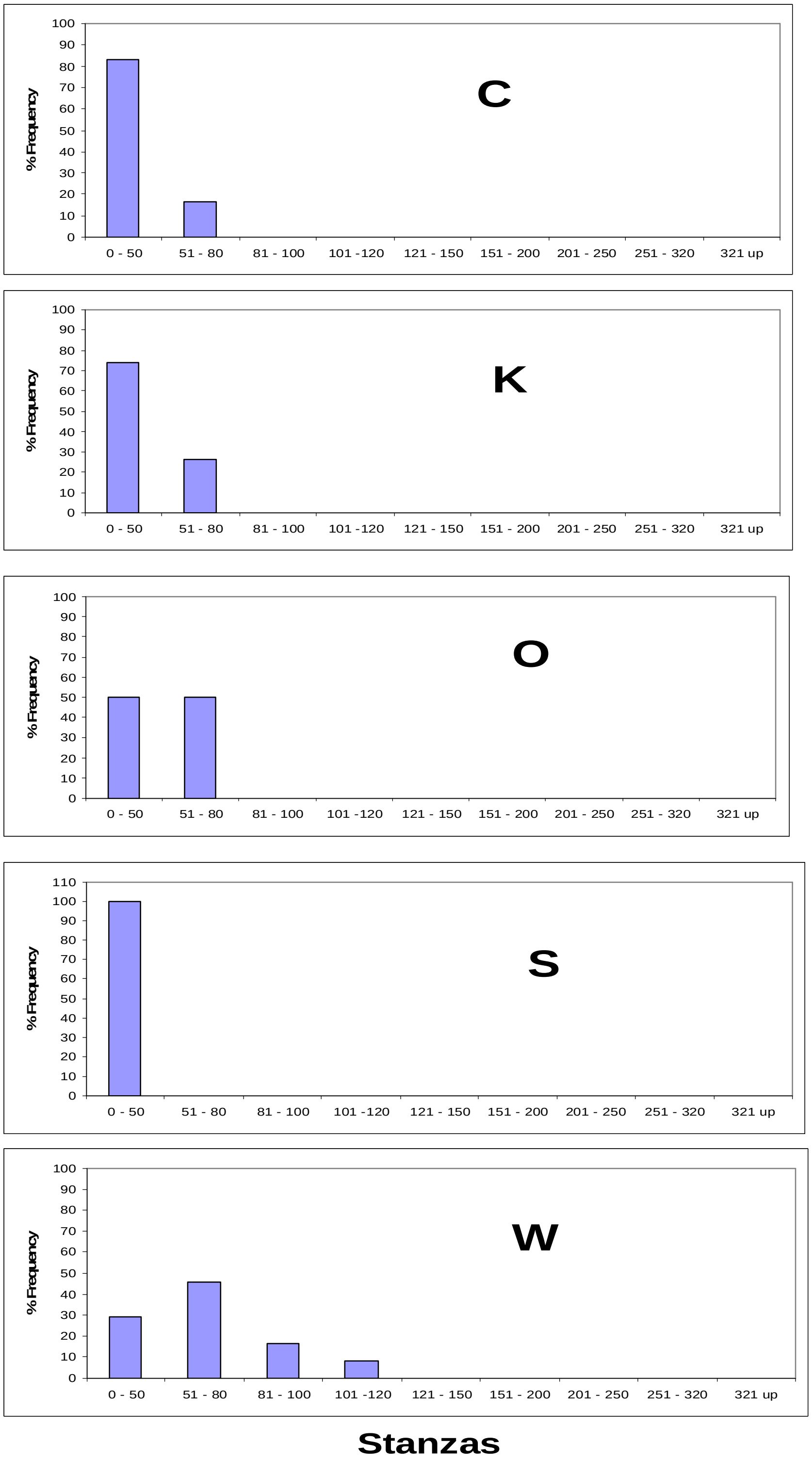

Figure 5.18: Frequency of occurrence of the stanzas of *Labeobarbus marequensis* in the slow-shallow biotopes (C = bedrock, K = cobble, O = gravel, S = sand and W = silt). Stanza sizes (mm) are shown on the X –axis.

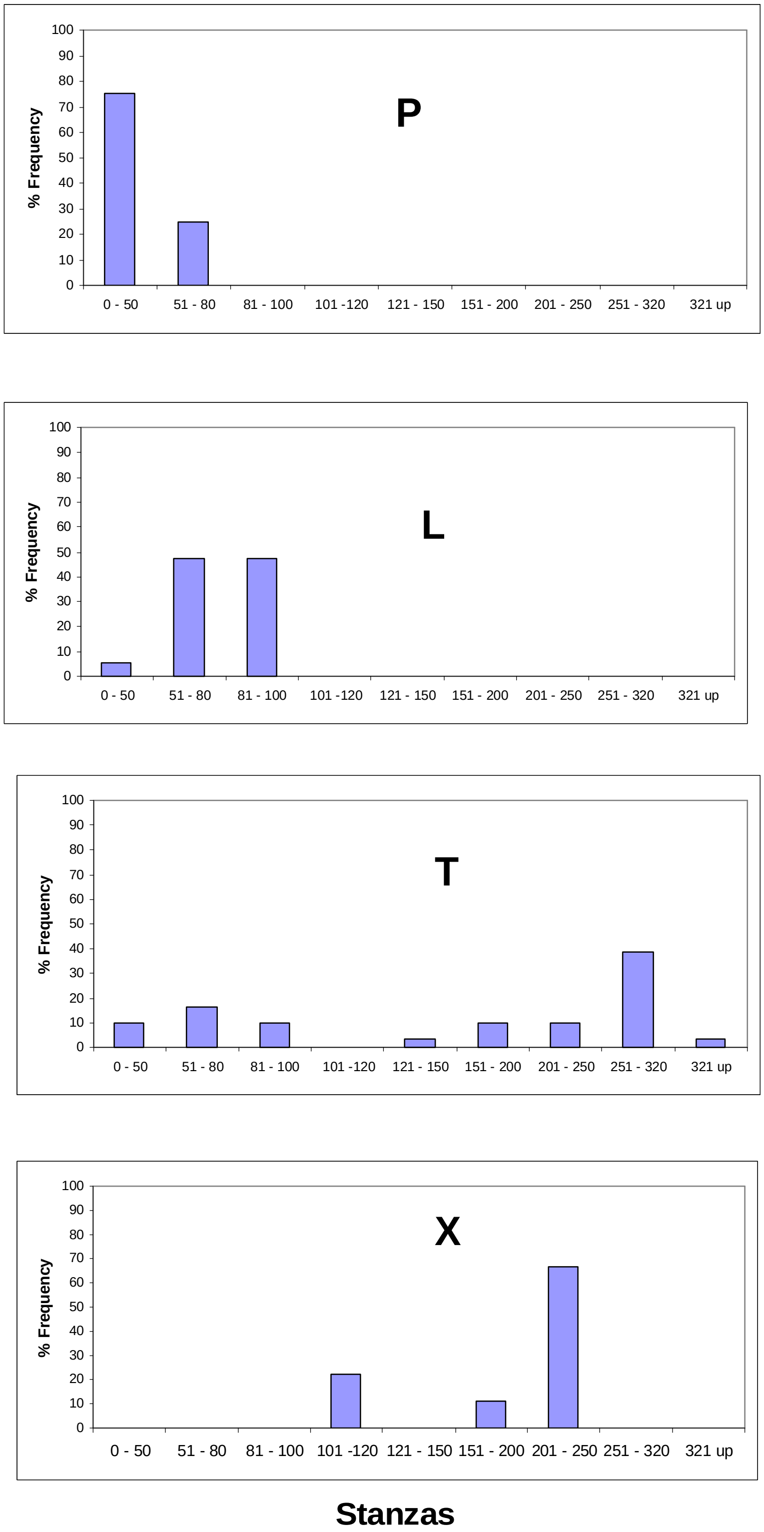

Figure 5.19: Frequency of occurrence of the size classes of *Labeobarbus marequensis* in the slow-deep biotopes. (P = gravel, L = cobbles, T = sand and X = silt). Stanza sizes (mm) are shown on the X –axis.

5.3.9 Statistical analyses of the habitat preference of the size classes of *L. marequensis*.

Cluster analysis, using hierarchical agglomerative cluster analysis with group average linking in Primer v.6., identified six groups of closely related biotope types in which *L. marequensis* were collected (Figure 5. 20). Non-metric multi-dimensional scaling (MDS) used to display the unconstrained relationships between fish stanzas and biotopes (Figures 5.21 to 5.29) show that a definite relationship exists. However none of the biotope preference of the stanzas was completely distinct and overlaps in varying degrees did occur. For example, while figures 5.21 and 5.22 show that only a slight degree of overlap could be detected between stanzas 1 and 2, a comparison between figures 5.21 and 5.22 shows considerable overlap between stanzas 2 and 3. Although there is no clear cut division in the biotope:stanza relationship it can be accepted that a change in habitat preference occurs during the life cycle of the species.

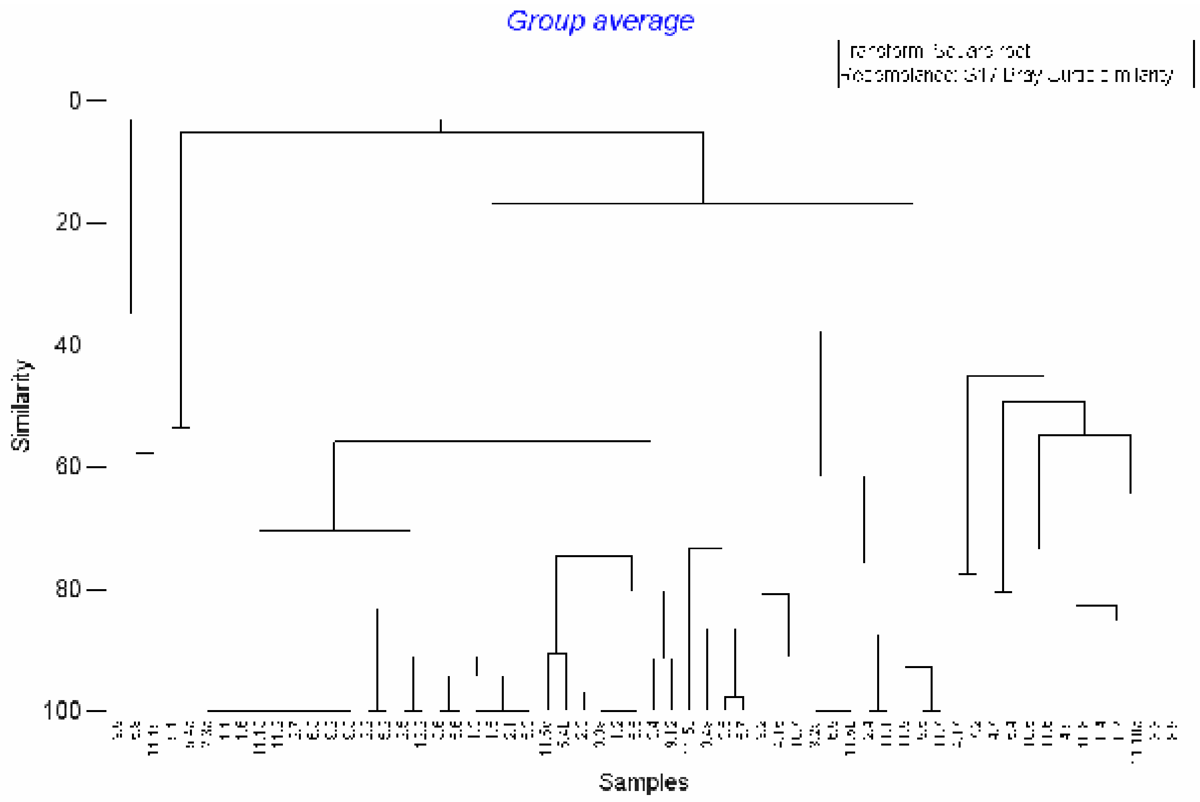

Figure 5.20: Hierarchical agglomerative cluster analysis based on group average linking for all biotopes.

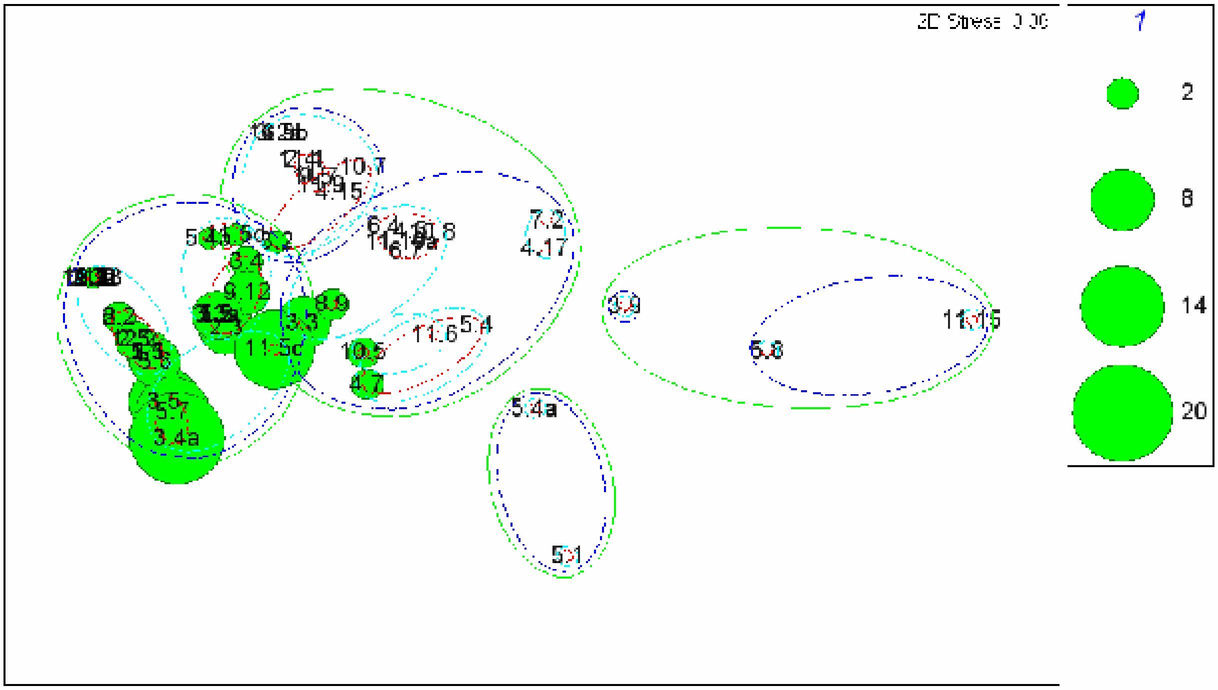

Figure 5.21: MDS ordination of stanza 1 (specimens less than 50mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

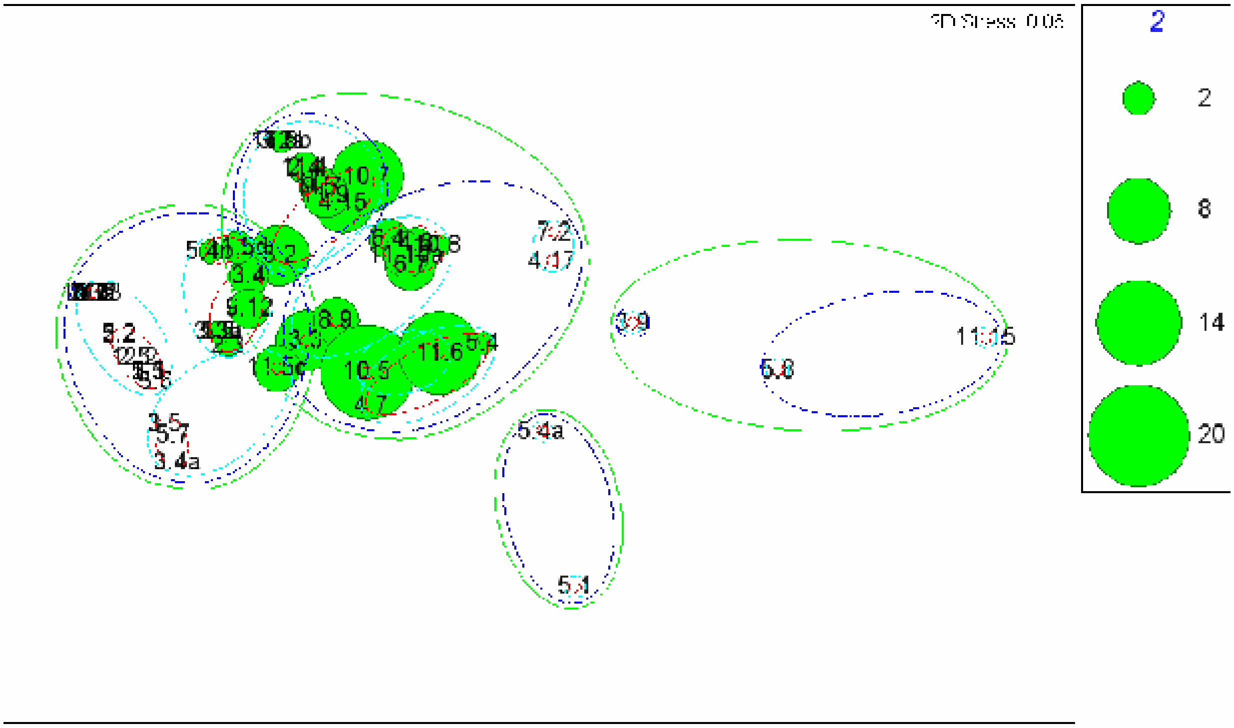

Figure 5.22: MDS ordination of stanza 2 (specimens ranging from 51 to 80mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

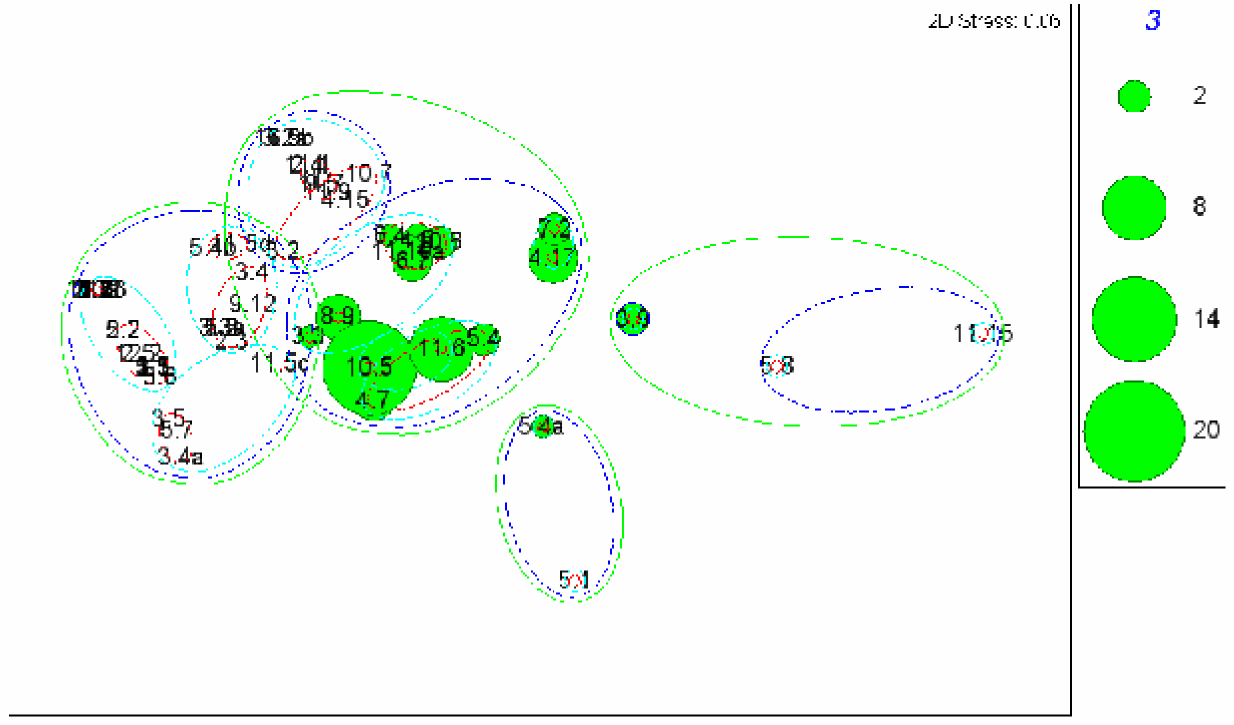

Figure 5.23: MDS ordination of stanza 3 (specimens ranging from 81 to 100mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

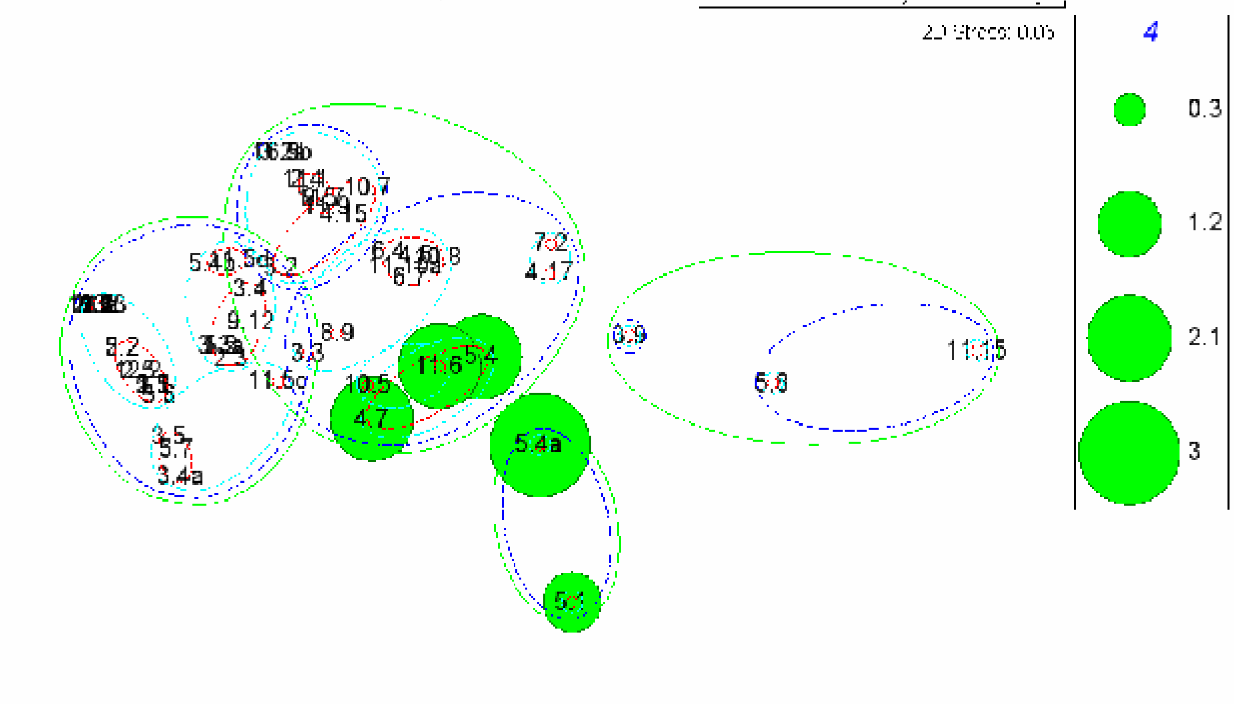

Figure 5.24: MDS ordination of stanza 4 (specimens ranging from 101 to 120mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

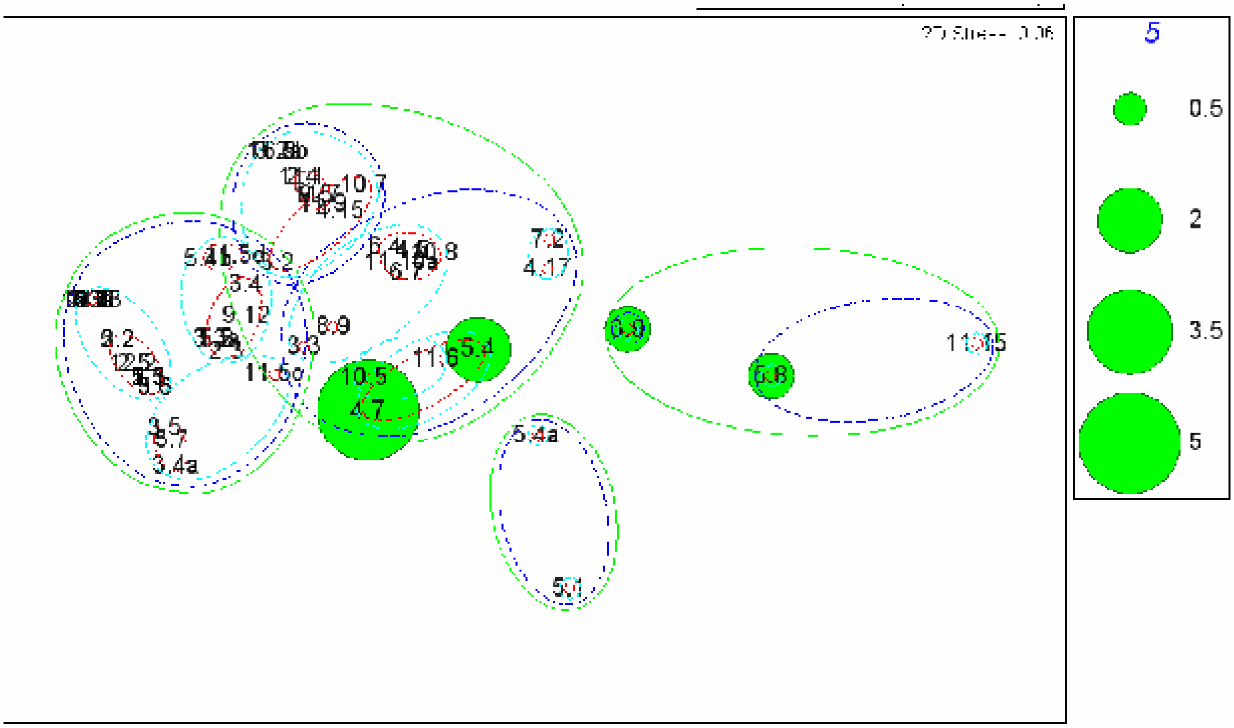

Figure 5.25: MDS ordination of stanza 5 (specimens ranging from 121 to 150mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

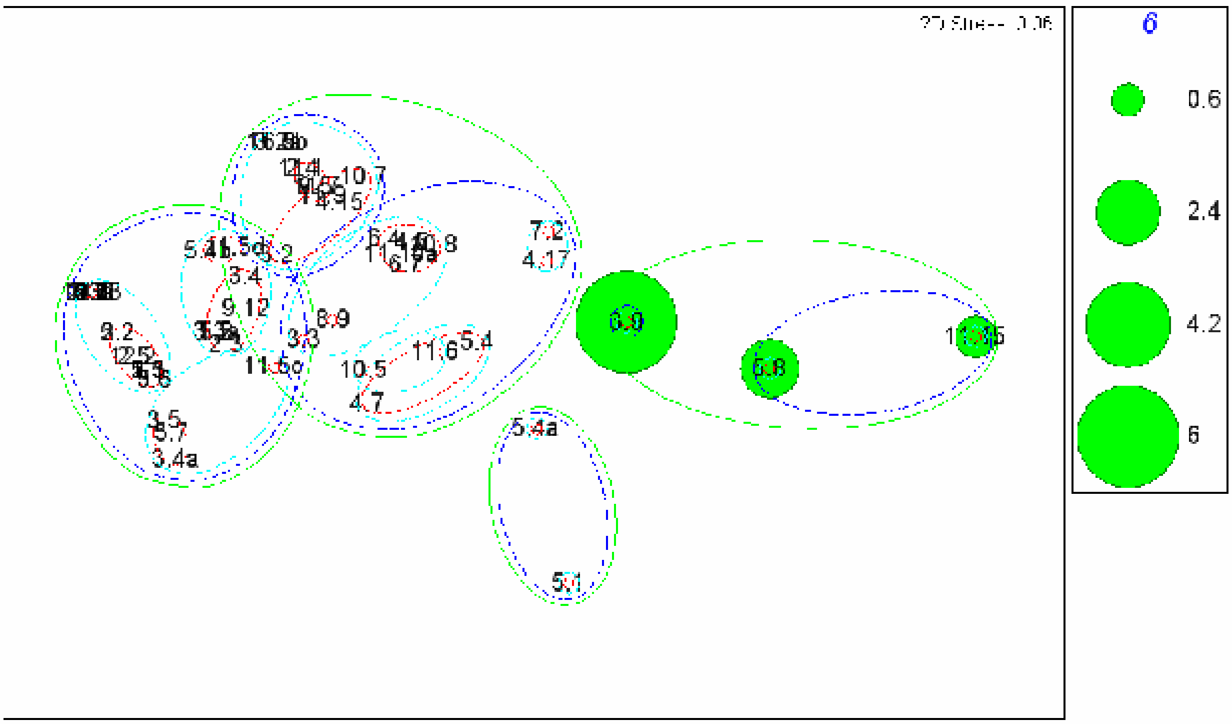

Figure 5.26: MDS ordination of stanza 6 (specimens ranging from 151 to 200mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

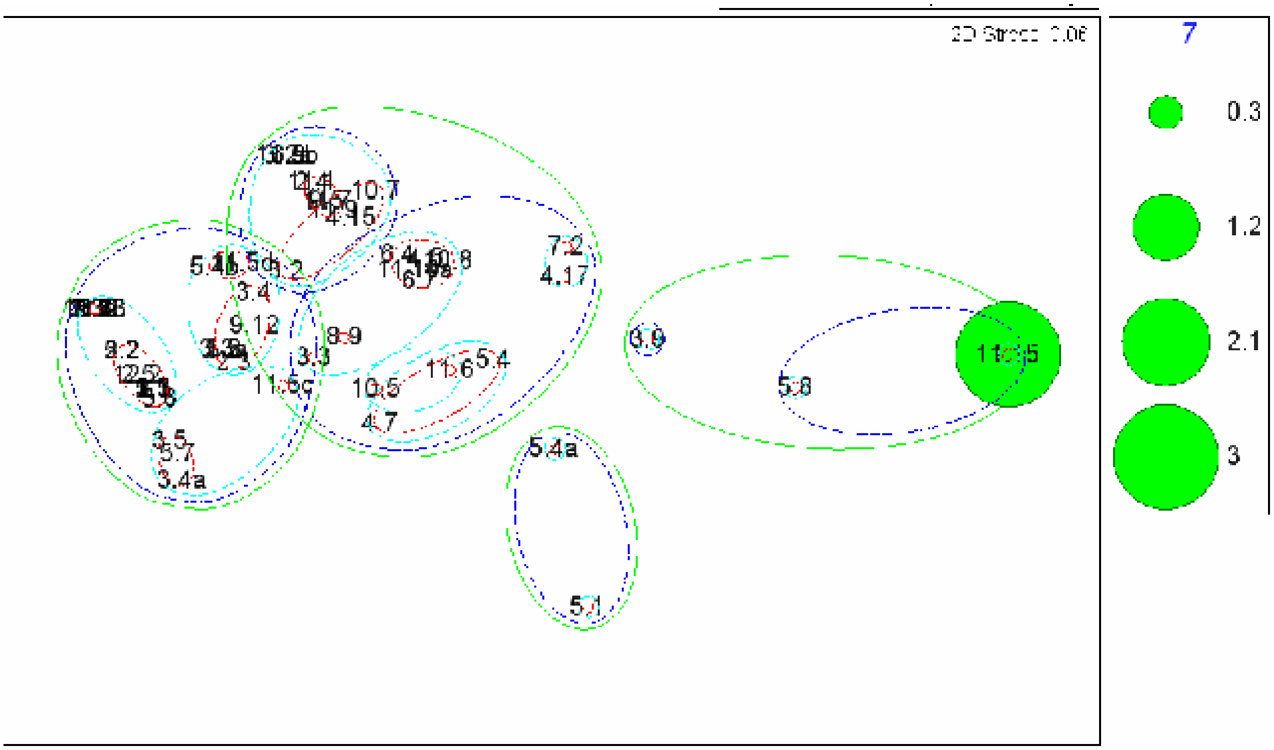

Figure 5.27: MDS ordination of stanza 7 (specimens ranging from 201 to 250mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

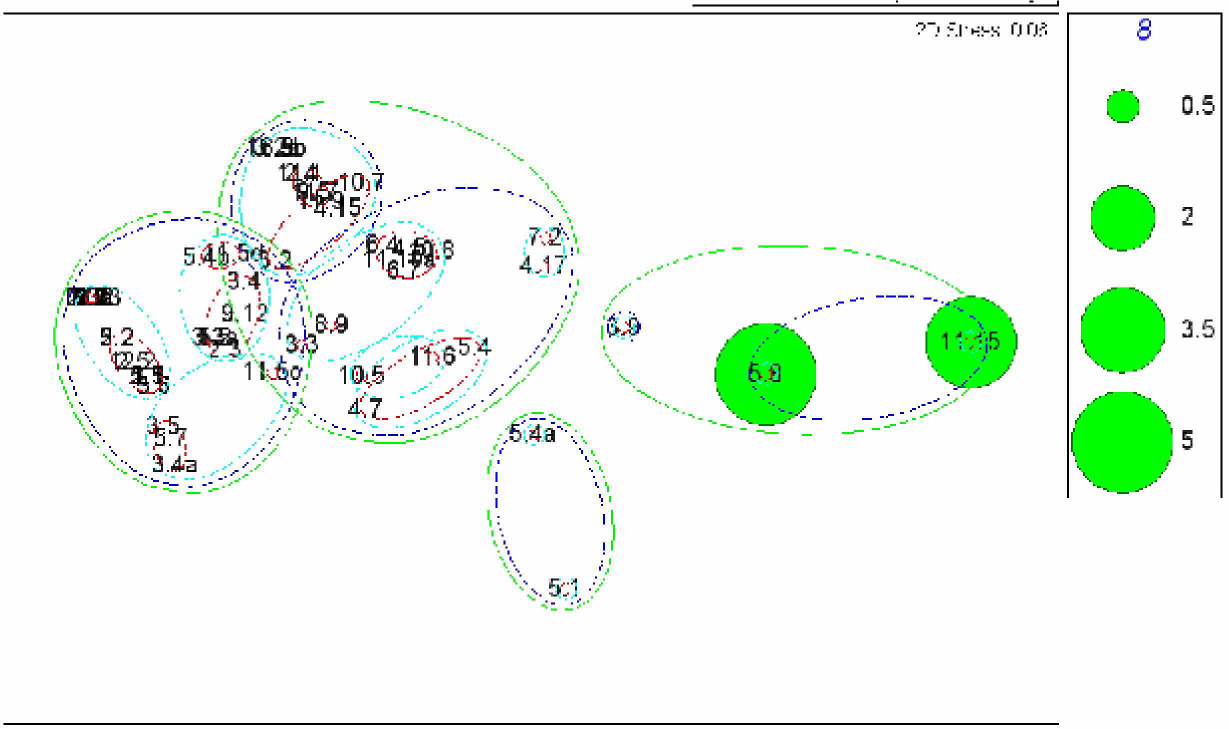

Figure 5.28: MDS ordination of stanza 8 (specimens ranging from 251 to 320mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

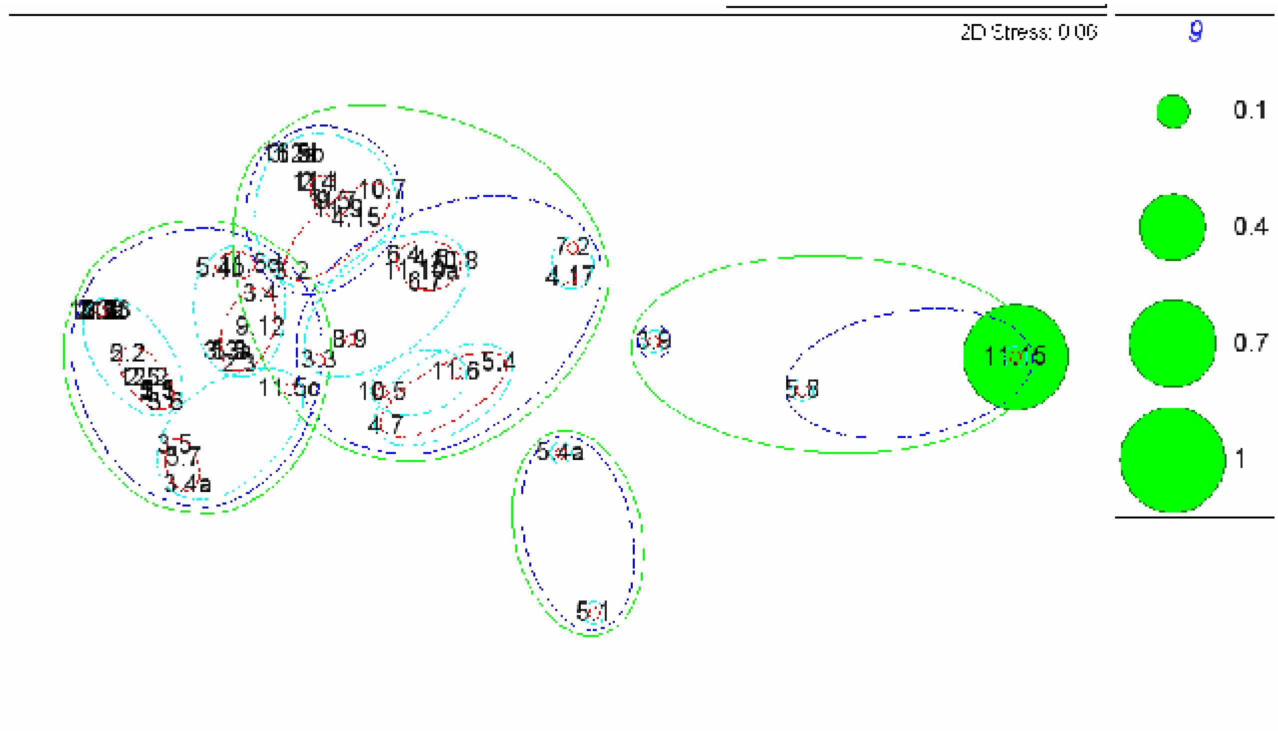

Figure 5.29: MDS ordination of stanza 9 (specimens longer than 320mm in fork length) of *Labeobarbus marequensis* in the biotopes. The bubble size represents frequency of occurrence.

The relationship between fish and the habitat components were examined with redundancy analysis RDA and the results are represented as a tri-plot (Figure 5.30). This tri-plot shows that gravel dominates in the biotopes in which stanza 1 occurs, while cobbles and boulders dominate in biotopes that stanzas 2 and 3 prefer. For the rest of the stanzas there is a better relationship between sand and silt. With regard to the other habitat components, maximum depth related best to the stanzas from 4 upwards as was the case with root wads, overhanging vegetation and undercut banks. The strong relationship between maximum velocity and the stanzas, and in particular stanzas 2 and 3, should be noted. The distance between the biotopes, indicated as small circles in figure 5.30, and the arrows representing the environmental variables indicates the relationship with shorter distances representing a closer relationship and vice versa. The clustering of the biotopes in figure 5.30 is the same as figure 5.20.

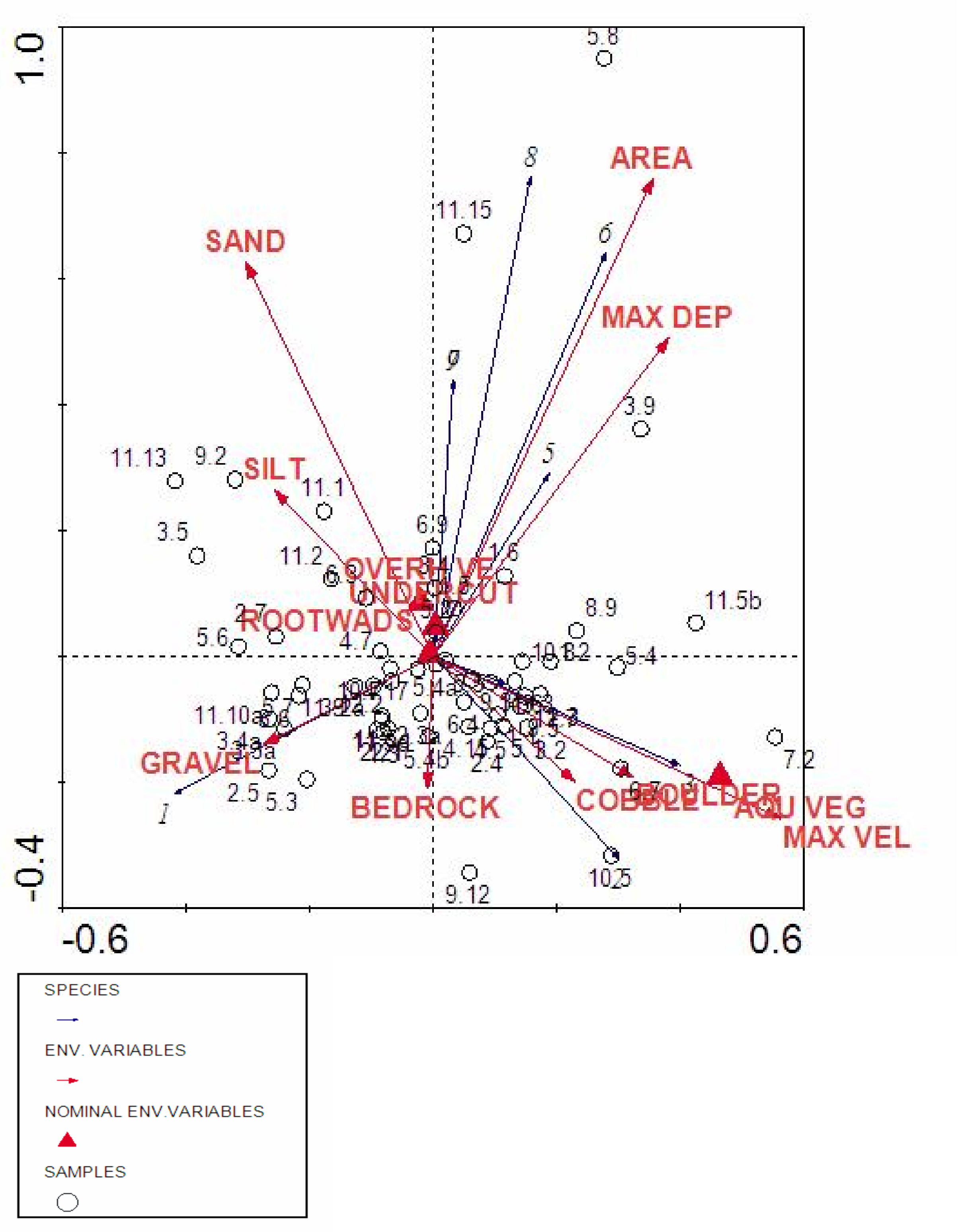

Figure 5.30: Tri-plot of biotopes, abundance and environmental variables, based on RDA analysis. The length of the arrows indicates direction of increase in values.

5.4 Discussion.

As stated in the introduction to this chapter it was regarded important to establish the biotope requirements of the species, despite the fact that some aspects of the larger scale habitat preferences of the species is known. Consequently this gave rise to a number of questions from which aims were formulated. The answer to these questions and stated aims will now form the core of the discussions.

The distribution of most species is “limited by its environmental tolerances and within these limits distribution is related to preferences for given environmental conditions” (Felley and Hill, 1983). In addition these authors (Felley and Hill, op cit.) stated that one way to characterize the distribution of a species relative to an environmental variable would be by the mean of that variable over all the collected individuals. This mean is the state of that variable found at locations where individuals are the most likely to occur and can be used as an estimate of a species’ preference for the variable.

As is shown in figure 5.31 the Luvuvhu River system, in which *L. marequensis* were collected, is an impacted system with the average rating of impacts at the majority of sampled sites scored as “extensive” or “very extensive”. The exceptions are however the Mukhase River (site 8) in the Mphaphuli Cycad Reserve and the upper Mutale River (site 12) where the average impact rating was regarded as “small”. These findings conform with the findings regarding the impact rating of the reaches of the river reported earlier in the text (see 5.3.2). It should however be noted that none of the sites, and this in effect implies the relevant river reaches, could be rated as “critically” impacted. The majority of impacts used in the rating for the study were aspects such as grazing, commercial farming and the presence of roads and bridges that all have an influence on water quality and habitat diversity.

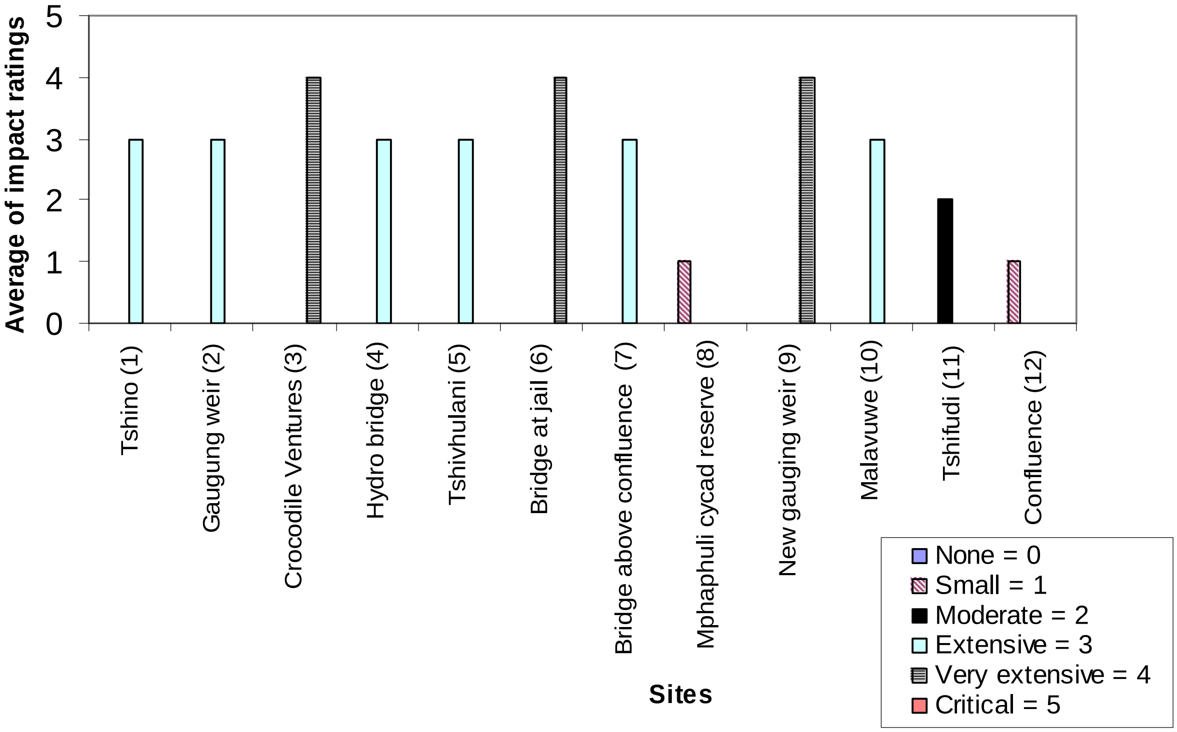

Figure 5.31: An illustration of the "average" impact ratings at the surveyed sites in the Luvuvhu River.

"The physical attributes of the freshwater *inter alia* include temperature, dissolved gases, hydraulics, light and sediments. The latter attribute is responsible for the turbidity and the amount of total suspended solids (TSS). The chemical constituents on the other hand include the major and minor ions, nutrients, numerous organics and hydrogen which determine the pH. Limiting factors are important role players in freshwater ecosystems and include temperature, transparency and chemical composition" (Dallas *et al*., 1994). Mathews and Hill (1979) found that temperature and velocity had the greatest impact, whilst gradients of total dissolved solids (TDS) and pH also have a distinct influence. Although temperature varies less in water than in air it is still important as fish are temperature sensitive and often stenothermic. Light penetration in water is determined by the suspended sediments and turbidity is therefore a limiting factor. Water circulation determines and affects the distribution of gasses as well as the dissolved and suspended solids. Oxygen and carbon dioxide as well as nitrates and phosphates can also be limiting factors. Water temperature affects the available oxygen resulting in less available oxygen in warmer water and vice versa. The oxygen requirements of fish vary between species, with life stages and with different life processes. Juvenile stages are more sensitive than adults to physiological stress, caused for example by oxygen depletion, and in particular to secondary effects such as increased vulnerability to predation. Continuous exposure to concentrations of less than 80% saturation is most harmful and is likely to have acute effects. The values in table 5.21 provide limits and targets that will ensure protection of aquatic biota from the adverse effects of oxygen depletion. The Minimum Allowable Values (MAV) aims to protect sensitive life stages but takes the resilience of biota to short term oxygen depletion in account.

Table 5.21: Target Water Quality Range (TWQR) and Minimum Allowable Values (MAV) for dissolved oxygen concentrations in water bodies (adapted from Dallas and Day, 2004).

Criteria	Concentration	Condition
TWQR	80 – 120% of saturation	Will protect all life stages of most southern African aquatic organisms endemic to, or adapted to, aerobic warm water habitats
MAV	< 60% (sub-lethal)	Violation of these minimum values is likely to cause toxic effects
	< 40% (lethal)	

The pH of unpolluted water is determined by geological and atmospheric influences and most freshwater bodies are well buffered and more or less neutral. The pH *inter alia* determines the concentration of chemical constituents and therefore also the potential toxicity of water. Changes in pH can also affect the osmotic balance of aquatic organisms and although small changes in pH are seldomly lethal they can lead to physiological stress and eventual death.

Suspensoids are the particles suspended in water as part of a dynamic situation. The dynamism, which is directly related to the turbulence and velocity of water, consists of a continuous exchange between suspension and sedimentation. The turbidity of water describes the amount of suspensoids with the turbidity increase positively related to suspensoids increase. Because turbidity causes physical interference with the passage of light, a decrease in passage or clarity is associated with a turbidity increase. This can affect predator-prey relationships as visually hunting predators are impaired by a lack of light.

Conductivity is a measure of the ability of water to conduct an electric current and is directly related to the number of ions in solution. Where conductivity is the sum of the inorganic ions and the organic ions, the total dissolved solids (TDS) results from the sum of the dissolved non-ionic organics (Dallas and Day, 2004).

Most of the nitrogen in aquatic ecosystems is present in gaseous form and the more biological important nitrate, ammonia and nitrite are less abundant. Whereas nitrate is non-toxic with a concentration that normally does not exceed 0,1 mgl^{-1}, nitrite is more toxic and specifically toxic to fish. Ammonia, present in aquatic systems mainly in the dissociated ammonium form, has the highest toxicity of the three nitrogen containing components.

Table 5.22 shows some of the criteria listed by Kempster *et al.* (1980) for the protection of aquatic life. The median value can be regarded as the recommended limit for the constituent and the maximum value reportedly the upper end of the scale of tolerance. The median value is the most reported value and it should be noted that only ammonium is toxic. According the Kempster *et al.* (op cit.) is it regarded as moderately toxic. In addition Dallas *et al.* (1994) reports that freshwater fish and in particular in clear, mountain streams are adapted to TDS values below 200 mgl^{-1} and may not survive at values in excess of 500 mgl^{-1}.

Table 5.22: Quality criteria for the protection of aquatic life in dams (Kempster *et al.*, 1980).

Criteria	Unit	Minimum value	Median value	Maximum value
pH		6	6 – 8	9
TSS	*mgl*$^{-1}$	25		80
Dissolved oxygen	*mgl*$^{-1}$	4,0	5,0	5,8
Ammonium	*mgl*$^{-1}$	0,016	0,016	124

Each aquatic species is adapted to living in water of a certain quality and this adaptation is species specific and varies from species to species. Where some species can tolerate wide differences in concentrations, others can not. The ranges over which a species can survive are known as its tolerance ranges with the upper and lower values known as tolerance limits. Within the tolerance limits the optimal range is that to which organisms are most ideally suited and in which all measures of health or well being are the greatest. Tolerance limits are defined by the evolutionary history and the physiological capacities of each species, but are modified by factors such as the life cycle stages.

During the sampling period certain physico-chemical water quality parameters (Table 5.15) were outside the acceptable ranges (Table 5.22) for the protection of aquatic life. Even so the species was found at all the sites throughout the survey. This serves as an indication that they have adapted to these environmental conditions and underscores the decision to regard them as "non-sensitive" (Kleynhans, 1999). In addition Kleynhans (2007) rated the average tolerance of *L. marequensis* as "moderately" tolerant with a score of 2.6 on a scale of 1 to 5 with its specific tolerance for unmodified water quality rated at 2.1. This latter score is the lowest score in the category of "moderately tolerant". The tolerance of the species is also well illustrated by the findings of Pollard *et al.* (1996) who showed that during periods of drought in the Sabie River specimens of *L. marequensis* were observed to survive in pools where the electrical conductivity ranged between 200 and 800µScm^{-1} and the dissolved oxygen saturation was as low as 50%. It should however be noted that these conditions probably lasted for short periods.

According to Helfman *et al.* (2000) the presence of a particular species in a particular habitat implies an active choice by individuals. The habitat they prefer are the ones in which the species is most often found and the preferred habitat are those in which they can successfully feed, avoid predators and reproduce. In other words, habitat choice is an evolved aspect of a species' niche. Habitat choice is however dynamic within a species and varies with age, sex, reproductive condition and environmental conditions. Habitat choice changing with size or age is known as an ontogenetic habitat shift. The abovementioned is summarised well by Greene and Stamps (2001) who stated that "habitat selection occurs when an individual chooses an area in which to live from among sites that differ with respect to characteristics affecting growth, survivorship and reproduction." The terms "habitat use", "habitat preference", "habitat selection", "habitat utilization", "habitat partitioning" have all been used to describe habitat choice and over the years the phenomenon of "choice", as well as the factors influencing it, has been extensively investigated. Allan (1995) mentioned that in the period 1940 to 1983 one hundred and sixteen studies on "resource partitioning" in freshwater fish had been carried out. This trend was observed in the literature study of this project with numerous studies addressing aspects of the habitat selection phenomenon ranging from marine and estuarine fish to those species found in freshwater (Gaigher, 1973; Schrenk and Whiteside, 1976; Kynard,1979; Matthews and Hill,1979; Paine *et al.*, 1982; Felley and Hill, 1983; Werner *et al.,* 1983; Jones *et al.*, 1984; Gatz *et al.,* 1987; Wikramanayake and Moyle,1989; Harvey, 1991; DeWald and Wilzbach, 1992; Facey and Grossman, 1992; Ross *et al.,* 1992; Harding *et al.,* 1998; Snodgrass and Meffe, 1999; Walsh *et al.,* 1999; Dahlgren and Eggleston, 2000; Greene and Stamps, 2001; Fouché *et al*., 2003; Paxton, 2004;Vagelli, 2004. Kadye and Moyo, 2008).

It stands to reason that in order to survive in a river reach, and in the process select their preferred habitat, the habitat should be available. In determining the habitat components Jones *et al.* (1984) coined the phrase “habitat availability”. This might not always be the case. Gatz *et al.* (1987) showed that in case of competitive influences, rainbow trout habitat availability had no effect on habitat use.

Not only did the results of this study show that the species was present throughout the system but it also illustrated that the population structure of *L. marequensis* was well balanced with specimens of the most of the fork lengths classes and stanzas present (Figure 5.15). As could be expected the larger numbers of specimens were collected in the shorter length classes with only a few of the larger specimens present. In addition the river also supported a high degree of fish biodiversity (Table 5.18). The sites surveyed in this study displayed well developed pool-riffle sequences. All the sites displayed a high degree of instream heterogeneity but as is shown in tables 5.9 to 5.14 and 5.19 the substrate is dominated by boulders and cobbles. A typical pattern of downstream change in habitat, where the coarser alluvial material decreases, was observed. Despite this change the species was collected at all the sites surveyed. Although cover types other than substrate were present at the surveyed sites, their abundance throughout the river was rated as either “rare” or “sparse”, as illustrated by the summary of the average abundance rating of overhanging and aquatic vegetation (Table 5.23). In addition it is clear that although other aspects are statistically well related to the presence of the species (Table 5.20 and Figure 5.30) these cover types are not as important as the substrate composition.

This correlates with the findings of Russell (1997) and Fouché *et al*. (2005) and underpins the preference ratings awarded to the species by Kleynhans (2007). These preference ratings indicate that *L. marequensis* has a "low" and "very low" preference for overhanging and aquatic vegetation respectively.

Table 5.23: Abundance rating of overhanging and aquatic vegetation in the biotopes of the surveyed sites in the Luvuvhu River (The numbers represent the number of biotopes within the scoring class and averages rated as in table 5.4).

	Abundance scoring of cover type							
Site name and no.	**None**	**Rare**	**Sparse**	**Common**	**Abundant**	**Very abundant**	**Total number of biotopes present**	**Average rating at the site.**
Gauging weir (1)							6	None
Tshino 2 (2)							8	None
Crocodile Ventures (3)			1	1			12	Sparse
Hydro bridge (4)		2	1	1			14	Common
Tshivhulani (5)		1		1		1	11	Common
Bridge at jail (6)			1	1			9	Sparse
Bridge above confluence (7)			2				7	Common
Mphaphuli cycad reserve (8)					1		11	Sparse
New gauging weir (9)			3				14	Sparse
Malavuwe (10)		2					8	Common
Tshifudi (11)					4	1	19	Common
Confluence (12)		1		1			9	Sparse

Traditionally the physical habitat characteristics of fish were described on a macro-scale level in terms of physical stream units which included riffles, runs and pools (Harding *et al.*,1989). This was also the case with *L. marequensis* studies in southern Africa. The addition of parameters such as discharge, water velocity, water depth, substrate composition and gradient were later added as habitat characteristics. As previously stated this study investigated the habitat preference of *L. marequensis* on a micro-scale by dividing the physical stream units into biotopes.

The habitat type preferred by fish is often assumed to be the habitat type with the highest catch of specimens (Meng and Powell, 1999). Habitat selection modeling on the other hand involves two components: 1) the frequency with which the animals use various habitats and 2) the availability of the habitat types is used. The ratio of habitat use to habitat availability is then transformed into a measure of habitat selection (Railsback *et al.*, 2003). In this study this transformation was not employed but frequency of occurrence rather than abundance (Schrenk and Whiteside, 1973) was used. The use of frequency of occurrence was based on the argument used by Gaigher (1973) that density or abundance should not be used because of the selectivity of the collecting gear. Johnson (1967) also used this argument and further reported that species with the highest frequency of occurrence for a certain habitat type tend to be the most abundant in that habitat.

Specimens of *L. marequensis* were collected in all the physical stream units, biotopes, at all the sites (Figures 5.16 to 5.19). These units were also referred to as velocity-depth classes (Table 5.1) and included shallow-pools and back-waters, deep pools, rapids and riffles. This agrees with the findings of Gaigher (1973) and in particular when the altitudes in the range of 306 to 610m and 611 to 915 (Table 2.2) that correspond with the altitudes of this study are taken into consideration. But it should be noted that Gaigher (op cit.) did not distinguish between the depth classes of the fast flowing habitats. In as far as all the size groups of the species are concerned, no “partiality to deep rocky pools” as described by Pienaar (1978), Bruton *et al.* (1982) and Bell-Cross and Minshull (1988) could be illustrated. The results of this study rather indicate a partiality for flowing water with high frequencies of occurrence recorded in both fast-shallow and fast-deep habitats. Fouché *et al*. (2003) found in their study in the Luvuvhu River that 85 % and 11% of the specimens collected were in the fast-shallow and fast deep units respectively. Weeks *et al*. (1996) regarded the species as one that "needs shoots and rapids" and recent work by Kadye and Moyo (2008) underpins these findings by placing *L. marequensis* in a group of fish of "associated with riffles".

The work by Lancaster and Belyea (1997) on "flow refugium use" highlights the concept of nested hierarchies and shows that the habitats referred to in the preceding text could actually be referred to as habitat patches within a habitat type. For example a riffle would be a habitat patch within a mountain stream, the habitat type. The biotopes are now nested within the habitat patch and this would for example be a fast-shallow biotope within a riffle. Poff (1997) pointed out that species, and in particular fish, have a functional relationship to habitat selective forces which occur at these hierarchical landscape levels which ranged from watersheds to microhabitats.

Results of the microhabitat scale investigations in this study, when all the measured habitat components are considered, show that velocity was best correlated to the habitat preference of the species as it features in all combinations tested. This was also the case with the coarse alluvial material, namely boulders and cobbles. It can therefore be concluded that the preferred habitat of the species should at least contain fast flowing water over a coarse substrate which then constitutes the habitat availability. Similar results were obtained by Fouché *et al.* (2005) at one specific site in the Luvuvhu River where the combination of velocity, depth and boulders correlated best.

Ontogenetic changes in resource use have been described in many fish species (Helfman *et al.,* 1982). Coral reef Apogonids showed a marked shift in habitat and microhabitat use with new recruits differing slightly from older juveniles and adults (Vagelli, 2004). Stream fish, and in particular their life stages, often use different habitats for the various life processes related to the stages. These processes can include reproduction, feeding or refuge from harsh environmental condition. The "spatial distance" that separate habitats are not huge and could, as shown by Vagelli (op cit.) occurs within a small area and at the same depth. Studies have shown that the use of nursery biotopes by juvenile fish is very specific for different species (Nagelkerken *et al.*, 2000). Although most fish described as having ontogenetic shifts show a defined habitat choice for reproduction purposes, which is usually separate from both recruitment and juvenile habitats. Vagelli (op cit.) also stated that a shift need not be related to a shift in feeding habits or to intraspecific competition.

The spatial distribution of age classes of fish can be explained in terms of the habitat needs of the different life stages. Studies have shown size related habitat selectivity where juvenile, or physically smaller fish, in general display a preference for shallow lateral habitat whereas large fish prefer deeper habitat (Paxton, 2004). This was shared by Harvey and Stewart (1991) who observed "a strong relationship between pool depth and the size of the largest fish in the pool which consequently implied that the bigger fish are found in the deepest pool". Eggleston (1995), Nagelkerken *et al.* (2000) and Laegdsgaard and Johnson (2001) said that "although size is also a significant factor in determining ontogenetic shifts in fish individuals could remain in their juvenile microhabitats after attaining adult or close to adult size". Because of these ontogenic shifts in habitat use, the spatial mosaic of habitats in a stream reach is likely to influence the population dynamics of fish (Snodgrass and Meffe, 1999).

The causes or drivers of habitat shift are complex and have been studied by various researchers. Habitat shift normally occur because of foraging needs, predation risk and productive conditions that change during the ontogeny of animals and occurs in ways that meet their changing needs (Payne *et al.*, 1982). In addition this would imply that shifts could also be seasonal (Payne *et al.*, op cit.). DeWald and Wilzbach (1992) positively linked competition to habitat shifts and Harvey (1991) showed that in order to survive, small fish tended to occupy shallower water when predators are around. In addition shallower water is warmer than the water of deeper biotopes which allows for higher metabolism and subsequently faster growth (Vlok, pers com.[3]). Although it is often postulated that increasing population densities could lead to habitat shifts Byström *et al.* (2003) found that shifts were not always related to densities. Dahlgren and Eggleston (2000) investigated whether size-

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[3] Vlok, W. Freshwater ecologist and Ichthyologist, BioAssets Consulting, Polokwane, South Africa.

specific habitat shifts maximize growth rate and came to the conclusion that shifts were often a trade-off between safety and growth rates

Analyses of the habitat preference of the various stanzas or size classes clearly show a distinct difference in habitat preference (Figures 5.16 to 5.19). These results show that where the small fish, up to a maximum size of 150mm fork length prefer shallow water habitat dominated by coarse material the same size groups were also found in fast deep habitat.

Fish longer than 150 mm were only observed in deep pools but smaller fish were occasionally observed in pools where the substrate provided shelter. These findings are underpinned by the MDS ordination (Figure 5.26 – 5.29). The hierarchical agglomerative cluster analysis of the biotopes (Figure 5.20) indicates how similar biotopes are clustered together and illustrates the degree of similarity. These aspects, and in particular the degree of similarity are also reflected by the MDS ordination results. From this it can be concluded that habitat selection is carried out by the various size classes or stanzas.

An ontogenetic shift in biotopes as the species becomes older was evident (Figures 5.21 to 5.29) but overlaps in biotope preference of the consecutive stanzas were evident. Where these overlaps do occur the low frequency of occurrence, indicates by the bubble size, are an indication that these overlaps may be incidental. This argument is strengthened by the fact that the major portion of the frequency of occurrence is within the closely related biotopes.

Substrate heterogeneity causes water flow perturbations or eddies that create shelters or areas with lower velocity and these area are known as “velocity shelters” (VS) (Harding ***et al.***, 1998). Although ***L. marequensis*** is most commonly found in riffles, they seek biotopes assumed to be localized areas of lower velocity or “velocity shelters” along the stream bed within and around substrate features as is provided by the coarse substrate material. Harding *et al.* (op cit.) regarded microhabitat velocity shelters (VS) or flow refugia within the stream bed as important habitat components for benthic fish.

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5.4 Appendices.

APPENDIX III : Field form 1 : Site data

PROJECT ……………………………… DATE ………………….. TIME …………………..

RIVER ………………………….. Site no and name …………………………………………

a) Geographical Position

Atm. Press	Altitude	South	East	Remarks

b) Weather

Rain				Cloud cover								Wind direction
Yes		no		1/8	2/8	3/8	4/8	5/8	6/8	7/8	8/8	

c) Habitat types present (mark appropriate with cross and indicate number present

Fast-shallow		Fast deep		Slow-shallow		Slow-deep	

d) Active channel dimensions **e) Macro-channel dimensions**

Width (m)	Depth max (m)	Depth max (m)		Width (m)	Flow level	

e) Physical characteristics of water

pH	Conduct µS	Water temp. °C	Oxygen mgl^{-1}	Oxygen %	

f) Chemical characteristics of the water (lab results)

TSS	TDS	Turbidity	NO_3	NO_2	PO_4	Ca	Alkalinity	Hardness

g) Checklist of samples taken (indicate YES/NO)

Fish				Water	Diatoms	Insects	Grab	Photo (Stiffy + no's)
Voucher		FL Measured						

SITE MAP: a) indicate biotopes in table below *and on map* and b) indicate habitats on map

N ↑

HABITATS

Number ……….. and position on map ………..

i) Physical parameters

Velocity (m/sec)				Depth (cm)				Width (m)	Length (m)	
Max		min		Max		min				

ii) Available microhabitat

Substrate present (**Cross and ring dominant**)						Vegetation		Other	
B/rock	Boulder	Cobble	Gravel	Sand	Silt	Aquatic	Overh	Root wads	U/cut banks

Number ……….. and position on map ………..

i) Physical parameters

Velocity (m/sec)				Depth (cm)				Width (m)	Length (m)	.
Max		min		Max		min				

ii) Available microhabitat

Substrate present (**Cross and ring dominant**)						Vegetation		Other	
B/rock	Boulder	Cobble	Gravel	Sand	Silt	Aquatic	Overh	Root wads	U/cut banks

Number ……….. and position on map ………..

i) Physical parameters

Velocity (m/sec)				Depth (cm)				Width (m)	Length (m)	
Max		min		Max		min				

ii) Available microhabitat

Substrate present (**Cross and ring dominant**)						Vegetation		other	
B/rock	Boulder	Cobble	Gravel	Sand	Silt	Aquatic	Overh	Root wads	U/cut banks

Number ……….. and position on map ………..

i) Physical parameters

Velocity (m/sec)				Depth (cm)				Width (m)	Length (m)	.
Max		min		Max		min				

ii) Available microhabitat

Substrate present (**Cross and ring dominant**)						Vegetation		other	
B/rock	Boulder	Cobble	Gravel	Sand	Silt	Aquatic	Overh	Root wads	U/cut banks

Number ……….. and position on map ………..

i) Physical parameters

Velocity (m/sec)				Depth (cm)				Width (m)	Length (m)	
Max		min		Max		min				

ii) Available microhabitat

Substrate present (**Cross and ring dominant**)						Vegetation		other	
B/rock	Boulder	Cobble	Gravel	Sand	Silt	Aquatic	Overh	Root wads	U/cut banks

Recording of fish data collected in the biotopes.

Biotope no	Species	Fork length of LMAR

Chapter 6: The reproductive biology and ecology of the *Labeobarbus marequensis* in the Luvuvhu River.

6.1 Introduction.

As is the case with any animal, the life cycle of a fish consists of a number of periods starting at the embryo and ending in a period of senility, during which the organism slows down and eventually dies (Nikolsky, 1963). One of the periods, namely the adult period occurs when the organism is able to reproduce, forms the focus of this component of the study. Reproduction is regarded as the link in the life cycle of a fish which, in connection with other links, ensures the continuation of the species (Nikolsky, op cit.). Although each of the periods and specifically the adult period has its own adaptive significance in each species, there are features common to all species.

In the process of preparing for spawning, the gonads and in particular the gametes within them grow and ripen or mature up to a point where the gametes are ready to be released in order to facilitate fertilization. It should also be pointed out that the maturing process is cyclic and consists of a continuous but with repeating steps in which the gametes start to develop, then grow in size after which they are discarded. This process is repeated between spawning events and the length of time between spawning events varies between species. Although the maturing process is continuous, various stages in the process have been identified. A number of scales, such as the one proposed by Nikolsky (op cit.) have been developed to describe the stages which represent the state of the gonads in general terms. Nikolsky (op cit.) recognized the following six stages: Stage I which consists of young individuals that have not spawned yet; Stage II, known as the "*quiescent stage*", is where the gonads are small due to the fact that the gametes have either been discarded or have not developed yet; Stage III, the "*ripening stage*", is when the gonads have increased in size and in the females the gametes become visible to the naked eye; Stage IV is the "*ripeness stage*" and although the gonads have reached their maximum size and some of the gametes are fully grown, spawning does not yet occur; Stage V, the "*reproduction stage*", is when the mature gametes are expelled; the last stage is the "*spent stage*", Stage VI, where the gametes have been extruded and the gonads start decreasing in size. This classification has been adapted and applied by various authors over the years (Tómasson et al., 1984; Knight and Ross, 1992; Layman, 1993; Albanese, 2000) and table 6.1 shows two of the derived classification systems that have been applied in South Africa. During the life cycle of fish stages II to VI are repeated between spawning events and the reproductive biology of the species can investigated by studying the state of the gonads.

Table 6.1: A comparison of classes used to express the fish gonadal maturity of fish.

(De Villiers, 1991)		(Cambray, 1992)	
Maturity classes	Description	Description	Gonadal development or maturation stages
		Gonads small, thin and translucent, no visible oocytes	Stage 1 Juveniles
1. Virgin	Sexual organs small, both ovaries and testis are white, no eggs visible	Ovaries small to moderate Oocytes (<0,2mm) visible at 10X)	Stage 2 (inactive) Virgins and recovering spent fish
2. Developing	Size increase in both, colour changes to cream, eggs visible, eggs are of various sizes	Ovaries enlarged, oocytes visible to naked eye (0,2 – 0,6). Testes whiter	Stage 3 (Maturing)
		Ovaries almost fill body cavity, more mature oocytes, Ovaries distinct yellow. Testes more enlarged No sexual products extruded on light pressure.	Stage 4 (late maturing)
3. Ripe	Size increase in both, testis appear swollen and is cream in colour Ovaries increase dramatic and occupy large amount/volume of abdominal cavity. Large eggs are visible	Gonads at maximum size, ovaries distend body cavity, oocytes are large and eggs are shed upon slight handling Testis white, milt is extruded under slight abdominal pressure.	Stage 5 (Ripe-running)
		Ovaries noticeably smaller than stage 5, less mature oocytes. Very similar to stage 4	Stage 6a (partially spent females)
		Not distinguishable from stage 5	Stage 6b (Spent males)
4. Spent	A marginal size decrease in testis, but still cream in colour. Ovaries decrease in size and in size of eggs.	Stage 7 (Fully spent)	

Various other names and descriptions for the stages have been proposed and applied over the years (Sanz and Uriarte, 1989, Knight and Ross, 1992; Layman 1993; Albanese, 2000) and these for example include the terms “latent” instead of virgin or inactive and “maturing 1” and “maturing 2” for maturing and late maturing respectively.

Fecundity in fish is regarded as an adaptation that ensures the survival of the species under conditions in which it originated and exists. Although on the whole fishes have a higher fecundity or number of produced eggs (often referred to as ova) compared to terrestrial vertebrates, the number of eggs spawned by various species of fish varies considerably. Individual fecundity, which is the number of eggs contained in an ovary, is increased by fractional spawning, which is when only groups of the eggs in an ovary are "ripe" at different times. Fecundity is often, for practical reasons, measured as the number of eggs present in the ovary immediately before spawning (Cambray, 1992). Fractional spawning, and for that matter prolonged spawning periods are often characteristic of tropical and sub-tropical fish where no clear distinction between seasons are recorded. Even when the gonads are mature not all the eggs within the ovaries are of the same size and a mixture of large (fully developed) and smaller (undeveloped) oocytes, are often present at the same time. Nikolsky (1963) stresses the fact that the "occurrence of small eggs together with large ones in the ovary are not always indicative of fractional spawning". Many of the small eggs that remain in the ovary are reabsorbed. The size of eggs varies from species to species and the diameter of ripe eggs can vary from a fraction of a millimetre to as big as few millimetres (Helfman *et al.*, 2000).

A further important link in the life cycle of fish is its growth or increase in size. Over and above the fact that fish increase in length and mass as it ages, growth is characterized by seasonal variation. Variations in both linear and seasonal growth are observed between seasons and between life stages (Helfman, op cit.). Closely related to growth are the dynamics of fat deposition and the condition status of the fish. Both of these aspects display distinct seasonal characteristics. The concept "condition" refers to the mass to length ratio of the fish and it is generally accepted that the larger this ratio is, the better the general condition of the fish is said to be. Fat deposition not only plays a quantitative role in the condition of the fish, but forms an important stored reserve for periods of high activity such as migration and reproduction (Shul'man, 1974).

One of the most important indicators of the condition or state of the gonads is their mass and in particular this mass in relation to the body weight of the specimen. Nikolsky (1963) referred to this relation where the gonad mass is expressed as a percentage of body mass as the "coefficient of maturity" but in more recent work this term is replaced by the term Gonado-Somatic Index or GSI (Branson, 1962; Knight and Ross, 1992; Layman, 1993). Helfman *et al*. (1997) aptly refers to the GSI as a "popular, simple and instantaneous measure of reproductive effort". When the seasonal changes in the GSI are studied, it is observed that the period when the calculated index score increase up to peak is followed by a sharp decrease. Knight and Ross (op cit.) stated that "spawning occurs on this down slope from the peak". The period of decrease is followed by a period during which the GSI remains at a low level and this represents the quiescent or latent stage.

In conjunction with the development in the gonads, secondary sexual characteristics also develop. The most visible characteristic is size-related with the females that are larger than the males. Other aspects such as "nuptial dress" may develop and this can include changes in colour and the development of nuptial tubercles (Branson, 1962; Settles and Hoyt, 1978).

Over the years the various aspects of reproductive biology of freshwater fish, and in particular the Cyprinidae in developed countries, have been extensively studied (Hubbs and Walker, 1942; Lachner, 1952; Settles and Hoyt, 1978; Duarte and Alcaraz, 1989; Blinn *et al*., 1998; Platania and Altenbach, 1998; Albanese, 2000). To an extent this is also the case in the indigenous cyprinids where the larger and the reportedly economically more important species, have received preference (Göldner, 1969; Baird, 1971; Mulder, 1971; Bloemhoff, 1974; Hamman, 1974 and 1981; Koch, 1975; Tómasson *et al*., 1984).

As is shown in appendix I (Chapter 1), very little detailed knowledge is available about the reproductive biology of *Labeobarbus marequensis*. Bell-Cross and Minshull (1988) studied gonadal development and reported that adults migrated up swollen rivers to spawn in rapids between October and April and further mentioned that these migrations correlate with the timing and possibly the onset of rainy seasons. Crass (1964), Pienaar (1978) and Skelton (2001) also reported that migrations, most likely associated with spawning, occur during spring and summer. This behaviour was similar with that of *L. natalensis* and *Barbus capensis* that migrate upstream to reproduce (Tómasson *et al*., 1984). A distinct difference in the size at breeding is reported. According to Crass (1964) and confirmed by Gaigher (1969), males mature at a fork-length of approximately 70 mm while females mature at a length of 280mm (Gaigher, op cit.; Skelton, 2001). The work done by Gaigher (op cit.) in the Incomati River system reported ripe males during all four seasons with the first ripe females occurring from October onwards. Gaigher (op cit.) did however point out that not all eggs ripen at same time. As far as the size of the eggs are concerned the only reference found was that of Hecht (1982) who reported that the egg sizes ranged from 0,644mm to 1,818 mm.

This limited information on the breeding biology and ecology of *L. marequensis* suggested that additional studies were needed to enhance the understanding of the species. The first aim of this section of the project was to investigate the breeding biology of the species using traditional methods that *inter alia* included an investigation of gonadal structure and development. Secondly, the breeding ecology was investigated through a seasonal investigation of selected breeding sites in the river system.

6.2 Materials and methods

6.2.1 Recruitment.

By using length-frequency analyses, periods of recruitment can be established from which the times of spawning can be inferred. Tómasson *et al*. (1984) inferred the time of spawning of both *Labeobarbus kimberleyensis* and *L. aeneus* from the time of capture of juveniles. Weatherly (1972) stated that "if reproduction in fish populations occurs so that there is a regular influx of new recruits over a relative short period of time, the population will display a length-frequency distribution that features modes. Each of these modes reflects the annual influx of new recruits". Merron and Tómasson (1984) showed that a bi-modal distribution could be related to two spawning events and conversely a uni-modal distribution reflected a single event.

In order to determine breeding trends, and specifically to establish during which period of the year a concentrated survey should be conducted, it was decided to survey one site for a period of twelve months. The selected site was at Ha-Nesengani in the Luvuvhu River. This survey formed part of the “Pilot Study Phase” of the project and will be referred to as such in the text. At this site the shallow habitat preferred by small and juvenile specimens of the species (See Figures 5.16 and 5.18 in chapter 5) were sampled thoroughly. The fish were electro-narcotized during the surveys the slow-shallow and fast-shallow biotopes (Kleynhans, 2007), using a 220V AC generator, and collected with scoop nets. The fork lengths of the specimens collected were measured to the nearest millimeter on a measuring board and all the fish were returned to the environment after measurement.

In addition it was decided to analyze the fish length data of the specimens collected in the “migration component” (Chapter 8) of this study. During this component fish was collected once a month for a period from May to November 2004. This data will be referred to as the “migration component” in the text.

6.2.2 Breeding ecology.

Jubb (1961 and 1967) as well as Gaigher (1969) is of the opinion that the species is dependent on fast flowing habitats for breeding. Gaigher (1973) specifically referred to the dependency of the species on “rapids” for breeding. In essence both the concepts rapid and riffle would imply the presence of coarser alluvial material (Rowntree and Wadeson, 2000). Vlok (pers com.)[4] indicated that both *Labeobarbus polylepis* and *L. marequensis* have been observed to move onto cobble beds, where flow occurs, and that breeding activity has been observed in these habitats.

Based on the above it was decided to use sites in the Luvuvhu River and selected tributaries, where *L. marequensis* historically occur, that contain what could be regarded as the necessary breeding habitat requirements of the species. This implied that the sites should have: a) pools, where the adults and specifically the larger females are reported to occur (Angliss pers com.)[5], b) fast flowing water, which would suit the adult males, and c) flowing water over cobble beds where the spawning presumably would take place. The selected sites were also used in the habitat studies that formed part of the project (see Chapter 5). In order

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to establish whether the species prepared for breeding it was decided that the sites would be monitored once a month from July until October which would include the period during which spring rain normally occur.

Using the classification proposed by Rowntree and Wadeson (2000) the substrate of the instream habitat was classified and based on this, the site was subdivided into homogenous ***"areas"*** according to the dominant substrate types. In essence this implied that in some areas biotopes with a similar substrate composition, as identified in the habitat preference component of this study, were combined. A sketch map, demarcating the different areas, was drawn of each site. At each site the on-site impacts were rated using the Instream Habitat Integrity (IHI) protocol and methods prescribed by Kleynhans (2007) and used by Fouché et al. (2005).

During each survey, fish was collected in each "area" and kept separate. Except for the pools, where gill nets were used, all other areas were electro-fished twice over a 24 hour period. Gill nets, with stretched mesh sizes of 25, 45, 65 and 85 mm were placed in the pools for 24 hours. Because the gill nets used in impoundments usually are 30 meters long and cumbersome to use in restricted areas such as pools in rivers, shorter nets were constructed for the project. These nets were 15 metres long and 1,5 metres deep. Two sets of nets of each size class of were used per survey. While one set was placed set to form barriers across the pool in order trap fish migrating towards the riffles and rapids, the other set was placed along the edges of the pools. The nets were monitored every 2 hours, or when net activity indicated that fish had been trapped. In order to reduce disturbance, a rubber dingy, that was rowed, was used to place and service the nets.

The fish collected were identified using the key proposed by Skelton (2001) and placed in containers with aerated water until the survey was complete. The fork length of each *L. marequensis* specimen was determined to the nearest millimeter on a measuring board. All the fish, except for the representative specimens of the 10mm interval fork length size classes, needed for gonadal studies, were returned to the river. The representative specimens were preserved in 10% formalin and transported to the laboratory.

During each survey the velocity and depth were recorded in each demarked "area". The velocity was measured with a Pasco Explorer PS 2000 velocity meter and the depth with a steel meter rule at four randomly selected points throughout the area. Velocity was measured at 0,6 of the depth of the water column (Jones *et al.*, 1984; Facey and Grossman, 1992; Harding *et al.*, 1998) and directly above the substrate. At each site the pH, temperature, conductivity, total dissolved solids and dissolved oxygen were determined with handheld Eutech Cyberscan meters.

Because the existing official gauging weirs were not situated in close proximity to the sites it was decided to utilize natural hydraulic controls for flow measurement. Barnard and Rooseboom (2004), who set out to explore the possibilities and accuracy of these measurements found that "step-pool controls" (Wadeson, 1994; Rowntree and Wadeson, 2000) were robust controls that provided efficient critical controls for a wide range of flows. Barnard and Rooseboom (op cit.) concluded that these controls were suitable for flow measurements but pointed out that at these measurements were on average ten percent less accurate than constructed gauging weirs. Since this component of the study was aimed at establishing trends in flow and discharge, rather than exact flow magnitudes, it was decided to apply their method. At each site a step-pool control or controls was selected with the proviso that all the flow at that point in the river should flow through the control or combination of controls. In the selection process preference was given to controls where the substrate, or the floor of the control, consisted of bedrock or was as smooth as possible. During each survey the water depth in the control was determined at four randomly selected points in the control and at these points the water velocities was determined with a Pasco Explorer PS 2000 velocity meter in meters per second. The average depth and velocity was then calculated. The width of the active flow in the control was measured during each survey. The discharge (Q) was calculated using the formula below and expressed in cumecs (cubic meters per second):

Q = (average depth in metres) X (width of control in metres) X (average velocity).

6.2.3 Breeding biology.

The specimens used in this component included fish collected at the sites selected for the habitat preference studies (Chapter 5), migration studies (Chapter 8) as well as those collected as described in 6.2.2 above.

6.2.3.1 Dissection and observation of gonads.

Each specimen was dissected with an incision on the mid-ventral line from just anterior of the anal opening through the pelvic and pectoral girdles to posterior of the branchiostomal membrane (Willers, 1991). In order to express the condition of the fish two aspects namely i) the level or scale of intestinal fat content deposition and ii) the condition factor were considered. After the viscera have been exposed, the fat deposits surrounding the intestines were assessed and classified according to the scale (Table 6.2) proposed by Nikolsky (1963). Once exposed, the gross assessment of gonadal development was based on the classification proposed by De Villiers (1991) with the exception that is was decided to replace the terms virgin, developing and ripe with the terms “Latent” (LA), “Maturing” (MA) and “Mature” (M) respectively. Fish that were in classes 2, 3 and 4 (De Villiers, op cit.) was regarded as sexually mature and this corresponds with the decision of Tómasson *et al.* (1984). The results were recorded on data form II (Appendix IV). The number of specimens in each of the visually classified gonad stages observed during monthly collections was recorded and this is presented in tabular form to illustrate the seasonal changes.

Table 6.2: The scale of fat deposition (Adapted from Nikolsky, 1963)

Fat content scale	Description of the visual appearance
Unit 0	No fat present
Unit 1	Thin cord-like strips/globules of fat appearing between the segments/folds of the intestines.
Unit 2	Strips start joining to form dense fat.
Unit 3	Strips that have joined started “growing over” the intestines. Intestines are being covered by fat.
Unit 4	Intestines almost completely covered by fat. No gaps seen.
Unit 5	Intestines completely covered by fat. No gaps seen.

The condition of the fish was expressed by calculating the body mass to body length ratio as a percentage. The Gross Condition Factor (GCF) of each specimen was calculated using the formula (De Villiers, 1991):

$$\mathbf{GCF = \frac{Fish\ mass}{L^{b}} \times 100}$$

Where b = exponential derivative that refers to the length mass relationship.

In most cyprinids the length to mass relationship is usually expressed as follows $L^{b} = L^{3}$ (Hamman, 1981; Fouché, 1995). As is shown in chapter 4 this was the case for *L. marequensis* where regression analysis of the length mass relationship showed a power trend with the formula $M = 0{,}023\ L^{\mathbf{3{,}0575}}$ (Where M is the mass and L is the fork length) This would imply that an exponential derivative of 3 could be applied in this study.

However when whole body mass is used in calculating the condition factor, it is impossible to exclude the effect of the gonads which can make up to as much as fifteen percent of the body mass (Nikolsky, 1963). Therefore, in order to reflect the true dynamics of the condition of the fish it was decided to calculate a second condition factor that excluded the mass of the gonads. In the following text this is referred to as the condition factor (CF) and was calculated as follows:

$$\mathbf{CF = \frac{(Fish\ mass - Gonad\ mass)}{L^{b}} \times 100}$$

During dissection one of the gonads was removed, blotted dry, the mass determined to the nearest 0,0001g, and divided into two equal halves that were separately stored in 4% formalin for future analyses.

To establish the seasonal trends, or reproductive seasonality, a number of methods were employed:

a) The monthly Gonadosomatic Index (GSI) values were calculated:

$$\mathbf{GSI = \frac{gonad\ mass}{Total\ fish\ mass} \times 100}$$

From this the mean GSI and mean gonad masses were calculated for each collection date (Glazier and Taber, 1980).

b) The Maturity Coefficient (MC) of the fish sampled was then determined using the following method described by Gaigher (1969 and 1976):

$$\mathbf{MC} = \frac{\mathbf{Gonad\ mass\ (g)}}{\mathbf{Fish\ length\ (cm)^3}} \times 10^4$$

These two methods were combined with the technique used by Gaigher (1976) who used the size frequency distribution of the ova to determine whether the species is a total or multiple spawner.

6.2.3.2 Laboratory and data analyses.

Histology.

Because the fish specimens had been stored in 10% formalin for an extensive period, preliminary fixation, performed in formol saline (Baker and Silverton, 1980) was regarded as superfluous. Two sub-samples, that each did not exceed a thickness of *ca* 3mm, of the gonad were sectioned out, placed in a test tube and covered with Helly's fluid (Bancroft and Stevens, 1982), the secondary fixation medium, for 12 hours. The secondary fixative was then decanted and the tissue covered with distilled water for one hour after which it was washed in running tap water for 12 hours. This was followed by a dehydration process through a series of increasing alcohol concentrations. One sub-sample was then de-alcoholised in toluene and the other in xylene. The tissue was then impregnated, with two wax changes, and embedded in wax. Thin, 7µm, sections (Buxton, 1990) were then cut on a rotary microtome, mounted on glass slides and stained with a protein stain, Haematoxylin, and a cytoplasmic stain, Yellow eosin (Baker and Silverton, op cit.).

Counting and measurement of ova**.**

The mass of a third sub-sample of the gonad was determined to the nearest milligram. This sub-sample was then placed in a vial containing 4% formalin and shaken vigorously to separate the ova and the connective tissue. The excess connective tissue was then physically removed with tweezers and by repeated filling and decanting with water. After separation, all the liquid was decanted and 1*ml* distilled water added to the sample. The sample was then thoroughly stirred and a 0,2*ml* sub-sample removed with a micro-pipette and transferred to a counting chamber (Gaigher, 1976). The counting chamber consisted of a Petri dish with a grid consisting of 1 mm^2 squares attached to exterior surface of the floor. All oocytes with yolked nuclei or fully yolked ova were counted with the aid of a dissecting microscope at 10X or 30X magnification. Because of the large numbers of grid blocks that were covered by the liquid, ova were counted in a minimum of 10% of the blocks. Standard cell enumeration protocols, as described by Baker and Silverton (1980) for erythrocyte counts on calibrated chambers were applied. The number of ova in the 0,2*ml* sub-sample was then calculated as follows:

$$\frac{\textbf{Total number of ova counted}}{\textbf{Number of blocks counted}} = \textbf{average number of ova per block.}$$

This average was then multiplied by number of grid blocks covered by the 0,2*ml* sub-sample. The total number of ova in the ovaries was calculated using the formula adapted from Mulder (1971):

$$T_o = \frac{T_s}{B} \quad X \quad A$$

Where : T_o is the total number of ova in gonad, T_s the number of ova in sub-sample, A the mass of gonad/ovary and B the mass of sub-sample.

A second sub-sample of 0,1 *ml* was then extracted and placed on a microscope slide and the diameter of the ova was then measured with a calibrated ocular micrometer at 400X magnification on a light microscope. A minimum of 50 ova were measured to the nearest 0,01mm. After measuring, the ova were grouped into 0,125mm egg size categories and the data presented in a histogram to establish the modalities of egg size distribution (Gaigher, 1976). All the data were recorded on data form III (Appendix V).

Where Knight and Ross (1992) suggested that the mean ovum diameter should be determined by averaging the diameter of ten ova from the largest size category and five ova from the smaller size categories, Heins (1991) as well as Layman (1993) suggested actually measuring ten ova that were selected at random. It was however decided to use the actual measured diameters of the fifty measured ova to calculate the mean for each specimen. The mean diameter was determined for the different stanzas and multivariate analyses of covariance, using ANOVA was used to test for differences (Knight and Ross, 1992).

Length at sexual maturity

As proposed by Tómasson *et al.* (1984) fish with gonads in classes 2, 3 and 4 (De Villiers, 1991) (Table 6.1) were regarded as sexually mature as opposed to fish with gonads in class 1 that were regarded as immature. Length at sexual maturity was determined as the length at which 50 % of the fish had maturing or mature gonads and was calculated to determine the relationship between fish length and fecundity. In addition, length at sexual maturity was calculated by plotting the calculated maturity coefficient as a function of fork length (Gaigher, 1969).

Fecundity.

The number of eggs contained in the ovary is termed the individual, absolute or total fecundity of the fish (Nikolsky, 1963) and is usually determined by physically counting the number of eggs in a known sub-sample of the ovary. The relative fecundity on the other hand relates to the mass or length of the fish (Gaigher, 1969; De Villiers, 1991). In this study it was expressed as the number of mature ova per gram of fish and was correlated to the fork length of the fish and presented as such.

Spawning chronology.

In order to determine the spawning chronology and to establish whether the species spawned at different times the GSI and the ova diameters were used as suggested by Settles and Hoyt (1978).

The influence of environmental parameters on breeding.

All the relevant breeding characteristics which included the GSI, maturity coefficient, spawning seasonality and fecundity were correlated with measured environmental data parameters to investigate the possible effects of environmental parameters on gonadal recrudescence.

6.3 Results

6.3.1 Recruitment

For logistical reasons one of the historical sites at Ha-Nesengani in the Luvuvhu River was the most accessible and was surveyed for the period January 2005 to November 2005 as part of the “Pilot study”. Although it was envisaged in the initial planning to survey monthly, the site was only surveyed in January, March, May, July, September and November. The results (Figure 6.1) show that during the September 2005 survey specimens in the smallest fork length class (11 – 20mm) were collected for the first time. This is an indication that breeding had occurred in the period between the previous survey in May and this survey in September. This agrees with findings of Fouché *et al.* (2005) in the Luvuvhu River who found specimens smaller than 20mm in fork length during the September 2004 survey but in addition also observed small specimens during the May 2004 survey. Heath *et al.* (2005) reported that at the Xikundu fishway, gonadal growth peaked in September. This is in general consensus that breeding is correlated with the onset spring rains (Bell-Cross and Minshull, 1988). According to Skelton (2001) the species breeds twice a year, in summer and spring, to coincide with the rainy seasons. Based on the findings and the work of these authors it was decided to concentrate the breeding ecology component of this study in a period that would reflect both instances.

The results obtained from the analyses of the length frequency data collected in the “migration component” (Chapter 8) at the Xikundu fishway of this study is not as conclusive. The best results were obtained in the first year of the survey. Figure 6.2 shows that no fish from the smallest fork length classes of 11 - 20 and 21 – 30mm were collected. The smallest fish collected were from the 41 – 50 mm size class and specimens of this class were collected during the July 2004 survey and then again during the September, October and November 2004 surveys with the highest frequency of occurrence during the November survey. From this it can be deducted that two spawning events had occurred with the first in May before the June survey and then again in the period starting in September. The absence of the very small juveniles in the fishway can be ascribed to the lack of suitable habitat within the fishway. Linked to this is the inability and constraints caused by their morphological features, which such small juveniles have in negotiating the fast flowing environment.

Analyses of the length frequency distribution of small fish, less than 110mm fork length, collected at the breeding ecology sites from June to October 2007 (Figure 6.16) show that small fish in the 11-20mm class were collected August, September and October 2007.

6.3.2 Breeding ecology

a) A description of the sites monitored.

Based on historic distribution data and information collected as part of the habitat preference component of this study, four sites with all the necessary habitat and biotopes were selected. Two of the sites were in the main stem of the Luvuvhu River, while one was in the Dzindi River and the fourth in the Mutshindudi River (Figure 5.1). The location and general aspects of the sites is shown in table 6.3. The numbering of the sites conforms with the numbers awarded to the sites in the habitat preference component (Chapter 5) of this study.

Table 6.3: Possible *Labeobarbus marequensis* breeding sites in the Luvuvhu River system monitored in the period June – October 2007.

No	**Site name**	**River**	**Altitude (m.a.s.l)**	**Coordinates**			
				South		**East**	
				Degrees	**Minutes**	**Degrees**	**Minutes**
2	Tshino	Luvuvhu	576	23	06.800	30	33.385
11	Tshifudi	Luvuvhu	447	22	50.600	30	44.282
5	Tshivhulani	Mutshindudi	550	22	54.328	30	31.120
3	Crocodile ventures	Dzindi	549	23	00.300	30	28.410

Site 2: Tshino.

This site in the main stem of the Luvuvhu River was selected because of its habitat diversity and the presence of what is commonly regarded as breeding areas. Biotopes ranged from slow, deep pools to slow shallow cobble beds and from fast flowing deep rapids to shallow riffles over boulders and cobbles. It was regarded that this habitat

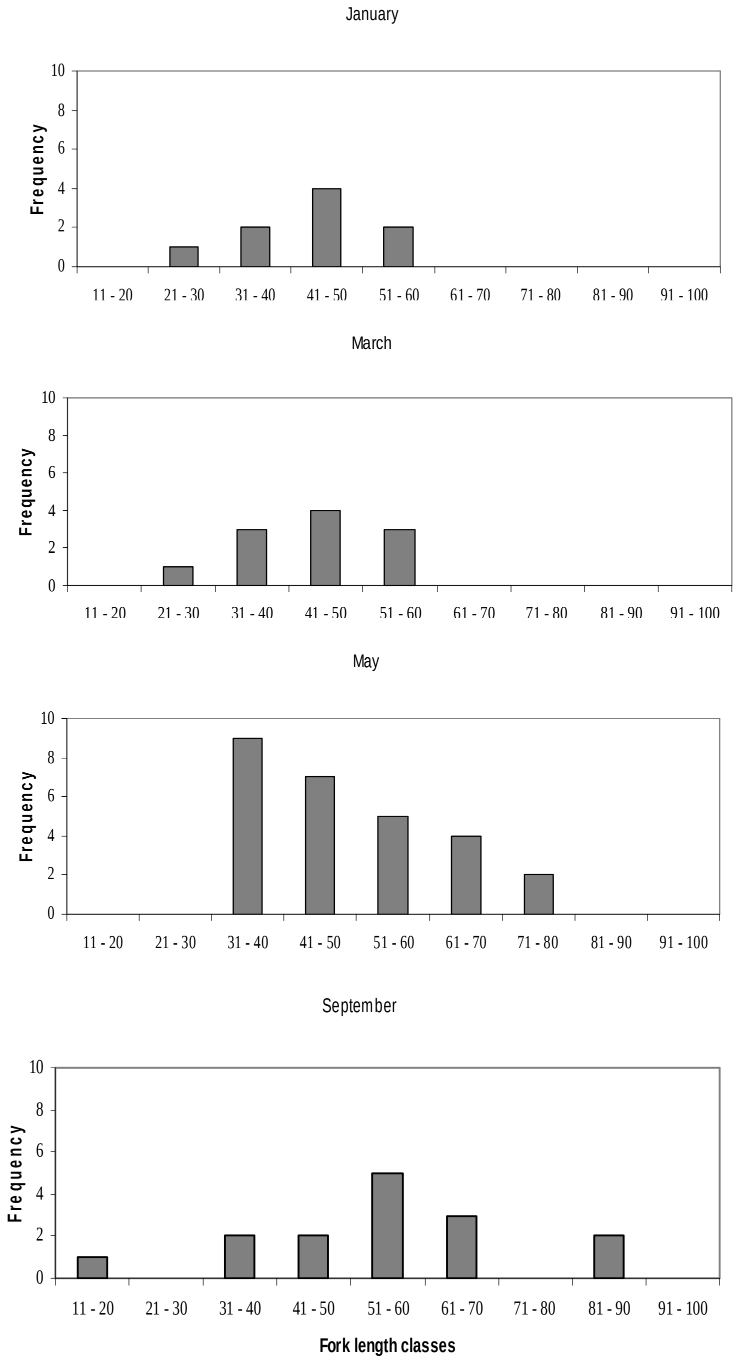

Figure 6.1: Length frequency distribution of *Labeobarbus marequensis* specimens collected in the shallow biotopes at the Ha-Nesengani site, in the pilot study, in the Luvuvhu River in the pilot study during 2005. The fork length classes are shown on the X-axis.

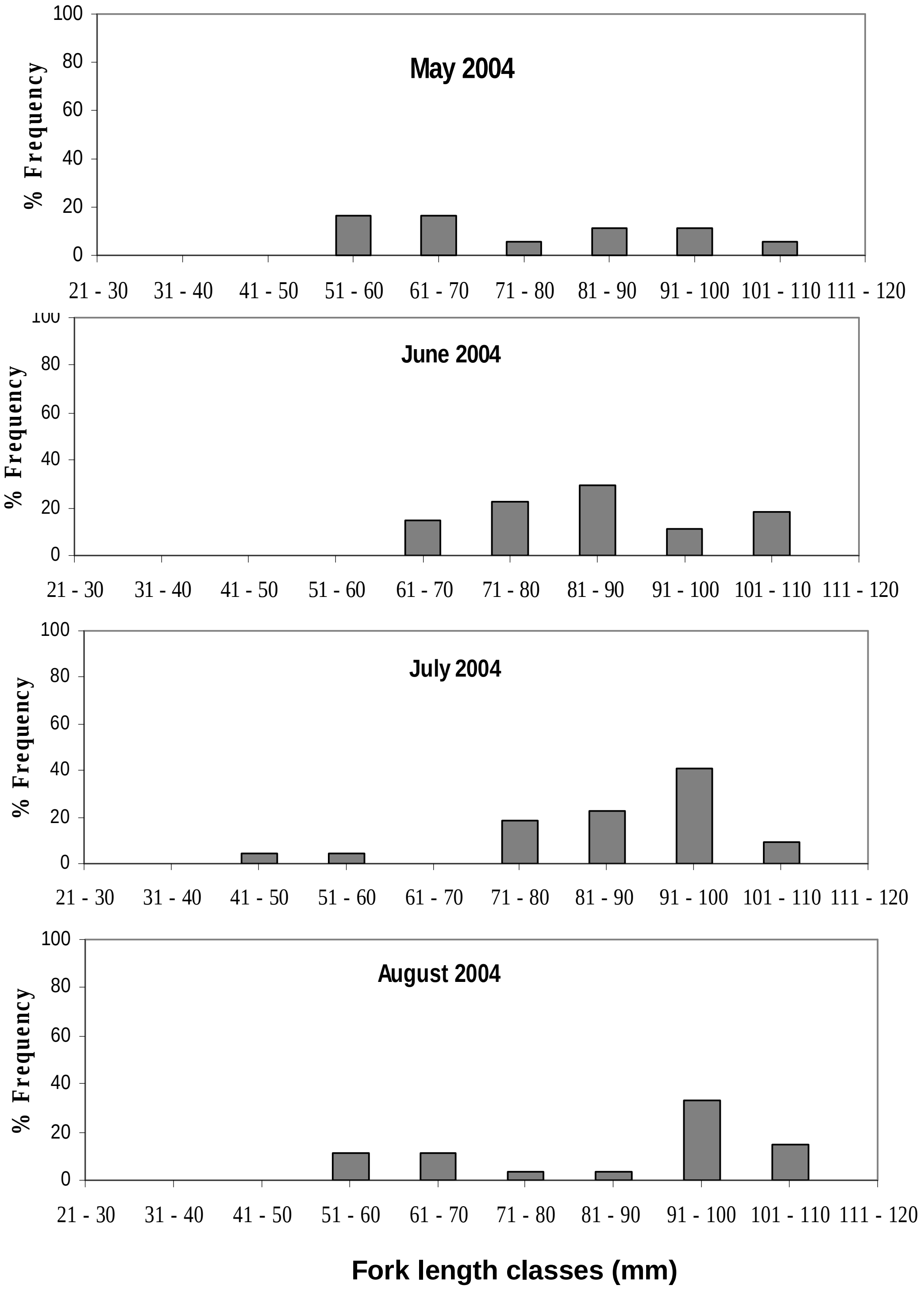

Figure 6.2: Length frequency distribution of *Labeobarbus marequensis* specimens collected in the Xikundu fishway as part of the "migration studies" in the Luvuvhu River during May to November 2004. The fork length classes are shown on the X-axis.

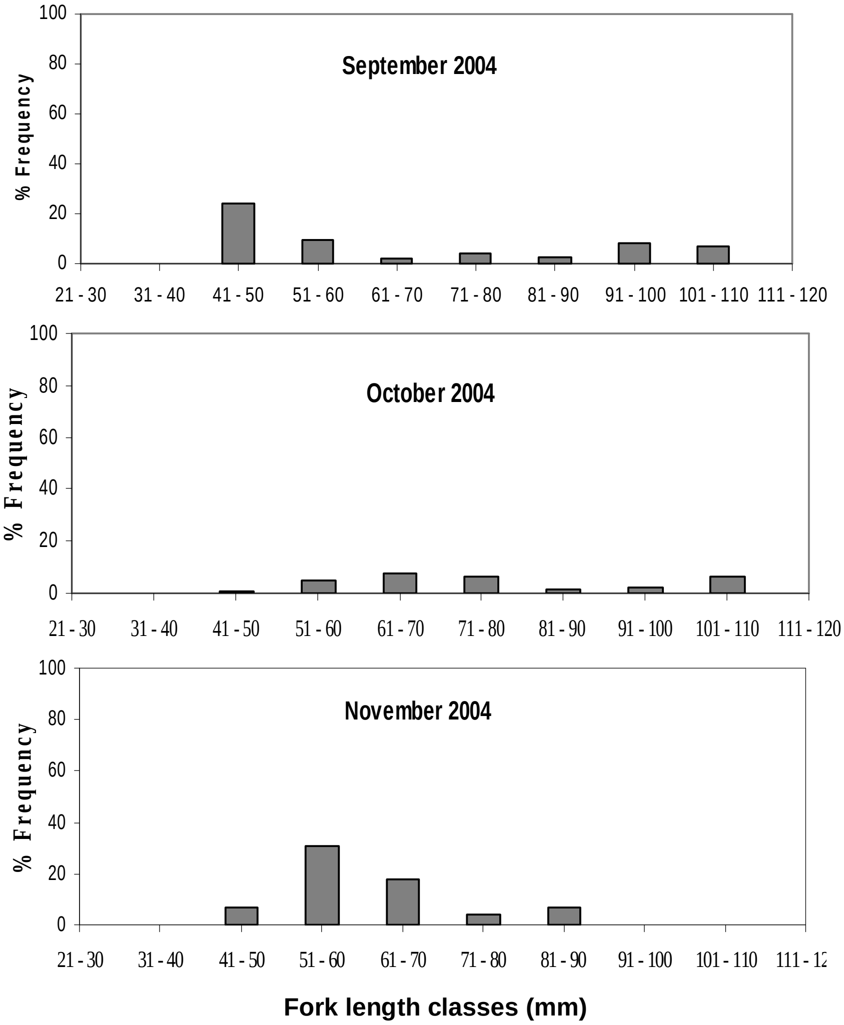

Figure 6.2 (cont.): Length frequency distribution of *Labeobarbus marequensis* specimens collected in the Xikundu fishway as part of the "migration studies" in the Luvuvhu River during May to November 2004. The fork length classes are shown on the X-axis.

diversity would therefore provide habitat for both large and smaller specimens. The distribution of the biotopes based on areas with similar substrate composition within the site is shown in figure 6.3. Area 1 was regarded as the most suitable for breeding. Although figures 6.4 and 6.5 show that the substrate is dominated by cobbles there was also some gravel and sand in the interstitial spaces. In addition area 3 and the deep rapids (area 2) could function as habitat for larger breeding adults. Impacts at the site were rated as "moderate" and included washing, fishing and trampling by cattle.

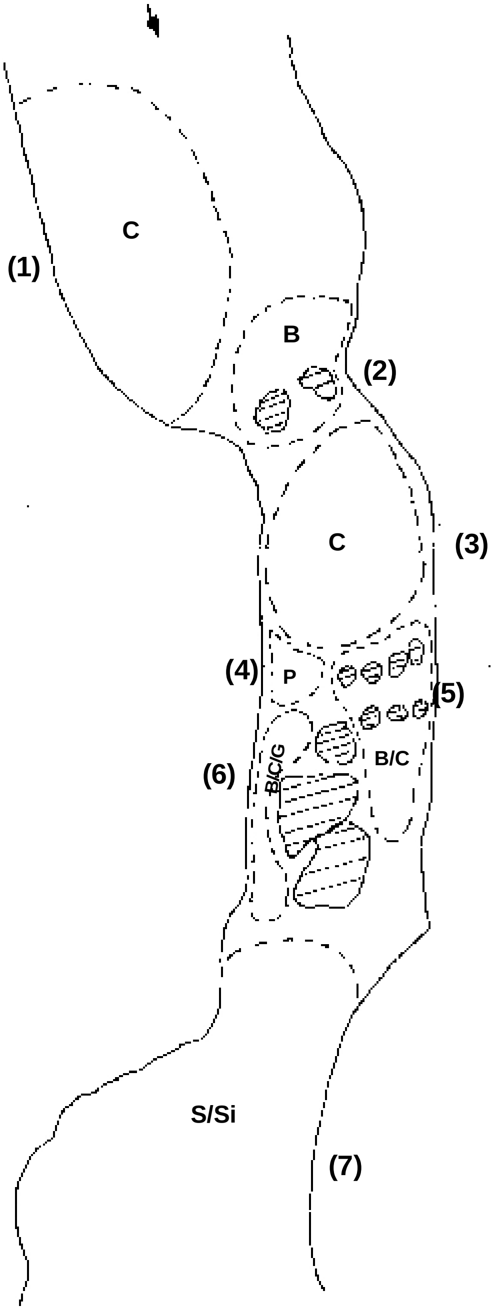

Figure 6.3: Sketch map showing the areas (1 – 7), based on dominant in-stream substrate type, surveyed at site 2, Tshino, in the Luvuvhu River. (Where BR; bedrock, B; boulders, C; cobble, G; gravel, S; sand and Si; silt. The non-aquatic areas with diagonal lines are rock outcrops and the dotted areas are sandy or vegetated. The arrow indicates flow direction.)

Figure 6.4: A potential breeding location at site 2, Tshino during periods of low flow.

Figure 6.5: The breeding location at site 2, Tshino during periods of higher flow.

Site 11: Tshifudi

The site is situated in the lower reaches of the Luvuvhu River downstream of the Nandoni Dam. In this reach of the Luvuvhu River the gradient has declined and rapids with suitable boulder and cobble substrates are rare. These characteristics are however present at the site. Because of its length the site was sub divided into two sub-sites during the habitat studies and it was decided to maintain the *status quo*. Both sub-sites were suitably diverse and figures 6.6 and 6.7 shows the distribution of the substrate types within each sub-site. The possible breeding locations (areas 8 and 17) are shown in figures 6.8 and 6.9 respectively. Area 8 seemed more favourable because the substrate was dominated by cobbles and an adjacent deep rapid was present. The substrate composition of area 17 was not as favourable but a very deep pool was located immediately downstream of it. Impacts at the site, which ranged from water extraction at sub-site 2b to washing at the drift in sub-site 2a, were rated as "moderate". The substrate in the pools consisted of sand and mud.

Site 5: Tshivhulani

This is a historic site in the Mutshindudi River where *L. marequensis* has been collected (State of the Rivers Report, 2001) and is typical of the lower section of a *mountain reach zone* of the river system that is steep and narrow and dominated by cobble riffles and bedrock rapids. The occasional pools are deep with boulders and mud as substrate. The necessary habitat diversity is present at the site (Figure 6.11) and the impacts at the site, which included washing, angling and water extraction, were rated as "small" to "moderate". Figure 6.10 shows that area 21 has the best potential as breeding habitat but area 22 could also be suitable. Although the substrate is dominated by cobbles there are boulders present as well with sand and gravel in the interstitial spaces. In addition area 24, a deep boulder rapid, and the deep pool (area 27) would provide habitat for breeding adults.

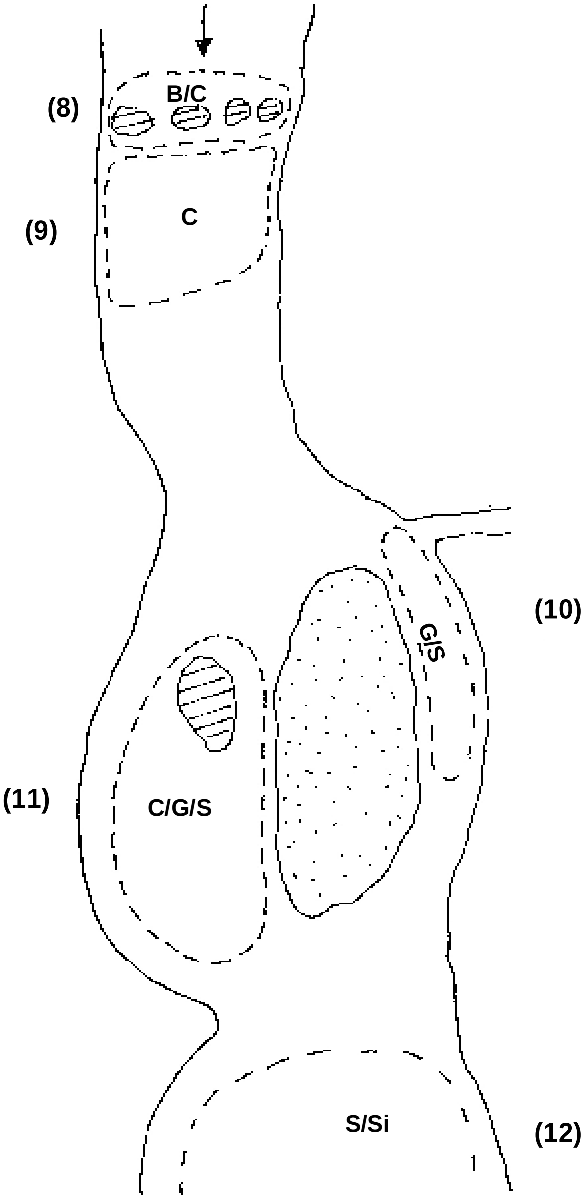

Figure 6.6: Sketch map showing the areas (8 – 12) , based on dominant in-stream substrate type, surveyed at sub-site a at site 11, Tshifudi, in the Luvuvhu River. (Where BR; bedrock, B; boulders, C; cobble, G; gravel, S; sand and Si; silt. The non aquatic areas with diagonal lines are rock outcrops and the dotted areas are sandy or vegetated. The arrow indicates flow direction.)

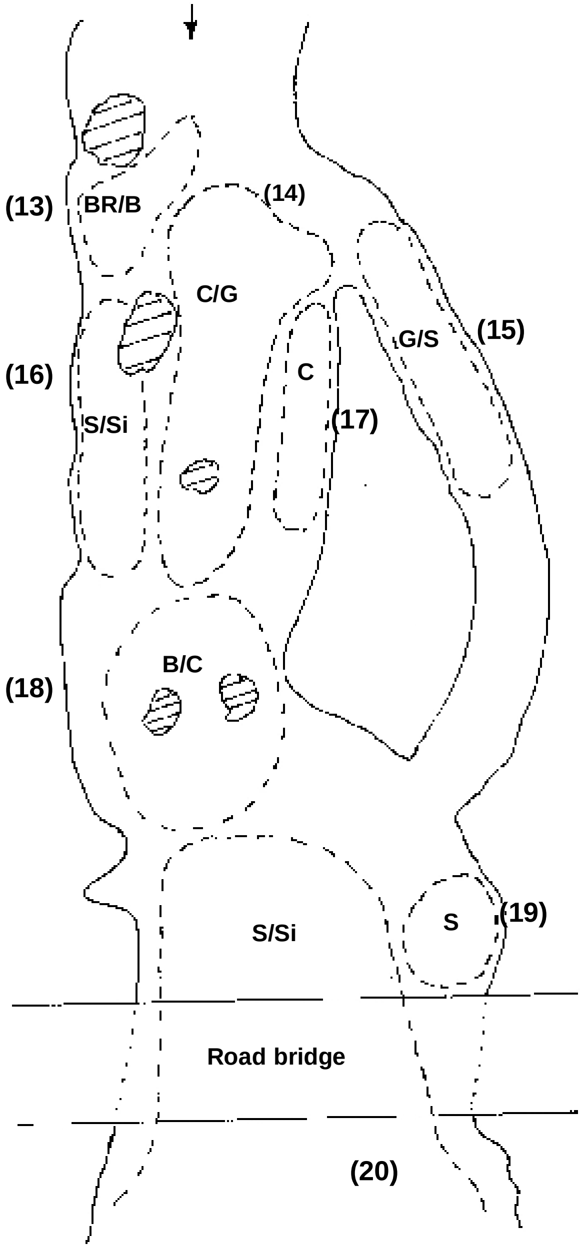

Figure 6.7: Sketch map showing the areas (13 – 20), based on dominant in-stream substrate type, surveyed at sub-site b at site 11, Tshifudi, in the Luvuvhu River. (Where BR; bedrock, B; boulders, C; cobble, G; gravel, S; sand and Si; silt. The non-aquatic areas with diagonal lines are rock outcrops and the dotted areas are sandy or vegetated. The arrow indicates flow direction)

Figure 6.8: Possible breeding location at sub-site “a” of site 11, Tshifudi in the Luvuvhu River

Figure 6.9 : Possible breeding location at sub-site “b” of site 11, Tshifudi in the Luvuvhu River

Site: Crocodile ventures

This historic site (State of the Rivers Report, 2001) is probably the most impacted of the four sites with on-site impacts such as water extraction and sedimentation ranging from “extensive” to “very extensive”. There was evidence of solid waste pollution, such as plastic bags. The site is situated in the Dzindi River upstream of the Nandoni Dam in the Luvuvhu River and straddles one of the bedrock hydraulic controls encountered after the river has entered the lower foothills. The site is diverse with areas 29 and 34 (Figure 6.12) regarded possible breeding areas. The characteristics of area 34, which was regarded as the better of the two areas (Figure 6.13) not only included both boulders and cobbles in the substrate but was immediately upstream of the largest pool (area 35) at the site.

Figure 6.10: Possible breeding location (area 21) at site 5, Tshivhulani, in the Mutshindudi River.

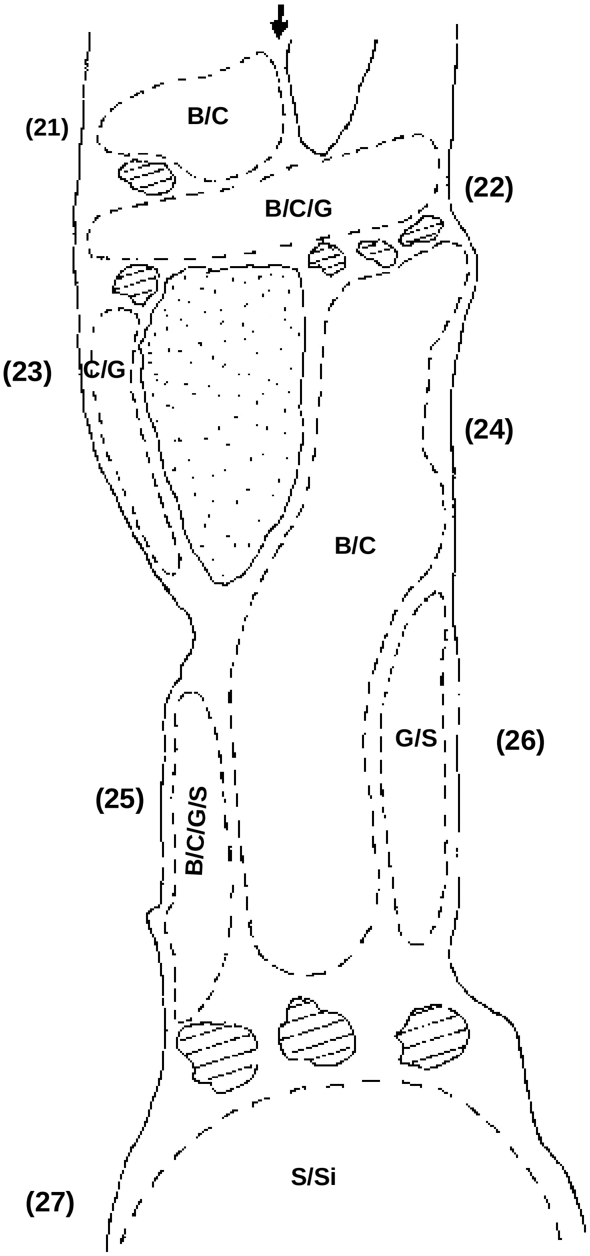

Figure 6.11: Sketch map showing the areas (21 – 27) , based on dominant in-stream substrate type, surveyed at site 5, Tshivhulani, in the Luvuvhu River. (Where BR; bedrock, B; boulders, C; cobble, G; gravel, S; sand and Si; silt. The non-aquatic areas with diagonal lines are rock outcrops and the dotted areas are sandy or vegetated. The arrow indicates flow direction.)

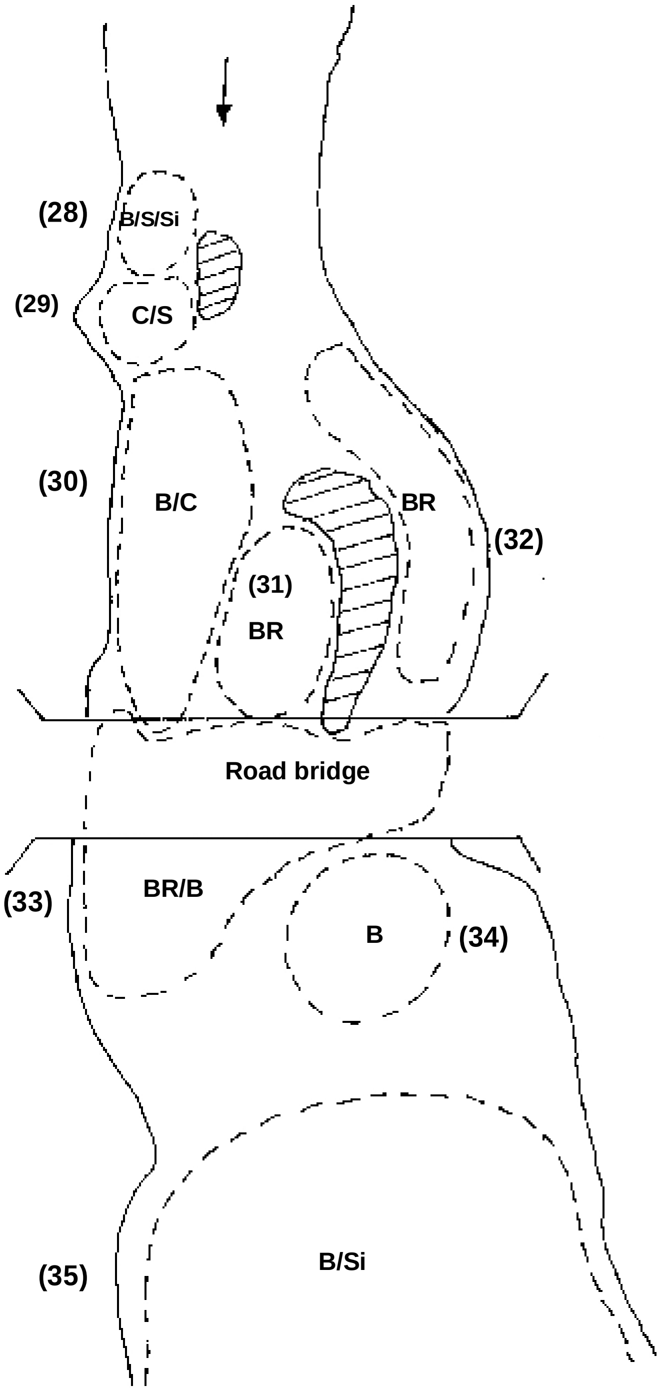

Figure 6.12: Sketch map showing the areas, based on dominant in-stream substrate type, surveyed at site 3, Croc Ventures, in the Luvuvhu River. (Where BR; bedrock, B; boulders, C; cobble, G; gravel, S; sand and Si; silt. Non aquatic areas with diagonal lines = rock outcrops, dotted area = sandy or vegetated area. The arrow indicates the direction of flow.)

Figure 6.13: Possible breeding location at site 3, Crocodile Ventures in the Dzindi River.

b) The monitoring period

Each of the four sites was monitored for a 24-hour period once a month from July to October 2007 on the dates shown in Table 6.4.

Table 6.4: Dates on which the selected *Labeobarbus marequensis* breeding sites in the Luvuvhu River were monitored.

No	Site name	July 2007	August 2007	September 2007	October 2007
1	Tshino	9 – 10	3-4	7 – 8	4 – 5
11	Tshifudi	11 – 12	5 – 6	8 – 9	18 – 19
5	Tshivhulani	12 – 13	6 – 7	9 – 10	17 – 18
3	Crocodile ventures	10 – 11	4 – 5	10 – 11	5 – 6

c) The selected physico-chemical aspects over the monitoring period.

The physico-chemical aspects determined *in situ* and the time of monitoring are shown in tables 6.5 – 6.8. These results show that in general the water in the main stem is warmer than the water in the two tributaries. As expected, the Mutshindudi has the lowest conductivity and amount of total dissolved solids (TDS). The values of the TDS readings at site 3 (Croc Ventures) in the Dzindi River is indicative of the high amount of sedimentation in the river. The dissolved oxygen levels at all the sites were above the required median values for aquatic life (Kempster *et al.*, 1980) and did not even reach sub-lethal levels (Dallas and Day, 2004). The same phenomenon was observed with the range of pH values but it should be noted that at all the sites a distinct increase (Figure 6.14) was observed over the survey period.

Table 6.5: The physico-chemical aspects recorded at site 2 (Tshino).

Parameters	Units	July 2007	August 2007	September 2007	October 2007
Dissolved oxygen	*mg/l*	**8,77**	**9,86**	**8,2**	**6,9**
	%	**88,0**	**99,8**	**90,7**	**92,4**
Temperature	*°C*	**14,7**	**15,5**	**21,8**	**25,6**
pH	▨	**7,2**	**6,56**	**8,75**	**8,02**
TDS	*ppm*	**63,0**	**63,4**	**65,7**	**67,7**
Conductivity	*µS/cm*	**128,0**	**125,7**	**131,3**	**134,4**
Time sampled		**10h00**	**8h30**	**9h15**	**10h30**

Table 6.6: The physico-chemical aspects recorded at site 11 (Tshifudi).

Parameters	Units	July 2007	August 2007	September 2007	October 2007
Dissolved oxygen	*mg/l*	**9,69**	**10,3**	**8,95**	**8,3**
	%	**100,1**	**105,7**	**112,0**	**100,5**
Temperature	*°C*	**15,7**	**16,5**	**24,1**	**24,6**
pH	▨	**6,61**	**6,79**	**8,5**	**7,3**
TDS	*ppm*	**74,3**	**81,3**	**80,0**	**78,1**
Conductivity	*µS/cm*	**148,6**	**162,9**	**159,3**	**156,0**
Time sampled		**10h00**	**10h00**	**11h00**	**9h30**

Table 6.7: The physico-chemical aspects recorded at site 3 (Croc ventures).

Parameters	Units	July 2007	August 2007	September 2007	October 2007
Dissolved oxygen	*mg/l*	**9,1**	**7,41**	**6,8**	**8,4**
	%	**92,0**	**87,1**	**78,5**	**95,5**
Temperature	*°C*	**14,3**	**13,9**	**19,6**	**22,7**
pH	▨	**6,6**	**6,64**	**7,14**	**7,12**
TDS	*ppm*	**69,0**	**79,4**	**74,7**	**62,3**
Conductivity	*µS/cm*	**139,2**	**144,3**	**148,6**	**124,6**
Time sampled		**9h15**	**11h00**	**9h45**	**11h50**

Table 6.8: The physico-chemical aspects recorded at site 5 (Tshivhulani).

Parameters	Units	July 2007	August 2007	September 2007	October 2007
Dissolved oxygen	*mg/l*	8,61	11,86	8,8	6,5
	%	92,0	106,9	88,6	92,2
Temperature	*°C*	15,6	14,4	18,7	23,5
pH	▨	6,8	6,61	6,91	6.54
TDS	*ppm*	52,3	50,3	55,7	50,0
Conductivity	*µS/cm*	101,4	100,7	111,3	100,0
Time sampled		10h00	9h00	9h30	11h00

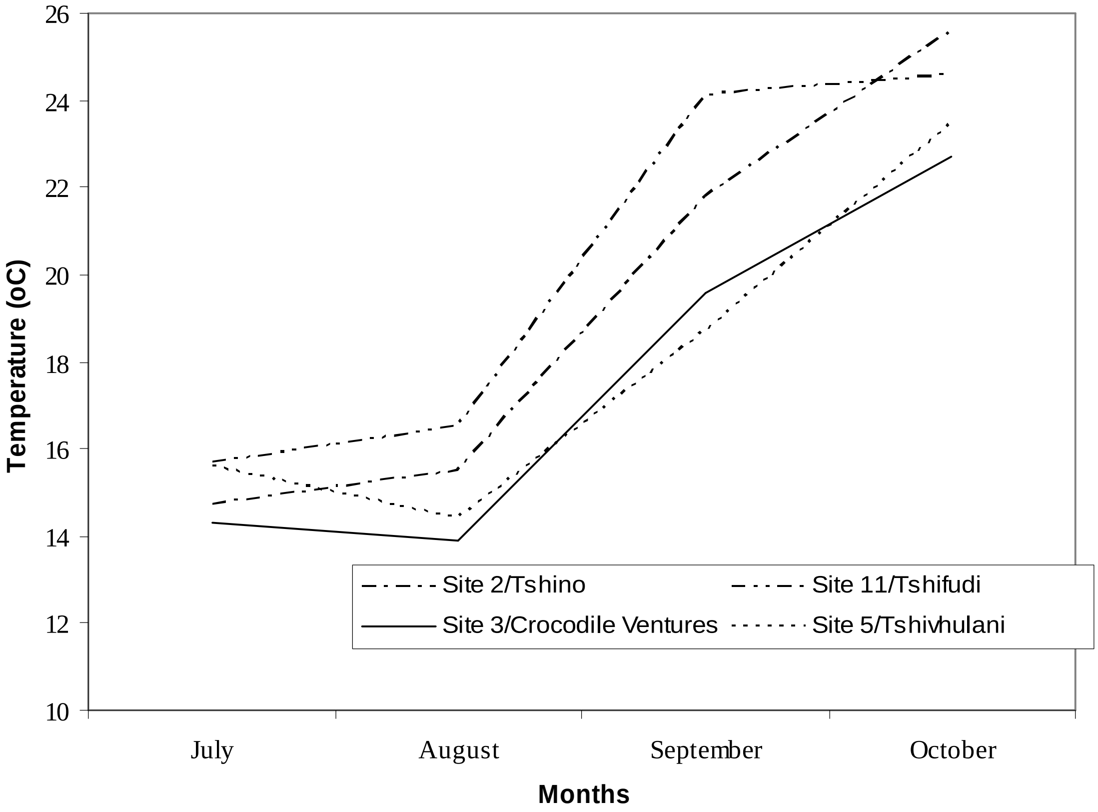

Figure 6.14: The water temperature measured at the potential *Labeobarbus marequensis* "breeding sites" surveyed in the Luvuvhu River during the period July to October 2007.

d) Observed trends in the discharge and flow during the survey.

The discharges calculated from the data collected in the natural hydraulic controls at the four sites during the four surveys in 2007 (Figure 6.15) shows that from August the flow increased dramatically at three sites. The exception was site 2 which was situated downstream of a weir. The observed increase peaked at sites 5 and 11 in September after which the increase slowed down. At site 3 the August to September increase was not as dramatic but continued up to October.

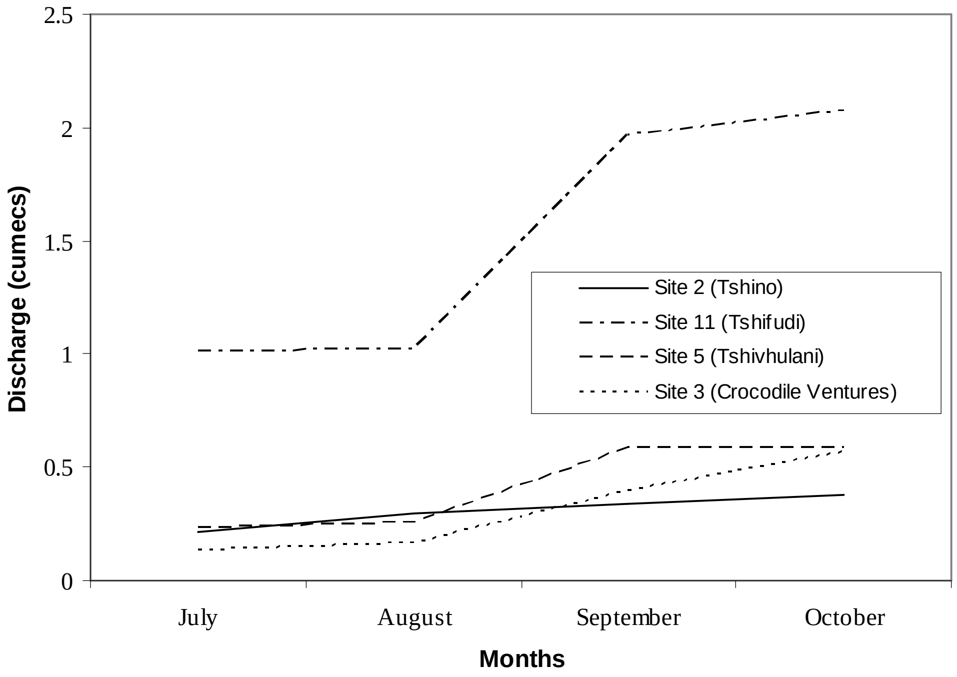

Figure 6.15: Calculated discharges (Q) in the natural hydraulic controls surveyed at the four breeding sites in the Luvuvhu River system during the period July to October 2007.

During the periods of flow increase the character of the breeding areas changed. At site 2 (Tshino) and site 5 (Tshivhulani) the rise in water level, that began in August, covered the substrate in areas 1 and 21 and increased flow was observed among the cobbles and boulders. Changes in the breeding areas at the other two sites (areas 29 and 34 at site 3 and areas 8 and 17 at site 11) were not as dramatic where only increases in surface area, water depth and velocity were observed.

e) The specimens collected during the survey period.

In chapter 4 it was illustrated that, based on changes in the length-mass relationship, nine stanzas or growth phases, each with its own distinct relationship, could be identified in the life cycle of the species. In this chapter stanza 1, which included specimens up to 50mm in fork length were subdivided into 3 subgroups namely J_1 which included fish up to 20mm in length, J_2 which included fish up to 30mm and subgroup 1 which contained the rest of the original stanza 1.

The results based on the presence or absence of specimens of the different stanzas of the species (Table 6.10) show that small specimens of below 30mm fork length were collected in the identified "breeding areas" at the sites. The exception was areas 22 and 24 (Figure 6.11) where some juveniles of the J_2 group were collected. In retrospect, area 22 should have been regarded as a breeding area when the available habitat is re-considered. It is important to note that a few very small specimens of the J_1 sub-group were collected in area 8 at the Tshifudi site during August and in area 21 at the Tshivhulani site in September. The majority of specimens in this sub-group was collected in October at sites 2, 11 and 5. It should further be noted that although no specimens of this sub-group was collected at site 3, the fact that specimens of the J_2 group occurred was a clear indication that spawning had occurred at the site.

High velocity areas are utilised as spawning areas by many species partly because of the higher oxygen concentration in these areas relative to other habitats. This results from turbulence and the entrainment of air bubbles near the surface and make them ideal for incubating fish eggs (Soulsby *et al*., 2001). Although the breeding areas were selected because of the presence of suitable breeding substrate, namely boulders and cobbles, and flowing water, cognizance of two further aspects should be taken. First of all, these areas were in close proximity to suitable habitat for adult specimens. This implies that both rapids and pools had to be present. The second aspect concerns the physical areas, which could be termed **"micro-areas"**, within the breeding areas in which the very small specimens of the sub-groups J_1 and J_2, were collected. Table 6.9 shows the recorded physico-chemical parameters recorded in the micro-areas. With exception of temperature which was higher in the micro-areas all other characteristics were the same as in the rest of the area. In most of these areas the substrate that consisted of both cobbles and boulders offered sufficient protection against predators. These micro-areas, which were usually at the outer edges of the larger area, which were characterized by very low water velocities or even absence of flow, were regarded as the “nursery areas” and will be referred to as such in the text. In addition the higher water temperatures of these nursery area will lead to the production of more food which together with the accelerated metabolism of the juveniles will result in a better growth rate of the juveniles.

Table 6.9: A comparison of the physico-chemical parameters measured in the “micro-areas” and in the general breeding area during the October 2007 survey in the Luvuvhu River. (Sub-area = the micro-areas, General = the rest of the area)

Site name and number		pH	DO (%)	DO (mgl^{-1})	Electrical Conductivity (μScm^{-1})	TDS (ppm)	Temperature (^{o}C)	Date
Site 2 Tshino	Sub-area	8.02	107	9.0	130.0	67.7	26.1	Oct.
	General	8.02	92.4	6.9	134.4	67.7	25.6	
Site 11 Tshifudi	Sub-area	7.3	95.0	7.6	154.0	77.0	25.0	Oct.
	General	7.3	100	8.3	156.1	78.1	24.6	
Site 5 Tshivhulani	Sub-area	6.5	90.0	6.2	104.9	52	23.8	Oct
	General	6.54	92.2	6.5	100.0	50	22.5	
Site 3 Croc ventures	Sub-area	7.29	93.3	7.34	127.7	63.7	23.9	Oct
	General	7.12	95.5	8.37	124.6	62.3	22.7	

Table 6.10 further shows a second trend that is related to recruitment and spawning. With the exception of site 2 (Tshino) where no specimens longer than 80mm were collected, fish from stanza 4 and above were collected during August and September at all the other sites. Because the sampling protocol remained unchanged, their presence in the nets can only be related to an increase in activity and in particular to a spawning event. This was even the case at site 5 (Crocodile Ventures) where no J_1 specimens were collected. As was expected the large specimens in stanzas 7, 8 and 9 were females and were present in the deeper pools. Special mention need to be made of the male specimens, of stanzas 3, 4 and 5 that were collected in area 24 at site 5 (Tshivhulani). The chronology of their presence prior to the spawning event should be noted. This area is immediately downstream of the initially identified breeding area (area 21) and area 22 which later proved to be suitable for breeding.

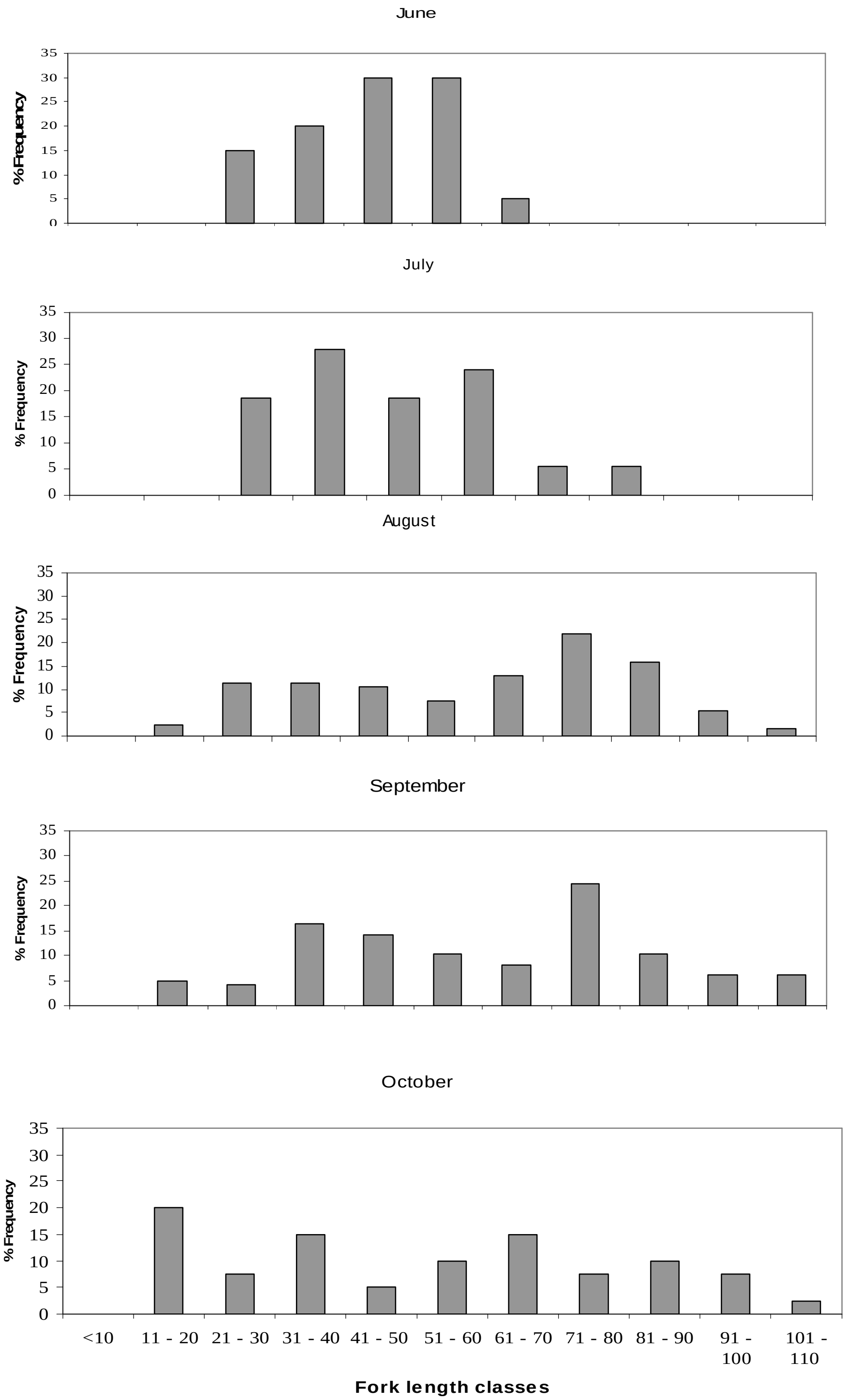

Figure 6.16: Percentage frequency of occurrence of *Labeobarbus marequensis* in the fork length classes smaller than 110 mm collected at the breeding ecology sites in the Luvuvhu River during the period June 2007 to October 2007.

Table 6.10: Presence of specimens of the different stanzas *Labeobarbus marequensis* collected in the period July to October 2007 in the identified "areas" related to breeding at the four sites in the Luvuvhu River system. (The numbering of the areas relate to figures 6.3, 6.6, 6.7 and 6.11. The abbreviations for the hydraulic biotopes are: B = breeding area, RI = riffle, RA = rapid, SP = shallow pool, P = pool. The stanzas identified are: 2 = 51 – 80mm, 3 = 81 – 100mm, 4 = 101 – 120mm , 5 = 121 – 150mm, 6 = 151 – 200mm, 7 = 201 – 250mm, 8 = 251 – 320mm and 9 = > 321mm. Stanza 1 was subdivided in J_1 = 11 -20mm, J_2 = 21 – 30 mm and 1 = 31 – 50mm.

Site name	Area	Velocity depth class	Hydraulic Biotope	July 2007	August 2007	September 2007	October 2007
Site 2 Tshino	1	SS	**B**				J_1
	2	FS	RI				2
	3	FS	RI			J_2, 1	
	4	FS	RI	J_2, 1			
	5	FS	RA	J_2, 1	1,2		
	6	SS	SP		J_2	J_2, 1	
Site 11 Tshifudi	8	FS	**B**	2	J_1, J_2	J_1, J_2	J_1
	9	FS	RI				
	11	SS	RI	J_2, 1,2			2
	13	FS	RI	2		2	2
	14	FS	RA			2,3	2,3
	15	SS	SP				J_1
	16	SS	SP	2		2	3
	17	SS	SP	2		2	
	20	SD	P	7		6,7,8	8,9
Site 5 Tshivhu-lani	21	SS	**B**		J_2	J_1, J_2	J_2
	22	SS	SP	J_2	J_2	1	
	23	FS	RI			J_2, 2	J_2, 1
	24	FS	RA			J_2, 2,3,4,5	J_1J_2 ,2
	25	SS	RI		J_2		J1
	26	FS	RI				4
	27	SD	P			5,6,8	
Site 3 Crocodile Ventures	28	SS	**B**		J_2	J_2	
	30	FS	RI	1,2		2	J_2
	31	SS	SP	1,2		1	
	32	FD	RA	2		1	2
	33	FS	RI	1,2		1,2	
	34	SS	**B**		1		J_2
	35	SD	P			6	3,5,6
	38	SS	**B**	J_2, 1			

6.3.3. Size distribution and the male to female ratio.

In the specimens collected in the Luvuvhu River and tributaries as part of the breeding ecology component of this project a male to female ratio of 77:23 was observed. This is similar to the results of the migration component at the Xikundu fishway in the Luvuvhu River where a ration of 79:21 was recorded.

6.3.4 Breeding biology.

The results of the seasonal gonadal development analyses of the *L. marequensis* specimens collected in the Xikundu fishway are shown in table 6.11 and figure 6.17. Although these calculations were done on fish longer than 80mm, no distinction was made between males and females. This is illustrated by the difference between the minimum and maximum values in all the months (Table 6.11). Figure 6.17 does however show that at the onset of the survey, (in May 2004) the GSI was on a downward trend and according to Knight and Ross (1992) which could be an indication of a spawning event. It should however be treated with caution and the trend should not be regarded as a spawning event but rather a continuation of a spawning event that occurred in the February or March. Similar results were obtained by Vlok (1992) in his investigation of testicular development of *L. marequensis.* The downward trend was followed by a period (starting in July 2004) during which the GSI increased and peaked in August which according to Knight and Ross (op cit.) indicates that reproductive readiness was at a maximal. The peak was then followed by a downward trend that gradually started in September and ended in November. This downward trend presents a second spawning event. A second increasing trend, that commenced after November and continued towards April, indicates that the gonads were again preparing for a spawning event.

Table 6.11: Average values of the calculated Gonadosomatic Index of specimens of *Labeobarbus marequensis* > 80mm fork length collected in the Xikundu Fishway in the Luvuvhu River during the period May 2004 to April 2005.

	May	**June**	**July**	**August**	**September**	**October**	**November**	**January**	**February**	**March**	**April**
Average	0.7,646	0,6517	0,6109	1,0893	1,0478	0,7140	0,2390	0,5310	0,4727	0,6465	0,5622
SD	0,5127	0,5353	0,2117	0,8540	0,7608	0,6790	0,1540	0,2056	0,3187	0,3726	0,2294
Minimum	0,1855	0,1281	0,3550	0,4080	0,1629	0,0656	0,0490	0,3260	0,1559	0,2167	0,1274
Maximum	1,6890	1,7923	1,0940	3,9500	5,3027	3,4618	0,5970	0,9359	0,7935	0,8998	1,1098

Table 6. 12: Average values of the calculated Condition Factor of all the *Labeobarbus marequensis* specimens collected in the Xikundu Fishway in the Luvuvhu River during the period May 2004 to April 2005.

	May	**June**	**July**	**August**	**September**	**October**	**November**	**January**	**February**	**March**	**April**
Average	1,4203	1,5002	1,6935	1,6074	1,7996	1,8264	1.9708	1,7844	1,7948	1,5973	1,6561
SD	0,4092	0,1344	0,1355	0,1437	0,2479	0,1846	0,2581	0,1684	0,0744	0,2210	0,1189
Minimum	0,6591	1,2461	1,4548	1,2563	1,0230	1,1610	1,6559	1,4408	1,7909	1,4509	1,4856
Maximum	1,8583	1,6527	1,9149	1,9090	2,3171	2,1405	2,4433	1,9838	1,9838	1,8516	1,7927

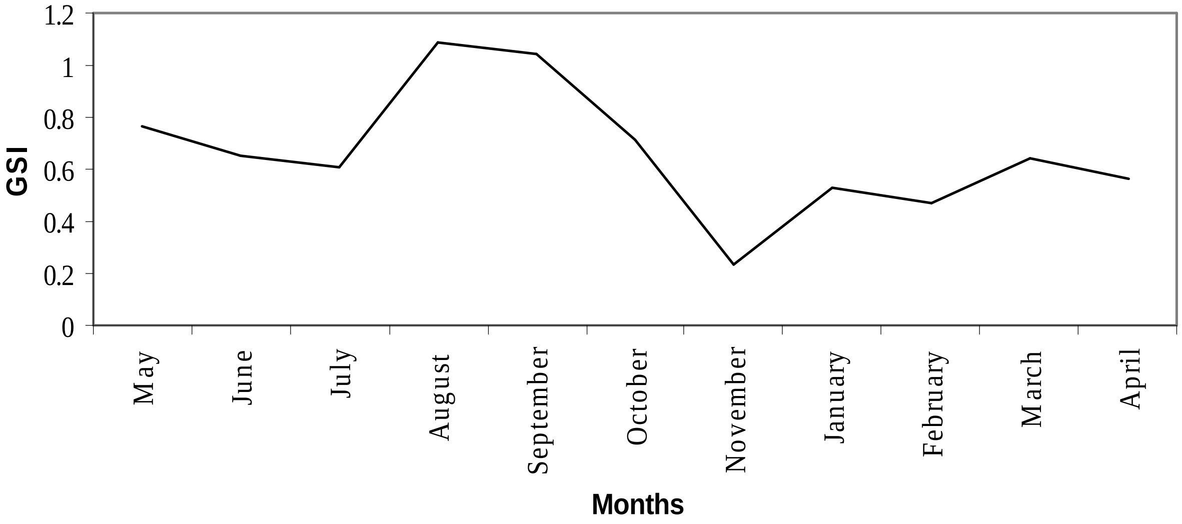

Figure 6.17: Seasonal changes in the Gonadosomatic Index of specimens of *Labeobarbus marequensis* > 80mm in fork length collected in the Xikundu Fishway in the Luvuvhu River during the period May 2004 to April 2005.

The analyzed data of the fish collected in the "breeding component" of the study (Figure 6.18) shows that during the July 2007 survey the GSI values where on a downward trend followed by an increase which commenced in September and showed a slight decreases to October. The downward trend is indicative of the end of a spawning event while the deceleration in the increasing trend could be an indication of the start of a second spawning event. The downward trend was not observed when the Maturity Coefficient (Figure 6.19) was calculated for the same specimens. In the case of these specimens the males and females where gonadal activity had been recorded, were separated and the results are shown in figure 6.20. In these results the initial downward trend is not exhibited but the GSI of both males and females increased from July onwards indicating a preparation towards a spawning event. The relative smaller size of the testis is illustrated by the difference in the calculated values shown in figure 6.20. The lower GSI observed in the males also reflects the lower effort directly expended in males (Helfman *et al.*, 2000).

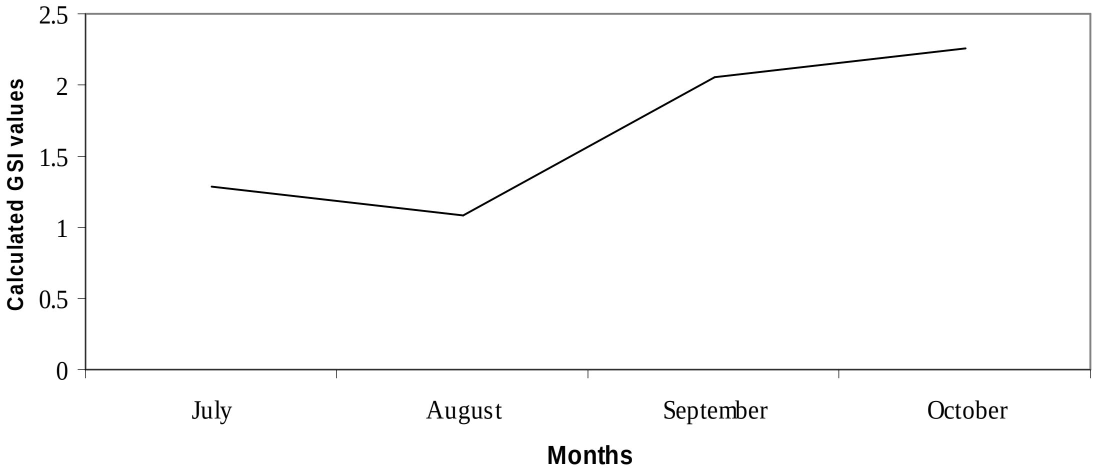

Figure 6.18: Calculated GSI values of all the specimens of *Labeobarbus marequensis* collected at the breeding sites in the Luvuvhu River during the period July to October 2007.

The values of the calculated Condition Factor of the fish collected in the Xikundu Fishway (Figure 6.21) show that the condition of the fish increased steadily after the first spawning event that occurred prior to May 2004. This increase continued to November 2004 which was after the second spawning event which occurred from September 2004. The Condition Factor data collected in the breeding ecology component (Figure 6.22 and Table 6.13) also show an increase starting in August 2007 which continued up to October. Figure 6.23 shows examples of the degrees of the fat deposition observed in the fish collected at the Xikundu fishway. Figure 6.24, which shows the percentage frequency of the visually observed fat deposition classes, mirrors the situation observed with the calculated condition factors. It is observed that during the pre-spawning phase (from June to August) the extent of fat accumulation decreases while in the spawning phase (in September and October) the fat deposition increases. The spawning event is followed by a period, from November to February, during which the frequency of occurrence of fat deposition is dominated by unit 4 and 5 situations of fat deposition.

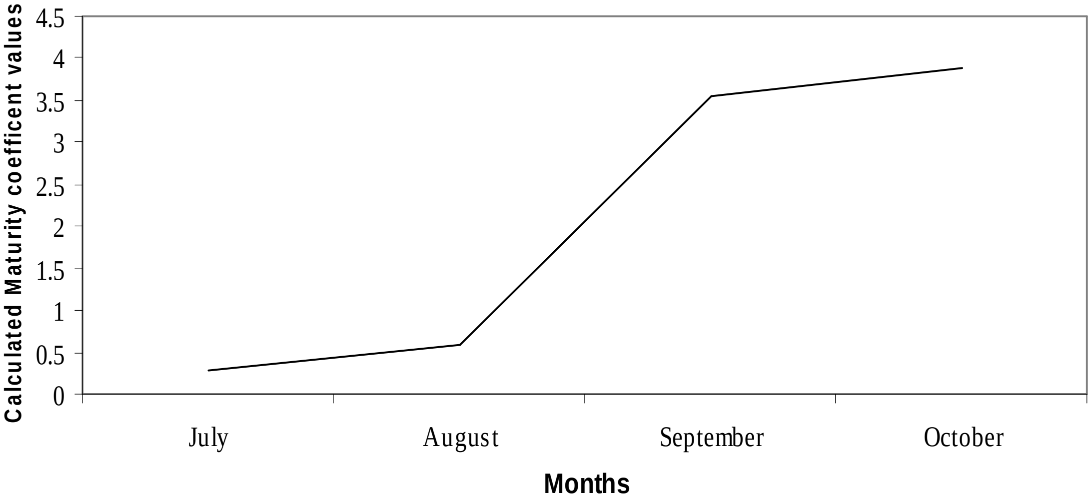

Figure 6.19: Calculated maturity coefficient values of all the specimens of *Labeobarbus marequensis* collected at the breeding sites in the Luvuvhu River during the period July to October 2007.

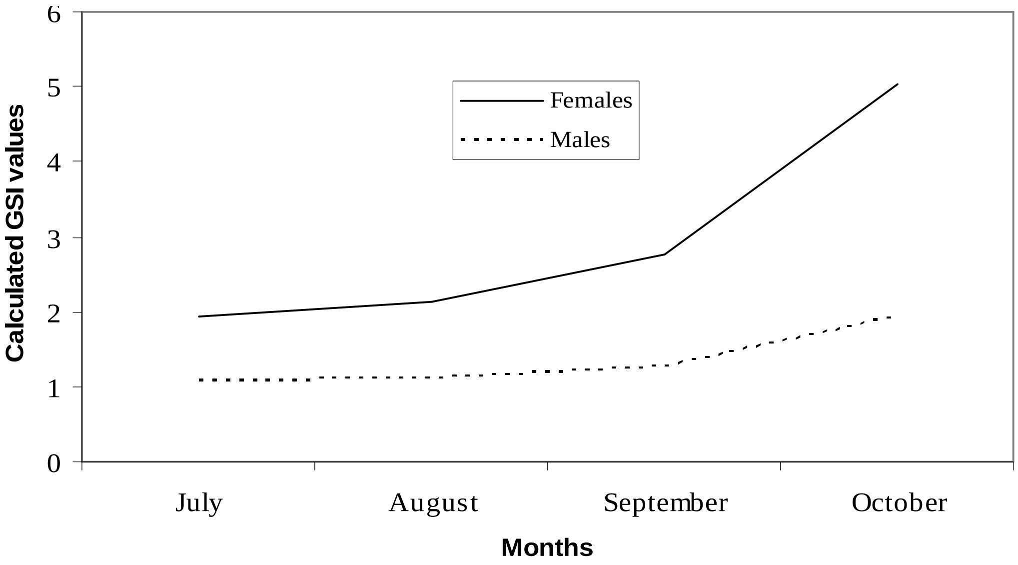

Figure 6.20: Calculated GSI values of the male and female specimens of *Labeobarbus marequensis* collected at the breeding sites in the Luvuvhu River during the period July to October 2007.

Table 6.13 : Summary of the descriptive statically analyses of the calculated Condition Factor , Gonadosomatic Index and Maturity coefficient values for all the specimens of *Labeobarbus marequensis* collected in the Luvuvhu River during the period July to October 2007.

	July	August	September	October
Gross Condition Factor (GCF)				
Mean	1.397	1.3785	1.7087	1.72
Standard deviation	0.1778	0.1793	0.2345	0.375
Minimum	1.1686	0.9713	1.4927	0.7558
Maximum	1.65547	1.7634	2.5051	2.1835
Condition Factor (CF)				
Mean	1.4287	1.366	1.673	1.6832
Standard deviation	0,1164	0.1761	0.2078	0.375
Minimum	1.2084	0.9718	1.4876	0.7559
Maximum	1.6547	1.7539	2.505	2.1835
Maturity coefficient (MC)				
Mean	0.2751	0.581	3.544	3.887
Standard deviation	0.0953	1.517	4.776	4.720
Minimum	0.270	0.13	0.3810	0.0612
Maximum	4.572	7.379	16.05	16.222
Gonadosomatic Index (GSI)				
Mean	1.2049	1.0821	2.0636	2.2566
Standard deviation	1.3058	1.5619	2.15265	2.7854
Minimum	0.191	0.0972	0.21907	0.0821
Maximum	3.393	5.3483	6.4241	8.119

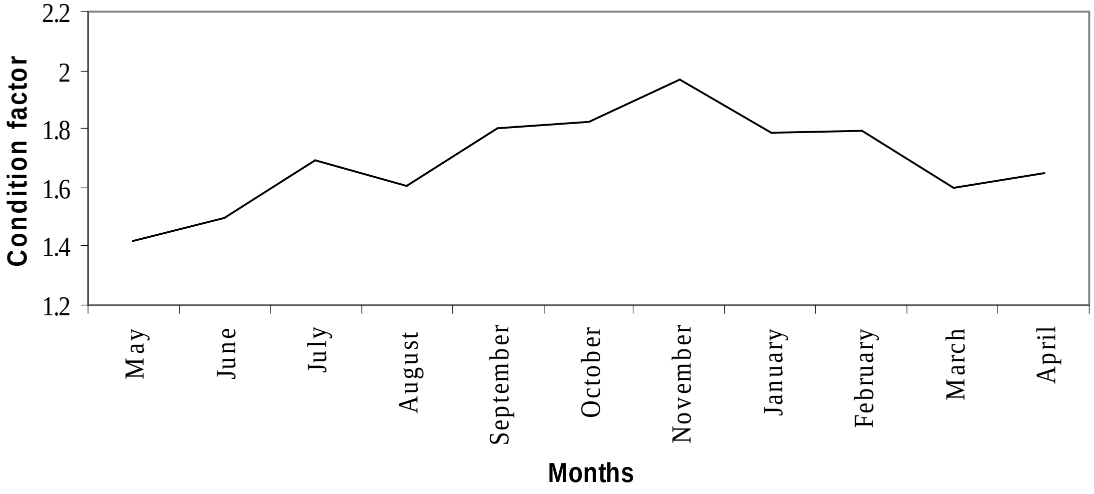

Figure 6.21: Seasonal changes in the condition (GCF) of specimens of *Labeobarbus marequensis* > 80mm in fork length collected in the Xikundu Fishway in the Luvuvhu River during the period May 2004 to April 2005.

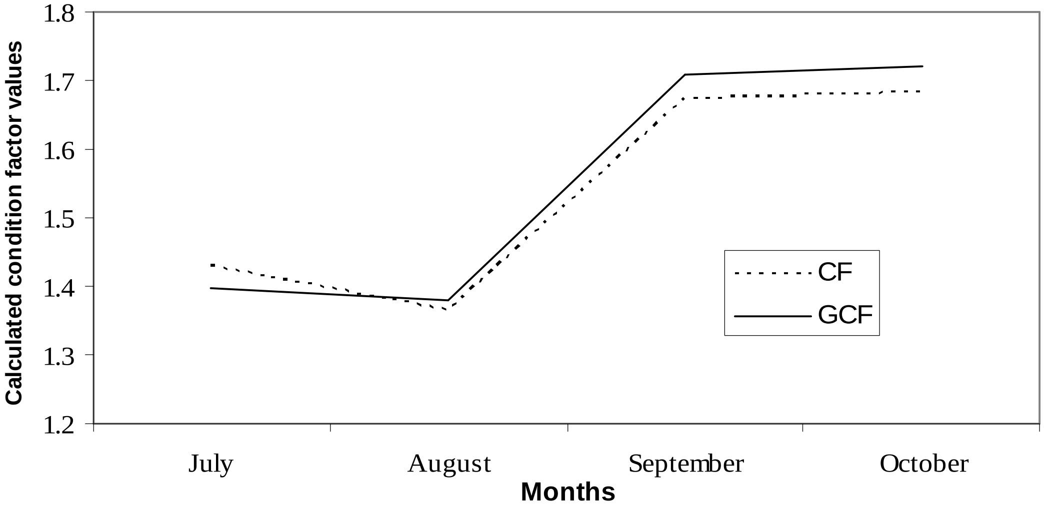

Figure 6.22: **Seasonal changes in the Condition Factor of specimens of *Labeobarbus marequensis* collected at the breeding sites in the Luvuvhu River during the period July to October 2007.**

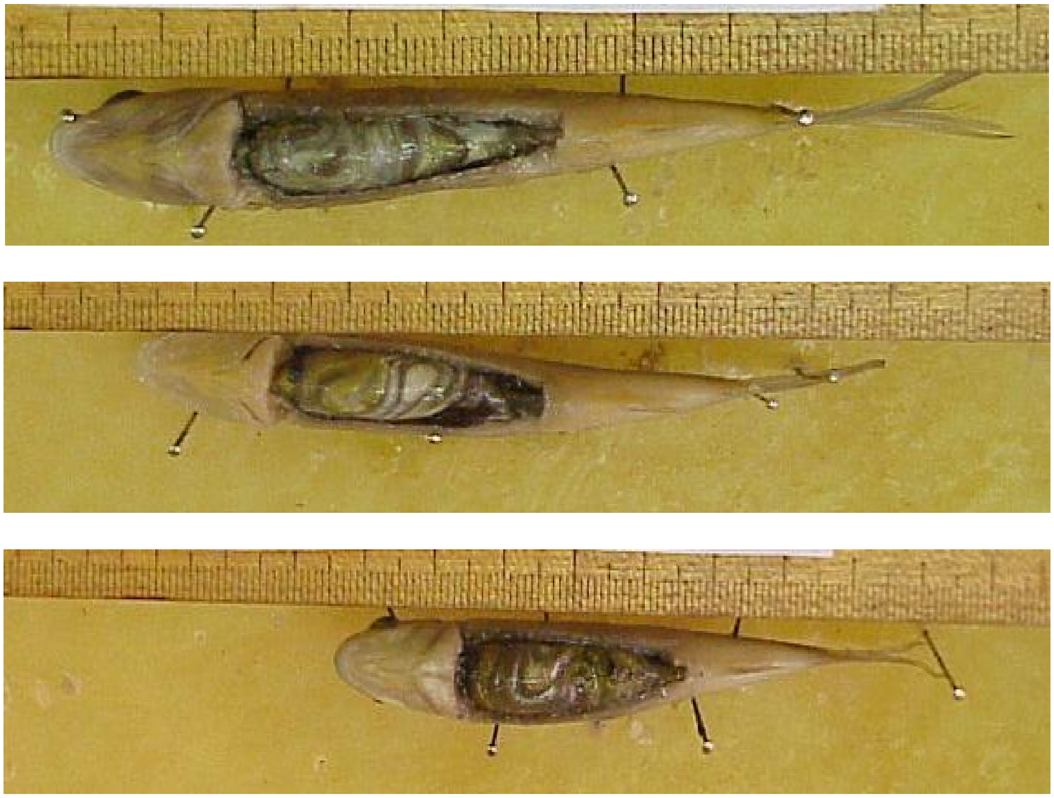

Figure 6.23: **Examples of the degrees of fat deposition observed in specimens of *Labeobarbus. marequensis* collected at the Xikundu fishway during the period May 2004 to April 2005.**

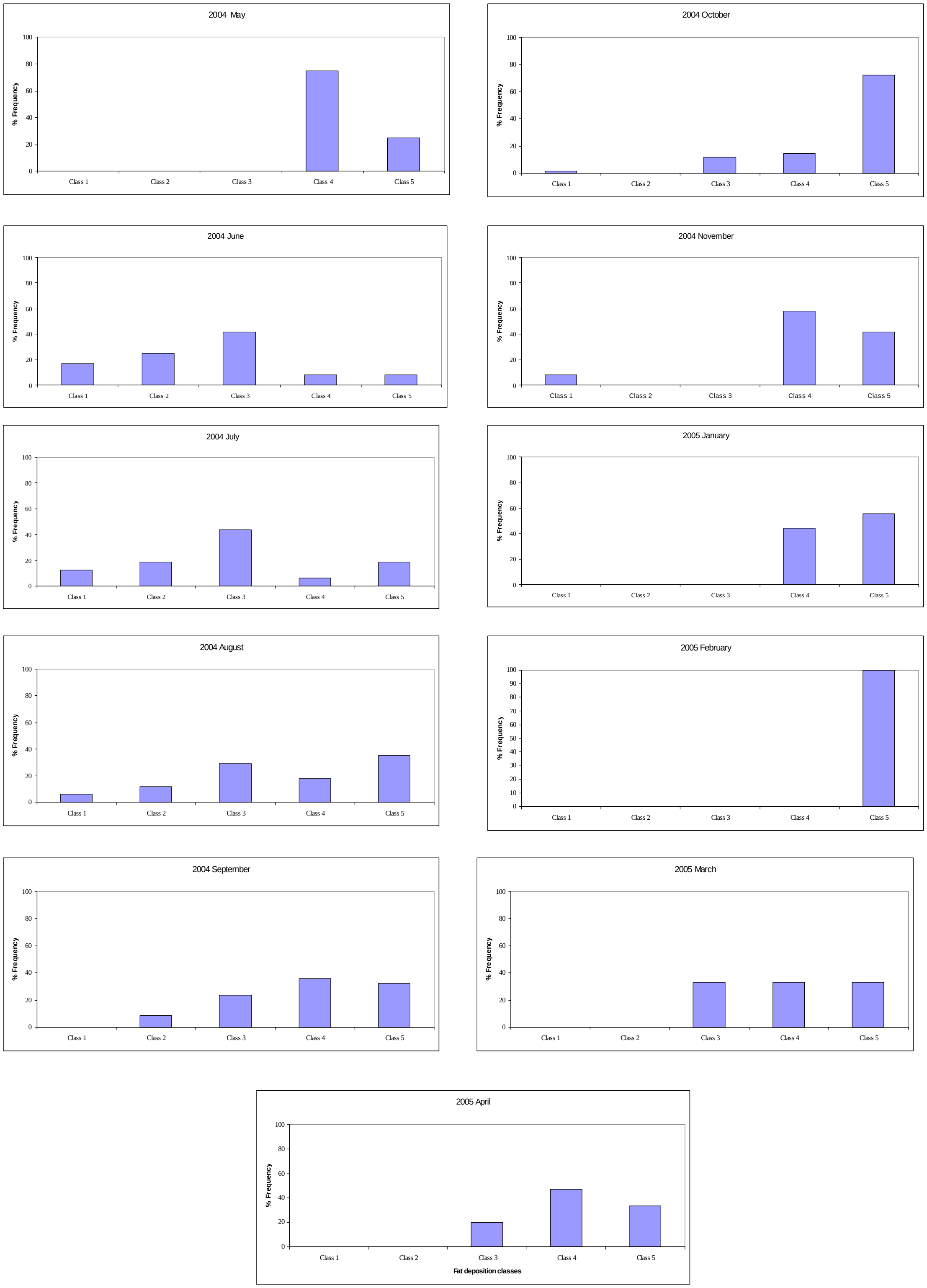

Figure 6.24: Frequency of the fat classes observed in *Labeobarbus marequensis* collected in the Xikundu fishway during the period May 2004 to April 2005. The fat deposition classes are shown on the X-axes.

The length at sexual maturity was recognized as the length at which fifty percent of the fish had maturing or mature gonads. To establish these lengths the data of all the specimens of *L. marequensis* collected in all the aspects of this study were pooled. Figure 6.25 shows that at fork lengths longer than 90mm fifty percent and more of the males had gonads that were classified as either as maturing or mature.

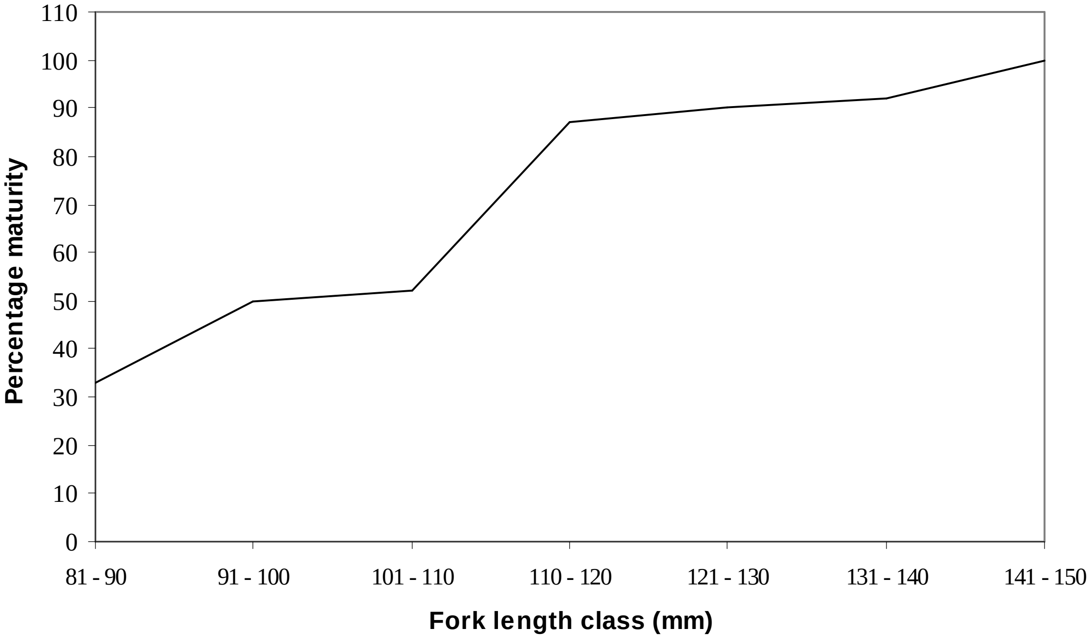

Figure 6.25: The length at sexual maturity of all the identified male *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries.

These findings are confirmed by the maturity coefficients of the male specimens of *L. marequensis* collected in the breeding ecology component of this study. Figure 6.26 shows two inflection points in the graph when the maturity coefficient is plotted against the fork length classes. The first inflection coincides with the 71 – 80 mm fork length classes while the second inflection point coincides with the 91 – 100 mm class.

Because of the low number of female specimens collected in some of the fork length classes, the length at sexual maturity results could not be graphically displayed as is the case with the

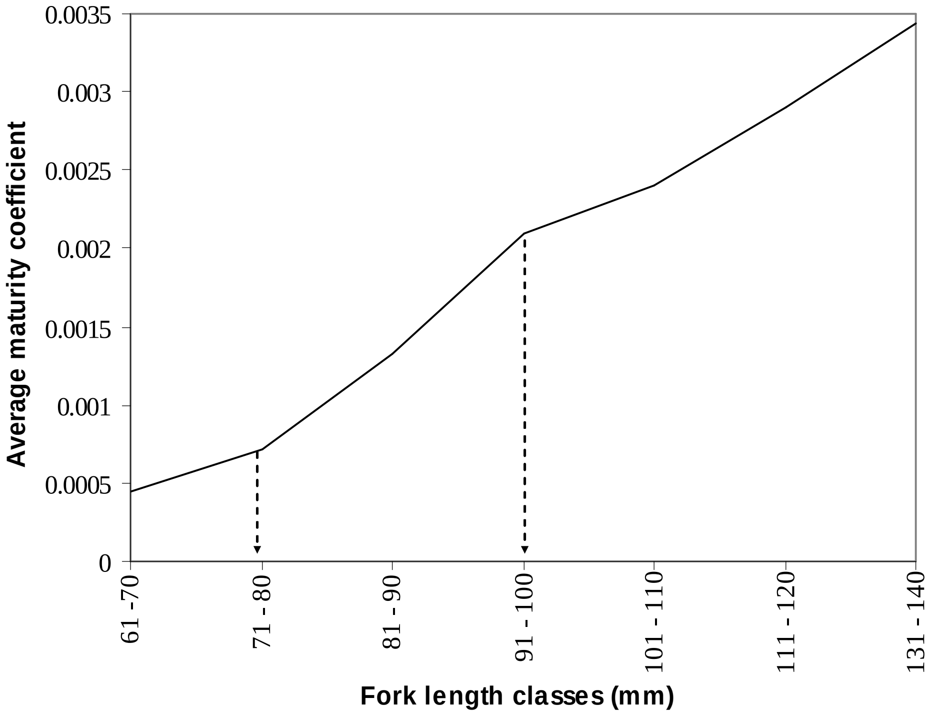

Figure 6.26: Average maturity coefficient of the male specimens collected at the breeding ecology sites in the Luvuvhu River from July to October 2007. (The arrows indicate the inflection points in the graph).

male specimens collected. It should however be noted that no sexually mature ovaries were observed in fish with fork lengths shorter than 120mm. All these ovaries were classified as "virgin" (Table 6.1) or Latent. In the fork length classes between 121 and 140mm ovaries classified as "maturing" were observed in sixty percent of the specimens investigated. Although the first two "mature" (class 3) ovaries were observed in specimens in the 151 – 180 mm class, the majority of "ripe" ovaries were observed in specimens with a fork length longer than 200mm..

However, when the values of the calculated Maturity Coefficients of the females collected in the breeding ecology component are plotted as a function of fork length, as proposed by Gaigher (1969), the results show an increasing trend as fork length increases. This trend is indicated by the fitted trend line in Figure 6.27. In this figure three distinct peaks are observed that correlate with the findings in the paragraph above. It is however noteworthy that it would appear that females in this instance obtain sexual maturity after obtaining a fork length of 240mm.

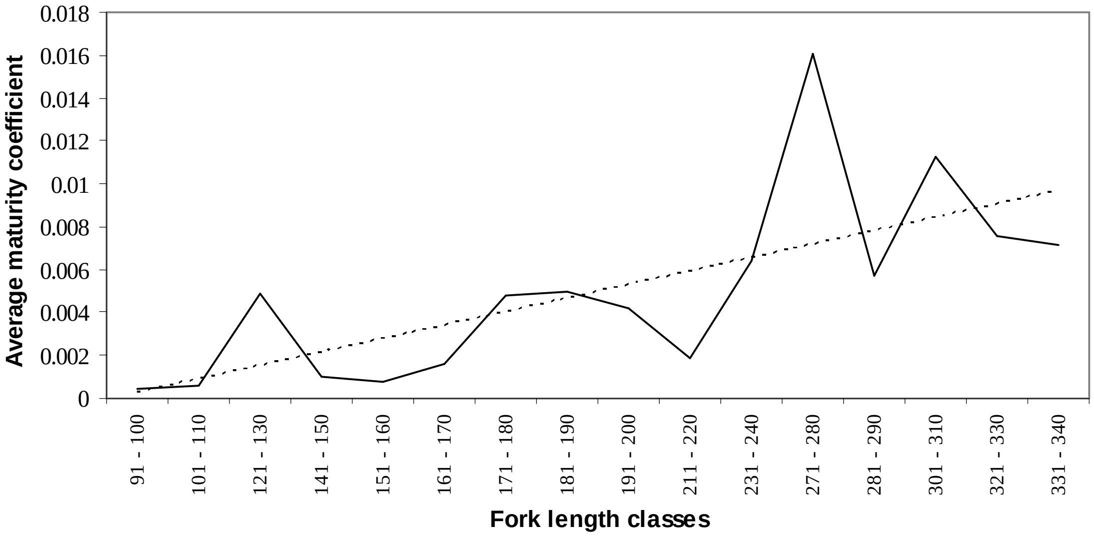

Figure 6.27: Average maturity coefficient of the female specimens of *Labeobarbus marequensis* collected at the breeding ecology sites in the Luvuvhu River from July to October 2007. (The dotted line in the illustrates a fitted trend line to illustrate the increase in the maturity coefficient with an increase in fork length).

Measured egg diameters ranged from 0,125 to 2,25mm and the microscopical investigation, at 30X magnification, of the eggs during the counting and measuring process showed distinct morphological differences between “eggs” of various sizes. These morphological differences were used to classify the eggs into three different egg size classes. The smallest size class (from 0,125 up to 0,5mm in diameter) consisted of eggs where no distinctive features could be observed. In the second size class (with diameters up to 1,0 mm) the cytoplasmic component took on a grainy appearance, while in the largest size class, where eggs with a diameter up to 2,25mm were measured, a distinctive dark area was visible in the cytoplasm.

These findings were confirmed by the results of the histological investigation which indicated that eggs less than 0,5mm in diameter were recruitment eggs with no signs of yolk development. These eggs were regarded as the oocytes. Eggs that ranged from 0,5 to 1,0mm

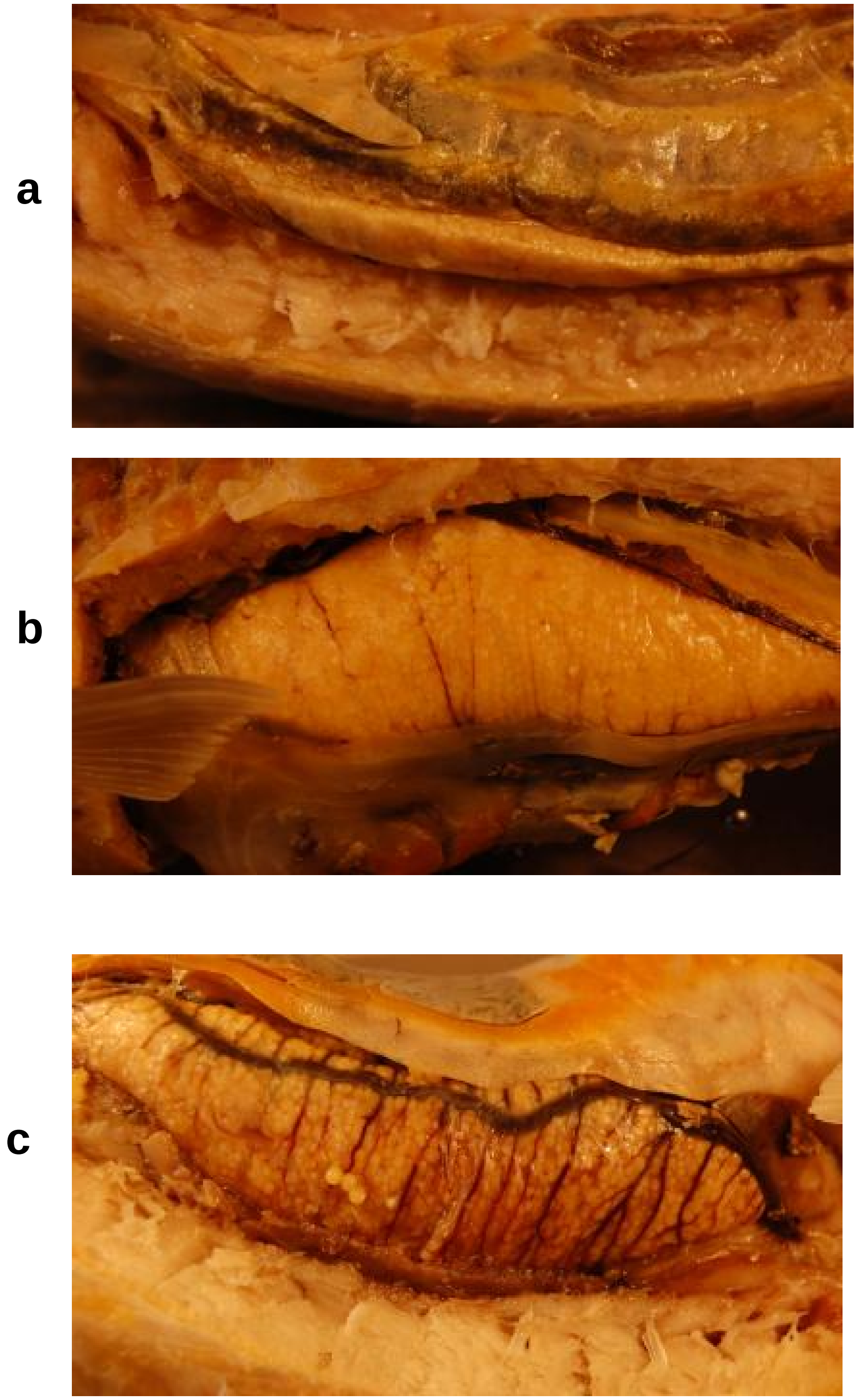

Figure 6.28: Examples of some of the stages in the gonadal development observed in *Labeobarbus marequensis* female specimens in stanzas 8 and 9 collected in the Luvuvhu River during 2007. (a = maturity class 2 and b and c = maturity class 3)

in diameter were classified as developing or maturing eggs or ova and were in various stages of vitellogegenesis (De Villiers, 1991) with proteinaceous yolk granules forming in the cytoplasm (Helfman *et al.*, 1997). Eggs over 1mm in diameter, exhibiting dense yolks, were regarded as the mature eggs or ova.

Different egg sizes were identified in the ovaries of *L. marequensis*. Latent ovaries (maturity class 1) as well as spent ovaries (maturity class 4) only contained recruitment eggs or oocytes. Ovaries in maturity class 2 (the maturing phase), contained both oocytes and developing ova while in the mature ovaries (maturity class 3) eggs of all three size classes were present.

The Fecundity and Relative Fecundity was calculated using only maturing and mature eggs observed and counted in mature females. Although this included specimens from the 161 – 170 fork length class the majority of the specimens were of a fork length longer than 270mm. Table 6.14 shows that the mean relative fecundity of *L. marequensis* was 44,7 ova per gram of body mass. It should however be pointed out that in the majority of the fork length classes only one or two specimens were observed which could account for the difference between the averages. No trend related to an increase in fork length could be established. On the other hand Bagenal (1957) pointed out that fecundity differences such as fecundity-length relationships did not remain constant.

Table 6.14: The calculated average Relative Fecundity (RF) of mature (Maturity class 3) females of *Labeobarbus marequensis* collected in the Luvuvhu River during the period July to October 2007.

Fork length classes	Average RF based on egg classes 2 and 3	Average RF based on egg class 3
161-170	111.4	59.9
271 – 280	57.8	44.6
281 – 290	77.8	55.3
301 – 310	64.0	54.8
311 – 320	57.0	31.9
321 – 330	63.1	48.7
331 – 340	87.7	42.9
371 – 380	38.2	28.4
391 – 400	46.6	36.2
Mean	**67.0**	**44.7**

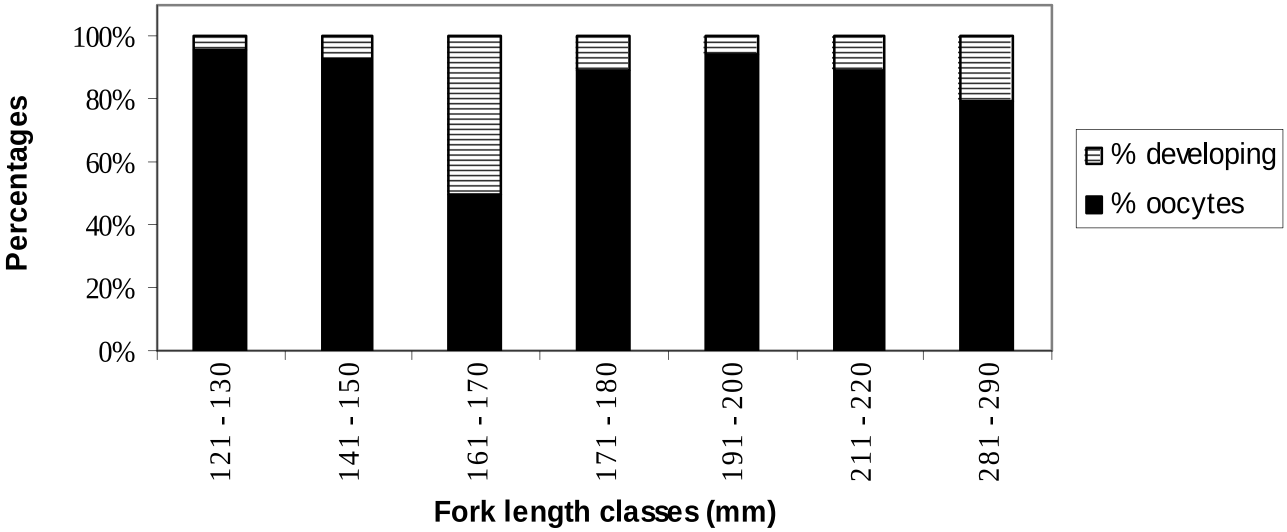

Figure 6.29: **Calculated percentages of egg size classes recorded in female specimens of *Labeobarbus marequensis* in maturity class 2 (Maturing) collected in the Luvuvhu River and tributaries in the period July to October 2007.**

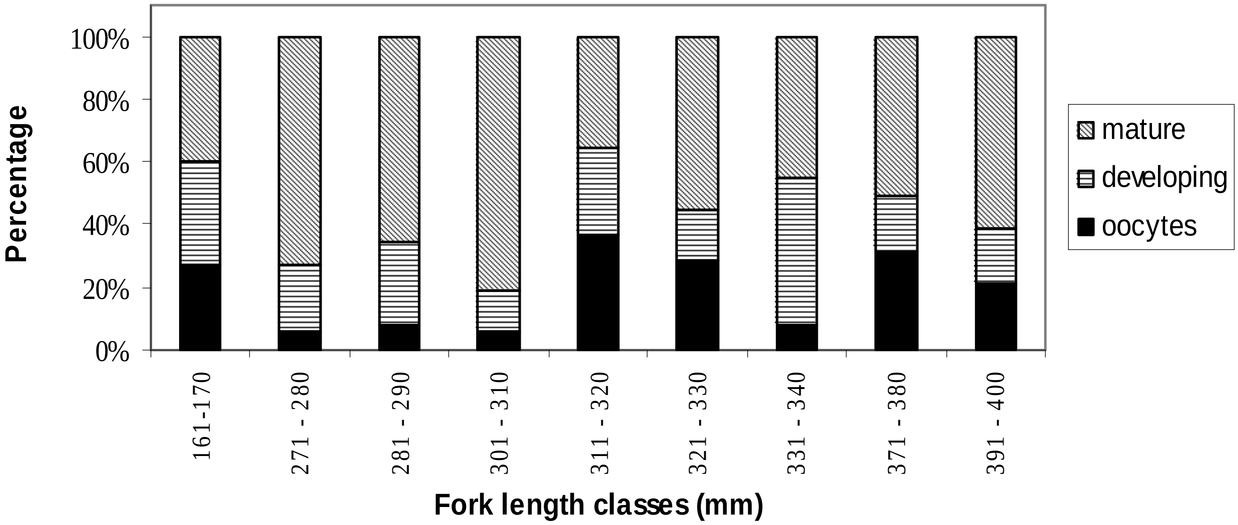

Figure 6.30: **Calculated percentages of egg size classes recorded in female specimens of *Labeobarbus marequensis* in maturity class 3 (Mature) collected in the Luvuvhu River and tributaries in the period July to October 2007.**

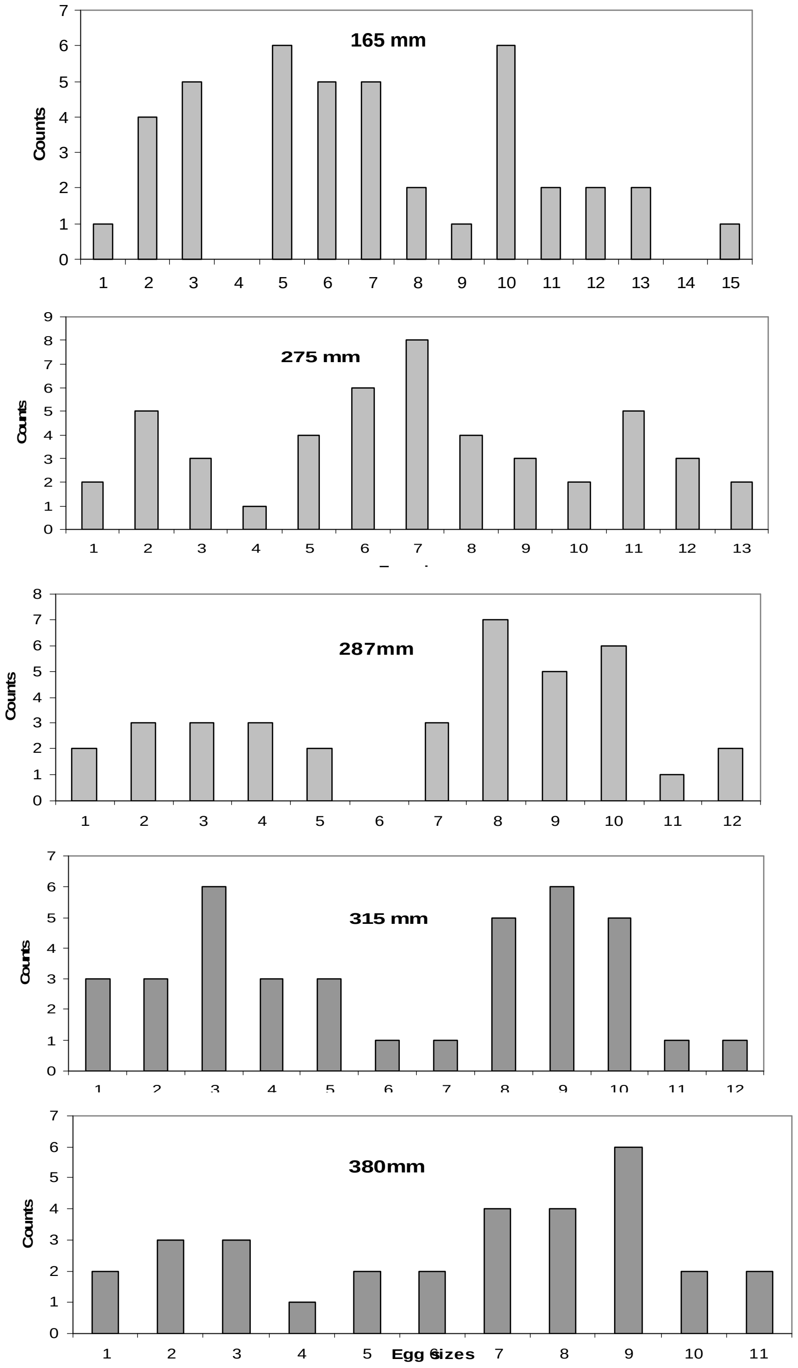

Figure 6.31: Histograms displaying the distribution in egg size counts of five "mature" *Labeobarbus marequensis* females collected in the Luvuvhu River in the period from July to October 2007. Each of the units of the x-axis represents a 0,125 mm interval, with unit 1 for example being eggs of 0 – 0,125 mm.

The ovaries of the mature (Maturity class 3) females, of which examples are shown in figure 6.28 contained eggs of all three the distinct egg size classes. This included oocytes that were smaller than 0,5mm, maturing ova of between 0,5 and 1,0 mm as well as mature ova that were larger than 1,0mm in diameter. Figure 6.30 shows that the composition of this “mixture”, based on each egg size presented as a percentage of the total number of eggs, varied between the size classes with no distinct pattern or trend that could be identified. This mixture suggests that ***L. marequensis*** is a serial spawner with probably a prolonged spawning period. The prolonged spawning aspect is supported by the fact that juveniles that were classified as J_1 (11 – 20mm) were collected over long periods at some of the breeding ecology sites (Table 6.10). The best example is the site at Tshifudi (site 11) where J_1 juveniles were collected during the August, September and October 2007 surveys. At the Tshivhulani site (site 5) these juveniles were collected in September as well as in October of the same year.

The distribution in egg size, as illustrated by the histograms of four randomly selected mature females, displayed bi-modal distributions (Figure 6.31). This reconfirms to illustrate that the species is a serial spawner as the “number of modes in a size distribution of ova is often considered to represent the number of spawnings individual females are capable of ” (Bagenal and Braun in Tómmason *et al.*, 1984).

Only two egg sizes were observed in the females classified as “maturing” (Figure 6.29) including oocytes and maturing or developing ova. With exception of some specimens in the 161 – 170mm fork length class, the majority of the eggs in this maturity class consisted of oocytes smaller than 0,5 mm in diameter. From the results (Figure 6.29) it does however appear that the number of maturing ova increases as the fork length increases.

In all the specimens where the gonads were classified as latent (maturity class 1) or spent, (maturity class 4) all the eggs were smaller than 0,5mm in diameter and were classified as oocytes.

6.4 Discussion.

6.4.1 Size and sex ratio of the species.

As is the case with many fish species, females of ***L. marequensis*** grow faster, attain a greater length and on average become older than males (Gaigher, 1969) and the male to female ratio of 25:75 observed by Göldner (1969) seem to underpin this. However in this study a male to female ratio of 77:23 was observed in the specimens collected in the Luvuvhu River and tributaries as part of the breeding ecology of this project. Similar results were found during the migration component at the Xikundu fishway in the Luvuvhu River where a ratio of 79:21 was obtained.

Gaigher (op cit.) established that the size obtained by the fish was related to their distribution and specifically altitude and found that in areas higher than 610mASL, the dominant fork length was 160mm while at an altitude lower than 610mASL specimens of lengths up to 300mm were common. A similar situation was reported by Groenewald (1960) who found that 69,8% of the specimens he collected were less than 170mm and only 4,2 % longer than 280mm. Russell (1997) found that in the Olifants River within the KNP the modes with the highest frequency within the length frequency distribution were in the 20–30, 101-120, 161-170 and 240 – 250mm classes respectively.

Figure 6.32 shows that results similar to those mentioned above were obtained in the Luvuvhu River and tributaries. Here the majority of the specimens collected (78,9 %) had fork lengths shorter than 170mm.

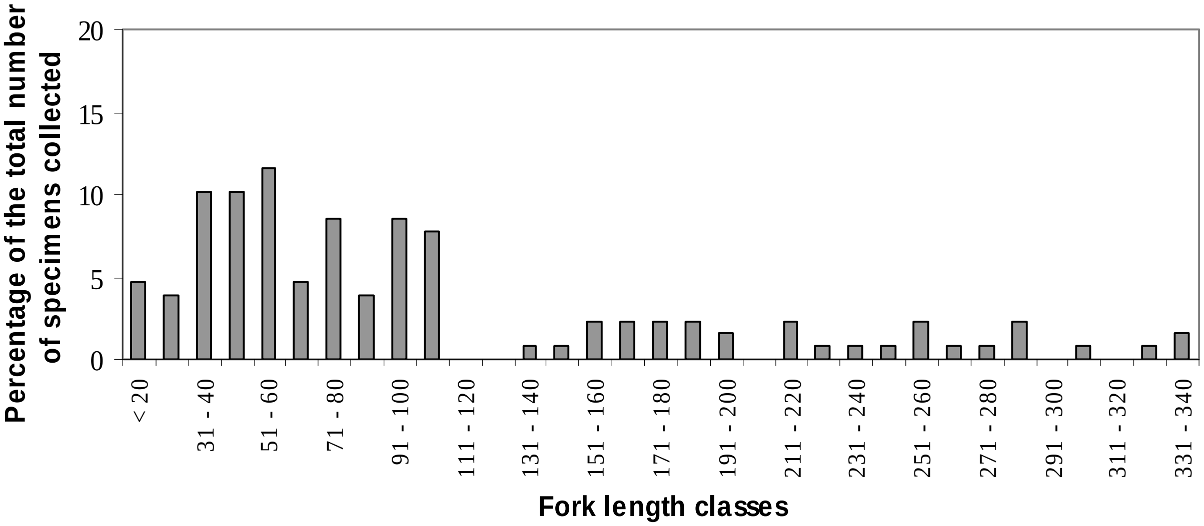

Figure 6.32: The numbers of specimens of *Labeobarbus marequensis*, presented as a percentage of the total number of specimens, collected in the different size classes in the Luvuvhu River during the period July to October 2007.

6.4.2 Recruitment and time of breeding

Length-frequency and modal progression analyses are usually part of the indirect methods of age determination as is illustrated in Weatherly and Gill (1989) who stated that "if reproduction in fish populations occurs such that there is a regular influx of new recruits over a relative short period of time, they will display a length-frequency distribution that features modes". A *proviso* set by them was that if the sample included a broad size range and at least contain an adequate number of the youngest fish each new mode would reflect the influx of new recruits. The method using the time of capture of small juveniles to infer the time of first spawning since the previous summer has been used extensively over the years (Lachner, 1952; Platania and Altenbach, 1998) and has been applied to South African yellowfish (Mulder, 1971; Hamman, 1974 and 1981; Tómasson *et al.*,1984). The method however demands that some knowledge of the growth and size of the species should be available. A survey of the literature reveals that very little is known about the length-at-age of the species (Fouché *et al.*, 2005). Russell (1997) pointed out that the same applies to knowledge concerning the growth rate of the species. The work by Göldner (1969) who found that in Loskop Dam the species attained a fork length of 110mm at the end of the first year and 150

– 160 mm at the end of the second year was the only reference that could be found on these aspects. Results presented by Tómasson ***et al***. (1984), who studied the two larger yellowfish species, ***L. kimberleyensis and L. aeneus*** showed that 10 mm long fish were approximately 10 days old. Based on the findings of Göldner (1969) and Tómasson ***et al***. (op cit.) it was therefore accepted, for this study that in the case of ***L. marequensis***, a morphologically smaller species than the two mentioned above, specimens up to 20mm in length would be about a month old and those up to 30mm a month older.

The results of the "pilot study" component (Figure 6.1) of this project showed that small specimens of the J_1 sub-group were collected in September which is evident of one spawning event. Analyses of the Xikundu data (Figure 6.2) indicate spawning events that occurred prior to May and the again in early spring. This is supported by the analyses of the Gonadosomatic Index of the specimens collected at the Xikundu fishway. The selective nature of the fishway and the resultant absence of specimens of the J_1 and J_2 sub-groups should be borne in mind. The results of the "breeding ecology" component (Figure 6.16 and in particular table 6.9) provide evidence of two spawning events that occurred. Although no specimens of the J_1 subgroup was collected during the July survey (Table 6.10), the presence of specimens of the J_2 subgroup are indicative of a spawning event that occurred two months prior to the survey, i.e. in May. The presence of J_1 subgroup specimens in August, September and October (Table 6.10) confirms the results obtained in the pilot study and Xikundu studies. Based on these results it can therefore be deducted that the species spawn twice during a year and that each of the spawning event is an extended one.

It was observed that the second spawning event (August, September and October 2007) coincided with an increase in both the temperature and discharge (Figures 6.14 and 6.15). This agrees with the findings of Tómmason ***et al.*** (1984) who stated that "time of first spawning of ***L. kimberleyensis*** and ***L. aeneus*** was influenced by the effect of the hydrological regime and increase in temperature".

Analyses of the GSI of the fish collected in the Xikundu fishway, and the resultant bi-modal pattern underpins (Settles and Hoyt, 1978) that the species spawns twice a year. This is not fully supported by the development of the maturity coefficient. The latter aspect does however clearly indicate that a Spring spawning event occurs. The average GSI of both male and female *L. marequensis* determined in this study was found to be lower than that of the larger yellowfish, *L. kimberleyensis* and *L. aeneus.*

Analyses of the fat deposition and the condition factors show that the condition of *L. marequensis* improves in conjunction with gonadal development in the pre-spawning period. The pre-spawning period is characterized by intensive protein synthesis associated with differentiation and growth of the gonads for which material is derived from food and stored reserves (Shul'man, 1974). The principal sources of energy are the fat reserves and the fat content of fish declines as the gonads develop. This decline is especially marked in the first stages of gonad development and by the time the gametes are fully formed the fat reserves are greatly depleted. This is also the case in *L. marequensis* where very few unit 5 fat deposition classes (Table 6.2) where the intestines are covered with fat deposits are recorded in the period from June to October 2004 (Figure 6.24). During and after the September 2004 spawning event a marked increase in the number of unit 4 and 5 situations are observed. As is the case with most fish, *L. marequensis* shows an increase in fat deposition during the spawning period and intensive accumulation of fat reserves in the post-spawning period. This latter accumulation is not achieved by a reduction in activity of the fish but rather the maintenance of high levels of activity and vigorous feeding, termed hyperphagy (Nikolsky, 1963).

6.4.3 Breeding habitat and habitat requirements of the small juveniles.

The results of this study have shown that breeding occurs at sites where flow of water occurs over boulders and cobbles. It was pointed out that it was essential that these breeding habitats should be in close proximity to both fast-deep biotopes (deep riffles and rapids) as well as slow-deep biotopes (pools). These deep habitats can be regarded as resting areas where specimens mature out for breeding (Vlok, 1992)

The presence and utilization of nursery areas have been pointed out in this study. These are the areas where very small (J_1), 10 – 20mm in fork length, juveniles occur. It was shown that over and above the fact that these nursery areas should have a cobble or boulder substrate, which provide the necessary protection and shelter, the water velocity in these areas are very low or even non existent. It was also found that these nursery areas were shallow and subsequently warmer than the surrounding biotopes. These aspects would not only lead to increased food production but would create an ideal situation for improved metabolism of the juveniles. This will result in an increase in the growth rate which would make the juveniles less vulnerable to predation. It is also important to note these nursery areas were in close proximity to identified breeding areas.

6.4.4 Length at sexual maturity.

Analyses of the maturity classes awarded to gonadal development (Figure 6.20) showed that male *L. marequensis* collected in this study became sexually mature when their fork lengths exceeded 90mm. This is slightly longer than the 70mm observed by Gaigher (1969) for male *L. marequensis* in the Incomati River. The results of the analyses of the observed gonadal maturity classes of the females in this study showed that although mature females, based on analyses of the maturity coefficient, with fork lengths shorter that 200mm were observed, the majority of sexually mature females were observed in fish longer than 200mm. This is substantially lower than the findings of Gaigher (op cit.) who found that the females with the highest maturity coefficients recorded were in specimens exceeding 280mm. When the calculated Maturity Coefficients of the females collected in the breeding ecology component of this study are considered it would appear that females in this instance obtain sexual maturity after obtaining a fork length of 240mm.

The lack or absence of large specimens, and in particular females, during surveys of the Luvuvhu River has been a matter of concern for a number of years. (Angliss, pers com.; Fouché, 2002; Fouché and Gaigher, 2001; Fouché *et al.*, 2003; Fouché *et al.*; 2005; Gaigher and Fouché, 2000). Despite the perceived absence of large specimens, young and juvenile specimens have always been abundant in the Luvuvhu River and its tributaries. If the findings of this study are regarded, where it points out that the females are sexually mature at a smaller size, it would go a long way to explain the presence of the juveniles in the system.

The measured egg diameters that ranged from 0,125 to 2,25mm are larger than the maximum of 1,818mm reported by Hecht (1982). Because Marriott *et al*. (1997) referred to the eggs of *Amphilius natalensis*, which are 1,5mm in diameter, as large, the eggs of *L. marequensis* can also be referred to as "large". The egg size is larger than that of similar sized cyprinids such as the common carp (*Cyprinus carpio*) where the mature egg size ranges between 0,9 and 1,2 mm (Nikolsky, 1963) and that of *Labeobarbus aeneus* where mature ova ranges between 1,26 and 1,5mm. Schulz and Schoonbee (1999) reported that in the smaller cyprinids such as *Barbus brevipinnis* egg size varied between 0,25 and 1,25mm whereas in *Barbus treurensis* it varied between 0,44 and 1,15mm.The size of eggs varies from species to species and can range from fractions of millimeters to 7mm in diameter as observed in salmon (Nikolsky, op cit.). The size of ova is often regarded as a reproductive tactic of fish where larger egg and their larvae are more likely to survive than smaller ones (Duarte and Alcaraz, 1989). In larger eggs the size is related to the amount of yolk present in the mature egg which is available for the embryo. In essence it implies that the more yolk is present the longer the embryo can rely on this internal feed source and thus grow larger in size, before switching to external feeding. Not only are the eggs of *L. marequensis* regarded as large, they also contain substantial amounts of yolk that would sustain the embryo for some time.

This mixture of egg sizes observed in the mature females (maturity class 3) suggests that *L. marequensis* is a serial spawner and it can be accepted that it probably has a prolonged spawning period. The bi-modal distribution in egg diameters confirms that is a serial spawner. Tómmason *et al.* (1984) found that *L. aeneus* displayed a uni-modal distribution while Gaigher (1976) reported that bi-modal distributions were observed in some specimens of *L. kimberleyensis.* As stated previously fractional or serial spawning, and for that matter prolonged spawning periods, are often characteristic of fish that occur in areas where no clear distinction between seasons are recorded. The area in which *L. marequensis* is distributed, which starts at the middle Zambezi River system (Jubb 1967) and stretches south to the Phongolo River system (Skelton, 2001) is an area where no clear distinction between seasons exist.

6.4.5 Relative fecundity

Although only a few specimens were suitable for this analysis it was calculated that the mean relative fecundity of *L. marequensis* was 44,7 ova per gram of body mass. In the case of sexually mature specimens, that longer than 270mm and weigh in excess of 400 grams, it would imply that more than 17,000 mature ova are present in the ovaria. This is distinctly more than in the smaller cyprinids where 1341 eggs are reported in *Barbus. brevipinnis*, 2040 in *B. treurensis* and 3000 in *B. anoplus* (Schulz and Schoonbee, 1999). It is however similar average egg counts of ca 18400 calculated by Hamman (1981) in four mature female *L. kimberleyensis*. Mulder (1971) calculated that the egg counts ranged between 16000 and 52000 for large (52 to 79cm) female *L. kimberleyensis* and between 29000 and 45000 for large (42 – 53cm) female *L. aeneus* in the Vaal River.

Although relative fecundity is not truly an indication of the reproductive capacity of a species it does however give some indication of its reproductive capability. According to Nikolsky (1963) the mean relative fecundity of *L. marequensis* would place it in a group of fish where the relative fecundity ranges from 10 – 50 which is an intermediate group which is adapted to a median rate of mortality due to predation on eggs.

Based on the above it can be concluded that *L. marequensis* is a fractional spawner that spawns twice per year. These spawning events coincide with an increase in flow caused by the onset of rains. In spring and early summer the flow increase is accompanied by an increase in temperature. The moderate fecundity and relative large eggs of *L. marequensis* forms part of its adaptive capability. In order to breed the habitat should consist of a mixture of fast flowing water over coarse substrate and deeper habitat with low velocities. It was also observed that nursery areas, where the small juveniles survive, should be in close proximity to the actual breeding areas, which are situated in cobble and boulder areas where slow flow occurs.

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APPENDIX IV :

Data form II : Gonads & Condition

PROJECT ……………………………… RIVER …………………………..

				Measured values			Visual observations		Calculations					
Site	Date	Fish no	Male/ female	Length (mm) **A**	Tot. mass (g) **B**	Gonad mass (g) **C**	Fat class	Maturity class	Basic Body mass **D = B - C**	GSI **(C/B X 100)**	Maturity coefficient **MC = (C/A^3) X 10^4**	Raw condition factor **RCF = (B/A) X 100**	Condition factor **CF = D X 100/A^3**	Gross Condition factor **CFG = B X 100/A^3**

Appendix V

Data Form III: Egg counts and sizes.

		Masses		Fecundity determination (Grid count) (0,2ml)							Size distribution (Light microscope) Size (counts) where 40 counts = 1 mm										
FL	Sex	Mass of both gonads (g)	Sub-sample mass (g)	Number of blocks covered (A)	Number of blocks counted = 10% (B)	Total ova counted (X)	Ave ova (X/B)	W) No of ova in 0,2ml C X A	Ts = W x 5	To = Ts/Y x Z	N/Y	N/Y	N/Y	N/Y	N/Y	N/Y	N/Y	N/Y	N/Y	N/Y	
											0 to 5	6 to 10	11 to 15	16 to 20	21 to 25	26 to 30	31 to 35	36 to 40	41 to 45	46 to 50	Tot.
											0 - .125	to .250	to .375	to .500	to .625	to .750	to .875	to 1.00	to 1.125	to 1.250	

Chapter 7: The feeding biology of *Labeobarbus marequensis* in the Luvuvhu River.

7.1 Introduction.

The basic functions of an organism, namely growth, development and reproduction, all take place at the expense of energy which enters the body in the form of food. Feeding is therefore one of the most important functions of an organism. As stated by Weatherly (1972) "digestion and absorption of food are necessary conditions of growth while growth, and therefore feeding, forms an important component of the understanding of the processes and in particular the bioenergetics, within ecosystems". To understand fish bioenergetics it should be determined precisely which kind of food fish consume and this calls for a dietary survey. Fish differ greatly in the character of food they consume and the amount of variation in food types consumed leads to fish being classified either as euryphagic or stenophagic (Nikolsky, 1963). Where the latter refers to feeding on a few different food types, the former refers to feeding on a wide variety of food. Although species may appear to show preferences for certain food types these are often determined by the predominant food items among those available. Both the size and the systematic position of the food organisms are extremely variable and the range of the food types consumed by fish is greater than that of other groups of vertebrates.

"Morphology is related to ecology and morphological differences have often been related to resources utilization" (Paine *et al.*, 1981). In some instances it may be generally simple to relate the general nature of a fish's food from the knowledge of its functional morphology. Herbivorous fish have specialized alimentary systems that from appearance alone indicate their food habits (Hildebrand, 1974). They often lack true stomachs that are highly muscular and acid secreting, but possess a long thin-walled intestine. These stomach types are often referred to as pseudogasters (Fouché *et al.*, 2001). Carnivorous fish on the other hand have true stomachs and short intestines, while omnivorous fish have alimentary systems that are more or less intermediate between those of the true herbivores and carnivores. The length of the digestive tract is closely related to the type of food. Among the fish that have no stomach are the cyprinoids where the intestine length is more than 100% of the body length in herbivores (Nikolsky, op cit.). Studies of the dietary composition of fish should therefore ideally be accompanied by an examination of the functional morphology and physiology of the alimentary system.

Ontogenetic changes occur and food habits may change as fish grow. For example Castillo-Rivera *et al.* (2000) found that in the flatfish (*Citharichthys spilotterus*) there were significant ontogenic differences in the diet where larger specimens became entirely herbivorous. At the same time marked changes can occur in the morphology of the alimentary systems from early life on (Eccles, 1986) and these changes could be related to changes in the diet. The change in the composition of food with age is often accompanied by a substantial adaptation toward increasing the food range. Nikolsky (1963) regards euryphagism as an adaptation that evolved to deal with unstable food supplies. In the majority of fish there is a widening of the food spectrum and an increase in the number of components in the food as they grow and change from one stage to the next (Helfman *et al.*, 2000)

In their review of the food habits of African freshwater fish Lauzanne and Gaigher (1988) recognized two important types, *Labeo* and *Brill* within the family *Cyprinidae* and place *L. marequensis* within the latter. They state that within the *Brill* group "the larger species have very eclectic diets based mainly on benthic food resources which include aquatic larvae of insects, mollusks, plants and seeds as well as copepods, ostracods, fibrous algae, shrimps and crabs, terrestrial insects and small fish. These varying diets vary qualitatively in terms of seasons, the biotipes and the size of the fish."

Gaigher (1979) expressed concern about the fact that feeding was one of the neglected aspects of freshwater fish biology. A survey of the literature reveals that to a large extent this is still the case. Studies of the food and feeding habits of the larger South African cyprinid species and in particular the genus *Labeobarbus* include the work of Gaigher and Fourie (1984), Eccles (1986) and Dörgeloh (1996) on *L. aeneus.* As is illustrated in appendix I, the lack of knowledge also extends to *Labeobarbus marequensis*. While Jubb (1961, 1967), Jackson (1961), Crass (1964), Gaigher (1979), Bell-Cross and Minshull (1988) and Skelton (2001) to a certain extent addresses the diet of the species, very few detailed studies have been carried out on the alimentary morphology and the ontogenetic differences of the species (Fouché and Gaigher, 2001; Fouché *et al*., 2003).

While *L. marequensis* feeds mostly on plant material (Gaigher, 1979) the most dominant component, as found by Gaigher (1969) in the Incomati River system is filamentous algae that was dominant in all seasons and all habitats. Chironomidae and Ephemeroptera were second in importance while terrestrial insects, pelecypods, gastropods and fish were also consumed. In the study of Fouché and Gaigher (op cit.) an investigation of the stomach contents included invertebrates of groups Chironomidae and Simuliidae, as well as organic matter and "even algae". Based on the above both these authors regarded the species as "unspecialized facultative feeders".

The objective of the study was to describe the trophic biology of this species in the Luvuvhu River and its catchment. The aim of this section of the project was to investigate alimentary morphology and the related ontogenetic differences between the various size classes of the species and to relate these differences to the diets through the investigation of the stomach contents.

7.2 Materials and methods

7.2.1 The fish used in the investigation.

Only the specimens of *L. marequensis* collected during the four monthly surveys, from July to October 2007 that formed part of the "breeding ecology" (Chapter 6) component of this study were used for the stomach content and alimentary tract analyses.

In the deep biotopes, such as the pools, gill nets were used for collection while the shallow biotopes were electro-fished twice over a 24 hour period. The gill nets, with stretched mesh sizes of 25, 45, 65 and 85 mm were placed in the pools for 24 hours. The fish collected were identified using the key proposed by Skelton (2001) and all the fish, except for the representative specimens of *L. marequensis* of the 10mm interval fork length size classes were returned to the river. The representative specimens were preserved in 10% formalin and transported to the laboratory. A slit was made into the abdominal wall of all the specimens to allow the formalin to enter the abdominal cavity and preserve the intestines.

7.2.2 Laboratory analyses.

In the laboratory each specimen was measured to the nearest millimeter on a measuring board. The fish specimens were dissected to expose the viscera, using the method described by Willers (1991). The intestines were then severed at point of entry, which is at the anterior part of the esophagus, and at the point of exit of the abdominal cavity (Eccles, 1986). Care was taken to dissect and remove the intestines within 24 hours after the field survey. After removal from the visceral cavity the fatty tissue, mesenteries, liver, spleen and gall bladder were dissected away and the "cleaned" alimentary canal was then preserved in 4 % formalin in a marked container.

Intestinal morphology

The lengths and diameters of the pseudogaster/stomach and intestines were measured after dividing the gut into sections on the basis of consistent points of inflection. For total length measurement the intestines were measured at full length on a measuring board from the posterior end of the pseudogaster/stomach to anus or vent, taking care not to over-stretch the organ. In smaller specimens calipers were used. The measurements were recorded on a data form III (Appendix V). In order to facilitate comparison, the stomach, intestine and total gut length were converted into relative lengths, which is a percentage ratio of the body length. In the case of gut lengths, mean relative gut lengths (mRGL) where the length of the whole alimentary tract in relation to fork length was calculated (Wootton, 1990; De Silva & Andersson, 1995). As stated in Kleynhans (1983) the relationship between RGL and the fish length is an important indication of the feeding habits of a fish. In addition, and because of the digestive functional role of the intestines in "stomachless" fish (Nikolsky, 1963), it was decided to also determine the mean relative intestinal length (mRIL) in the same way. In this study the relationship was determined through regression analyses. This relationship and possible changes in it during the various life stages, which in the case of this study is represented by the fork length classes, was used to supply an indication of possible changes in feeding biology of this species. These envisaged changes were statistically related to the findings of the pseudogaster/stomach content analyses.

Stomach fullness

The state of nourishment of the fish is usually expressed in terms of the index of fullness of the gut where the ratio of dry weight of the contents to the body mass of the fish is determined (Nikolsky, 1963) and this is expressed either as percentage or decimilles ($^{0}/_{000}$) (Charalambos and Economidis, 1989). Other methods include a system where a score was awarded on the basis of stomach fullness (Paine *et al.,* 1982).

The pseudogaster fullness was estimated by holding the pseudogaster up against a strong light source and then classified as empty, less than ¼, ½, ¾, or completely full (Bowen, 1976). Where size permitted the volume of the contents was determined using the water replacement method described by Göldner (1964). The pseudogaster/stomach contents were then preserved in 4% formalin for stomach content analyses. The results were recorded on the data form III (Appendix V).

Analysis of stomach contents

After the volume of the stomach contents were determined a sub-sample of the contents were taken for content analyses. For diet analyses the pseudogaster contents were microscopically investigated under a stereomicroscope at 40X magnification and individual food items identified. Because food is rapidly digested the investigation was limited to foregut contents to restrict bias towards organisms which are resistant to digestion (Gaigher and Fourie, 1984). The following food items (Fouché and Gaigher, 2001) were expected: diatoms, insects, algae and plant material. The volumes of each food type was be estimated with the aid of a grid (Gaigher, 1969) and expressed as a percentage of the stomach content. The foregut contents of individual fish were identified to the lowest possible taxon (Marriott *et al*., 1997) and divided in predetermined taxonomic groups (Gaigher and Fourie, op cit.). The percentage by volume was visually estimated (Marriott *et al*., op cit.) by counting number of grid blocks covered by the food item and expressed as a percentage of the total number of blocks covered by the sub-sample. Charalambos and Economidis (1989) when investigating stomach contents used percentage frequency of occurrence and percentage contribution to the content of each component. Each estimated percentage was multiplied by the fullness factor for that specific stomach and these figures added to determine composition by bulk (Gaigher and Fourie, 1984). The frequency of occurrence of the food types were determined by tabulating the food types identified in the stomachs of each size class. To determine the percentage frequency of occurrence, the individual food items were sorted and the number of the stomachs in which it occurred expressed as a percentage of the total number of stomachs of the specific species (Gaigher, 1969; Windell, 1971).

As a measure of food resemblance between two size classes an index of similarity was calculated by adding the smallest percentages of each food type (Fouché and Gaigher, 2001). Rather than using the dried mass as suggested by Windell (op cit.), these calculations were based on the percentage volume of each food item. Where detritus formed part of the stomach contents, cellulose-specific staining methods as described by Cranford *et al.* (1987) were used to quantify the cellulose present. An index of relative importance (IRI) was calculated for each prey item according to Hyslop in Mariott *et al.* (1997) using the formula:

$$\mathbf{IRI = (\%N + \%V) \times \% FO}$$

Where N is the number, V is the volume and FO is the frequency of occurrence.

7.3 Results.

7.3.1 Sample sizes.

In his estimation of the reliability of diet overlap measures Smith (1985) recommended that samples sizes examined to detect gross changes in diet overlap should consist of at least twenty stomachs for each length group This sample size was not achieved in all length groups especially of fish longer than 100 mm in fork length. Therefore instead of merely increasing length intervals to include more fish (Dörgeloh, 1996) it was decided to use the “stanzas” identified in chapter 4 of this study. Even though there were still stanzas that consisted of less than twenty specimens the numbers in each stanza increased substantially. The use of stanzas was initially applied in all the data analyses of this chapter prior to statistical analyses of the results.

7.3.2 Morphology of the digestive tract.

Although every effort was made to preserve the intestines in the best possible way, the digestive tracts of a large number of specimens had become hard and brittle. Because of this it was decided not to use the sections used by Eccles (1986) and to only distinguish between the foregut (the stomach) and the hindgut (the rest of the intestines).

Tables 7.1 and 7.2 show that both the mRIL and mRGL increased from stanza 1 up to stanza 5. This was followed by a gradual decrease up to stanza 9. Because this differed from what is reported in the literature (Eccles, op cit.) and was probably due to the small sample size (Smith, 1985) it was decided to statistically investigate the differences and similarities between the stanzas.

The analyses of variance (ANOVA) of both the relative intestinal lengths and relative gut lengths showed that the differences between stanzas 3 and 4, 5 and 6, as well as 7 and 8 were not significant (Table 7.5). This was also the case in the relative gut length between stanzas 8 and 9. Based on these findings the following combinations were done: the data of stanza 3 and stanza 4, the data of stanza 5 and stanza 6 and the data of stanzas 7, 8 and 9. In the following text the stanza groupings will be referred to as stanza groups A (stanza 1), B (stanza 2), C (stanzas 3 and 4), D (stanzas 5 and 6) and E (stanzas 7, 8 and 9). Although the numbers of specimens in some of the stanza groups were still lower than twenty, the data does give some indication of the food overlap. Table 7.6 and figure 7.1 show that when the stanzas are combined as above, the mean Relative Gut Length does increase with fork length. This is confirmed by regression analysis of the mRGL length relationship of the data of all the specimens measured (Figure 7.2). The obtained R-squared value shows the amount of variability observed within the length classes and in particular the increase in variability that accompanies growth in length. In the case of the mean Relative Intestinal Length (mRIL) the increase did not extend to the last combined group (Table 7.7) and the fitted trend lines for both the means (Figure 7.3) and the actual measured data (Figure 7.4) did not show the same degree of fit but showed a greater degree of variance.

Table 7.1: Mean Relative Intestinal Lengths (mRIL) of the nine stanzas of *Labeobarbus marequensis* collected at the four sites in the Luvuvhu River and tributaries during the period July to October 2007.

Descriptive statistics	Stanza 1	Stanza 2	Stanza 3	Stanza 4	Stanza 5	Stanza 6	Stanza 7	Stanza 8	Stanza 9
Mean	62.16	142.07	210.57	223.27	278.05	253.49	210.85	205.09	176.55
Standard Deviation	12.78	43.20	35.38	16.57	34.88	27.38	7.38	9.92	10.05
Minimum	36.0	51.9	128.0	199.0	235.1	212.1	202.9	193.2	167.8
Maximum	81.3	234.6	253.0	254.9	327.5	290.9	217.4	220.4	191.0
Count	27	53	25	15	11	6	3	6	4

Table 7.2: Mean Relative Gut Lengths (mRGL) of the nine stanzas of *Labeobarbus marequensis* collected at the four sites in the Luvuvhu River and tributaries during the period July to October 2007.

Descriptive statistics	Stanza 1	Stanza 2	Stanza 3	Stanza 4	Stanza 5	Stanza 6	Stanza 7	Stanza 8	Stanza 9
Mean	89.87	174.00	243.90	254.65	310.70	287.91	243.29	236.14	202.95
Standard Deviation	15.18	45.72	35.60	16.55	37.51	26.86	13.59	10.36	11.05
Minimum	56.0	78.8	162.2	228.2	264.9	249.7	227.9	225.4	194.3
Maximum	115.6	265.4	291.6	292.2	365.2	325.5	253.6	253.7	219.1
Count	27	53	25	15	11	6	3	6	4

Table 7.3 : Mean stomach volumes of the nine stanzas of *Labeobarbus marequensis* collected at the four sites in the Luvuvhu River and tributaries during the period July to October 2007.

Descriptive statistics	Stanza 1	Stanza 2	Stanza 3	Stanza 4	Stanza 5	Stanza 6	Stanza 7	Stanza 8	Stanza 9
Mean	0.051	0.118	0.240	0.307	0.591	0.717	1.467	3.050	3.700
Standard Deviation	0.025	0.062	0.091	0.110	0.370	0.133	0.702	1.279	1.711
Minimum	0.01	0.03	0.1	0.2	0.3	0.5	0.8	1.5	2.3
Maximum	0.1	0.3	0.4	0.5	1.5	0.9	2.2	4.5	6
Count	27	53	25	15	11	6	3	6	4

Table 7.4 : Mean Relative stomach volumes of the nine stanzas of *Labeobarbus marequensis* collected at the four sites in the Luvuvhu River and tributaries during the period July to October 2007.

Descriptive statistics	Stanza 1	Stanza 2	Stanza 3	Stanza 4	Stanza 5	Stanza 6	Stanza 7	Stanza 8	Stanza 9
Mean	0.1322	0.1768	0.2701	0.2880	0.445	0.41987	0.6593	1.0838	1.01389
Standard Deviation	0.069	0.080	0.100	0.099	0.206	0.089	0.275	0.463	0.417
Minimum	0.029	0.059	0.115	0.179	0.225	0.286	0.385	0.509	0.654
Maximum	0.370	0.448	0.476	0.455	1.02	0.545	0.937	1.698	0.575
Count	27	53	25	15	11	6	3	6	4

Table 7.5: Results of the analyses of variance between the means of the relative intestinal length (RIL) and the relative gut length (RGL) of consecutive stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007.

	Relative intestinal length		Relative gut length	
Stanzas	**F-crit.**	**F-score**	**F-crit.**	**F-score**
1:2	3.9634	87.95	3.9634	86.09
2:3	3.9966	47.65	3.9817	45.34
3:4	4.0981	1.60	4.9819	1.20
4:5	4.2596	28.53	4.2597	26.73
5:6	4.5430	2.21	4.5307	1.71
6:7	5.5914	6.60	5.5914	23.47
7:8	5.5914	0.77	5.5914	5.52
8:9	5.3176	19.67	5.3176	0.16

Table 7.6: Mean Relative Gut Lengths (mRGL) of the combined stanzas of *Labeobarbus marequensis* collected at the four sites in the Luvuvhu River and tributaries during the period July to October 2007.

Descriptive statistics	Stanza 1	Stanza 2	Stanzas 3 and 4	Stanzas 5 and 6	Stanzas 7,8 and 9
Mean	89.87	174.00	247.93	302.66	331.34
Standard Deviation	15.18	45.72	30.10	35.08	60.85
Minimum	56.0	78.8	162.19	249.69	254.13
Maximum	115.6	265.4	292.15	365.22	449.08
Count	27	53	40	17	13

Table 7.7: Mean Relative Intestinal Lengths (mRIL) of the combined stanzas of *Labeobarbus marequensis* collected at the four sites in the Luvuvhu River and tributaries during the period July to October 2007.

Descriptive statistics	Stanza 1	Stanza 2	Stanzas 3 and 4	Stanzas 5 and 6	Stanzas 7,8 and 9
Mean	62.16	142.07	215.00	269.38	197.64
Standard Deviation	12.78	43.20	30.13	33.78	17.17
Minimum	36.0	51.9	128.05	212.12	167.84
Maximum	81.3	234.6	254.9	327.54	220.37
Count	27	53	40	17	13

Figure 7.1: Graphic illustration of the relationship between the mean Relative Gut Lengths (mRGL) and the mean fork lengths of the combined stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007.

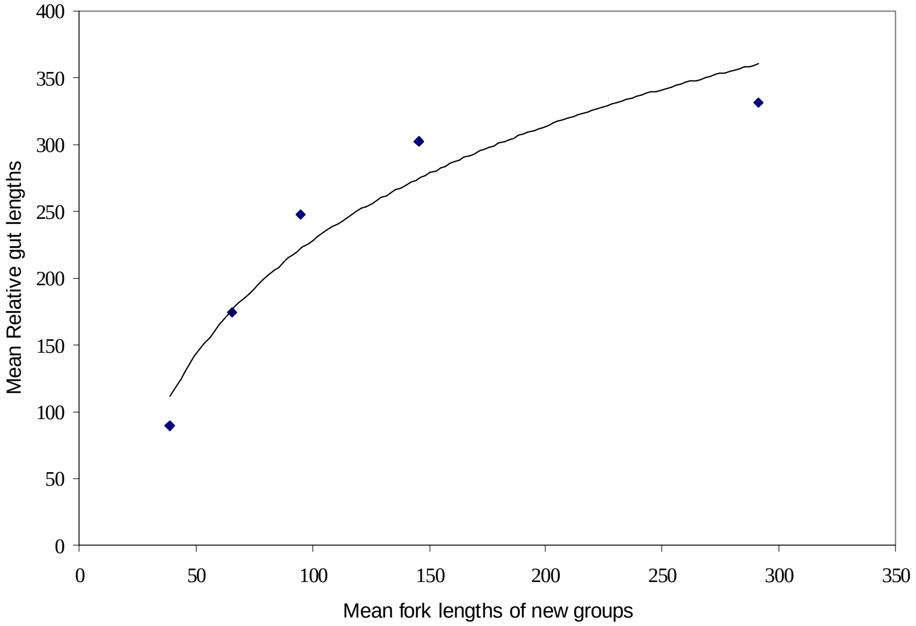


Figure 7.2: Regression analyses of the fork length: mean Relative Gut Length (mRGL) relationship of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007

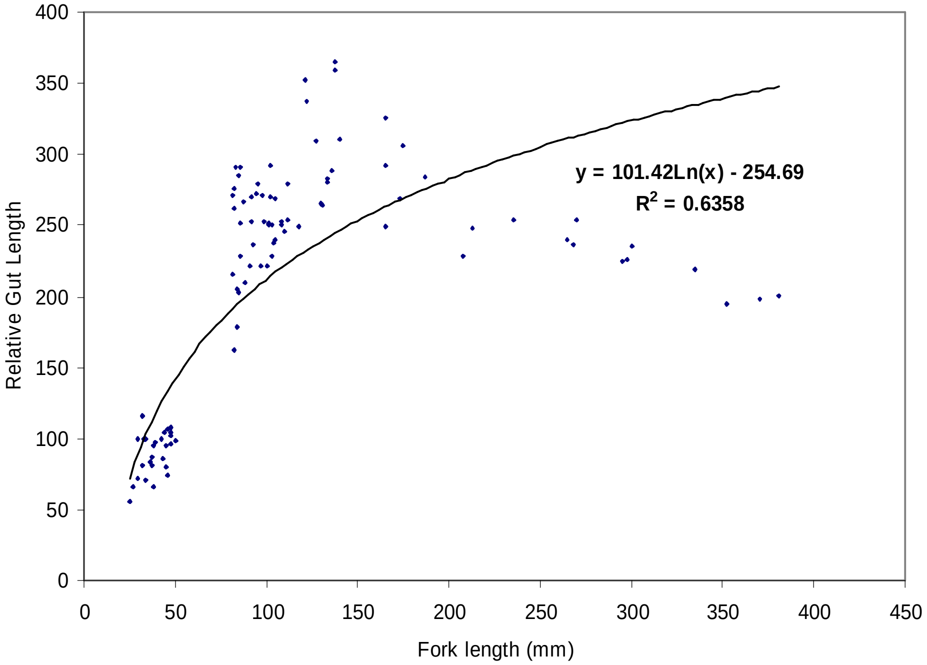


Figure 7.3: Graphic illustration of the relationship between the mean Relative Intestinal Lengths (mRIL) and the mean fork lengths of the combined stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007.

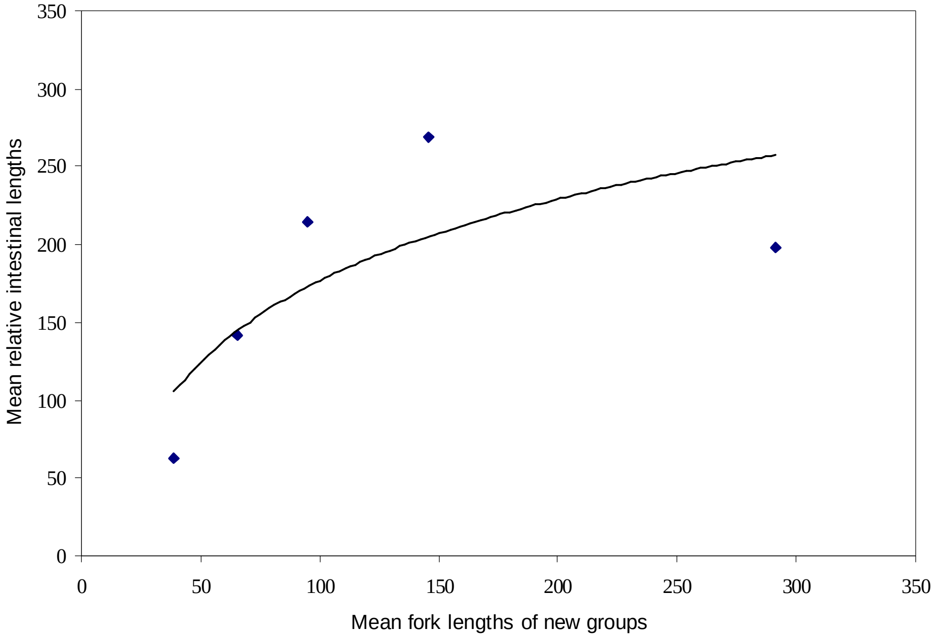
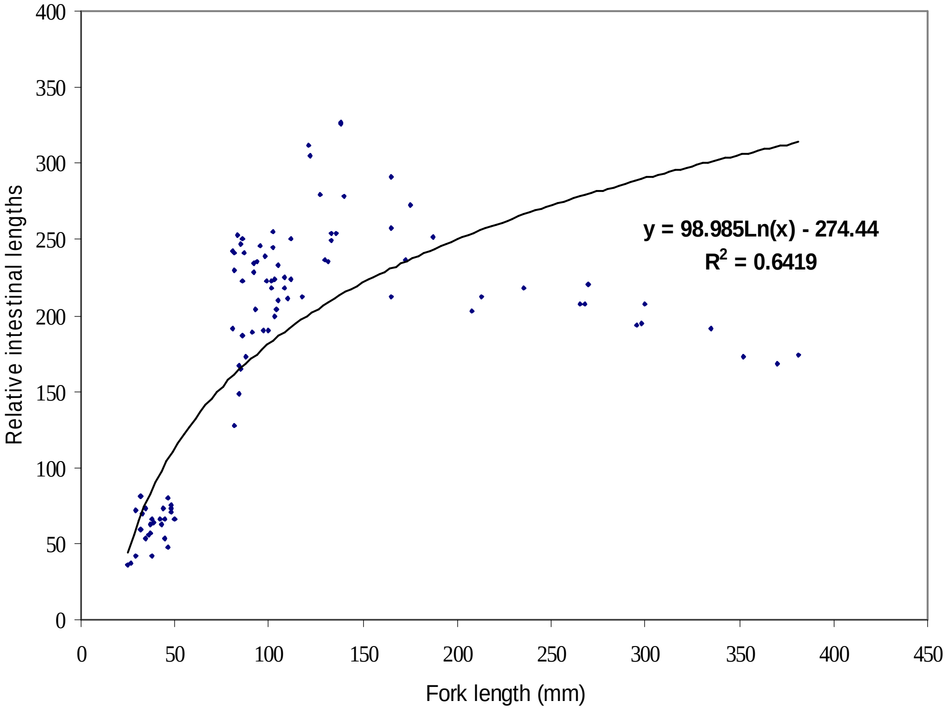


Figure 7.4: Regression analyses of the fork length: mean Relative Intestinal Lengths (mRIL) relationship of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007



Despite the small size of the stomachs, or pseudogasters, of the fish specimens particularly in stanzas 1 and 2 all the volumes were determined with the water displacement method (Göldner, 1964). Table 7.3 shows that the means of the actual measured volumes do increase with an increase in length. However if the volumes are transformed into relative volumes and expressed as a percentage of the fork length, the increases are between stanzas 1 and 2, stanzas 2 and 3, stanzas 4 and 5, 6 and 7 and 8. Between stanzas 3 and 4 the value was the same as between 2 and 3 while there was actually an increase between 5 and 6 and from 8 to 9 (Table 7.4). Results of the analyses of variance (Table 7.8) show that the only significant differences (p = 0,05) were in the smaller specimens up to stanza 4.

Table 7.8: Results of the analyses of variance between the means of the relative Stomach volumes of consecutive stanzas of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007.

	Relative intestinal length	
Stanzas	**F-crit.**	**F-score**
1:2	3.963	6.02
2:3	3.968	114.42
3:4	4.098	31.55
4:5	4.259	0.28
5:6	4.543	0.51
6:7	5.987	3.14
7:8	5.529	2.06
8:9	3.317	0.59

7.3.3 Stomach morphology.

Macroscopic investigations suggest that *L. marequensis* does not have a stomach in the true classical sense. It has a straight-tubed monogastric stomach that is actually a widened part of the digestive tract that is wide at the anterior end and tapers to the posterior. At the anterior end the changeover from esophagus is distinct, but at the posterior end the change-over is difficult to detect from the outside. The “stomach” is funnel shaped with a sharp curve towards the intestinal side. For this study this curve was regarded as the posterior boundary of the stomach. Microscopical investigations vindicated this decision as the curve proved to be an area with distinct changes in the internal topography where the rugae changed into the typical form of rugae in the intestines i.e. lower and more widely spaced (Figure 7.5).

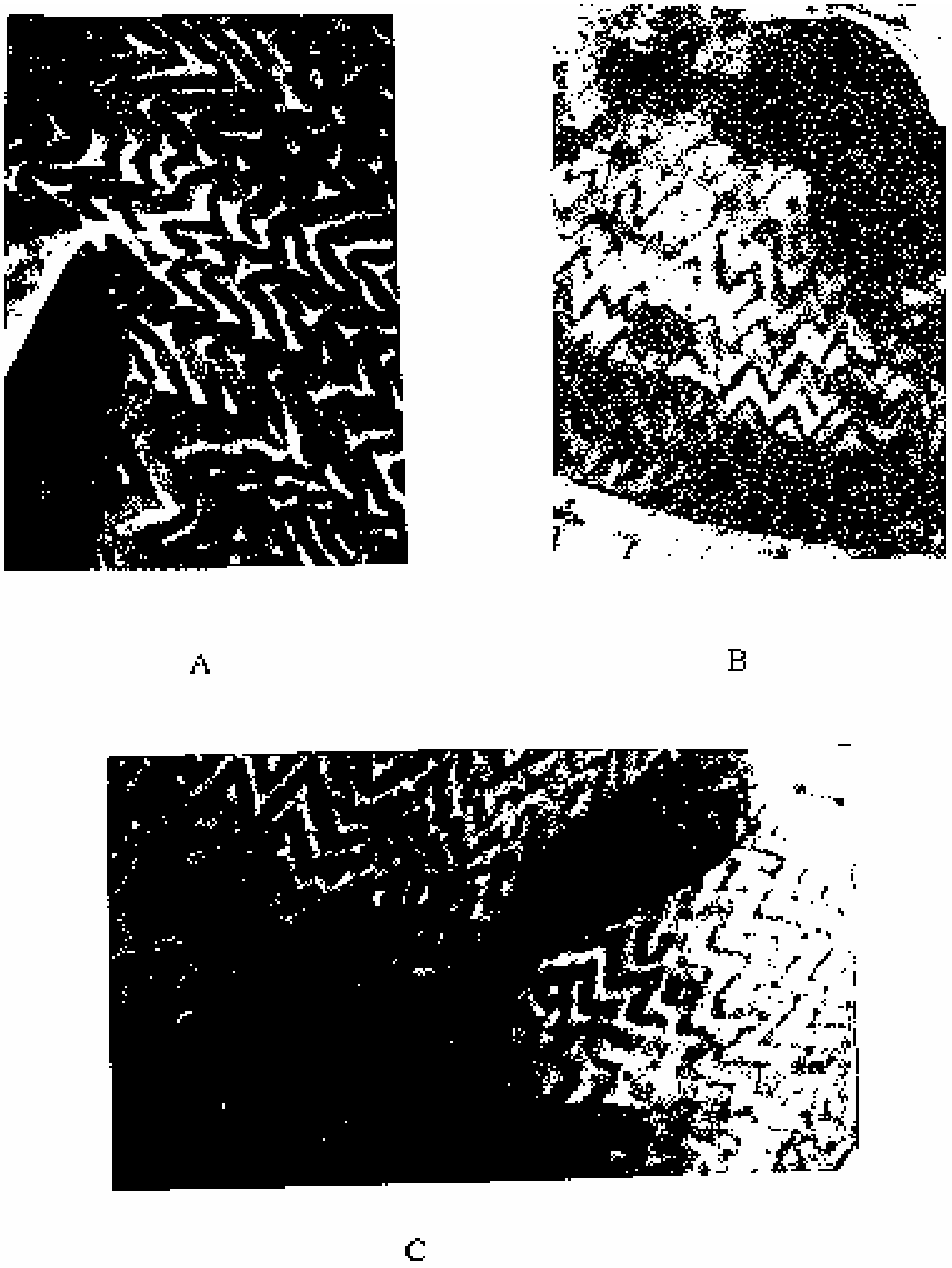


Figure 7.5 : **Micrographs of the rugae observed on the inner surface of the digestive tract of *Labeobarbus marequensis* (X30). A) typical rugae observed in "stomach" or pseudogaster. B) Typical rugae observed in the intestines. C) The change in the rugae at the first curvature.**

7.3.4 Analyses of the stomach content.

In order to facilitate comparison between stomach contents and intestinal morphology it was decided to apply the same combinations of stanzas as was applied in the intestinal morphology (7.3.2) were used in analyses of the stomach content.

Stomach fullness

Table 7.9 shows that in general very few of the stomachs, namely 4.48%, of the specimens collected had full stomachs followed by stomachs that were ¾ full. The highest percentage of stomachs were less than ¼ full with an even spread among empty stomachs and those ¼ and ½ full. When the "stanza groups" are concerned no distinct pattern regarding stomach fullness could be established.

Table 7.9: Calculated percentage of stomachs in the "fullness categories" in the stanza groups of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007 (The numbers in parentheses reflect the numbers of the original stanzas).

Stanza groups	Empty	< 1/4	¼	1/2	¾	Full	Total number of stomachs
A (1)	33.33	37.04	22.22	7.41	0.00	0.00	27
B (2)	7.69	55.77	13.46	17.31	3.85	1.92	52
C (3,4)	23.08	41.03	20.51	7.69	5.13	2.56	39
D (5,6)	5.88	64.71	5.88	17.65	5.88	0.00	17
E (7,8,9)	7.69	15.38	23.08	38.46	15.38	0.00	13

Stomach contents

As was the case with the short fin minnow *Barbus brevipinnis* (Schultz and Schoonbee, 1999) the stomach contents were digested and it was only possible to describe the contents in terms of the frequency of occurrence and the estimated volume of the dietary items. It should also be noted that the percentage volumes are based only on discernable food types, namely plant, invertebrate and algal material that could be identified. The macrophyte category consisted of leaf fragments probably from aquatic vascular plants from the emergent and terrestrial vegetation. The detritus category consisted mostly of plant material and insect parts that were too fragmented to be categorised with confidence. As was the case in

Wikramanayake and Moyle (1989) detritus, sand and silt were excluded from the analysis and the proportions of the other categories were adjusted accordingly.

Table 7.10 shows that based on percentage volume, plant material dominated the stomach contents of the first four stanza groups. The dominance by plant material is underpinned by the general frequency of occurrence as shown in table 7.11. The only exception was the stanza group E (stanzas 7, 8 and 9) where approximately equal values were obtained for plant and animal (invertebrate) material.

Stanza groups A and B, which consisted of fish with fork lengths up to 80 mm, had the largest percentage of invertebrate material of all the stanza groups. In stanza group C the percentage volume of invertebrate material was larger than the percentage volume of algae. In the two last stanza groups the animal content formed the smallest volume by percentage (Table 7.10).

Table 7.12 shows that based on the frequency of occurrence of food types in stomachs that did contain food, plant material was again identified as the dominant component as it was present in nearly all the pseudogasters investigated in all the stanza groups. Because the numbers of the food types items could not be determined the index of relative importance (IRI) proposed by Hyslop in Marriott *et al.*(1997) could not be calculated and was adapted to only include the percentage frequency of occurrence and the percentage volume of each food item.

The calculated index of relative importance (Table 7.14) also confirms that plant material is the most dominant component of all the ingested food types in all the stanza groups.

When the pseudogaster components of executive stanzas are compared the calculated index scores (Table 7.13) show that the contents of the first two stanza groups are the most comparable. The index score decrease with an increase in fork length.

Table 7.10: Percentage volume, based on visual identification, of the discernable food types observed in the pseudogasters of the stanza groups of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007 (The numbers in parentheses reflect the numbers of the original stanzas).

Stanza group	% Algae	% Plant material	% Animal material
A (1)	1.611	5.542	2.694
B (2)	1.449	5.007	2.24
C (3, 4)	1.111	4.341	1.608
D (5, 6)	1.422	6.422	0.891
E (7, 8, 9)	1.792	4.41	1.528

Table 7.11: Percentage frequency of occurrence of the identified food types in the pseudogasters of all the specimens of *Labeobarbus. marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007

Food types	% Present	% Absent
Algae	57.94	41.27
Plants	97.62	1.59
Invertebrate	50.00	50.00

Table 7.12: Percentage frequency of occurrence of the identified food types in the pseudogasters of the stanza groups of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007 (The numbers in parentheses reflect the numbers of the original stanzas).

	Food types					
	Algae		Plants		Invertebrates	
Stanza group	% Absent	% Present	% Absent	% Present	% Absent	% Present
A (1)	38.89	61.11	0.00	100.00	55.56	44.44
B (2)	41.67	56.25	29.17	97.92	43.75	56.25
C (3,4)	34.48	65.52	0.00	100.00	55.17	48.28
D (5,6)	43.75	56.25	0.00	100.00	50.00	50.00
E (7,8,9)	41.67	58.33	8.33	91.67	41.67	58.33

Table 7.13: Calculated indices of food similarity of the consecutive stanza groups of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007.

Food types	Stanza groups A : B	Stanza groups B : C	Stanza groups C: D	Stanza groups D : E
% vol. Algae	1.611	1.11	1.11	0.792
% vol. Plants	5.007	4.341	4.341	1.41
% vol. Inverts	2.24	1.608	0.891	0.891
Calculated index scores	8.858	7.059	6.342	3.093

Table 7.14: Calculated indices of relative importance (IRI) of the food items in the pseudogasters of the stanza groups of *Labeobarbus marequensis* collected in the Luvuvhu River and tributaries during the period July to October 2007 (The numbers in parentheses reflect the numbers of the original stanzas).

Stanza group	Algae	Plants	Invertebrates
A (1)	0.985	5.542	1.197
B (2)	0.815	4.903	1.260
C (3, 4)	0.728	4.341	0.776
D (5, 6)	0.800	6.422	0.446
E (7,8, 9)	0.462	1.293	0.891

7.4 Discussion.

As stated before *L. marequensis* does not have a stomach in the true classical sense with a thick muscular wall with secretory abilities. The stomach is straight-tubed and monogastric. It has a funnel shape that is wide at the anterior end and tapers towards the posterior. Externally no distinct change-over from stomach to intestine is observed and internally there is no sphincter present at this point. There is however, as pointed out in the results, a distinct difference between the rugae of the stomach and the intestines and the structural changeover indicates the posterior boundary of the stomach. This is similar to the findings of Eccles (1986) who found that in *Labeobarbus aeneus* there is no sphincter that delimits the foregut from the hindgut and controls the passage of food from the stomach. Eccles (op cit.) also found that the straight anterior portion of the intestine is expanded and muscular. Similar results were reported by Fouché and Gaigher (2001) who found that the stomach wall of *L. marequensis* had a thicker muscular layer than the rest of the intestines.

The investigation of the intestinal morphology and in particular the relative lengths of both the total alimentary tract and small intestines showed that in general these relative lengths were longer than the fork length of the specimens involved. The exception is however the smallest specimens in stanza 1 (< 50mm) where the relative lengths were less than the fork length. After obtaining a length of 80 mm both the RGL and RIL were in excess of twice the fork length. Statistically analyses of these results showed that the increase in length up to stanza 5, when the fish reach a fork length of 150mm, were significant different ($p \leq 0{,}05$). The results obtained by Fouché and Gaigher (2001) showed that the relative gut length of *L. marequensis* was on average twice as long as the fork length with the mean relative gut length at 224% and the relative intestine length at 197 % of the fork length. Fouché *et al.* (2003) showed that the mean relative gut length (mRGL) in specimens with fork length of up to 110mm ranged from 109,5% to 202,7% with an average of 197,1 %.

The observed ontogenetic changes in gut morphology could be indicative of ontogenetic changes in the diet of the species. It could be inferred that the smaller specimens with their relative short intestines tended towards carnivory (Hildebrand, 1974). On the other end of the scale the larger specimens from stanza 4 onwards had the long intestines typical of animals that have to deal with the digestive problems encountered when plant material is ingested. Stanza 2, with relative intestinal (RIL) and relative gut lengths (RGL) of *ca* 140 and 170 % respectively were seemingly more omnivorous in nature and possibly represent a change over from a more carnivorous to more a herbivorous diet. To an extent this is confirmed by the analyses of the stomach contents where it was found that highest percentage volume of animal material was observed in stanzas 1 and 2 (less than 100mm in fork length). This was confirmed by the scores obtained by the indices of food similarity and relative importance.

In general the domination of the stomach content by plant and algal material (Table 7.11) is proven by the frequency of occurrence in all the stanzas investigated. As a percentage volume plant material dominated the stomach contents in all the stanza groups investigated. These results obtained from stomach content analyses and the data on the intestinal morphology seem to indicate that *L. marequensis* is an opportunistic feeder with a tendency towards the ingestion of plant and algal material. Because the animal matter observed in the intestines, and in particular in the small intestines, were in various stages of digestion it should however be emphasized that this clearly indicate that animal material is selected as prey and not merely ingested as an unselected component of the food.

When the fork length to mass ratio (Figure 4.2) is considered, it is seen in the smaller specimens that initially the increase in length exceeds the increase in mass. This is an important survival strategy to negate predatory pressure but is highly energy demanding. It is hypothesized that the energy needs, with regard to magnitude and availability, for this growth process, is met by ingestion and digestion of animal matter that has higher energy content and is more readily digested than the cellulose containing plant and algal matter. This is underpinned by the fact that the animal material in the stomach contents were in advanced stages of digestion.

Gaigher (1979) found that while *L. marequensis* feeds mostly on plant material the most dominant component in specimens collected in the Incomati River system (Gaigher, 1969) was filamentous algae. Fouché and Gaigher (2001) found that the stomach contents of *L. marequensis* collected in the Mutshindudi River included invertebrate material and algae. Based on the above the species was regarded as an “unspecialized facultative feeder” by these authors but the results obtained in the current research, and in particular the ontogenetic changes rather seem to indicate that to a certain extent the species is not an unspecialized feeder.

Fouché *et al.* (2003) showed that when the combined stomach contents of all the length class are considered, *L. marequensis* feeds primarily on algae and detritus. Invertebrates and specifically the adult stages, form a very small component of the diet. However when the length classes were viewed individually a slightly different pattern emerges (Table 7.15)

Table 7.15: The estimated % volume of food components in the stomach content of the size classes of *Labeobarbus marequensis* collected in the Mutale River during 2001 to 2002 (Adapted from Fouché *et al.*, 2003).

			Invertebrates	
Fork length classes	**Algae**	**Detritus**	**Larvae**	**Adults**
31 – 40	63	29	a	a
41 – 50	61	40		
51 – 60	55	38	a	
61 – 70	20	70	a	
71 – 80	30	58	a	a
81 – 90	40	60		
91 – 100	35	55		a
101– 110	25	60	a	a

These findings do not fully agree with what is reported in other sources. Bell-Cross and Minshull (1988) stated that *L. marequensis* has a varied diet that includes larval and adult forms of aquatic insects, shrimps crustacean, mollusks, terrestrial insects and small fish. They furthermore stated that “vegetation has been recorded but ingestion may have been incidental during foraging”. This is similar to the findings of Jackson (1961) who reported that the diet of adults was of a carnivorous nature and that the stomach contents consisted of aquatic insects, molluscs and small fish. However Crass (1964) did report the presence of plant detritus but stated that mainly insects were observed in the stomach. It should however be borne in mind that in the abovementioned sources (Jackson, 1961; Crass, 1964; Bell -Cross and Minshull, 1988) no mention is made of the method used to quantify the stomach contents and it can thus be argued that their reports, although useful, are merely observations.

When compared to other species in the same genus there are some similarities. Kruger and Mulder (1975) classified *L. aeneus* as an omnivore and found that vegetable material formed an increasing proportion of the diet after the fish attained a fork length of about 200mm and noted that the ratio of gut length to fork length increased as the fish grew. Gaigher and Fourie (1984) regarded *L. aeneus* as a facultative feeder and found that fish under 200 mm in length feed mainly on benthic and planktonic invertebrates while larger fish show a preference for filamentous algae and macrophytes.

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Appendix VI: Data form III: Intestinal dimensions and stomach contents.

			Intestinal dimensions			Stomach contents							
			Measured			Total content	Small particles		Identifiable large particles (list names identified)				
Fish no	Code	Fork length	Volume	Sect 1	Sect 2	Total grid count of covered blocks	Grid count of particles	%	Grid count of particles	%	Algae	Plant	Invert

Chapter 8: The movement and migration of *Labeobarbus marequensis* as observed in the Luvuvhu River.

8.1 Introduction.

The distribution of a fish species, as is the case with most animals, is limited by its tolerance of environmental conditions and within these limits its distribution is related to preferences for given environmental conditions (Nikolsky, 1963; Felley and Hill, 1983). If these conditions change it may lead to changes in the spatial distribution which results from movement of the species. Various forms and degrees of movement or changes in spatial distribution in fish have been observed and described (Whitfield and Blaber, 1978; Bell-Cross and Minshull, 1998; Wurtsbaugh and Li, 1985; Freeman, 1995; James and Kelso, 1995; Lucas and Batley, 1996; Helfman *et al.,* 2000; Yako *et al.,* 2002). In fish three major forms of spatial behaviour or movement are recognised. Firstly there are local movements confined to a single geographic area, secondly there are dispersals which are more extensive and entail an expansion from an identifiable area and lastly there are "true migrations" in which organisms move between widely separated geographical regions. Paxton (2004) distinguishes between ontogenetic habitat shifts and seasonal movements. Ontogenetic shifts include the movement of eggs and larvae on the one hand and juveniles and sub-adults on the other hand. In the case of the juveniles and sub-adults shifts occur once the fish are beyond a certain age or body size in nursery areas and they then move to other habitats. These shifts may be local, within the same river reach, or more extensive and can either be "gradual dispersal or it may take on the form of a well-timed synchronized migration by a large number of individuals" (Paxton, op cit.). Where ontogenetic shifts may occur only once during the lifetime of an organism, seasonal movements on the other hand tend to occur periodically.

Migration has been described in various ways where Nikolsky (op cit.) describes it as an "active, though sometimes passive, mass movement from one habitat to another". Paxton (2004) in his review refers to it as "movements resulting in an alteration between two or more separate habitats occurring with regular periodicity but certainly within the lifespan of a individual and involving a large fraction of the population". It should be noted that in this latter description the term alteration refers to a moving away followed by a return whereas the term periodicity reflects that the movement is usually seasonal or annual. Skelton (2001) emphasises that migrations are distinct movements of whole populations or sections of populations for a specific purpose such as breeding, feeding or dispersal. In addition it can be accepted that migration denotes any form of active movement which is synchronized and undertaken by a large fraction of a population between one location and another (Paxton, 2004). In this respect local movements relating to foraging activity, passive dispersal of eggs or larvae, or random wandering do not constitute migratory behaviour. Migration, like other properties of a species, has some adaptive significance and ensures favourable conditions for the existence and reproduction of species (Nikolsky, 1963). Migrations take on several forms and according to Nikolsky (op cit.) the "cycle of migrations" consists of a) spawning migrations which is the movement from feeding or overwintering grounds to spawning grounds, b) feeding migrations that occur away from spawning or overwintering to the feeding grounds and c) wintering migrations away from either the feeding and spawning grounds from the above to overwintering grounds. Bok *et al.* (2007) aptly summarises it as follows: "*Aquatic species undertake migrations for a variety of reasons, including movement to habitats suitable for spawning and larval rearing, to access suitable, rich nursery or feeding areas that ensure good growth and high survival and to seek refuge from unfavourable or harmful environmental conditions such as low water levels, extreme temperatures or an abundance of predators*". Apart from migrations being part of the life cycle of fishes many migrations have a protective character where species for example move away from stormy weather, low water levels and severe decreases in water temperature (Nikolsky, op cit.). Migrations between various preferred aquatic habitats usually take place on a seasonal basis and are normally synchronised with the natural hydrology of the river to facilitate movement both up and downstream and to utilize seasonally favourable habitats, as discussed further below (Helfman *et al.*, 2000). Smaller fish (e.g. juveniles and post-larvae) often undertake upstream migrations into suitable nursery or feeding habitats under suitable flows throughout the year, while adult fish undertake upstream spawning migrations during the summer months when the rivers are swollen after rains (Skelton, 2001).

Many fish make seasonal migrations in accordance with seasonal flood cycles (Skelton, 2001) and various aspects, referred to as cues, that are related to migration or shifts have been identified. These range from light intensity (Bohl, 1980), discharge (Yako *et al.*, 2002, and temperature (Nikolsky, 1963). Although the cues that initiate or trigger movement have been debated and investigated (Nikolsky, op cit.; Shul'man, 1974; Durbin *et al.*, 1979; Bohl, 1980; Helfman *et al.*, 2000; Fishelson *et al.*, 1996; Turner *et al.*, 1994) the statement by Yako *et al.* (2002) that "the factors that trigger migration are not well understood" summarises the current state of affairs and highlights the complexity of the issue at hand. The complexity and the failure to fully understand the triggers of migration and specifically with regard to southern African freshwater fish are shared by Meyer (1974) and Bell-Cross and Minshull (1988).

Because spatial shifts and in particular migration are active movements, energy is expended. This energy is stored in the body inter alia as fat and migration can only be implemented if a certain level of fat reserves have been attained (Shul'man 1974). This would imply that periods prior to migration would be characterised by an increase in fat deposition or a general increase in the condition of the individual.

Although the migration of fish and in particular freshwater fish have been extensively investigated (Nikolsky, 1963; Shul'man, 1974; Durbin *et al.*, 1979; Bohl, 1980; Wurtsbaugh and Li, 1985; Chapman and Kramer 1991; Turner *et al.*, 1994; Freeman, 1995; James and Kelso, 1995; Fishelson *et al.*, 1996; Lucas and Batley, 1996; Helfman *et al.*, 2000 Yako *et al.*, 2002) a literature survey of the southern African freshwater environment shows that although a number of authors report in general on the migration patterns of fish (Jubb 1953; Bell-Cross, 1960; Jackson, 1961; Jubb, 1967; Pott, 1969; Bell-Cross and Minshull, 1988; Bok, 1990; van der Waal, 1996; Olivier, 2003; Paxton, 2004) very few studies dedicated to migration have been conducted. This latter group *inter alia* includes the work of Whitfield and Blaber (1978) and Meyer (1974) as well as the radio-telemetric studies of Økland *et al.* (2001), Thorstad *et al.* (2002) and Økland *et al.* (2002). This lack of knowledge in particular extends to *Labeobarbus marequensis*.

Where Skelton (2001) regarded *L. marequensis* as a local migrator that migrate for reproductive reasons during late spring or summer, it was shown by Fouché *et al*. (2005) and Heath *et al*. (2005) that movement of specimens of “non-reproducing” specimens also take place. It was therefore hypothesized that the species, in addition to breeding migrations, also participate in seasonal dispersal migrations or shifts.

The aim of this section of the project was to investigate migratory behaviour of the species to establish if different forms of migration took place. It furthermore aimed at investigating the seasonality of the migrations, the time of day at which the migration occurred, the cues involved in triggering migrations and finally the physiological preparation undergone prior to migrations. In addition it was decided to establish the physical parameters such as water velocity and other hydraulic characters that the species was able to negotiate.

According to Power *et al.* (1988) inadequate observation and for that matter sampling, may distort results. It should however be borne in mind that in a water body, and in particular in a river, the fish are relatively small and to track individuals in flowing water can be like “searching for moving needle in a complex haystack”. One suggested solution is to make the “needles” more detectable and this is done by methods such as telemetry. A second solution is to reduce the size of the haystack (Power *et al.*, op cit.) which would include the use of channeled structures such as fishways (Meyer, 1974). Because of the small size of juvenile and sub-adult *L. marequensis* specimens and the accompanying difficulty in attach telemetry equipment to the bodies it was decided to make use of the data collected at the Xikundu fishway in the Luvuvhu River to investigate migration of the species.

This component of the study formed part of an ongoing project that was aimed at establishing the effectiveness of the Xikundu fishway in the lower Luvuvhu River (Heath *et al.*, 2005). The project consisted of two phases and lasted over a period of 30 months. The first phase was a twelve month “long term low intensity” (LTLI) monitoring exercise that was primarily aimed at establishing migration patterns that occur in the river and to link this with environmental, hydraulic and biological parameters. The second phase was the “short term high intensity” (STHI) exercise and consisted of more intensive monitoring. The data collected in both the STHI and LTHI surveys were used to determine the cues, environmental or biological, for fish migration as well as the direction of migration.

8.2 Materials and methods.

8.2.1 Monitoring frequencies.

Two approaches, each with its own protocol, were used to monitor the movement and presence of fish in the fishway. The first approach (the long-term, low intensity phase or LTLI) was employed during the 12 months of the project. The second approach (the short-term, high intensity phase or STHI) protocol was employed in the second 12 months of the project. The term short stems from the fact that in the initial planning the STHI phase was intended to be only applied during the *short periods* when breeding migration reportedly occur or in periods of the LTLI phase during which an increase of fish activity was observed. It was however decided to extend it to a full 12 months. In the LTLI phase the fishway was sampled once per month for a 24-hour period at four-hour intervals. In the STHI phase protocol, the fishway was sampled for a 48-hour period once per month and this aspect is reflected in the term "high intensity".

8.2.2 Monitoring of biota.

a) Long-term, low intensity monitoring.

Fish were collected from a set of three pools in each of the middle and lower sections and from two three-pools sets in the upper section of the fishway. Each set of three pools was closed off at the upstream end with a steel-framed net that fitted in the pre-cast grooves of the notch. At the downstream end of the set of pools a metal-framed net with a long bag, which in essence was a funnel trap (Freeman, 1995), was fitted into the groove. Each set of pools was shocked for ten minutes and the fish that washed into the long bag were removed and placed in plastic buckets with water (Figure 8.1). During shocking long - handled scoop nets were used to scoop up the fish that did not wash into the net and these specimens were added to the buckets.

b) Short-term, high intensity monitoring.

In the first 24-hour period the same protocol as in the LTLI phase was used. Thereafter a trapping cage was placed near the entrance of the fishway (Figure 8.2) and a long-bagged net near the exit (Figure 8.3). The entrance of the cage faced downstream and trapped fish moving upstream. The entrance of the long-bagged net faced upstream to trap fish moving downstream. The fish collected in the trap and net were collected every 6 hours and placed in plastic buckets.

Figure 8.1: Electrofishing the pools in the Xikundu fishway.

Figure 8.2: The placement of the trapping cage in the notch in the baffle of the fishway at Xikundu.

Figure 8.3: Placement of the long bagged net in the Xikundu Fishway.

In both the above monitoring methods the buckets containing the fish were taken to a sorting table and the species identified using the key provided by Skelton (2001). Each individual specimen of *L. marequensis* was then weighed and measured and specimens of each 10mm length class preserved. All other specimens were returned to the river. The length was determined to the nearest millimeter on a measuring board and the mass to the nearest milligram.

8.2.3 On-site determinations

The following on-site determinations were carried out:

During each survey the extent of cloud cover, wind direction and rain was established on site and recorded at the start of each monitoring session. At the same time the atmospheric temperature and atmospheric pressure was recorded using a handheld Pasco Explorer data logger. At the onset of the survey the pH, water temperature, conductivity, total dissolved solids (TDS) and dissolved oxygen were determined once-off *in situ* in the middle section of the fishway using handheld Eutech meters. As part of the STHI component a HOBO temperature logger was placed below the water surface in the pool closest to the exit of the fishway and the data downloaded during the following monthly survey.

8.2.4 Physical aspects and hydraulic data of the fishway

The depth of the water in the pools and over the crest of the notch of was determined with a steel meter ruler at points where the least surface turbulence was observed. The velocities were determined with a Pasco Explorer 2000 velocity meter in ms^{-1}. The depths and velocity was measured once during each site visit. The width of the notch of the fishway was measured once-off at the start of the project and during each survey the height of the water flowing through this notch was measured and recorded. The discharge (Q) was then calculated and expressed in cumecs (cubic meters per second):

Q = (Water height in notch in metres) X (width of notch in metre) X (maximum velocity in m/s).

A second method to monitor the hydraulic parameters of the fishway was employed from October 2005 (Fouché in Heath *et al*., 2005). This involved the measurement of the height of the head of water (H_1) above the "crest" of the notch (Figure 8.4). In this method three rulers were affixed to the walls upstream of the notch in three separate pools in the fishway (Figure 8.3). The "zero" mark of each ruler was at the exact level of the crest of the baffle (Figure 8.5). During each survey from October 2005 to October 2006 of the height of the head of water backing up (H_1) at each "ruler" was measured. The maximum and minimum readings were used and an average calculated. Using the calculated average the discharge through the fishway and turbulence in the pools, expressed as the power dissipation per cubic meter ($watt/m^3$), was read from table 8.1. All the data collected was recorded on field form 2 (Appendix VII).

8.2.5 The condition factor.

The lengths and mass of the specimens of *L. marequensis* collected in each survey was used to determine the condition of each species. The condition of the fish was then expressed by calculating the body mass to body length ratio as a percentage (De Villiers, 1991) and this is referred as the gross condition factor (GCF).

8.2.6 Statistical analyses.

The temporal structuring of the fish assemblages and environmental characteristics were visually examined using MDS (Multidimensional Scaling) with Primer v.6 (Plymouth Routines in Marine Ecological Research) that constructs matrices summarising Bray- Curtis similarities Clarke and Ainsworth, 1993). The BIOENV procedure in Primer was used to investigate combinations of environmental variables that best explain the observed fish assemblage structure. The ability of environmental variables to predict the abundance of individual species were analysed with multiple linear regression in Statistica. These analyses were used to determine the environmental cues and were done for all species that formed 4% or more of the fish collected in the fishway.

Figure 8.4: A view of a "ruler" that was painted on side walls of the Xikundu fishway in the Luvuvhu River.

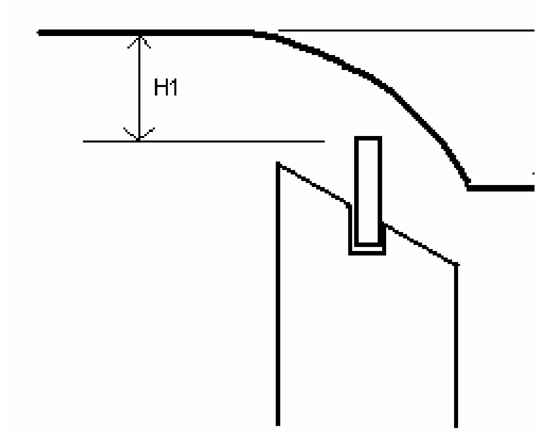

Figure 8.5: Illustration of the measurement of the head (H_1) at a baffle in the fishway (Adapted from Fouché in Heath *et al.*, 2005).

Table 8.1 : Fishway hydraulics with the baffle crest 200mm above the crest of the notch where H1 = height, Q = discharge and Pv the power (Fouché in Heath *et al*, 2005).

H1 (m)	**Q** (l/s)	**Pv** (watt/m^3)		**H1** (m)	**Q** (l/s)	**Pv** (watt/m^3)
0.02	3	1		0.24	101	44
0.04	7	4		0.26	112	48
0.06	13	7		0.28	123	52
0.08	20	10		0.30	134	55
0.10	28	14		0.32	145	59
0.12	37	18		0.34	156	62
0.08	20	10		0.36	167	65
0.10	28	14		0.38	178	69
0.12	37	18		0.40	189	72
0.14	46	22		0.42	210	78
0.16	57	27		0.44	239	88
0.18	68	31		0.46	273	98
0.20	79	36		0.48	311	110
0.22	90	40		0.50	353	123
0.24	101	44		0.52	398	137
0.26	112	48		0.54	446	151
0.28	123	52		0.56	496	166
0.22	90	40				

8.3 Results.

8.3.1 The location and structure of the Xikundu fishway.

The Xikundu Weir is situated in the Luvuvhu River *ca*. 30km upstream of the Kruger National Park and forms part of the greater water supply network of the Luvuvhu River and water is extracted and processed here for human consumption. It is one the larger weirs in Limpopo Province with an inundation surface area of about 3km² and a capacity of about 150 million m³.

The fishway at Xikundu (Figure 8.6) is a typical pool-type fishway with notched baffles. These notches are offset which allows for dissipation of the energy and creation of resting areas for the fish where the water velocity is greatly reduced (Figure 8.7). The notches are constructed to function in a way similar to Monk traps where the flow can be regulated by slotting in or taking out the specially constructed concrete slats. The fishway consists of three distinct sections with ten 1,2 m deep pools in the upper section, four pools in the middle section and nine pools in the lower section. The pools are all 2,4 meters wide and approximately 2,2 meters long. The sections are at right angles to one another with two slightly larger "joining pools" (2,6 X 2,35 m) between the upper and middle and two "joining pools" between the middle and lower sections. The entrance to the fishway is situated in the shallow water where fish movement reportedly occurs. Large boulders have been placed in the river to guide the fish into the fishway. The exit, which forms the link with the water body in the inundated area, is provided with a grid that prevents debris from entering.The slits in the grid is however wide enough to allow the free movement of fish. The flow of water into the exit can be regulated with a Monk trap.

8.3.2 The historic fish biodiversity at the Xikundu fishway.

Table 8.2 shows that 29 fish species have historically been recorded in the reach of the Luvuvhu River where the Xikundu Weir is situated. While Skelton (2001) indicates that thirteen of the species move or migrate in a river he regards only the catadromous *Anguilla mossambica* as a true migratory species that moves over long distances. The other eleven species *Chiloglanis paratus, C. pretoriae, Hydrocynus vittatus, Labeobarbus marequensis, Labeo congoro, L. cylindricus, L. molybdinus, L. rosae, L. ruddi, Micralestes acutidens, Marcusenius macrolepidotus* and *Petrocephalus wesselsi* are regarded as local migrators.

Figure 8. 6: The construction of the Xikundu fishway in the Luvuvhu River.

Figure 8.7 The observed flow pattern of the Xikundu fishway in the Luvuvhu River.

Table 8.2: General fish biodiversity of the reach of the Luvuvhu River where the Xikundu weir is situated (Adapted from Gaigher, 1998 ; Heath *et al.*, 2005).

Scientific name	English common name
Amphilius uranoscopus	Mountain catfish
Anguila mossambica	Longfin eel
Barbus paludinosus	Straightfin barb
Barbus toppini	East coast barb
Barbus trimaculatus	Threespot barb
Barbus unitaeniatus	Longbeard barb
Barbus viviparous	Bowstripe barb
Chiloglanis engiops	Lowveld suckermouth
Chiloglanis paratus	Sawfin suckermouth
Chiloglanis pretoriae	Shortspine suckermouth
Clarias gariepinus	Sharptooth catfish
Glossogobius giuris	Tank goby
Hydrocynus vittatus	Tigerfish
Labeobarbus marequensis	Lowveld largescale yellowfish
Labeo congoro	Purple labeo
Labeo cylindricus	Redeye labeo
Labeo molybdinus	Leaden labeo
Labeo rosae	Red nose labeo
Labeo ruddi	Silver labeo
Micralestes acutidens	Silver robber
Mesobola brevianalis	River sardine
Marcusenius macrolepidotus	Bulldog
Oreochromis mossambicus	Mozambique tilapia
Petrocephalus wesselsi	Churchill
Pseudocrenilabrus philander	Southern mouthbrooder
Schilbe intermedius	Butter barbel
Tilapia rendalli	Redbreast tilapia
Tilapia sparrmanii	Banded tilapia

During the surveys of this project, specimens of *Opsaridium peringueyi* were collected in the fishway during the 2004 and 2005 surveys (Tables 8.12 and 8.14). From historical records this was lowest downstream site in the Luvuvhu River where the species had been collected.

8.3.3. The extent of sampling period and the sampling frequency.

The project commenced in May 2004 and the survey dates are shown in table 8.3. The period May 2004 to April 2005 represents the LTLI phase and the period September 2005 to October 2006 the STHI phase. The period May 2005 to August 2005 was an interim period during which sampling methodology for the STHI phase was tested.

Table 8.3: The survey dates and the monitoring protocol used at the Xikundu fishway in the period May 2004 to October 2006.

	Protocols applied		
Date	**24 hours pool monitoring**	**Pool monitoring (one session)**	**a) 24 hours pool monitoring b) 24 hours cage and net monitoring**
17 – 18 May 2004	X		
17 – 18 June 2004	X		
22 – 23 July 2004	X		
26 – 27 August 2004	X		
27 – 28 September 2004	X		
29 – 30 October 2004	X		
20 – 21 November 2004	X		
19 – 20 January 2005	X		
23 – 24 February 2005	X		
17 – 18 March 2005	X		
20 – 21 April 2005	X		
24 May 2005		X	
29 June 2005		X	
29 July 2005		X	
25 – 26 August 2005		X	X
27 – 29 September 2005	X		X
25 – 27 October 2005	X		X
23 – 25 November 2005	X		X
31 January – 2 February 2006	X		X
23 – 25 March 2006	X		X
4 – 5 April 2006	X		X
29 – 31 May 2006	X		X
3 – 5 July 2006 (June survey)	X		X
26 – 28 July 2006	X		X
28 – 30 August 2006	X		X
28 – 30 September 2006	X		X
30 – 31 October 2006	X		X

8.3.4 The physical and physico-chemical parameters recorded on-site during the surveys.

The physico-chemical parameters recorded *in situ* during the surveys for the period May 2004 to October 2006 are shown in tables 8.4 and 8.5. These results show that the dissolved oxygen concentration and saturation was within accepted limits (Dallas and Day, 2004) throughout the whole period. The recorded pH values throughout the whole period remained within accepted limits with regard to aquatic life with the majority of the values within the median value range of 6 – 8 (Kempster *et al.,* 1980). Tables 8.8 and 8.9 show the measured physical characteristics and the calculated discharge through the fishway using the formula shown in 8.2.4. There were fluctuations in both the total dissolved solids and electrical conductivity values and figure 8.8 shows the relationship between these values and the calculated discharge for the same period.

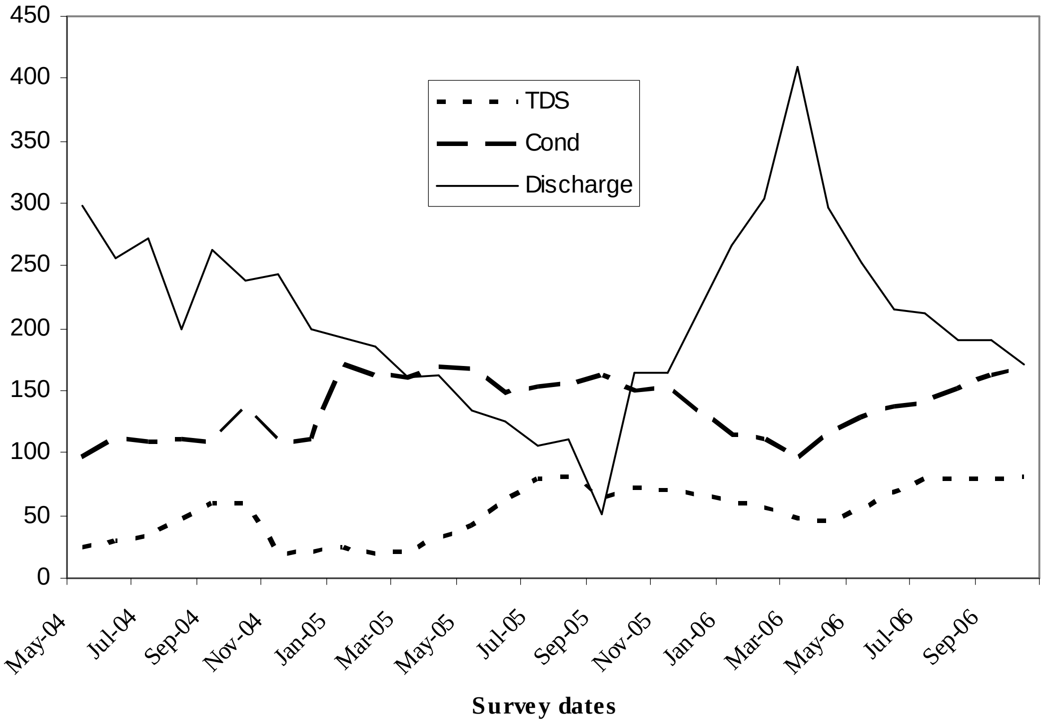

Figure 8.8: Graphic illustration of the relationship between the calculated discharge and the measured total dissolved solids (TDS) and Electrical Conductivity in the Xikundu fishway in the Luvuvhu River during the period May 2004 to September 2006. (TDS = ppm, Conductivity = μScm^{-1}, Discharge = ls^{-1})

Tables 8.6 and 8.7 show the atmospheric parameters observed and recorded during the same period. During the interim period the atmospheric, physico-chemical and hydraulic data was recorded and calculated and the data was interpreted independently. The recorded data is shown in tables 8.8 and 8.9. Table 8.10 shows the variation in the discharge and energy within the pools of the fishway read off from table 8.2. In figure 8.9 the calculated discharges obtained using both methods are compared. It is observed that although lower values were obtained when the formula was used, a similar trend was observed using both methods

It should also be borne in mind that both methods are prone to errors that in particular stem from incorrect height measurements due to the turbulence of the water surface. Because of the fact that the "table method" was designed by a professional hydrologist (Heath *et al.*, 2005) it can be accepted that it is the more accurate of the two.

The temperature data recorded by the data loggers are shown in figures 8.10 and 8.11 and summarized in table 8.11. When this is compared with the temperature data recorded during the site visits in the corresponding months (Tables 8.5 and Figure 8.12) the recorded trends are the same for both methods. The data obtained by the logger show that during November 2005 to February 2006, when an upward trend in the temperature existed, the fluctuations were not only more frequent but the changes often lasted for more than a day. This was not the case in the period with the downward trend that commenced in March 2006. In the coldest month, July 2006, the water temperature was at is most stable.

Table 8.4: The physico-chemical parameters of the water in the fishway determined during surveys at Xikundu fishway in the period from May 2004 to July 2005 .

Parameters	Units	May 2004	June 2004	July 2004	August 2004	Sept 2004	Oct 2004	Nov 2004	Jan 2005	Febr 2005	March 2005	April 2005	May 2005	Jun 2005	July 2005
Dissolved oxygen in still water.	*mg/l*	**8,3**	**8,2**	**8,5**	**7,2**	**7,9**	**6,7**	**7,6**	**7,5**	**6,42**	**7,38**	**7,7**	**7,75**	**8,64**	**8,57**
	%	**98,9**	**96,0**	**91,0**	**84,0**	**94,0**	**78,0**	**90,0**	**97,0**	**88,6**	**97,0**	**99,6**	**92,0**	**96,0**	**94,7**
Dissolved oxygen in turbulent water	*mg/l*	**8,6**	**9,4**	**8,9**	**7,3**	**8,4**	**6,8**	**8,3**	**7,6**	**7,06**	**7,56**	**8,11**	**7,86**	**8,73**	**8,7**
	%	**101,0**	**101**	**98,0**	**85,0**	**97,0**	**85,0**	**97,0**	**97,0**	**98,0**	**96,8**	**99,6**	**94,0**	**96,4**	**97,0**
Temperature	*°C*	**20,1 – 20,9**	**15,9 - 17,2**	**15,3 – 15,7**	**19,6 – 20,8**	**20,3 – 23,5**	**23,2 – 23,8**	**20,2 – 23,5**	**26,7 – 27,4**	**28,6 – 29,8**	**25,1 – 27,2**	**25,2 – 27,0**	**15,6 - 21,8**	**14,0 - 18,5**	**15,6 – 17,0**
pH		**7,6**	**8,7**	**7,8**	**7,9**	**7,9**	**8,0**	**7,9**	**7,0**	**7,0**	**7,3**	**7,5**	**7,5**	**7,3**	**7,27**
TDS	*ppm*	**25,0**	**30,0**	**34,0**	**48,0**	**60,0**	**60,0**	**20,0**	**25,0**	**20,0**	**22,0**	**32,0**	**43,5**	**62,0**	**79,4**
Conductivity	*µS/cm*	**97,0**	**112,0**	**109,0**	**112,0**	**110,0**	**138,0**	**110,0**	**171,0**	**163,0**	**160,0**	**170,0**	**168,0**	**148,0**	**154,0**

Table 8.5: The physico-chemical parameters of the water in the fishway determined during the field visits at Xikundu fishway in the period from August 2005 to October 2006.

Parameters	Units	Aug 2005	Sept 2005	Oct 2005	Nov 2005	Jan 2006	Febr 2006	March 2006	April 2006	May 2006	June 2006	July 2006	Aug 2006	Sept 2006	Oct 2006
Dissolved oxygen in still water.	*mg/l*	**7,5**	**7,32**	**7,3**	**7,6**	**7,3**	**7,7**	**7,32**	**8,2**	**8,3**	**8,38**	**7,56**	**7,7**	**7,84**	**6,68**
	%	**90,9**	**96,0**	**95,0**	**94,2**	**94,0**	**97,0**	**95,0**	**92,9**	**88,0**	**87,0**	**86,0**	**85,1**	**95,3**	**87,2**
Dissolved oxygen in turbulent water	*mg/l*	**7,8**	**7,34**	**7,4**	**7,8**	**7,7**	**8,0**	**7,4**	**8,6**	**8,5**	**8,56**	**7,88**	**7,80**	**7,87**	**6,75**
	%	**93,0**	**98,0**	**98,0**	**97,0**	**97,0**	**99,0**	**96,0**	**94,0**	**89,1**	**88,0**	**88,0**	**87,0**	**97,0**	**89,6**
Temperature	*°C*	**18,0-21,0**	**13,0 – 21,0**	**18,0 – 22,0**		**24,0 – 29,0**	**25,0 – 29,0**	**22,0 – 25,3**	**19,3 - 19,6**	**16,4 – 18,1**	**14,4 – 16,6**	**16,8 – 17,5**	**19,0– 21,2**	**19,9 – 22,7**	**25,6 – 27,1**
pH		**6,89**	**6,79**	**6,89**	**6,88**	**6,86**	**6,87**	**6,85**	**6,85**	**6,97**	**7,2**	**7,3**	**7,2**	**6,96**	**6,95**
TDS	*ppm*	**81,1**	**64**	**173**	**270**	**61**	**55,8**	**47,9**	**46,4**	**55**	**68**	**80**	**79**	**79,2**	**81,6**
Conductivity	*µS/cm*	**154,0**	**162,8**	**150**	**152,0**	**115**	**112**	**95,8**	**115,5**	**128,0**	**138,0**	**142,0**	**152,0**	**162,0**	**167,0**

Table 8.6 : Atmospheric parameters measured at the Xikundu fishway from May 2004 to July 2005.

Parameters	May 2004	June 2004	July 2004	Aug 2004	Sept 2004	Oct 2004	Nov 2004	Jan 2005	Febr 2005	March 2005	April 2005	May 2005	Jun 2005	July 2005
Max. air temperature (^{o}C)	**27.0**	**21,0**	**24,0**	**27,0**	**25,0**	**27,2**	**29,1**	**29,0**	**32,2**	**33,0**	**33,1**	**23,0**	**26,4**	**24,3**
Min. air temperature (^{o}C)	**11,0**	**9,1**	**5,0**	**15,0**	**18,0**	**16,0**	**19,0**	**21,1**	**22,0**	**22,2**	**21,1**	**12,0**	**10,5**	**14,0**
Range of cloud cover	**0/8 – 1/8**	**0/8 – 7/8**	**0/8**	**2/8 – 8/8**	**7/8**	**7/8 – 8/8**	**1/8 – 7/8**	**7/8 – 8/8**	**4/8 – 6/8**	**0/8 – 8/8**	**0/8 – 8/8**	**0/8 – 4/8**	**4/8 – 7/8**	**4/8 – 6/8**
Atmospheric pressure (mBar)											**961,2 - 967,9**	**966,7**	**979,6**	**977,7**
Rain	**No**	No	No	No	No	No	No	**Yes**	**No**	**No**	**Yes**	**No**	**No**	**No**
Prevalent wind direction	**SW/ NW**	**NW/ NE**	**N/ E**	**NW/ W**	**S/W**	**NW/S**	**S/NE**	**S/SW**	**E/SE**	**S/SE**	**S**	**SW**	**S**	**E**

Table 8.7 : Atmospheric parameters measured at the Xikundu fishway from September 2005 to October 2006.

Parameters	Aug 2005	Sept 2005	Oct 2005	Nov 2005	Jan 2006	Febr 2006	March 2006	April 2006	May 2006	June 2006	July 2006	Aug 2006	Sept 2006	Oct 2006
Max. air temperature (^{o}C)	**31,0**	**33,7**	**40,1**	**36,6**	**33,4**	**36,0**	**36,0**	**30,7**	**24,0**	**28,4**	**28,9**	**29,0**	**36,3**	**42,0**
Min. air temperature (^{o}C)	**18,0**	**15,7**	**15,0**	**21,3**	**23,4**	**22,0**	**22,0**	**18,3**	**12,5**	**9,9**	**13,8**	**14,4**	**15,7**	**19,8**
Range of cloud cover	**0/8**	**2/8 – 6/8**	**1/8 – 8/8**	**1/8 – 8/8**	**7/8 – 8/8**	**3/8 – 8/8**	**3/8 – 8/8**	**0/8 – 8/8**	**5/8 – 7/8**	**0/8**	**0/8**	**0/8 – 8/8**	**0/8 – 5/8**	**2/8 – 8/8**
Atmospheric pressure (mBar)	**964.0**	**954 – 977**	**956 - 977**	**961 – 966,4**	**959 - 966**	**960 – 968**	**960 - 968**	**963 – 967**	**938 - 975**	**968 - 974**	**975 -981**	**960 – 970**	**956 – 982**	**954,2 – 959,0**
Rain	**No**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**No**	**No**	**No**	**No**	**No**	**No**	**Yes**
Prevalent wind direction	**E**	**S**	**S**	**S – SE**	**SW**	**E – S/E**	**E – S/E**	**E**	**E**	**E - NE**	**E – N**	**S**	**E- NW**	**S – SE**

Table 8.8: Measured and calculated hydraulic characteristics of the Xikundu fishway during the period May 2004 to July 2005.

Parameters		May 2004	June 2004	July 2004	Aug 2004	Sept 2004	Oct 2004	Nov 2004	Jan 2005	Febr 2005	March 2005	April 2005	May 2005	Jun 2005	July 2005
Average depth of water in pools (m)		**1,17**	**1,11**	**1,15**	**1,06**	**1,10**	**1,09**	**1,10**	**1,03**	**1,00**	**1,02**	**1,04**	**0,99**	**0,99**	**0,94**
Water height of the over the crest of the notch. (m)		**0,33**	**0,33**	**0,30**	**0,22**	**0,29**	**0,27**	**0,29**	**0,24**	**0,24**	**0,22**	**0,25**	**0,21**	**0,20**	**0,16**
Velocity in notch (m/s)	Min.	**1,485**	**1,320**	**1,405**	**0,990**	**1,155**	**0,990**	**1,071**	**1,00**	**0,80**	**0,70**	**0,60**	**0,60**	**0,57**	**0,8**
	Max.	**1,815**	**1,550**	**1,815**	**1,815**	**1,815**	**1,650**	**1,683**	**1,600**	**1,540**	**1,46**	**1,310**	**1,280**	**1,26**	**1,32**
Q = Calculated discharge (cumec)		**0,299**	**0,256**	**0,272**	**0,200**	**0,263**	**0,239**	**0,244**	**0,192**	**0,185**	**0,161**	**0,163**	**0,134**	**0,126**	**0,106**

Table 8.9: Measured and calculated hydraulic characteristics of the Xikundu fishway during the period August 2005 to October 2006.

Parameters		Aug 2005	Sept 2005	Oct 2005	Nov 2005	Jan 2006	Febr 2006	March 2006	April 2006	May 2006	June 2006	July 2006	Aug 2006	Sept 2006	Oct 2006
Average depth of water in pools (m)		**1,03**	**0,81**	**1,03**	**1,00**	**1,15**	**1,15**	**1,18**	**1,11**	**1,08**	**1,07**	**1,02**	**1,02**	**1,00**	**1,03**
Water height of the over the crest of the notch. (m)		**0,16**	**0,10**	**0,22**	**0,21**	**0,35**	**0,40**	**0,46**	**0,37**	**0,28**	**0,26**	**0,25**	**0,23**	**0,23**	**0,22**
Velocity in notch (m/s)	Min.	**0,8**	**0,21**	**0,48**	**1,16**	**0,80**	**1,20**	**1,23**	**0,8**	**0,72**	**0,69**	**0,72**	**0,69**	**0,67**	**0,70**
	Max.	**1,4**	**1,02**	**1,50**	**1,57**	**1,52**	**1,52**	**1,78**	**1,60**	**1,80**	**1,66**	**1,69**	**1,66**	**1,65**	**1,56**
Q = Calculated discharge (cumec)		**0,112**	**0,051**	**0,165**	**0,165**	**0,266**	**0,304**	**0,409**	**0,296**	**0,252**	**0,216**	**0,211**	**0,191**	**0,190**	**0,172**

Table 8.10: Hydraulic data calculated from the water heights measured on the rulers at the Xikundu fishway during the period October 2005 to October 2006 . Q and Pv are read off Table 8.1.

Date	Max height on rulers (m)	Min height on rulers (m)	Average height on rulers (m)	Q (l/s)	Pv (watt/m^3)
October 2005	0,40	0,38	0,39	183	70
November 2005	0,38	0,37	0,375	178	69
January 2006	0,52	0,50	0,51	375	130
February 2006	0,56	0,54	0,55	471	158
March 2006	0,59	0,57	0,58	546	181
April 2006	0,54	0,52	0,53	422	135
May 2006	0,56	0,56	0,56	496	166
June 2006	0,55	0,55	0,55	471	159
July 2006	0,55	0,55	0,55	471	159
Aug 2006	0,55	0,53	0,54	446	151
September 2006	0,53	0,53	0,53	420	144
October 2006	0,53	0,52	0,525	410	140

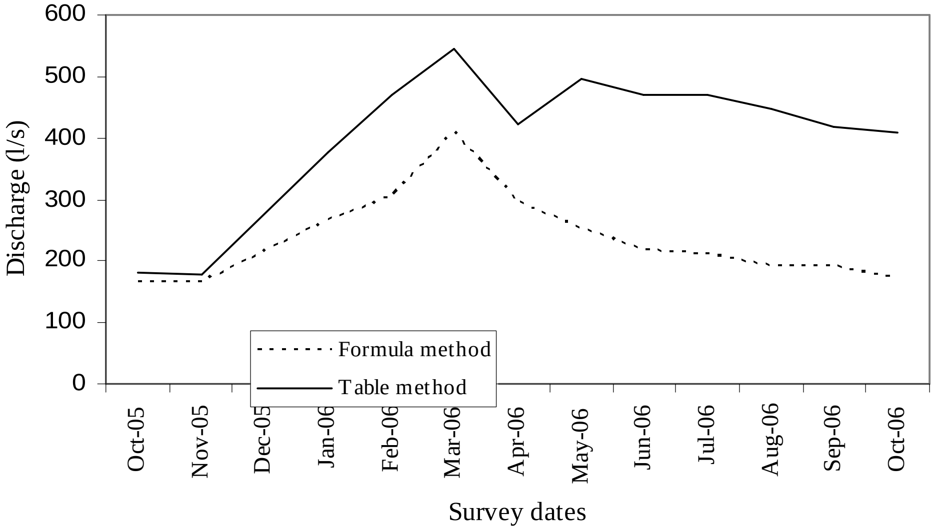

Figure 8.9: A graphic comparison of the calculated discharge results obtained from the two methods employed.

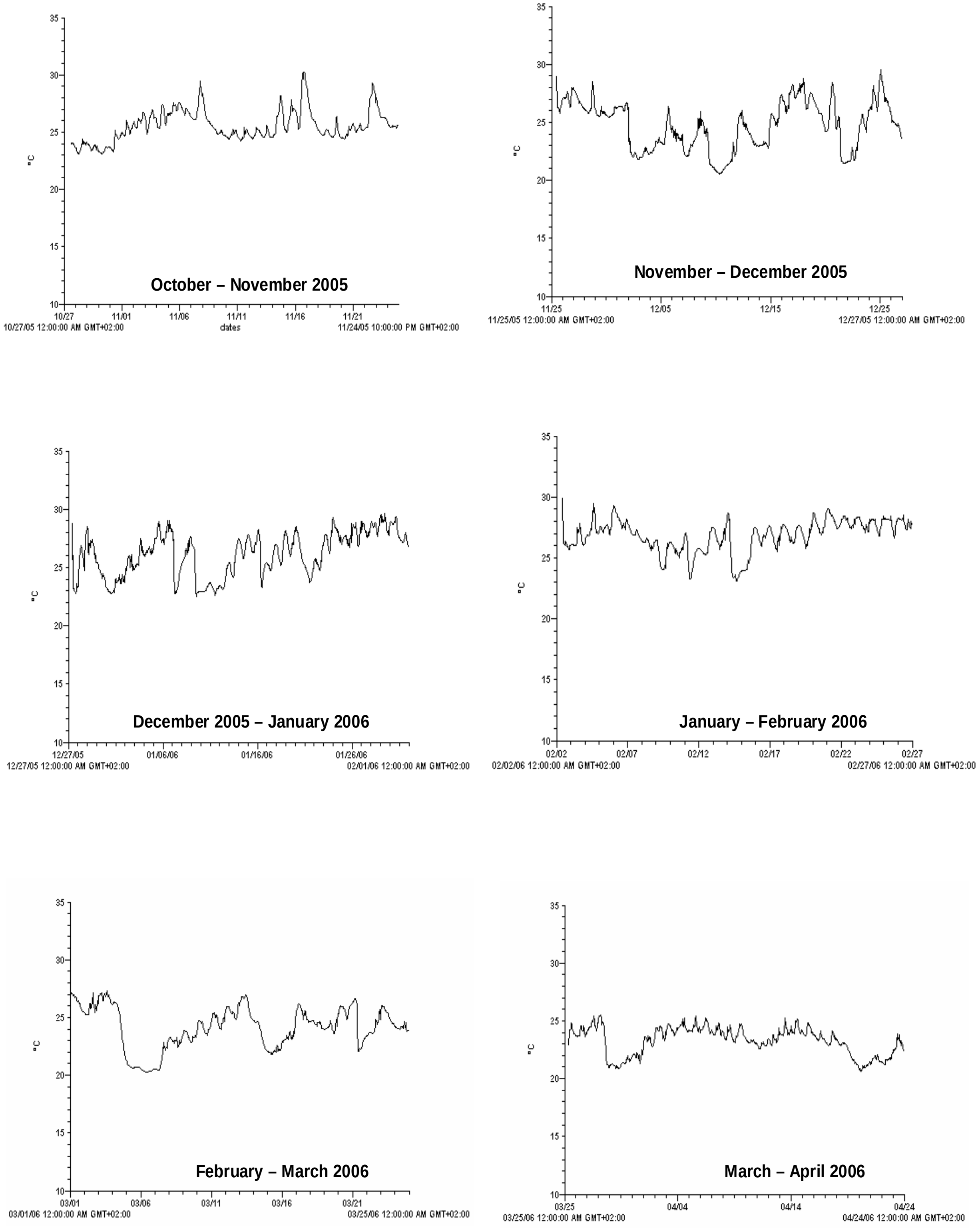

Figure 8.10: **Water temperature readings obtained from the Hobo Pendant data loggers installed in the Xikundu fishway in the period September 2005 to April 2006.**

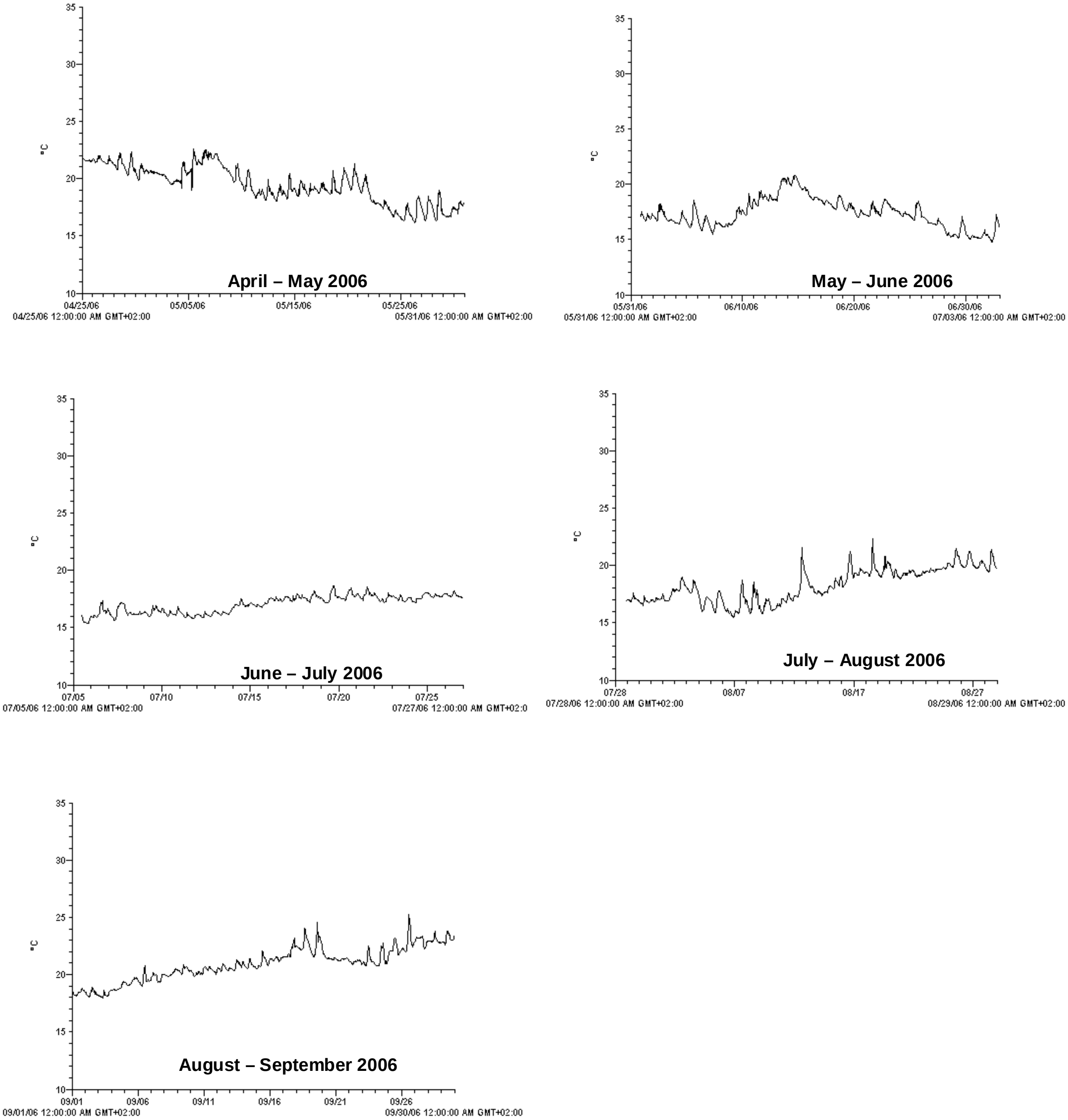

Figure 8.11: Water temperature readings obtained from the Hobo Pendant data loggers installed in the Xikundu fishway in the period April – September 2006.

Table 8.11 Summary of the water temperatures recorded by the data loggers.

Recording period	Temperatures recorded (°C)		
	Min.	**Max.**	**Average**
27/10 – 25/11 2005	23.12	30.36	25.56
25/11 – 27/12/2005	20.52	29.55	24.73
27/12 – 31/1/2006	22.05	32.39	26.17
31/1 – 28/2/2006	23.11	31.68	26.86
28/2 – 25/3/2006	20.23	27.28	22.47
25/3 – 25/4/2006	20.62	25.51	23.13
25/4 – 31/5/2006	16.01	22.75	18.45
31/5 – 28/6/2006	14.99	20.81	17.48
4/7 - 28/7/2006	13.27	18.62	17.48
28/7 – 29/8/2006	15.47	23.49	18.53
29/8 – 30/9/2006	17.95	25.22	20.22

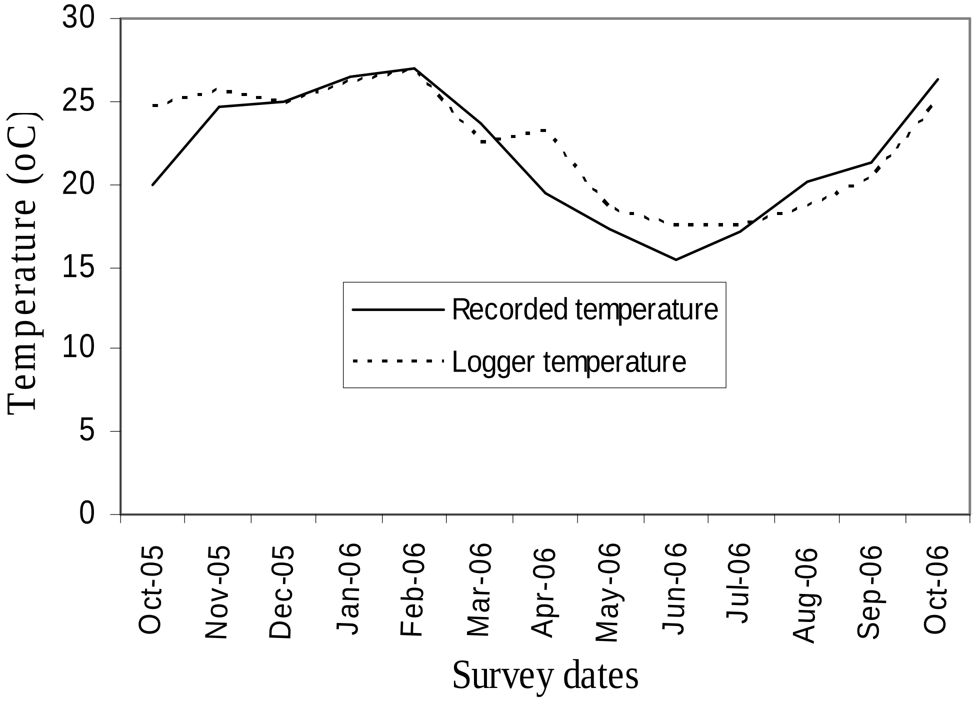

Figure 8.12: A graphic comparison of the recorded and measured temperatures in the Xikundu fishway in the period October 2005 to October 2006.

8.3.5 The fish collected in the Xikundu fishway.

Tables 8.12, 8.13 and 8.14 shows the diversity fish species and the numbers of each species collected during the LTLI, STHI and the interim period respectively.

Table 8.12: Fish collected in the Xikundu fishway during the period May 2004 to April 2005.

	Number of specimens collected										
Species	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Jan**	**Feb**	**Mar**	**Apr**
Amphilius uranoscopus				1							
Anguila mossambica					1						
Barbus trimaculatus		1				17	3	18		2	12
Barbus unitaeniatus				1							
Barbus viviparus	5			1	1	3	1	16	1	4	5
Chiloglanis engiops		8	1				18		1	6	7
Chiloglanis paratus	12	7	13	2	2	1	4	2		1	1
Chiloglanis pretoriae	147	180	42	80	24	11	20	27	4	12	18
Glossogobius giuris				2					1	1	5
Labeobarbus marequensis	17	27	23	28	145	168	45	51	26	51	88
Labeo cylindricus	28	23	7	5	1	5	48	47	24	52	22
Labeo molybdinus	21	18	3	3	5	70	23	22	26	10	31
Marcusenius macrolepidotus							14				
Micralestes acutidens	2	3			22	56	55	144	119	222	167
Mesobola brevianalis	26			26		73	13	57	82	100	159
Opsaridium peringueyi									4	3	10
Oreochromis mossambicus	6	3	42		1	16	6	7	29	24	2
Petrocephalus wesselsi							1				
Pseudocrenilabrus philander	1			1		4					
Tilapia rendalii						1					

Table 8.13: Fish collected in the Xikundu fishway during the period September 2005 to October 2006.

	Number of specimens collected												
Species	**Sept**	**Oct**	**Nov**	**Jan**	**Feb**	**Mr**	**Apr**	**May**	**Jun**	**Jul**	**Au**	**Sep**	**Oct**
Amphilius uranoscopus													
Anguila mossambica					2								
Barbus trimaculatus	6		6	1	2						4	4	60
Barbus unitaeniatus	2				9	4	1	1	4		5	5	19
Barbus viviparus	4	2	27	7	7	2					1	1	34
Chiloglanis Engiops				62	28	153	118	25	14	16	6	8	1
Chiloglanis paratus						73	130	40	10	9	4	4	
Chiloglanis pretoriae		5	2	5	21	75		74	21	23	9	15	16
Clarias gariepinus	2				2		2						
Glossogobius giuris				9	9	2		3	3	11	1	2	90
Labeobarbus marequensis	10	28	104	4				1	21	15	71	79	45
Labeo cylindricus		6	31	27	6	10	25	12	10	3	4	5	13
Labeo molybdinus		9	48	20	6	6	15	8	9	2	5	10	14
Marcusenius macrolepidotus	1		1		2	2	2						8
Micralestes acutidens	275	525	1616	1				1		1		27	415
Mesobola brevianalis	4	63	30	5	1	1			1	2	45	14	130
Opsaridium peringueyi													
Oreochromis mossambicus		3	42	1	7				1		1	24	19
Petrocephalus wesselsi													
Pseudocrenilabrus philander													
Tilapia rendalii													

Table 8.14: Fish collected in the Xikundu fishway during the interim period from May to July 2005.

	Number of specimens collected		
Species	**May**	**June**	**July**
Amphilius uranoscopus			
Anguila mossambica			
Barbus trimaculatus	48	2	
Barbus unitaeniatus			
Barbus viviparous	4	4	4
Chiloglanis engiops			
Chiloglanis paratus			
Chiloglanis pretoriae	4	16	4
Clarias gariepinus			
Glossogobius guiris			
Labeobarbus marequensis	131	125	66
Labeo cylindricus	12	7	2
Labeo molybdinus	11	8	7
Marcusenius macrolepidotus	9		
Micralestes acutidens	21	4	
Mesobola brevianalis	173	67	7
Opsaridium peringueyi	1	8	1
Oreochromis mossambicus	1	1	
Petrocephalus wesselsi			
Pseudocrenilabrus philander			
Tilapia rendalii			

To establish the temporal changes in the fish assemblages data collected during the period October 2005 to October 2006 were analysed using MDS (Multidimensional Scaling). The results (Figure 8.13) show a temporal structuring of the fish assemblages that suggest three clusters with a distinct annual pattern. The patterns could be positively related to ($p \leq 0,05$) to environmental characters and to discharge in particular. Cluster A had the lowest discharge (0.165 – 0.172 cumecs) but the highest average temperature, cluster B had the highest flow (0.252 – 0.409 cumecs) but a mix of high and low temperatures while cluster C had intermediate discharge values (0.190 – 0.216 cumecs) but the lowest temperatures.

The classification of fish species based on temporal change in their abundance suggest that some species have the same or similar temporal patterns of movement through the fishway (Figure 8.14). Note should be taken of *M. acutidens* which is unique and the two Labeos which are similar. The chiloglanids are distinctly grouped together and *L. marequensis* together with *M. brevianalis* are very similar and form a group. Figure 8.15 which shows the same results but here the boundaries of the groupings indicate the degrees of similarity which ranges from 20 to 80 percent.

Table 8.15 shows a summary of the number of *L. marequensis* specimens collected in the fishway in the surveys during the three years. Although the protocol applied during the interim period (May to August 2005) differed from the other protocols the number of specimens collected are included. With the exception of 2005, when the numbers increase commenced in April, the increase in numbers occurred at the onset of spring or in early summer.

The frequency distribution of the fork lengths of *L. marequensis* collected in the fishway during the period May 2004 to October 2006 is shown in Figures 8.16, 8.17 and 8.19. In all three years the majority of specimens moving through the fishway were specimens with fork lengths between 30mm and 150mm. No specimens shorter than 30mm were observed in the fishway. Larger specimens with fork lengths longer than 150 were collected in September, October and November 2004, April and May 2005 and from July to October in 2006.

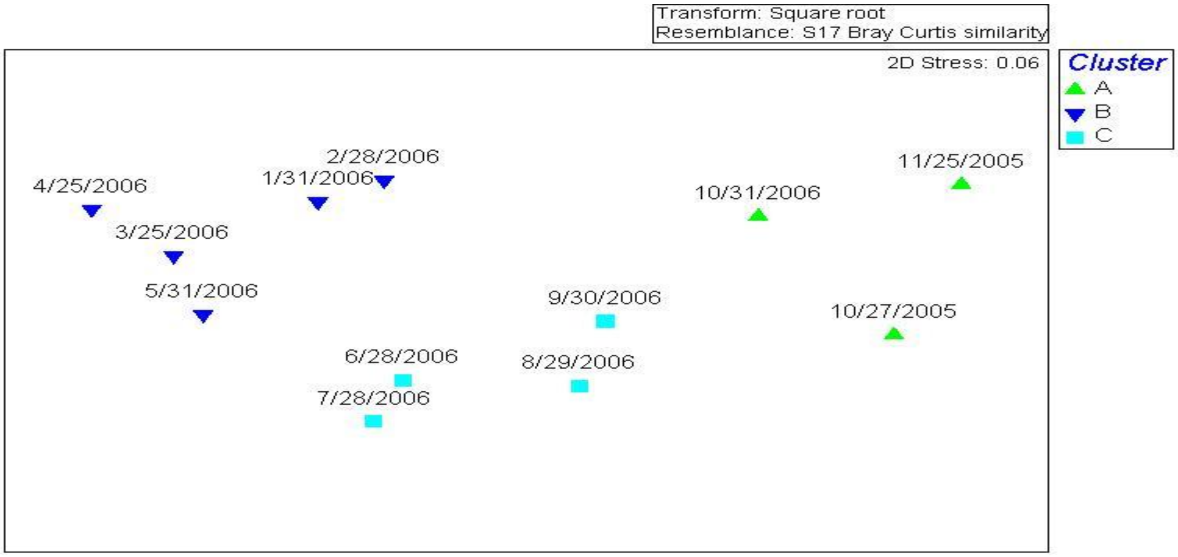

Figure 8.13: MDS ordination of all the dates of the surveys during which fish was collected in the Xikundu fishway from October 2005 to October 2006.

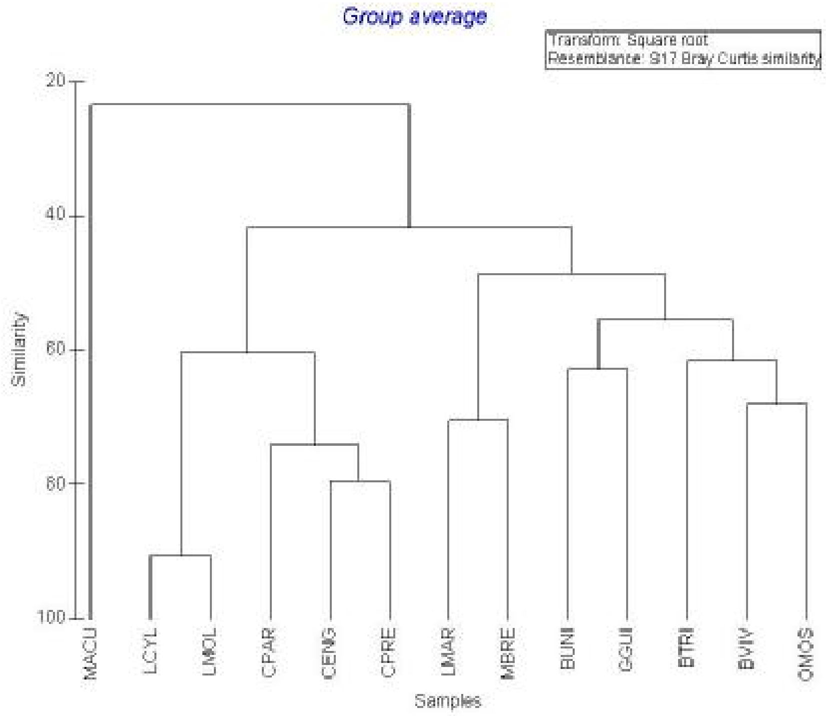

Figure 8.14: Cluster analysis of the similarities between the species collected in the Xikundu fishway from October 2005 to October 2006.

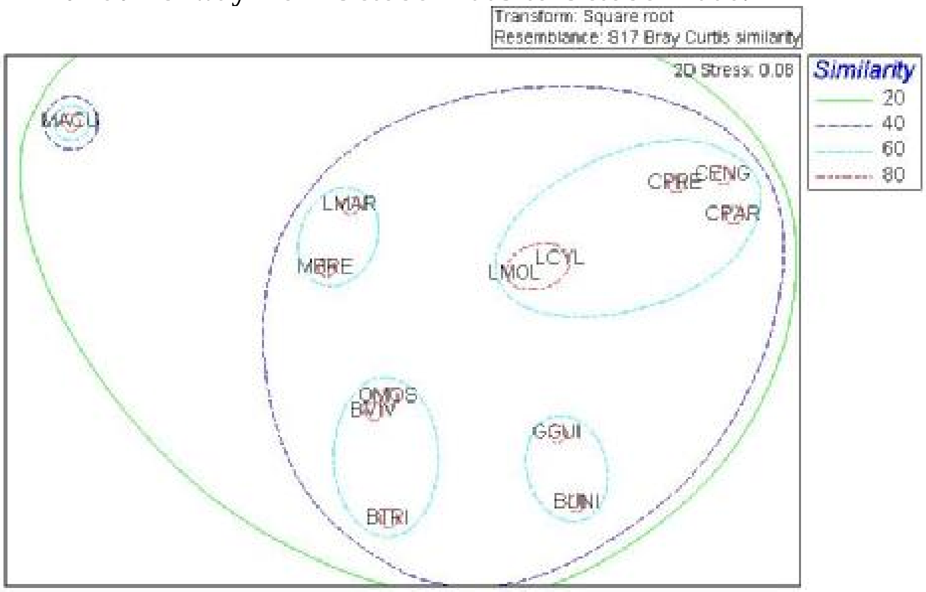

Figure 8.15: MDS ordination of all the dates of the surveys during which fish was collected in the Xikundu fishway from October 2005 to October 2006.

Table 8.15: The numbers of *Labeobarbus marequensis* collected in the period from 2004 to 2006 in the Xikundu fishway. (NS = not sampled)

Year	Jan	Feb	March	April	May	Jun	Jul	Aug	Sept	Oct	Nov
2004	NS	NS	NS	NS	17	27	23	28	145	168	45
2005	51	26	51	88	131	125	66	NS	10	28	104
2006	4	0	0	0	1	21	15	71	79	45	NS

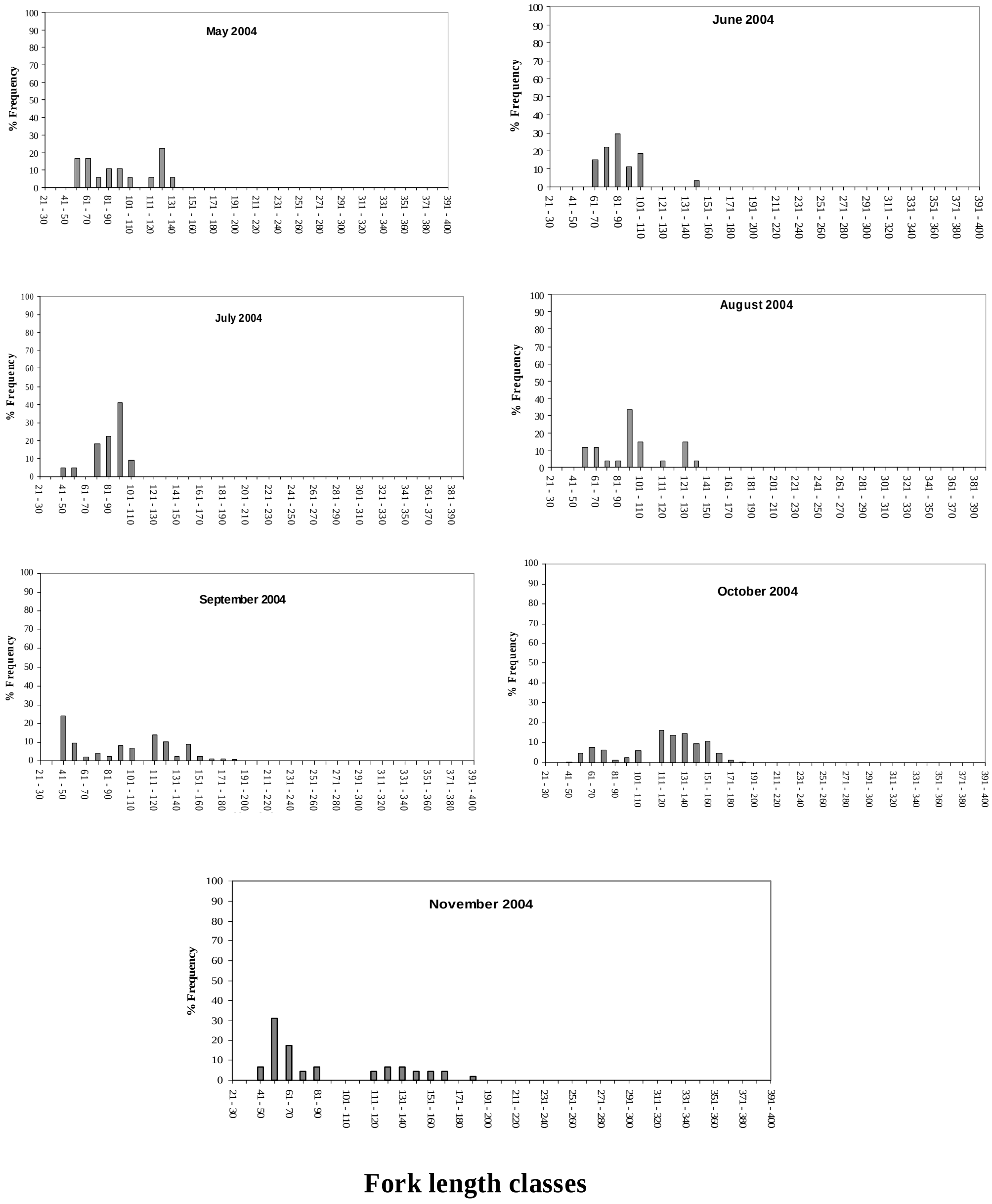

Figure 8.16: Length frequency distribution of the various sizes of *Labeobarbus marequensis* collected in the Xikundu fishway during 2004. The fork length classes (mm) are shown on the X-axes.

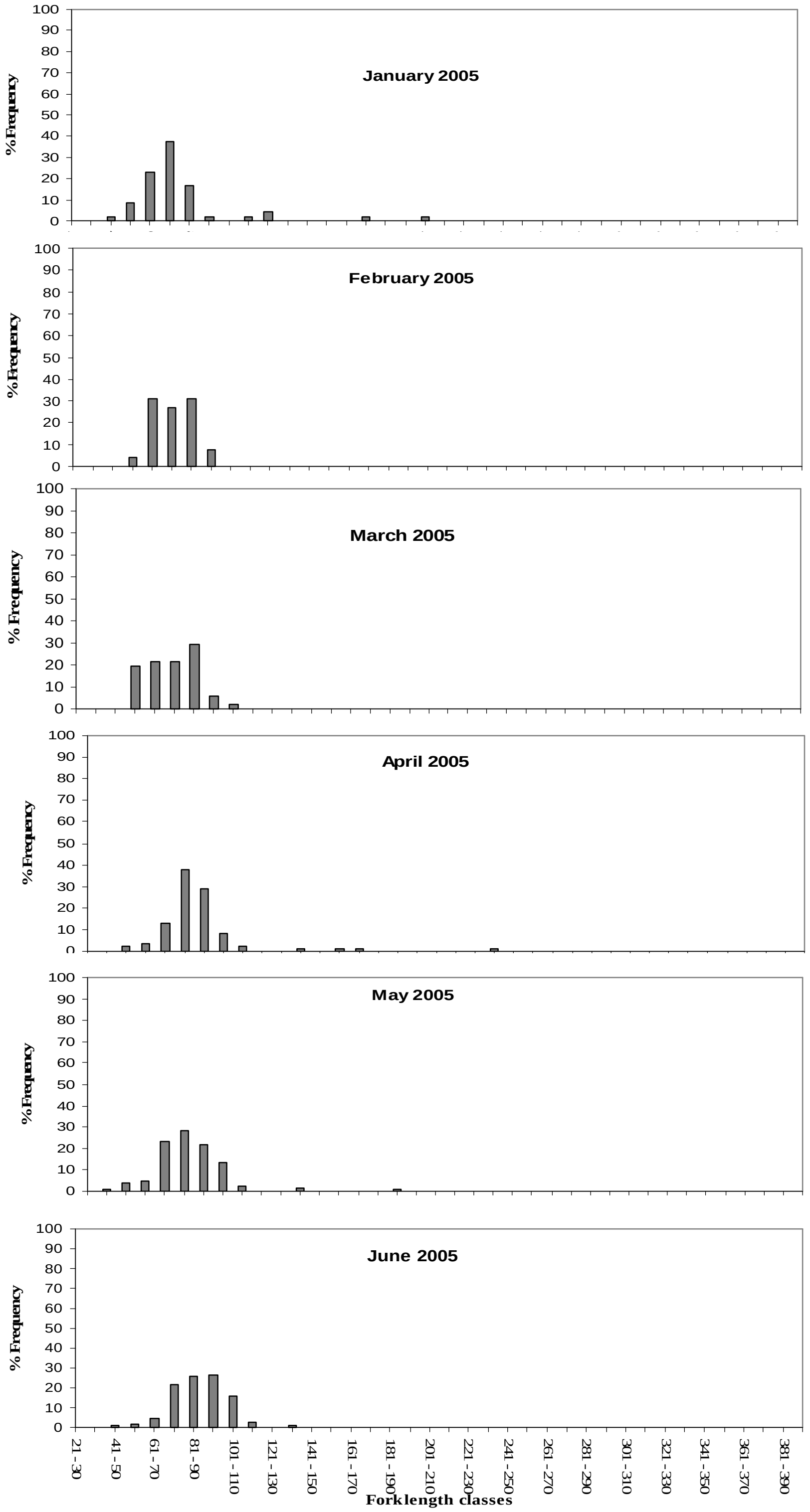

Figure 8.17: Length frequency distribution of the various sizes of *Labeobarbus marequensis* collected in the Xikundu fishway during 2005. The fork length classes (mm) are shown on the X-axes.

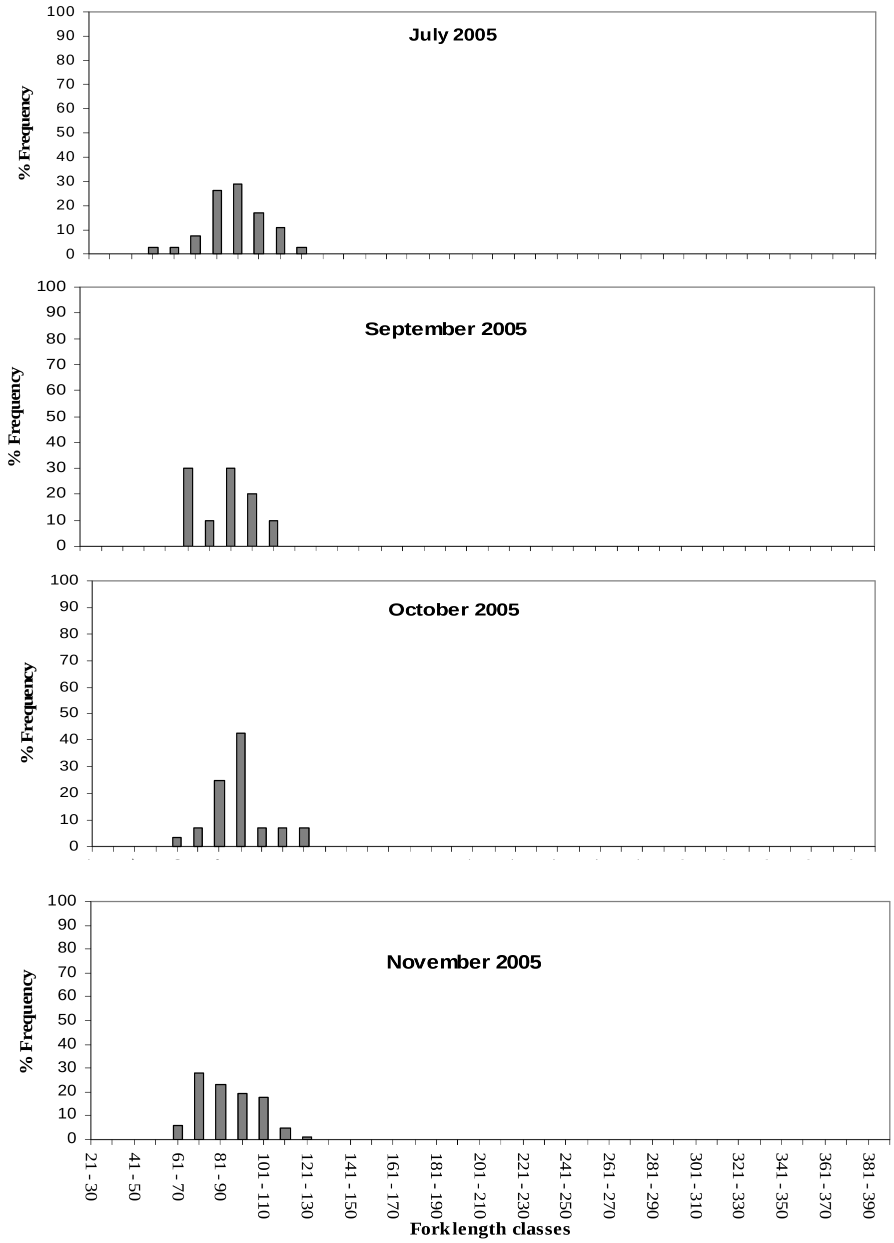

Figure 8.17 (cont.): **Length frequency distribution of the various sizes of *Labeobarbus marequensis* collected in the Xikundu fishway during 2005. The fork length classes (mm) are shown on the X-axes.**

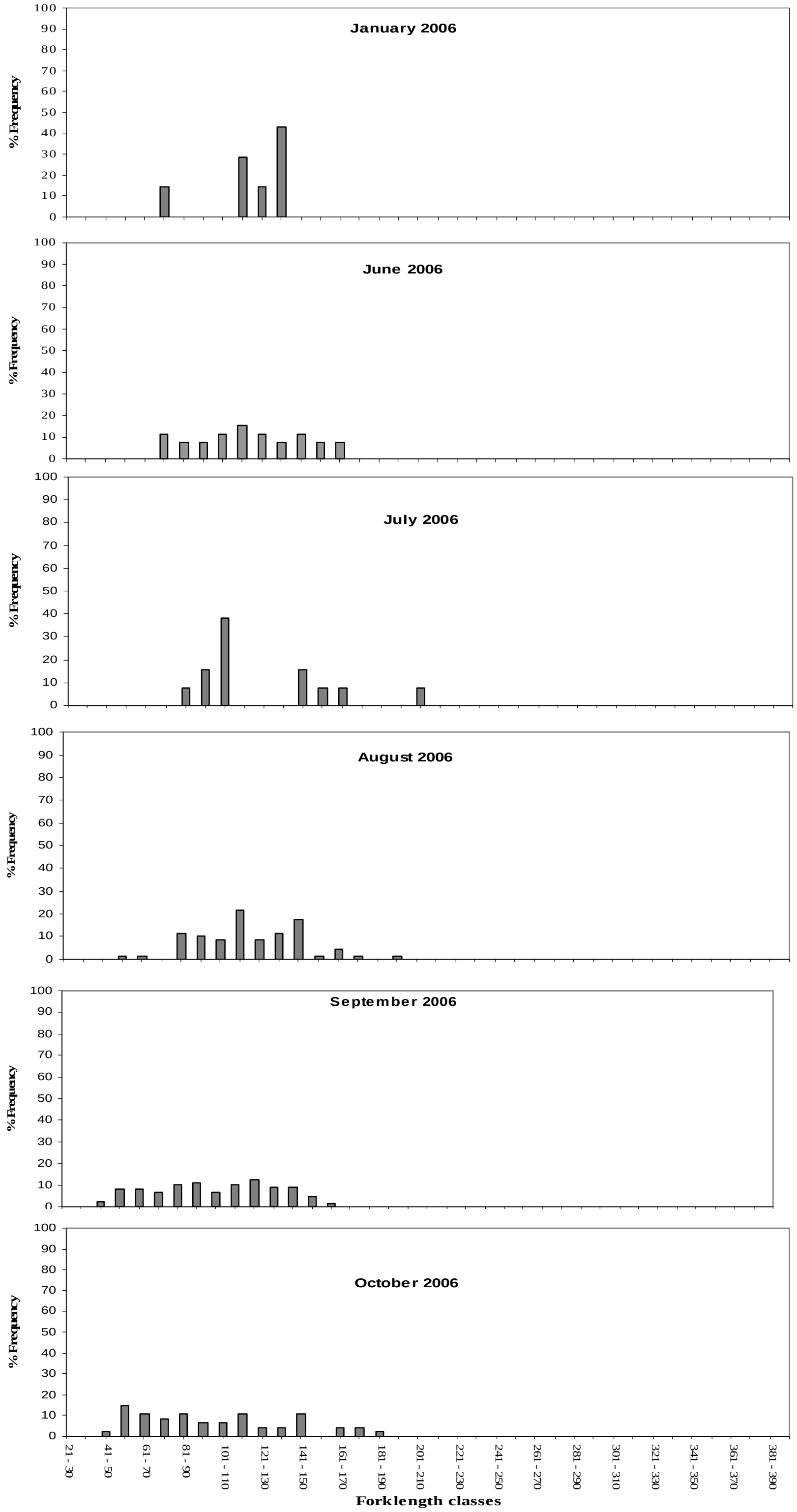

Figure 8.18: Frequency distribution of the various sizes of *Labeobarbus marequensis* collected in the Xikundu fishway during 2006. The fork length classes (mm) are shown on the X-axes.

8.3.6 The diel pattern of movement through the fishway.

In order to establish at which time of day *L. marequensis* frequented the fishway the time of day in which the species was collected in the fishway during the first 24-hour period of the STHI was recorded and analysed. The percentage frequency of occurrence was calculated and the results obtained (Table 8.16) show that the species most frequently moved though the fishway during daylight with the highest percentage in the period from 08h00 to late afternoon.

Table 8.16: The number and percentage frequency of occurrence of specimens of *Labeobarbus marequensis* collected at four hourly intervals in the Xikundu fishway during the period September 2005 to October 2006. The indicated times are at the start of the collection period. The numbers in parenthesis is the number collected expressed as a percentage of the total number of fish collected during the survey).

Date	08h00	10h00	12h00	16h00	20h00	24h00	04h00
Sept 2005			2 (33)	3 (50)		1 (17)	
Oct 2005	3 (13)		6 (26)	8 (35)	4 (17)	1 (4)	
Nov 2005	1 (1)		44 (49)	4 (6)	38 (42)	1 (1)	1 (1)
Jan 2005		2 (50)		1 (25)	1 (25)		
May 2006							
Jun 2006	1 (5)	13 (62)	3 (14)	3 (14)		1 (5)	
Jul 2006	2 (17)	6 (49)	2 (17)	2 (17)			
Aug 2006	4 (11)	4 (11)	14 (38)			5 (13)	10 (27)
Sept 2006	16 (19)	25 (29)	10 (12)	12 (14)		12 (14)	10 (12)
Oct 2006	4 (7)	35 (66)				5 (9)	9 (17)
Percentage frequency of occurrence	70	60	70	70	30	70	40

8.3.7 The direction of movement.

To establish the direction in which the fish move through the fishway the results obtained with the cage and long net collections were used. During the August 2005 survey the methods were not successful and only one specimen was collected. The results obtained are shown in table 8.17 and although not really conclusive, the results obtained from August to October 2006 does indicate that when the species migrates in larger numbers , the tendency is towards an upstream rather than a downstream movement.

Table 8.17: The number of *Labeobarbus marequensis* specimens collected using the cage and long-bagged net at Xikundu fishway during the period October 2005 to October 2006.

	2005		2006									
Method of collection	**Oct**	**Nov**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sep**	**Oct**
Long-bagged net	0	0	0	0	1	0	0	3	0	0	1	0
Cage collections	0	0	0	1	0	0	1	0	0	12	10	4

8.3.8 Environmental cues or triggers of migration or movement.

To determine single or individual cues that would predict the migration of fish proved to be very complex. It was rather shown that combinations of cues were responsible. Table 8.18 shows the results of a Spearman rank correlation of eight variables that was performed as part of BIOENV. From the results it is clear that none of the correlations were extremely good or close to 1,0. It should however be noted the best correlation was achieved with the calculated discharge. The position of the maximum temperature and temperature in general should be borne in mind. The best predictor of fish assemblage structure is however the calculated discharge (variable 8) and that discharge featured in each of the combinations. This is well illustrated when the discharge is superimposed on the clusters obtained in the MDS ordination (Figure 8.19).

Table 8.18: Correlation between the measured environmental variables and frequency of occurrence of *Labeobarbus marequensis*. (1 : minimum logger temperature, 2 : maximum logger temperature, 3 : average logger temperature, 4: minimum temperature measured *in situ*, 5: maximum temperature measured *in situ*, 6 : minimum velocity, 7: maximum velocity and 8 discharge).

Variables	Correlation
8	0,486
2 and 8	0,423
1 and 8	0.400
4 and 8	0,398
1, 2 and 8	0,390
2, 6 and 8	0,362
6 and 8	0,360
5 and 8	0,360
7 and 8	0,356

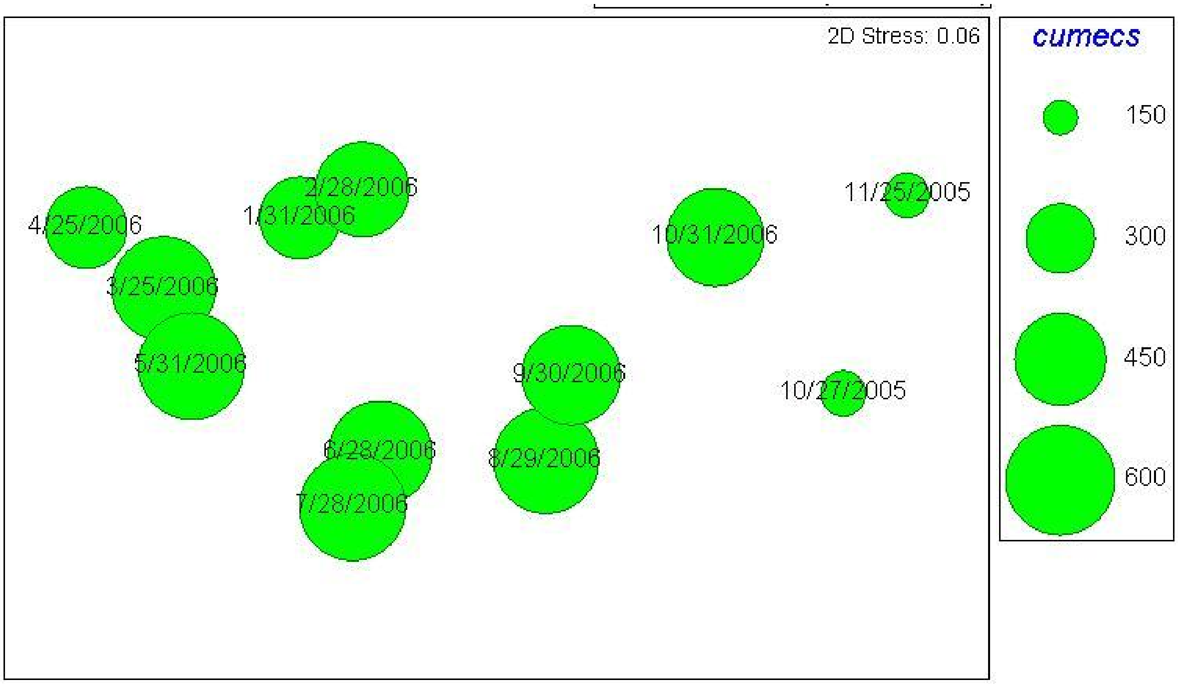

Figure 8.19: MDS ordination of the dates of the surveys during which fish was collected in the Xikundu fishway from October 2005 to October 2006 with the calculated discharge (cumecs) through the fishway superimposed on the clusters obtained in MDS ordination.

The suggested positive relationship between discharge and fish movement is illustrated in figure 8.20. This figure shows that particularly during the period August to October 2004 the increase in the number of *L. marequensis* collected in the fishway occurred at the same time that an increase in discharge was recorded. This was also the case in the period from September to November 2005. It should be pointed out that both periods during which the discharge increased was preceded by a period during which the discharge decreased. During 2006 a different pattern was observed. During the period from February to April the flow was extremely high with a peak in March. Very few specimens were collected during this period. This can be attributed to strength of the water and the high degree of turbidity of the water as is shown in figures 8.21 and 8.22. It should however be noted that the increase in the number of specimens in the fishway both during the April to June in 2005 and the August to September 2006 period coincided with a decrease in discharge.

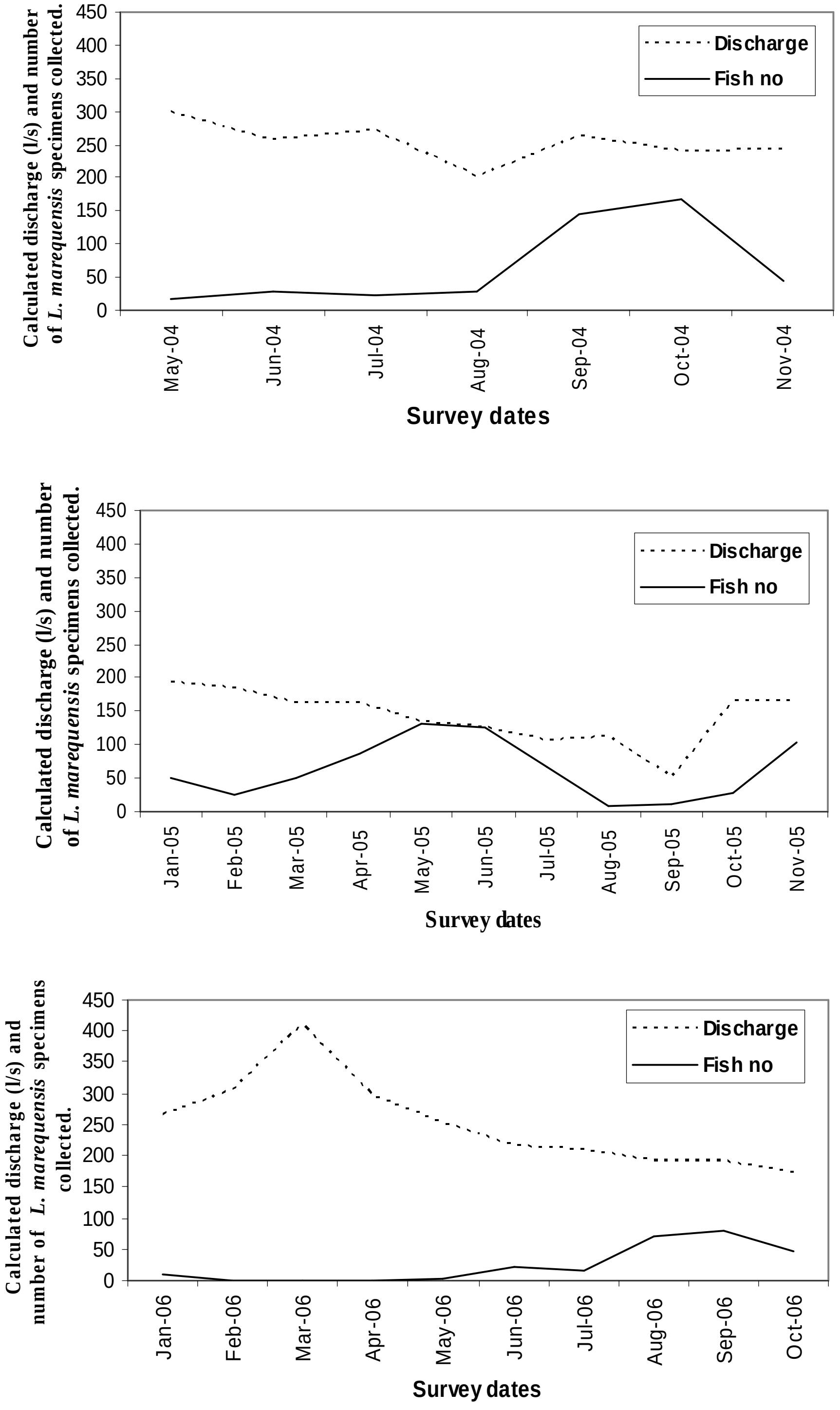

Figure 8.20: A comparison of the calculated discharge and numbers of *Labeobarbus marequensis* collected in the Xikundu fishway during the period May 2004 to October 2006.

Figure 8.21: An illustration of the volume of water in the Xikundu fishway during the February 2006 survey.

Figure 8.22: An illustration of the volume of water in the Xikundu fishway during the March 2006 survey.

8.3.9 Physiological preparation.

Figures 8.23 and 8.24 show the average of the calculated Condition Factor scores for specimens longer than 80mm in fork length. These scores were only calculated for selected periods within each year that preceded and coincided with periods when the numbers of specimens collected increased. In 2004 the index scores increased steadily from May onwards and a peak was reached in November. This was followed by a decrease and a later peak in July 2005 (Figure 8.23). Both these peaks coincided with the rise in numbers of in both September/October 2004 and May/June 2005.

In 2006 the average scores increased steadily from June and peaked in August (Figure 8.24). This was again followed by a period of decrease. Other than the case with the peaks observed in 2004 and 2005 the existence of the peak did not exceed migratory period.

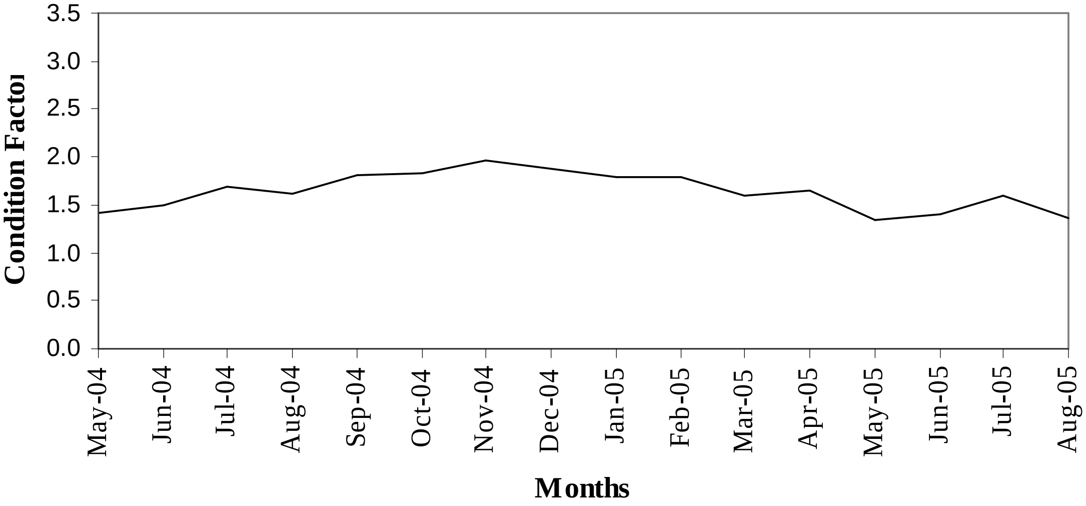

Figure 8.23: Average scores of the calculated Condition Factor of specimens of *Labeobarbus marequensis* longer than 80mm in fork length collected in the Xikundu fishway during the period May 2004 to August 2005.

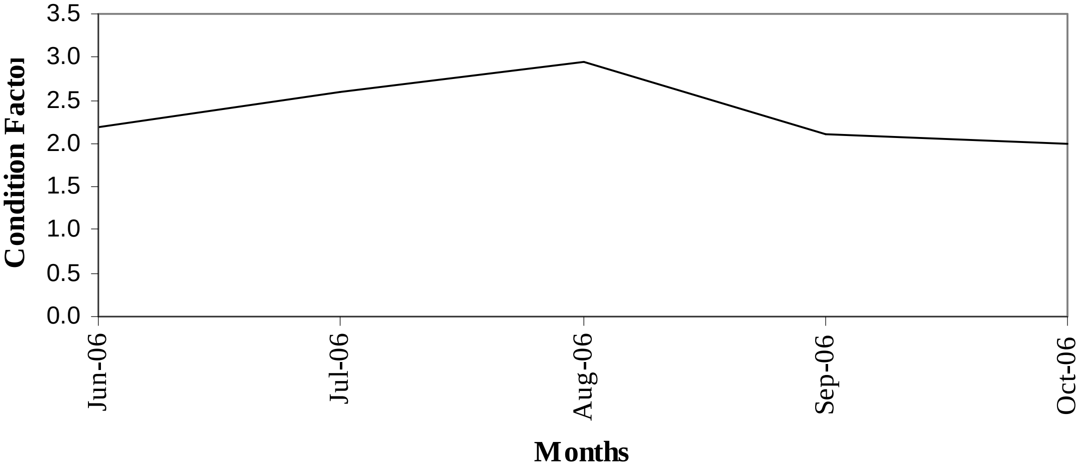

Figure 8.24: Average scores of the calculated Condition Factor of specimens of *Labeobarbus marequensis* longer than 80mm in fork length collected in the Xikundu fishway during the period June to October 2006.

8.4 Discussion.

The migratory behaviour of aquatic biota, and in particular that of fish, is categorized as either potamodromous or diadromous. While diadromous species alternate between fresh and saline or marine habitats, potamodromous species complete their entire life cycle in freshwater (Skelton, 2001). With the exception of the freshwater eels, *Anguilla* sp, all other species recorded in this study belong to the latter group. Paxton (2004) states that potamodromy "evolved where migration has conferred an adaptive advantage to species which complete their life history entirely within freshwater ecosystems".

The statement by Power *et al.* (1988) that "abiotic conditions of the local environment often determine whether lotic organisms can either colonise or persist in new or changing habitats and that inadequate knowledge of the rates and pathways of movements of stream organisms still confounds attempts to measure processes and interactions in lotic communities" is critical. Not only does it illustrate the importance of knowledge of processes such as migration but it also highlights the role that information of processes can play in management actions and decisions. The rates and pathways of movement combine to form what can be termed the "pattern of movement" and the understanding of these patterns is a problem central to ecology (Chapman and Kramer, 1991). Lucas and Batley (1996) found that the importance of natural migrations and seasonal activity patterns for *Barbus barbus* had probably been underestimated for a wide variety of river systems and postulated that this could apply to many other riverine cyprinids. Although not as spectacular as the fish migrations observed in the great South American rivers, the migration of Southern African freshwater fish is as important. This fact is illustrated by the findings of Olivier (2003) who showed that prior to the construction of the Kanniedood Weir in 1977 in the Kruger National Park *L. marequensis* historically occurred both up and downstream of the impoundment in the Shingwedzi River. Olivier (op cit.) goes on to show that despite the later addition of a fishway at the weir the species was never again collected in surveys conducted in 1989, 1997 and 1999 or in his survey of the fishway during 2002. The absence of the species is reconfirmed by Fouché and Vlok (2007) during recent surveys. Had knowledge of the migratory patterns and importance of migrations of *L. marequensis* been available, the existing situation could have been prevented.

In the literature *L. marequensis* has often been described as a potamodromous species that migrates for breeding reasons between river reaches when the rivers are rising or swollen due to heavy rain or increased flow (Jubb, 1967; Bell-Cross and Minshull, 1988; Skelton 2001). These flows seemingly act as cues for the migration that occurs in spring or early summer and usually occurs during daylight (Bell-Cross and Minshull, op cit.; Skelton op cit.). The species is reportedly a strong swimmer that can negotiate velocities of 1,3 m/s and jump up to a metre (Kotze and Niehaus, 2004; Kotze *et* al., 2004). Bok *et al*. (2007) also lists *L. marequensis* as a migratory species that occur in two of the defined inland migratory regions of South Africa (Table 8.19).

The results obtained in this study show that *L. marequensis* move through the Xikundu fishway and it can therefore be accepted that this species display spatial movements or changes in their geographic distribution. Because these movements display a pattern with peaks during certain periods of the season, their movement can be regarded as seasonal. This therefore answers to the periodicity or seasonality criterion to qualify as true migrators (Nikolsky, 1963; Paxton, 2004). The second criterion, which addresses the number of specimens participating, is met with the large numbers of specimens collected during seasonal movements. Although the third criterion, which refers to the alteration between habitats, was not tested the species can still be regarded as a true migrator.

Table 8.19: Inland migratory regions and the main migratory groups and species (Adapted from Bok *et al.*, 2007).

Migratory Region	Primary Rivers	Main Migratory Groups	Key Migratory Species
Orange-Vaal region	Main stem and tributaries of the Vaal- and Orange River	Potamodromous species of the genera *Labeobarbus*, *Labeo* and *Barbus*.	*Labeobarbus aeneus, L kimberleyensis, Labeo capensis, L. umbratus, B. paludinosus* and *B. trimaculatus.*
Upper Limpopo region	Upper Limpopo Crocodile-West, Marico, Mokolo Lephalala Mogalakwena	The catadromous eel, *Anguilla mossambica* and various potamodromous species, most importantly from the genera *Labeobarbus*, *Barbus* and *Labeo*.	*Anguilla mossambica,* various *Barbus* species, *Labeobarbus polylepis,* *L. marequensis, Labeo rosae, L. ruddi, L. cylindricus,* *L. molybdinus* and *Mesobola brevianalis.*
Lower Limpopo, Incomati and Pongola region	Lower Limpopo Luvuvhu, Letaba, Shingwedzi, Olifants, Komati Crocodile East, Sabie-Sand Pongola, Usuthu	The catadromous eels of the *Anguilla* family, and the catadromous macro crustaceans of the genus *Macrobrachium*. Also various potamodromous species, most importantly from the genera *Hydrocynus*, *Labeobarbus*, *Barbus* and *Labeo*.	*Anguilla mossambica, A. marmorata, A. bengalensis labiata, A. bicolor bicolor,* *Hydrocynus vittatus* various *Barbus species, Labeobarbus marequensis,* *L. polylepis Labeo rosae, L. ruddi, L. congoro, L. cylindricus, L.molybdinus, Chiloglanis anoterus,* *C. swierstrae*
KZN inland Region	Mkuze, Tugela Umfolozi Umtamvuna Mzimvubu	The catadromous eels of the Anguilla family and potamodromous species of the genera *Labeobarbus* and *Labeo*.	*Anguilla mossambica, A. marmorata, A. bengalensis labiata, A. bicolor bicolor,* *Labeobarbus natalensis,* *Labeo rubromaculatus.*
Cape inland region	Doorn, Kromme Gamtoos, Gouritz, Keiskama , Buffels.	*Barbus*, *Labeo* and *Pseudobarbus* species	*Barbus amatolicus, B. andrewi, B.erubescens, B. serra, B. trevelyani,* *Labeo seeberi*, various *Pseudobarbus* species, *Sandelia bainsii.*

From the data it is clear that although the species is found in the fishway during most months of the year, seasonal increases in the numbers were observed during spring and early summer of the three years that formed part of the study. In 2005 a second migration for that year was observed during May that was not observed in the other two years. If the length frequency distribution of the specimens collected is considered, it does appear that the September/October 2004 and the August/September 2006 increases in numbers can be regarded as spawning migrations. This is based on the presence of specimens longer than 200mm in fork length and according to the findings earlier in this study (Chapter 6) where this length was regarded as the minimum length at which females obtained sexual maturity in the Luvuvhu River. This could well then be "mature" females that were migrating to spawning habitat upstream of the weir. The timing of the "spawning migrations", namely May and September-November also agree with the times during which small juveniles were first observed (Chapter 6). The length of the period during which large numbers of specimens were collected during the spawning migrations underpins the findings that *L. marequensis* has an extended spawning period while the repetition in 2005 strengthens the conclusion that the species is a fractional spawner that spawns bi-annually.

Although the absence of large (>200mm fork length) specimens in the November 2005 migration events tends to disqualify it as a spawning migration, sight should not be lost of the fact that it could have been males that were in the process of migrating for breeding purposes. This is supported by the observed 79:21 female to male ratio that was observed in the Xikundu fishway.

Based on the size frequency distribution it is clear that the majority of specimens of *L. marequensis* utilising the fishway, and therefore participating in migration, are between the fork lengths of 70 and 120 mm in fork length, which constitutes a mix of sub-adult males and females. This could then be classified as a dispersal migration that is taking place. Because of the presence of specimens in the majority of the months it can be argued that the dispersal migration is an ongoing process during the year.

In all three years of this study the majority of specimens moving through the fishway were specimens with fork lengths between 30mm and 150mm. No specimens shorter than 30mm in fork length were observed in the fishway. This absence could be attributed to the hydraulic character of the fishway which implies that these small specimens were not able to negotiate the fishway because of the water velocity and turbulence within the pools. In the majority of the surveys the maximum velocity exceeded 1,3m/s. Larger specimens with fork lengths longer than 150 mm were collected in September, October and November 2004, April and May 2005 and from July to October in 2006. Meyer (1974) found that the majority of specimens, namely 60%, moving through the fishway in the Elands River during his 1972 to 1973 survey had fork lengths of between 100 and 200 mm.

As stated in the results the data obtained in this project during August to October 2006, using the funnel trap and the log-bagged net does seem to indicate that when the species migrate in larger numbers, the tendency is towards moving upstream rather than downstream. The majority of the specimens collected in the cage were collected during what is now regarded as a breeding migration. The findings of this study agrees with Meyer (op cit.) who found that in the fishway at the Engelhardt Dam the majority of *L. marequensis* moved upstream rather than downstream, with 253 specimens moving upstream as opposed to only four that moved downstream over in a period of twelve months. Similar patterns were observed in other cyprinids with Lucas and Batley (1996) finding that both males and females of *Barbus barbus* migrated upstream during their spring migrations and Fishelson *et al.* (1996) reporting the upstream movement of *Capoeta damascina* to their spawning grounds.

In this study, as was reported by Deacon *et al.* (in Bok, 1995) and Meyer (1974), it was found that the species most frequently moved though the fishway during daylight. The highest numbers of specimens collected in this study was also recorded in the period from 10h00 to late afternoon which is similar to the findings of Deacon *et al.* (in Bok, op cit.). In addition to their preference for moving in daylight Meyer (op cit.) found that the months of June, July and August, prior to the peak movement in November, were the exception when the species tended to move more at night. Although periods when large numbers of fish moved at night was observed in this study these periods were not always prior to spawning migrations (Tables 8.14 and 8.15).

In this study it was as difficult as reported by Yako *et al.* (2002) to single out one cue that was responsible for the onset of migrations. It was statistically shown that combinations rather than single factors were related to movement and even then these combinations did not score high. The best scores or ranking was obtained with calculated discharge which featured in all nine combinations of factors. This was followed by temperature and in particular maximum temperature as obtained with the data loggers. The maximum temperature recorded on site also featured strongly. It can however be accepted that flow and in particular flow increase would be the dominant cue (Table 8.17). This is well illustrated when the discharge is superimposed on the clusters obtained in the MDS ordination (Figure 8.19) and by the relationship between discharge and specimen numbers (Figure 8.20). As stated in the introduction of this chapter a wide variety of triggers for the onset of migration have been identified or suggested. Shul'man (1974) suggested that photoperiodism could be the main cue but went on to list what he referred to as "micro-stimulus signal factors" in which he *inter alia* included changes in temperature, current and water transparency. Paxton (2004) pointed out that in *B. barbus* in the River Nidd a positive correlation was observed with day length, i.e photoperiodism, and importantly a negative relation with mean monthly flows. This positive relation with light intensity was also observed by Bohl (1980). Turner *et al.* (1994) found that flow rates and to a lesser extent temperature were important correlates of the spatial dynamics and in particular of migration. Yako *et al.* (2002) differentiated between factors commonly associated with migration and those associated with peaks in migration with discharge the only listed factor in the latter category and aspects such as visibility and rainfall in the former.

As far as southern African fish are concerned Skelton (2001) states that "many riverine fishes make breeding migrations soon after the first major rains of spring or summer when the rivers are in spate and conditions are most favourable for the survival, growth and feeding of the offspring". *L. marequensis* is no exception and is reported by Gaigher (1969) as well as Bell-Cross and Minshull (1988) to migrate at the onset of the rainy season when the rivers rise. Although Meyer (1974) showed a positive relation between increased movement and patterns he also showed that very little activity of *L. marequensis* was observed at low temperatures in both the Letaba and Elands rivers.

Bell-Cross and Minshull (1988) mentions that although the factors triggering off these movements are as yet not fully understood they are of the opinion that two “main criteria” are probably involved as cues. As is the case in many of the cited literature (Jackson, 1961; Jubb, 1967; Skelton, 2001) Bell-Cross and Minshull (op cit.) also list the increase in water velocity as one factor but go then go on to add water quality as their second criterion. Bell-Cross and Minshull (op cit.) are of the opinion that a change in water quality alone can initiate movement and gives the example of the more dense Sanyanti River water that move along the lake floor of Lake Kariba which then cause fish movement upriver of a great number of species.

Although all of the factors mentioned above can be accepted as cues or triggers the question often arises on what specific component the fish are able to physically detect in the water. Various ideas have been discussed but the one put forward by Bell-Cross and Minshull (op cit.) deserves to be mentioned: “It has been suggested that rain falling on dry ground picks a substance referred to as petrichor which upon entering a river is detected by fish and initiates upstream migration”.

The effect of downstream migration, as well as the reasons for such a migration, into warmer areas is often not considered when migration is discussed. These downstream migrations allow the species to move to warmer areas in order to negate or escape the effect of cold water in the higher areas. Weirs, dams and other obstructions that do not allow for the movement of fish can therefore prevent this downstream migration and where this movement has been prevented the result is almost always tragic. One example is the Spekboom River in the Inkomati River system where bridge prevented downstream migration and with the result that thousands of *L. marequensis* consequently died (Engelbrecht in Fouché, 2008).

According to Nikolsky (1963) the start of migration in the majority of fish is connected with the attainment of a definite condition and fat content, but goes to point out that “preparedness for the migration does not mean the fish will immediately start to migrate as the migration begins only under particular conditions". Shul'man (1974) was of the opinion that the time and nature of spawning migration depends on the rate of maturation of the genital products and that this in turn is related to a certain level of fat reserves that have been attained.

The results obtained in the reproductive component of the study (chapter 6) show that highest frequency of occurrence of the fat class 5 was observed in October 2004 (Figure 6.24) and this coincided with a migratory event that was observed at the same time (Table 8.15). Further analyses of the calculated condition factors, which were done for selected periods, underscored these findings for the 2004 migration (Figure 8.23). Similar results were obtained for 2005 and 2006. It can therefore be concluded that *L. marequensis* physiologically prepares for a spawning migration. This preparation is shown both by the level of fat deposition and the general improvement in the condition of the fish. The observed decline in condition, following the migrations, (Figures 8.23 and 8.24) clearly indicates that these migrations are active because the energy reserves are well utilised.

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APPENDIX VII:
FISHWAY MONITORING - FIELD FORM 2

RIVER:____________________________ **FISHWAY SITE:**______________________.

DATE :_____________ **to** _____________. **MOON PHASE: ……………………….**

1. WEATHER

	Time of sampling						
▨							
Atm. temperature							
Cloud cover	**/8**	**/8**	**/8**	**/8**	**/8**	**/8**	**/8**
Wind direction							
Barometric pressure							
Rain							

2. FISHWAY

2.1 General data (Measured once)

Parameter	▨
pH	
Conductivity	
Velocity at exit	
Velocity at entrance	

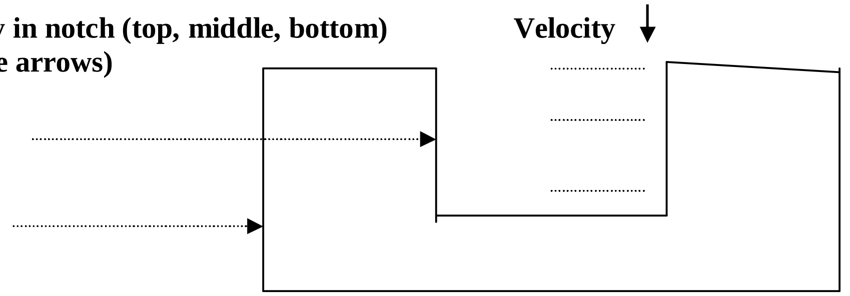
2.2 Changing data (At each sampling)

	Sampling periods						
Time	■						
H_2O temp	■						
DO (still water)	%		■	■	■	■	■
	g/l		■	■	■	■	■
DO (white water)	%		■	■	■	■	■
	g/l		■	■	■	■	■

2.3 Physical aspects (Hydraulic data)

Section	H1 at ruler 1 (m)		H1 at ruler 2 (m)		H1 at ruler 2 (m)		Average H1 (m)	Discharge Liter/s	Energy Watts/m^3
Baffle height							■	■	■
Baffle	In	Not in	In	Not in	In	Not in			
Top									
Middle									
Lower									

2.4 Diagram of velocity in notch (top, middle, bottom)
(Fill in heights opposite arrows)



2.5 Diagram of flow pattern (Indicate flow direction with an arrow - only do at very first site inspection). All other times only indicate pools that are functioning.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Top											
Middle											
Lower											

Field form 3: FISH DIRECTION DATA

RIVER:____________________________ **FISHWAY SITE:**______________________.

DATE :_____________ to _____________. **TIME SURVEYED ………………**

Method of collection: Cage/long bagged net that was facing:

Cage facing downstream	**Long bagged net facing upstream**

SECTION OF FISHWAY WHERE CAGE/NET WAS PLACED: (supply number of retaining wall or pool)

TOP (upper)	TOP (lower)	MIDDLE	LOWER

Specie	**length**	**mass**	**Specie**	**length**	**mass**

Chapter 9: Conclusion.

9.1 Introduction.

In the first chapter study it was shown that a survey of the relevant literature revealed “gaps” that existed within the knowledge base regarding the Lowveld largescale yellowfish, *L. marequensis*. These identified “gaps” were consequently transformed into research questions regarding the habitat preference of the species in general and the microhabitat preferences, the morphological adaptations and trophic niche differentiation of the various life stages. In order to obtain answers to these questions a hypothesis was formulated which in combined terms stated that “*The morphological adaptations of the various life stages of L. marequensis reflect their habitat preferences and niche differentiation and that changes in the macro-and microhabitat characteristics, which can be of physical or chemical origin, initiate or trigger reproductive behaviour in this fish and that at the onset of the reproductive process the species migrate to suitable microhabitats that will allow the spawning and developmental processes to take place”.*

As is traditionally done in the scientific method aims and objectives for this study were then formulated to guide the process and in the end test the hypothesis. These aims and objectives *inter alia* included the following:

a) To determine the habitat preference of the various size classes of the species.
b) To determine the trophic niche differentiation of its life stages.
c) To determine the environmental factors that trigger spawning behaviour.
d) To determine the preferred microhabitat and conditions required for spawning and the early developmental stages.
e) To determine the biological changes that precedes spawning and spawning behaviour.
f) To establish the community structure/fish assemblage composition at the sites.
g) To determine the morphological characteristics of the species and to infer the habitat preferences and trophic niche differentiation from this data.
h) To determine the migratory behaviour of the species.

In this chapter it will be investigated whether these aims and objectives were achieved and in the process the applicability of the generated data and the shortcomings of the study will be investigated.

9.2 Conclusions.

9.2.1 Conclusions regarding morphological characteristics of the species.

As is the case with many fish species, and in particular the larger cyprinids (Fouché, 1995; Hamman, 1974), the length-mass relationship, which is an indication of allometric growth, in this species is best described by the formula $M = 0{,}023L^{\mathbf{3{,}0575}}$ (r-squared of 0,9876). The slope at each length class-midpoint, which represented an inflection point, was calculated and from this nine stanzas could be identified. Each of the stanzas displayed its own distinctive length-mass relationship. Cyprinids have a body form that is closest to what is referred to by Helfman *et al.*, (2000) as a "sub-carangiform" body shape and *L. marequensis* can be included in this group. This body shape, which is characterized by a streamline body and broad V-shaped caudal fins, has the propulsive elements concentrated in the posterior part of the body where it is evident in the caudal fin aspect ratio and the length of the caudal peduncle. These elements gives the group the ability to accelerate rapidly and swim strongly therefore enabling *L. marequensis* to survive and thrive in fast flowing water. In addition to the adapted caudal fin the "short" posteriorly placed dorsal fin makes *L. marequensis* more suitable for flowing rather than non-flowing habitat. According to Helfman *et al.* (2000) sub-carangiform fish are regarded as locomotory generalists and "can switch between modes, depending on what is needed". This was shown to be the case in *L. marequensis*. Ontogenetic changes in the morphometry of caudal fin and related aspects were observed. These aspects included the relative caudal span and the caudal fin aspect ratio. Other observed ontogenetic changes involved the relative body depth and to an extent the body width. From these findings it was inferred that while the juveniles, stanzas 1 and 2, are adapted to slow flowing water, stanzas 3, 4, 5 and 6 were better adapted to the fast flowing water. Stanzas 7, 8 and 9 on the other hand were morphologically best adapted however to slow flowing water.

With regard to the eye diameter, mouth width and head length differences, and in particular ontogenetic changes, in both the actual and relative sizes were observed that could be linked to the stanzas of the species. From these findings it was inferred that stanzas 1 and 2 are better adapted to predation while the later stanzas were seemingly more herbivorous.

9.2.2 Conclusions regarding habitat and biotope preference of the various size classes of the species.

The surveyed sites all displayed well developed "pool-riffle sequences" and a high degree of instream heterogeneity with the substrate dominated by boulders and cobbles. A downstream change in habitat, where the less coarse alluvial material decreases, was observed. Cover types other than substrate were present at the sites but their abundance was rated throughout the river as either "rare" or "sparse".

No "partiality to deep rocky pools" as described by Pienaar (1978), Bruton *et al.* (1982) and Bell-Cross and Minshull (1988) could be illustrated. This could be ascribed to the lack of such pools within the particular river system but it should be pointed out that the later stanzas did frequent the slow-deep biotopes i.e. the pools. The results rather indicate a partiality for flowing water with high frequencies of occurrence recorded in both fast-shallow and fast-deep habitats.

At the onset of this discussion it should again be pointed out that in this study a distinction was made between habitat and biotope where the latter concept was at a finer scale and could be equated to a hydraulic biotope as it included the physical and velocity characteristics. A distinct difference in the biotope preference of various stanzas or size classes was observed. In addition an ontogenetic shift in biotopes, supported by the results of MDS ordination was evident. Overlaps in biotope preference, which do seem incidental, did however occur between consecutive stanzas. Sub-adult fish, up to a maximum fork length of 150mm (stanzas 1 to 5), preferred shallow water biotopes commonly dominated by coarse substrate material. Within this group the very young fish (< 80mm in fork length) in stanzas 1 and 2 were most commonly collected in slow-shallow biotopes where they are found in localized areas of lower velocity, known as "velocity shelters", along the stream bed within and around substrate features that is provided by the coarse substrate material. Specimens of stanzas 3 to 5 had a preference for fast flowing water that formed part of the group of shallow biotopes. Specimens of stanzas 6 to 9 were mostly collected in the slow-deep biotopes, such as the pools. These findings agree with the inferred biotope preferences

9.2.3 Conclusions regarding population structure of population structure of the species and the community structure/fish assemblage composition at the sites.

At a landscape scale it was observed that the species were present throughout the length of the river included in the study. Although many of the sites were impacted by anthropogenic actions, no relationship between these impacts and the distribution of the species could be illustrated. Although certain physico-chemical water quality parameters in the river were outside the acceptable ranges for the protection of aquatic life the species was found at all the sites throughout the survey. This indicates that the species has adapted to these environmental conditions and underscores the decision to regard them as “non-sensitive” and “moderately” tolerant (Kleynhans, 1999).

The results of this study not only showed that the species was present at all the sites surveyed in the Luvuvhu system but it also illustrated that the population structure of *L. marequensis* consisted of specimens of all the fork lengths. It should however be noted that large numbers of specimens were collected in the shorter length classes (< 180mm) and far less in the larger size classes (Figure 9.1). To an extent the lower number of large specimens could be ascribed the difference in surveying techniques and the difficulty in sampling of the deeper habitats such as pools. It could on the other hand be a true reflection of the population structure in the Luvuvhu River which would be a cause for concern. Based on personal observation the author is of the opinion that impacts, such as water extraction and flow modification, have changed the fluvial erosion patterns within the Luvuvhu River resulting in pools that are silted up with a decrease in water column (depth).

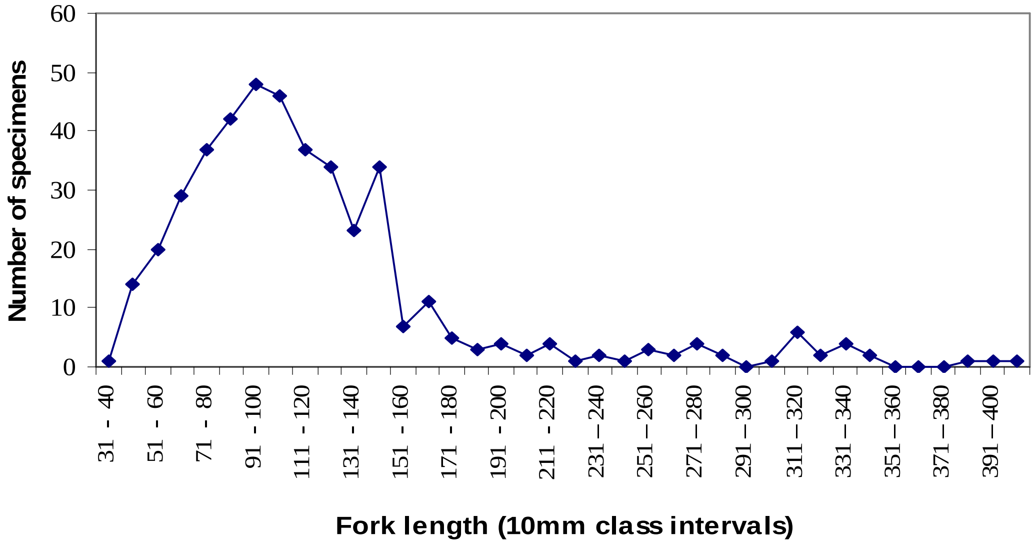


Figure 9.1: An illustration of the fork length distribution of *Labeobarbus marequensis* collected in the Luvuvhu River.

In addition the river also supported a high degree of fish biodiversity with a total twenty-four species collected. As could be expected the lowest diversity was observed at the high altitude in the mountain reaches (Site 12 in the Mutale River). The diversity at other sites, with the exception of site 11 and site 8, were similar.

9.2.4 Conclusions regarding the breeding biology and ecology of the species

The data of the surveys show that an average male to female ratio 78:22 was observed in the Luvuvhu River that differs distinctly from the 25: 75 ratio observed by Gőldner (1969). If the findings of Gaigher (1969) is considered in conjunction with the population structure, see 9.2.3 above, it can be deducted that it is the large females that are absent from the river system. The findings of this study showed that *L. marequensis* is a fractional spawner with two spawning events occurring per annum. The existences of the two spawning events, in May and in September, is based on the presence of juveniles observed during the monthly surveys and the bimodal gonado-somatic index (GSI) observed in the data collected at the Xikundu fishway. The May spawning event is however not fully supported by the maturity coefficient (MC) but it does however support September spawning event. In addition the September spawn is supported by the presence of J_1 juveniles (< 20mm in fork length) and coincided with increased temperature and discharge. It was observed that flow was a direct result of early spring rains. The results obtained in the migration component of this study not only underpinned the bi-modality of the spawning strategy of the species and the flow dependency of the spawning events but also showed that the species had an extended spawning period.

It was observed that a pre-spawning period, characterized by fat deposition and an increase in the Condition Factor (CF), occurred before the September spawn. These findings would seem to point out that the major, or important, spawning event is the one that occurs in September and agrees with the findings of Vlok (1992) on the testicular development of *L. marequensis.* The increased CF fully supports the existence of biological and physiological changes that precedes the spawning event.

It was found that breeding occurs at sites where water flows over boulders and cobbles. It was observed that these breeding biotopes were in close proximity to deeper rapids and pools where the mature males and females respectively occur. Nursery areas that are utilised by juveniles which are between ten and twenty millimeters in fork length were found to be important and it was pointed out that they should be in close proximity to breeding biotopes.. These nursery areas are shallow areas with consequent higher water temperatures than the surrounding areas and sufficient cover in the form of coarse substrates, such as cobbles and boulder.

Length at sex maturity, based on gonadal development was found to be at a fork length of 90 mm in males and 240 mm in females. The relative fecundity of the species was found to be 44,7 ova per gram of body mass which in effect would imply that *ca* 17000 mature ova are produced annually per female. Although the species produce a larger number of ova than smaller minnows (Schulz and Schoonbee, 1999) these counts are however similar to average egg counts of *L. kimberleyensis* and *L. aeneus* (Mulder, 1971; Hamman, 1981).

9.2.5 Conclusions regarding the feeding biology of the species.

The investigation of the intestinal morphology shows that in general the relative gut intestinal and lengths are longer than the fork length. This would imply that a tendency toward a herbivorous diet could be inferred. The exception was stanza 1 (< 50 mm in fork length) where the relatives intestinal lengths were less than the fork length, which could be regarded to display a tendency to carnivory. After obtaining a fork length of 80mm a significant increase (p = 0,05) in the intestinal up to fork length of 150mm occurred. These changes in the dimensions were accompanied by a shift towards a preference for plant material in the diet.

Plant and algal material in general dominate the stomach contents which vindicated the inferences based on the average relative intestinal lengths. As was also inferred the ontogenetic changes in intestinal morphology were indicative of changes in the diet with stanzas 1 and 2 having the highest percentage animal material in their stomach contents. From stanza 4 onwards the diet composition became more omnivorous with a tendency towards herbivory.

9.2.6 Conclusions regarding the migration of the species in the Luvuvhu River.

It was observed that *L. marequensis* displays spatial movements which could be regarded as “migrations” because of their seasonal patterns and the large numbers specimens that move during each of the events. Because the timing of the movements coincides with the spawning events observed in the reproductive studies, the migration is regarded as spawning migrations. The lengths of the periods during which these spawning migrations occur in addition indicate that the species has an extended spawning period and the bi-annual peaks in the migration events and number of participants underpin the findings that the species is a fractional spawner that spawns twice per annum.

Based on the size frequency distribution of the all the specimens collected in the fishway, which indicate that the majority of the specimens that moved through the fishway are less than 200mm in fork length, it was decided that some of the movement through the fishway could be classified as dispersal migrations because the majority of specimens were sub-adult specimens.

Migration tended to be upstream and most frequently occurred during daylight. The identification of single cues responsible for the onset of migrations proved to be problematic. This can be attributed to the complexity of the interrelationships between the cues in an aquatic ecosystem. However when combinations of cues were analysed, discharge and maximum temperatures were best related to the movement of the species. It is however accepted from the results that flow and in particular "increase in flow rate" was the most dominant cue and this agrees with the observations reported by earlier researchers (Gaigher, 1969; Bell-Cross and Minshull, 1988; Skelton, 2001).

If the set hypothesis is revisited it can be accepted that: a) the morphological adaptations of the various life stages of *L. marequensis* does reflect their habitat preferences and niche differentiation, b) that during the reproductive process the species use suitable microhabitats that will allow the spawning and developmental processes to take place and c) that changes in environmental conditions initiate or trigger reproductive behaviour in this fish

In summary it can be accepted that the majority of the set aims and objectives of the study have been achieved. In retrospect some shortcomings in the have been identified and these are discussed below.

9.3 Shortcomings of this study.

Because the seasonal investigation of the breeding of the species only extended over the five months that were identified as a "the most probable" season, the major shortcoming of this study could be regarded as of a logistic nature. If this investigation is extended to include a full calendar year with monthly surveys and is repeated during the next calendar year, the results obtained regarding the spawning events would be more conclusive. Because of the cost involved in such a long and intensive study it does warrant a separate and independent investigation. A second logistic shortcoming of the study involves the sampling of the pools. At the completion of the study it was realised that the results of the migration component of the study indicated that most movement of the species occurred during daylight. Even though the gill nets were set for a period of twelve hours and the period included both daylight and night time it may be that not enough daylight time was included in the survey. It is suggested that if the period of gillnet sampling had included more daylight hours more specimens would have been collected. Although it would not have changed the general outcome of the findings more confidence could have been attached to the results and in particular with regard to the mature females.

To underpin the GSI findings of this study an investigation of the gonadal histology of both males and females should be undertaken. The findings of such an investigation would in particular shed light on the first spawning event that occurs in May and will enhance the findings of the current study.

In addition to the above, two related aspects that need attention are identified. The first aspect concerns the hatching of the eggs, the size of the hatchlings and consequently the initial morphological changes that occurs with the first few days. It is suggested that these investigations be done in controlled laboratory conditions where harvested ova are hatched and the morphological characteristics of hatchlings are recorded on a daily basis. Knowledge of the morphological and morphometric characteristics would assist in the identification of the species during field survey and would be an invaluable conservation “tool”. The second aspect concerns the growth, growth rates and age determination of the species. As reported in the literature survey very little is known about these aspects of the species. Even though some inferences could made based on the results obtained in the monthly surveys it is suggested that a detailed study of these aspects would contribute to better understanding of the ecology of the species.

Although the findings of the study indicate that breeding migration occurs and suggest that some of the migrations are of a dispersal nature, there is still no clarity of the latter. Because of the size of the sub-adult and adult specimens of the species radio tagging is a definite possibility. Even though cues for migration have been identified, the aspects that trigger the movement needs to be investigated in greater detail. Knowledge of the cues would go a long way towards the correct management of fishways.

Although the current study did generate some data on the diet and trophic niche differences of the species a more detailed study of the stomach contents should be carried out. This should include an identification of the food items on a finer scale as well as a study of the small intestine content to determine the digestion of food and the rate of digestion. A study of intestinal histology will greatly enhance the findings.

9.4 Applicability of the generated data.

Despite the shortcomings listed above the study does contribute towards "filling of the gaps" in the knowledge base and underpins a number of aspects that are based on observational information. Valuable knowledge of the habitat preference both at landscape and local spatial level of the species has been obtained. If the fact that the species is often used as an indicator species in selected reaches of rivers during ecological reserve determinations is taken into consideration, this knowledge will go a long way in its selection. Kleynhans (2007) stated that "to relate drivers and the resulting fish habitat template to the stress response of fish, the life-history requirements and environmental preferences of species must be considered. This understanding is achieved by considering information on the life-history strategies and habitat preferences and requirements of each of the species in the assemblage. This includes consideration of breeding requirements and early life-history stages, frequency of occurrence in a river section, cover, health and condition and water quality". In essence this study contributes to knowledge to most of the aspects listed in 9.1. This includes the life-history strategies, habitat preferences, habitat requirements, breeding requirements, and frequency of occurrence in a river reach. In addition the knowledge concerning water quality and other physico-chemical at the sites and the presence of the species throughout the river vindicates the sensitivity and tolerance rating awarded to *L. marequensis* by Kleynhans (2008). The knowledge generated can to a great extent be applied to conservation recommendations on local, regional and on landscape scale. This implies that specific data with regard to flow requirements and the seasonality of flow within a river system can be supplied to managers of instream impoundments and reservoirs. With this knowledge the extent and seasonality of releases from such flow modifying structure can be managed to an extent that can contribute to the conservation of the species and the freshwater in general.

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