Towards an understanding of factors affecting the biotic integrity of rivers in the Limpopo Province: Niche partitioning, habitat preference and microbiological status in rheophilic biotopes of the Luvuvhu and Mutale rivers

Report to the Water Research Commission

by

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EXECUTIVE SUMMARY

1. INTRODUCTION AND BACKGROUND.

There are vast differences in the importance of rivers in underdeveloped rural areas as opposed to developed areas. Low income rural communities, have a high level of dependence on a river and its catchment. River ecosystems not only supply water but also food, medicine, fuel, building material, and play an important role in social and cultural lives of these people. General awareness of their effect on rivers, needs of other users, and potential employment opportunities related to services provided by rivers are, however, almost non existent.

River management should include aquatic awareness as well as community participation. All these activities are knowledge based and specifically include knowledge of threshold values of the factors that sustain riverine biota. However, very little is known about this aspect and how it is influenced by anthropogenic activities. Consequently the effects on diversity are still unclear. In trying to understand loss of diversity it is important to examine and understand fine scale determinants of freshwater rheophilic assemblages. These determinants include available habitat and changes in the physico-chemical parameters that influence habitat preference, distribution, niche partitioning, assemblage structure as well as nutrient pathways and feeding adaptations.

In rivers the fast-flowing or rheophilic habitats are the most sensitive because of their dependence on flow. This project aimed to determine thresholds of probable concern by explaining and modelling the loss of aquatic diversity in rheophilic biotopes through an understanding of niche differentiation and habitat requirements of selected biota.

It was envisaged the products of the project would include the proposal of TPCs and a neural network that describes niche partitioning and habitat requirements in rheophilic biotopes as well as applies TPCs.

2. SITE SELECTION AND GENERAL APPROACH.

Two rivers, the Luvuvhu and Mutale, were selected for study. The Luvuvhu River arises in the Soutpansberg Mountain complex and flows through a diverse landscape before it joins the Limpopo in the Kruger National Park. The Mutale River emerges as a "spring" below Lake Fundudzi and joins the Luvuvhu River in the Kruger National Park before its confluence with the Limpopo River.

The two rheophilic sites were selected to represent an anthropogenically "disturbed" and a "less-disturbed" reach. The "disturbed" site was in the Luvuvhu River close to Tshino village and is situated in what is regarded as Lowveld (ecoregion 5.04). The "less-disturbed" site was in the Mutale River and is situated in Central Highlands (ecoregion 2.01). Care was taken to ensure that the selected sites had the biotopes and habitats required by both fish and macro-invertebrates. Both sites are at similar altitudes which would imply that similar biota could be expected.

The sites were comprehensively sampled a total of nine times each in the period from August 2000 to May 2002. The microbiology team could not be present during all of these dates and sampled both sites on five separate occasions. Macro-invertebrate data generated in a separate survey during 1999 was also included.

The sampling protocol consisted of a grid of $4m^2$ quadrats marked out with metal stakes at each corner. Fish, macro-invertebrates and detritus for microbiological investigation, were collected in each of the quadrats. After all the quadrats had been sampled, different biotopes, such as a rapid, a riffle and pools, were identified outside the grid area and fish were collected in each. The sampling team consisted of at least one fish expert and one invertebrate expert. The physico-chemical aspects of the water, the river profile and substrate determination were done for each of the eighteen site visits.

3. THE PHYSICO-CHEMICAL CHARACTERISTICS

During each site visit pH, temperature, conductivity and dissolved oxygen were determined on site. A water sample was also collected for laboratory analyses.

In the laboratory the turbidity and the Total Suspended Solids (TSS) of the water were determined.

Maximum and minimum water depths, in flowing water, were recorded. Velocity was measured with a velocity meter at approximately 3 – 5cm above the substrate level and the maximum and minimum velocities for each site were recorded. Estimates of the dominant substrate types viz. boulders, cobble, gravel, sand and silt/clay in each block were noted.

4. MICROBIOLOGICAL ASPECTS

E. coli (45%) and *Salmonella* (45%) were isolated more frequently from the skin of the fish caught in the Mutale River while more *Staphylococcus areus* (54%) were isolated from fish skin from the Luvuvhu River. No *Listeria* isolates were cultured from any of the skin or gut samples of the fish from both rivers. From the gut content of the fish, *Salmonella* (45%) was the most frequently isolated organism in fish from both rivers. In both rivers during the study period *E. Coli, Salmonella* spp, *S. areus* and *Clostridium perfrigens* were detected.

5. MACROINVERTEBRATES

A total of 30 macroinvertebrate families and 4551 individuals were collected, three families were singletons and two families were doubletons. Statistically there were no significant differences between the assemblages of Tshino and Mutale. Functional feeding group analyses suggest that collectors dominated both sites while shredders were completely absent. Predators and scrapers comprised a small percentage of the total individuals caught and at Tshino (post 2000 floods) there was a moderate but probably not significant, increase in scrapers. The significant temporal seriation in the community assemblage of the macroinvertebrates structure suggests a successional recovery in communities after the floods of 2000. Habitat substrate, i.e. the presence of boulders, cobbles, bedrock and mud, played the most important part in invertebrate assemblages structuring at the micro scale. At macro-scale the abundance of one family, Leptophlebiidae, was significantly correlated with a combination of serial environmental factors.

6. FISH

Both sites were dominated by three rheophilic fish species, namely *Chiloglanis pretoriae, Labeo cylindricus* and *Amphilius uranoscopus* and one semi-rheophilic species, *Labeobarbus marequensis*. Some limnophilic species that are generally not found in rheophilic habitats were also collected. This included *Barbus trimaculatus, Petrocephalus wesselsii, Marcusenius macrolepidotus* and *Mesobola brevianalis.* The Tshino site appears to have a larger fish population that is completely dominated by *C. pretoriae*. The Mutale site has a higher fish diversity, which could be linked to the higher habitat integrity of the lesser-impacted Mutale River. During the study period continuous increase in fish numbers occurred at Tshino, caused mainly by the increase of *C. pretoriae*. The opposite is evident at Mutale where the total number of fish collected decreased. This difference was ascribed to recovery of the sites after the 2000 floods. Flood damage at the Mutale site was less severe, probably due to the buffering effect of Lake Fundudzi. As a whole there was a decrease in fish diversity, predominantly because of a significant decrease in evenness over the period. The absence of seasonal effects on fish abundance or composition indicates dynamic fish communities that are possibly not stable and still adjusting from the effects of the February 2000 flood.

The analyses of the stomach contents illustrated the differences between feeding types and trophic niches. In the case of the insectivores these differences were underpinned by the stomach morphology.

The results indicated that both *C. pretoriae* and *L. cylindricus* are distinctly rheophilic with the former more frequently observed in shallow water and the latter in water deeper than 0,5 m. *L. marequensis* showed no clear preference and is regarded as semi-rheophilic.

While the majority of *A. uranoscopus* were collected in fast flowing habitats at both sites their preference for shallow or deep water differed between the two sites. The species can however be classified as a true rheophilic species.

Monitoring projects can be used to outline the biotic integrity but little is still known about the factors and interaction of factors responsible for the decline in ecological integrity. Both fish and invertebrates have been used in monitoring but there still no agreement about which group is the most efficient.

Comparisons between the analyses of fish and macroinvertebrate data sets suggest that fish are better predictors of water quality, but no real seasonality was evidenced in assemblage structure and water quality variables were the best predictors of their composition. Macroinvertebrate assemblages seemed to be seasonal and respond more to characteristics of the habitat structure than to water quality variables.

Fish assemblages as a whole respond to water quality variables to a larger extent than invertebrates. However, when focusing on responses at finer taxonomic level, the macroinvertebrate family, Leptophlebidae, was the best indicator of the environmental conditions measured in this study.

7. A PREDICTIVE MODEL OF ANTHROPOGENIC INFLUENCES.

A simplified conceptual model was developed in an attempt to abstract important anthropogenic influences at increasing levels of detail. This model provides the essential framework for the understanding of the various influences on the river, as it captures those concepts that are important for the maintenance of biodiversity. A synthetic dataset was used to train a feed-forward back-propagation neural network consisting of one intermediate layer of 10 neurons, which was successful in classifying the dataset into six distinct groups. These preliminary results demonstrated that neural network modelling can be used as a diagnostic or classification tool. From this initial investigation it would appear that neural network analysis of river data will shed some light on river health and is an approach well worth pursuing. The preliminary model classified sites correctly and enabled the identification of sites impacted by anthropogenic influences. This kind of model needs to be investigated more fully and used for the examination of complex, multivariable water quality systems.

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CHAPTER ONE

INTRODUCTION AND BACKGROUND

1.1 Project title

"Towards an understanding of factors affecting the biotic integrity of rivers in the Limpopo Province: Niche partitioning, habitat preference and microbiological status in the rheophilic biotopes of the Luvuvhu and Mutale rivers."

1.2 Motivation

In underdeveloped rural communities, such as those that rely on a river like the Luvuvhu River, the role played by a river or the use of the river differs from what is encountered in developed areas. Because of their low per capita income these communities tend to have a high level of dependence on the river and its catchment.

These uses include fish and plants as food resources, animals and plants as medicine, plants as fuel and building material and finally water itself as a source for human and animal consumption and irrigation of crops. The aquatic environment and riparian zone furthermore plays a significant role in the social and cultural lives of the people. Rural communities are also unique because of the low level of general awareness that exist about the effects of their actions, direct or indirect, on the river and water quality. These communities do not perceive practices, such as riparian agriculture as wrong and are often suspicious of efforts to improve water quality.

It should be borne in mind that the needs of the Kruger National Park, as one end user of this water system, are not known by the people. This lack of understanding also exists as far as the rapid growing ecotourism industry is concerned. People are not aware of the vast number of job opportunities that this industry could provide. Neither are they knowledgeable about the negative influence that environmental destruction would have

on the industry. A study of the Mutshindudi River by Gaigher (2001) showed that any plans to manage rivers in these communities should not only take the abovementioned into consideration, but should also focus on community involvement and concentrated efforts to improve the level of environmental awareness. Any drive to improve aquatic awareness will require knowledge of the factors responsible for the decline in biodiversity. From a management viewpoint this implies knowledge of threshold values of factors sustaining the riverine biota. Although monitoring projects outline the biotic integrity, little is still known about the factors and interaction of factors responsible for the decline in integrity. Although the influence of anthropogenic events such as the removal of vegetation, over-grazing, cultivation and trampling of riparian zones as well as water extraction can be linked to changes in flow rate, water temperature and siltation, the dynamics of these factors on the aquatic biota are still unclear.

It is postulated that anthropogenic changes, caused by an increase in the human population and the subsequent increase in pressure on the river as a resource, have invariably led to a decrease in biotic diversity. To try and understand this loss of diversity it is important to examine and understand the fine scale determinants of freshwater rheophilic assemblages. These determinants could include aspects such as loss of available habitat and changes in the physico-chemical parameters. Such determinants will determine habitat preference, distribution, niche partitioning, assemblage structure as well as nutrient pathways and adaptations to feeding.

1.3 The aims of the project

The aims of this project were:

- To determine the influences of the above-mentioned factors or determinants on aquatic biota through an understanding of the habitat requirements and niche partitioning.
- To explain the loss of diversity in the rheophilic biotopes through an understanding and modeling of the aspects of niche differentiation and habitat requirements.
- To use this information to determine thresholds of probable concern.

1.4 The products envisaged by the project.

The items listed below are regarded as products that the project could produce:

a) Thresholds of probable concern (TPCs).

b) A neural network model that describes niche partitioning and habitat requirements in rheophilic biotopes and applies TPCs

1.5 The logistics of the project

It was envisaged that the project would consist of the following phases:

The first phase comprising of an in-depth study of the factors that affect the loss of biodiversity, an identification of the characteristics of biotopes and an assessment of the habitat requirements and niche differentiation and partitioning of selected biota. This is a multidisciplinary study and will include micro-organisms, macroinvertebrates and fish. Through the determination of the physico-chemical parameters the thresholds of probable concern will be determined.

During the second phase a management plan for the river will be developed, material for an aquatic awareness programme compiled and an aquatic system awareness plan will be launched.

This project report will only deal with the first phase and it is envisaged that the second phase will commence after completion of the first phase.

1.6 The Methodology employed.

1.6.1 The river system.

The Luvuvhu River system is an extremely complex and variable. In order to begin to understand the system and factors leading to a loss of diversity this project focused the attention of a team of investigators from different disciplines on certain key components of rheophilic biotopes.

The project aimed to identify the characteristics of rheophilic biotopes, quantify the important key variables and assess habitat requirements and niche partitioning. Mathematical modeling will be used to focus attention on key issues.

1.6.2 The study sites.

It was originally decided during the planning phase of this project to study at least two sites, with a possibility of a third, in the Luvuvhu River. On further deliberation one site in the Luvuvhu and another in the Mutale were selected as representative rheophilic sites. All field data were recorded in relation to a grid to enable overlays of the environmental characteristics and the biological data.

1.6.3 The teams involved.

Teams consisting of specialists were selected to focus with the different aspects. In the original planning phases of the project a team of algal experts were also included but due to the resignation of key role players at a critical stage of the project this component did not materialize.

Task one: The Physico-chemical properties of the water. (Mr. P.S.O. Fouché)
Objective:

a) Determine the physico-chemical properties of the water.

Task two: Microbes (Ms N. Potgieter)
Objectives:

a) Identify the pathogenic micro-organisms associated with detritus.
b) Identify the pathogenic micro-organisms found on the fish and in the fish intestines and relate it to micro-organisms associated with detritus

Task three: Macro-invertebrates (Dr. S.H. Foord)
Objectives:

a) Establish distribution of the macro-invertebrates and to group them into

Functional Feeding Groups.

b) Determine the factors that structure macro-invertebrate assemblages.

Task Four: Distribution, food habits and population composition of rheophilic fish (Prof. B.C.W. van der Waal and Mr. P.S.O. Fouche)

Objectives:

a) Establish the distribution of fish in rheophilic biotopes and to relate this to habitat preference.
b) Determine the structure composition of the fish populations
c) Determine the food habits of fish in order to establish their trophic niche differentiation.

Task 5: Statistical analyses.(Dr. S.H. Foord)

Objective:

a) Statistically establish the relationship between the environmental data and biotic data.

Task 6: Development of a predictive model for TPCs (Prof. T. van Ree)

Objective:

a) The development of the predictive model for TPCs

1.6.4 Reference list

GAIGHER, M.J. 2001. A socio-economic survey of the inhabitants of the Mutshindudi River catchment. In Gaigher, I.G. (Ed) " A Socio-biological study of the aquatic resources and their utilization in an underdeveloped rural region, the Mutshindudi River Catchment." *WRC Project Report 714/3/01,* 15 - 38.

CHAPTER TWO
SITE SELECTION AND GENERAL APPROACH

2.1 Introduction

This chapter details the rationale for selecting the two sites that were investigated while at the same time the reasons for this study are revisited by looking at the historic data and the results of the 1999 River Health Programme monitoring results. It also aims to supply a brief overview of the general approach and methodology adopted during the field surveys and to sketch a picture of the chain of events and procedures that followed the fieldwork.

2.2. Materials and methods

2.2.1 The rivers involved.

The Luvuvhu River arises in the Soutpansberg Mountain and flows through a diverse landscape before it joins the Limpopo in the Kruger National Park. The Mutale River emerges as a "spring" from below the landslide blockage that formed Lake Fundudzi. It joins the Luvuvhu River in the Kruger National Park before the latter forms the confluence with the Limpopo.

2.2.2 Sites and collection points.

The two sites were selected to represent an anthropogenically "disturbed" and an anthropogenically "less-disturbed" site. The first site selected is close to Tshino village (23^{o} 06.51' S and 30^{o} 23.26' E) in the Luvuvhu River. It is situated in what is regarded as Lowveld (ecoregion 5.04) and was deemed to represent ecological conditions that can be referred to as "disturbed". This was based partly on the fact that the site was situated downstream of a commercial farming settlement and partly because of the anthropogenic impacts that could be caused by the rural communities in the area. The second site is in the Mutale River (22^{o} 45.5' S and 30^{o} 23.47' E), and is situated in what is classified as Central Highlands (ecoregion 2.01). The site was selected to present a situation that can be referred to as "less disturbed" as it is in a sparsely populated area with no agricultural activities taking place upstream. During the selection process care was taken to ensure

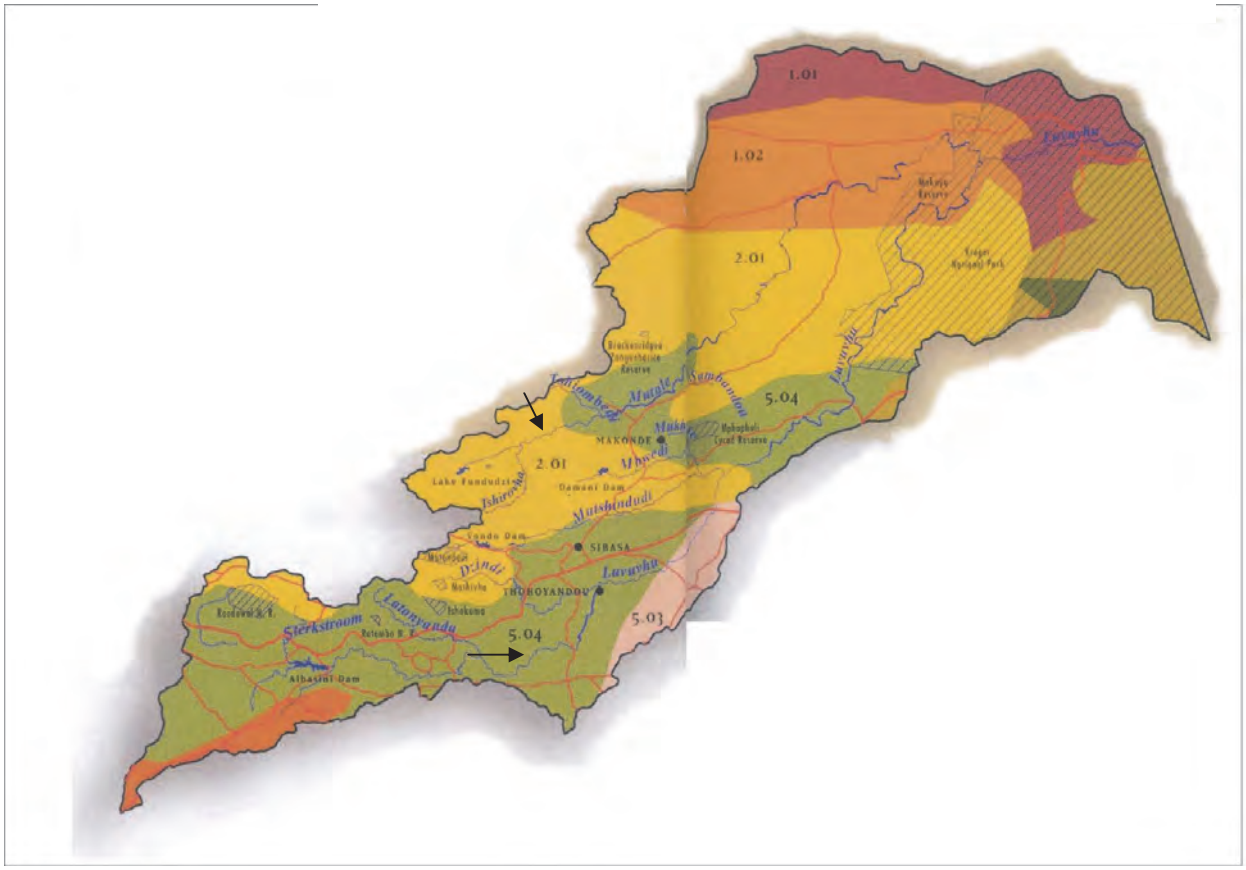

Figure 2.1 : The Luvuvhu River Catchment. Adapted from the State of the Rivers report: Letaba and Luvuvhu rivers (WRC, 2001). The two sites are indicated with the arrows.

that the selected sites had the biotopes and habitats required by both fish and macro-invertebrates. Both sites are at similar altitudes which would imply that similar fish species could be present (Gaigher, 1973).

Both sites were also biomonitoring sites in the 1999 National River Health Programme. The results of that programme are shown in Tables 2.1 (a) and 2.1 (b) below and these results supported the decision of the site selection.

Table 2.1 a) Scores obtained in 1999 at the two sites with the SASS 4 index.

	SASS 4 SCORE	SCORE PER TAXON (ASPT)	ECOLOGICAL RESERVE CLASS
TSHINO	169	7,04	C
MUTALE	195	7,05	B

Table 2.1 b) Scores obtained in 1999 at the two sites with the Fish Assemblage Integrity Index (FAII)

	FAII SCORE	ECOLOGICAL RESERVE CLASS
TSHINO	62	C
MUTALE	68	C

In Tables 2.2 and 2.3 the implications of the obtained Ecological Reserve Classes are explained and they indicate, as far as fish is concerned, that at both sites some loss of biodiversity has occurred. As far as the Fish Assemblage Integrity Index (FAII) scores are concerned it should be borne in mind that this index is not based only on the differences between presence and absence of fish species but that it also takes frequency of occurrence of the species in account. With both sites having been classified as class C the implication is clearly that some fish species might be absent and that the numbers of each fish species at the sites within the same river reach could also have diminished.

Table 2.2: Description of SASS4 condition classes. (Thirion, 1998)

CLASS	BIOTIC MODIFICATION RELATIVE TO CURRENT BEST ATTAINABLE CONDITION	DESCRIPTION	SASS4 SCORE (% OF REFERENCE CONDITION)	ASPT VALUE (% OF REFERENCE CONDITION
A	Unimpaired	Community structures and functions comparable to the best situation to be expected. Optimum community structure (composition and dominance) for stream size and habitat quality.	90 – 100 80 - 89	Variable >90
B	Minimally impaired	Largely natural with few modifications. A small change in community structure may have taken place but ecosystem functions are essentially unchanged	80 – 89 70 – 79 70 - 89	<75 >90 75 – 90
C	Moderately impaired	Community structure and function less than the reference condition. Community composition lower than expected due to loss of some sensitive forms. Basic ecosystem functions are still predominantly unchanged.	60 – 79 50 – 69 50 – 79	>75 60 - 75
D	Largely impaired	Fewer families present than expected, due to loss of most intolerant forms. Basic ecosystem functions have changed.	50 – 59 40 – 49	<60 Variable
E	Seriously impaired	Few aquatic families present, due to loss of most intolerant forms. An extensive loss of basic ecosystem functions has occurred.	20 – 39	Variable
F	Critically impaired	Few aquatic families present, with high densities of organisms, then dominated by a few taxa. Only tolerant organisms present.	0 - 19	Variable

Table 2.3: FAII Assessment classes (Kleynhans, 1999)

Class rating	Description of generally expected conditions for integrity classes	Relative FAII score (% of expected)
A	Unmodified, or approximate natural conditions closely	90 - 100
B	Largely natural with few modifications, A change in community characteristics may have taken place but species richness and presence of intolerant species indicate little modification.	80 - 89
C	Moderately modified. A lower than expected species richness and presence of most intolerant species. Some impairment of health may be evident at the lower limit of this class.	60 - 79
D	Largely modified. A clearly lower than expected species richness and absence or much lower presence of intolerant and moderately tolerant species. Impairment of health may become more evident at the lower limit of the class.	40 - 59
E	Seriously modified. A strikingly lower than expected species richness and general absence or much lower presence of intolerant and moderately tolerant species. Impairment of health may become very evident.	20 - 39
F	Critically modified. An extremely lowered species richness and absence or much lower presence of intolerant and moderately tolerant species. Only tolerant species may be present with a complete loss of species at the lower limit of the class. Impairment of health may become more evident at the lower limit of the class.	0 - 19

Table 2.4: The relationship between FIOBI and FAII scores and the Ecological Reserve classes.

FIOBI scores	Integrity classes	FAII scores
0,90 - 1,0	A	90 – 100
0,80 - 0,89	B	80 – 89
0,60 - 0,79	C	60 – 79
0,40 - 0,59	D	40 – 59
0,20 - 0,39	E	20 – 39
0 - 0,19	F	0 - 19

On the other hand the rapid method for habitat integrity assessment, the Fish Index Of Biotic Integrity or FIOBI, proposed by Gaigher and Fouche (2001) is based on abundance rather than frequency of occurrence as is the case with the FAII. This not only makes it

more suitable for determining the integrity of single sites but it also gives an indication whether the abundance of fish species have increased or not. When the fish data collected for the FAII was used to calculate the FIOBI, the scores were lower to what was obtained in the FAII (Table 2.5), thus strengthening the suggestion that the number of species and the abundance of each species has been impaired.

Table 2.5: Scores obtained in 1999 at the two sites with the Fish Index of Biotic Integrity (FIOBI)

	FIOBI SCORE	ECOLOGICAL RESERVE CLASS
TSHINO	0,45	D
MUTALE	0,61	C

2.2.3 Sampling frequency and site protocol.

The sites were sampled nine times each during the project which commenced in August 2000 and ended in May 2002. Due to logistical problems the microbiology team could not be present during all of these dates and sampled both sites on five separate occasions. (See Table 2.6). Macro-invertebrate data generated in a separate survey during 1999 was also included.

During each sampling event the river was divided into a grid consisting of $4m^2$ blocks using metal stakes. In each of these blocks the fish, macro-invertebrates and detritus were collected. After all the blocks had been sampled, different biotopes, such a rapid, a riffle and pools, were identified outside the grid area and fish were collected in each. The specific collection methods for the biota will be discussed in detail in the relevant chapters.

During each sampling event the team consisted of at least one fish expert and one invertebrate expert. Each of them was responsible for his/her own collection of the relevant data. A postgraduate assistant accompanied each of these experts. Upon arrival at the site the team as a whole commenced by setting up the grid.

The physico-chemical aspects of the water was then determined by one person while a second person commenced with the river profile and substrate determination. Only at completion of all this did the collection of samples commence.

2.2.4 The river profile

During each collection the river profile was determined by measuring the depth at 0,5 metre intervals with a wooden metre rule along a transect across the width of the active channel. At each half-metre interval the substrate immediately below the meter rule was classified and recorded. In order to standardize, the classification of the riparian substrate as supplied by Rowntree and Wadeson (2001) was used: Boulders: > 256mm (larger than an adult head), Cobble: 64 – 256mm (larger than a fist), Gravel: 2 – 64 mm (small pea to size of fist) and sand: < 2mm (but individual grains still visible). The description in brackets referring to the actual size reference used in the field.

2.2.5 Physico- chemical aspects.

In each block of the grid and in some of the selected biotopes the water velocity as well as the minimum and maximum depth were determined. The water velocity was determined with a Pasco Scientific velocity meter while the depth was determined with a metre rule.

At each site *in situ* determinations included dissolved oxygen, pH, conductivity and temperature. Water samples were also collected and in the laboratory the total suspended solids (TSS) and turbidity were determined. All the methods used will be discussed in detail in Chapter 3.

Table 2.6: Summary of dates of site visits and data collected in the Mutale and Luvuvhu rivers from 1999 - 2002. The asterisk (*) indicates that data was collected or analyzed while 0 indicates that no data was collected/analyzed

Site data		Physico-chemical data collected and analyzed				Fish data					Other organisms collected and analyzed	
						Fish collected		Fish data analysed				
Date	Site name	River pro-file	Sub-strate	Phys. pH/temp/cond/ DO/velocity	H_20 sample analysed	Fish in blocks	Fish in selected habitats	Fish in blocks	Fish in selected habitats	Age	Macro-inverte-brates	Micro-organi sms
30-9-99	TSHINO	0	0	0	0	0	0	0	0	0	*	0
8-8-00	TSHINO	*	*	*	*	*	0	*	0	*	0	0
15-8-00	MUTALE	*	*	*	*	*	*	*	*	*	*	0
29-8-00	TSHINO	0	*	*	*	*	*	*	*	*	*	0
22-8-00	MUTALE(B)	*	*	*	*	*	*	*	*	*	*	0
17-10-00	MUTALE	*	*	*	*	*	*	*	*	*	0	0
7-11-00	TSHINO	*	*	*	*	*	*	*	*	*	*	0
10-5-01	TSHINO	*	*	*	*	*	0	*	0	*	*	0
24-5-01	MUTALE	0	*	*	*	*	0	*	0	*	*	0
2-8-01	TSHINO	*	*	*	*	*	*	*	*	*	*	0
16-8-01	MUTALE	*	*	*	*	*	0	*	0	*	*	0
27-9-01	TSHINO	*	*	*	*	*	0	*	0	*	0	*
20-9-01	MUTALE	*	*	*	*	*	0	*	0	*	*	*
31-10-01	TSHINO	*	*	*	*	*	0	*	0	*	*	*
1-11-01	MUTALE	*	*	*	*	*	0	*	0	*	*	*
9-01-02	MUTALE	0	0	0	0	0	0	0	0	0	0	*
14-01-02	MUTALE	0	0	0	0	0	0	0	0	0	0	*
21-01-02	MUTALE	0	0	0	0	0	0	0	0	0	0	*
7-2-02	MUTALE	*	*	*	*	*	0	0	0	0	0	*
13-2-02	TSHINO	*	*	*	*	*	0	0	0	0	0	*
18-02-02	TSHINO	0	0	0	0	0	0	0	0	0	0	*
26-02-02	TSHINO	0	0	0	0	0	0	0	0	0	0	*
15-03-02	TSHINO	0	0	0	0	0	0	0	0	0	0	*
15-03-02	MUTALE	0	0	0	0	0	0	0	0	0	0	*
2-5-02	MUTALE	0	0	0	0	0	0	0	0	0	*	*
14-5-02	TSHINO	*	*	*	0	*	0	0	0	0	0	*
14-5-02	MUTALE	*	*	*	0	*	0	0	0	0	0	*

2.2.5 Reference list

GAIGHER, I.G. 1973. Habitat preferences of fishes from the Limpopo River system, Transvaal and Mocambique. *Koedoe* **16** , 103 – 116.

GAIGHER, I.G. 1998. The diversity, distribution, habitat preferences and conservation status of fishes of the Limpopo River system, South Africa. *Verh. Internat. Verein. Limnol*. **26**, 2237 – 2239.

GAIGHER, I.G. and FOUCHE, P.S.O. 2001. A rapid method for habitat integrity assessment of small perennial streams in the Northern Province, South African, based on rheophilic fish. *Verh. Internat. Verein. Limnol*. 27, 3455 – 3458.

KLEYNHANS, C.J. 1999. The development of a fish index to assess the biological integrity of South African rivers. Water SA **25** (3) 265 – 277.

ROWNTREE, K. and WADESON, R. 2001 *Field manual for channel classification and condition assessment.*

THIRION, C. 1998. An exploratory investigation of the instream biological integrity of the Crocodile River, Mpumalanga, as based on the assessment of macroinvertebrate communities. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.

WRC. 2001.State of the Rivers report: Letaba and Luvuvhu rivers. Report no:TT 165/01

CHAPTER THREE

THE PHYSICO-CHEMICAL CHARACTERISTICS OF THE TSHINO AND MUTALE RIVER SITES

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3.1 Introduction

Although water shortage is often regarded as one the greatest hazards to fish species (Gaigher, 1973) in fast-flowing rivers, it is postulated that other changes in water quantity will impact the situation even further. Reduced flow impacts negatively on water velocity and water depth. In the case of velocity a loss of available fast-flowing habitats will result and this in turn will have a negative impact on the rheophilic and semi-rheophilic fish. Reduced depth on the other hand will lessen the depth of the water column resulting in a consequent reduction of cover which leads to fewer habitats being available This will negative impact on the limnophilic or pool dwelling fish (Gaigher, 1998).

Changes in quantity can also lead to impacts on water quality. Negative impacts on quality, associated with a loss of velocity and depth include aspects such as changes in pH, increased turbidity and higher sediment loads (Kleynhans, 1996). The latter two aspects can for example lead to the filling of interstitial spaces in the substrate. This will not only impact negatively on the breeding opportunities of the rheophilic fish but will put severe stress on the macro-invertebrates.

3.2 Aims

In this project the physico-chemical characteristics of the two sites were determined in order to compare the two sites. The data generated was then incorporated into the findings of Chapters 4 (microorganisms), 5 (macro-invertebrates) and 6 (fish) in order to determine the relationship between the biota and the habitat.

In turn these findings will be utilized in the preparation of the predictive computer model.

3.3 Materials and method

During each site visit the pH, temperature, conductivity and dissolved oxygen was determined on site using a Hanna pHep 3 pH meter, a Checktemp thermocouple, a Hanna HI 8733 conductivity meter and a WTW Oxi 320 oxygen meter respectively. Care was taken to calibrate the oxygen and conductivity meters before each reading according to the measured temperature. A water sample was also collected and transported to the laboratory.

In the laboratory the turbidity of the water was determined from the water sample collected at each site with a Analite Novasino nephelometer using double distilled water as a standard. To determine the Total Suspended Solids (TSS) a 200ml sub-sample of the water sample was filtered through a filter paper of a predetermined mass. The filter paper was then dried overnight at $60^{o}C$ and the increase in mass determined on a Sartorius Handy micro balance to the fourth decimal. The increase in mass was then expressed as mgl^{-1}.

Water depth was determined with a wooden metre rule to the nearest centimeter and the maximum and minimum depths, in flowing water, was recorded. Velocity was determined with a Pasco Scientific velocity meter at approximately 3 – 5cm above the substrate level and the maximum and minimum velocities for each site was recorded where flow was observed.

3.4 Results

The results obtained are shown in Tables 3.1 and 3.2.

Table 3.1: Physico-chemical parameters of the Mutale River determined in the period from August 2000 – May 2002. Maximum and minimum depth and velocities were measured where flow occurred.

	Dates collected								
	M15/8/00	**M22/8/00**	**M17/10/00**	**M24/05/01**	**M16/08/01**	**M20/9/01**	**M01/11/01**	**M07/02/02**	**M14/05/02**
pH	7.3	6.7	7	7	6.8	7.2	8.7	7.85	7.2
Conductivity (μScm^{-1})	37	44	46	48	41.8	46	48.8	31	51
Dissolved O_2 (%)	95	99	81.8	98	94	92	94	82	98
Dissolved O_2 (mgl^{-1})	8.5	8.8	7.6	8.8	8.3	8.3	8.2	6.7	8.7
Temperature (^oC)	15.6	15.7	20.7	16	18.5	19.5	23.2	22.3	20.1
Max.depth (m)	0.74	0.6	0.7	0.6	0.6	0.52	0.36	0.82	0.39
Min. depth (m)	0.12	0	0.16	0.14	0.05	0	0	0.1	0
Max. velocity (ms^{-1})	1.22	1.3	1	1	1	0.67	0.56	1.29	0.36
Min. velocity (ms^{-1})	0.3	0.3	0.5	0.4	0.4	0.29	0.27	0.66	0.3
Total suspen-ded solids (mgl^{-1})	26	49.5	19	6.5	3.5	6.5	14	7.5	6
Turbidity (NTU)	20	20	25	8	2	3	3	2	8

Table 3.2: Physico-chemical parameters of the Luvuvhu River determined in the period from August 2000 – May 2002. Maximum and minimum depth and velocities were measured where flow occurred.

	Dates								
	T8/8/00	**T29/8/00**	**T7/11/00**	**T10/5/01**	**T02/08/01**	**T27/09/01**	**T31/10/01**	**T13/02/02**	**T14/05/02**
pH	7.4	7.8	7.95	7.36	7.1	8.1	9.4	7.8	8
Conductivity (μScm^{-1})	108	115	118	92	90	103	109	109	124
Dissolved O_2 (%)	95	85	90	94	92	90	90.5	87	93
Dissolved O_2 (mgl^{-1})	8.4	6.5	7.8	8.3	8.2	8.5	8.6	6.7	8.2
Temperature (^oC)	16	18.8	25	17	16.1	20.6	25.1	25.5	17.1
Max.depth (m)	0.9	0.85	0.82	0.9	0.7	0.6	0.52	0.66	0.5
Min. depth (m)	0.15	0.1	0.1	0.3	0.04	0.02	0.06	0.01	0.1
Max. velocity (ms^{-1})	2	1.82	1	1.67	0.86	1	1	0.7	0.88
Min. velocity (ms^{-1})	0.34	0.3	0.2	0.39	0.4	0.1	0.28	0.3	0.4
Total suspen-ded solids (mgl^{-1})	26	63.5	50.5	30	25	30.5	35	21	52.3
Turbidity (NTU)	31	21	36	14	9	12	10	7	20

3.4 Discussion

Figures 3.1 – 3.8 graphically illustrate the conditions, trends and differences between the two sites. As far as conductivity, total suspended solids (TSS) and turbidity is concerned the Luvuvhu River had the highest readings. This is in line with what was expected based on historic data (DWAF, 1990). Although the water at Tshino in the Luvuvhu River was more turbid and displayed a higher conductivity and TSS reading than the water from the Mutale site, the trend for these aspects at the two sites over the period of the survey were exactly the same.

The fact that the dissolved oxygen level in the water from the Mutale was higher than that at Tshino can be ascribed to temperature differences but more specifically to the fact that the site in the Mutale was closer, if not part of, to the headwaters. This is regarded as typical of the higher turbulence commonly observed in headwaters of rivers.

Being a wider river with less cover offered by the trees of the riparian zone the water of the Luvuvhu River at Tshino is warmer than that of the Mutale with the exception of August 2001 when the water of the Mutale River was 2,4 ^{o}C warmer. This could be ascribed to the August surveys not being carried out on the same date but could also be the result of the stabilizing effect of Lake Fundudzi upstream of the site.

The water velocity (Figure 3.8) and maximum depth (Figure 3.5) graphs show that the Luvuvhu River is deeper and faster than the Mutale River. This is with the exception of the February 2002 data when the Mutale River was deeper and flowing faster than the Luvuvhu River. This was due to localised rain that had occurred in the catchment of the Mutale River.

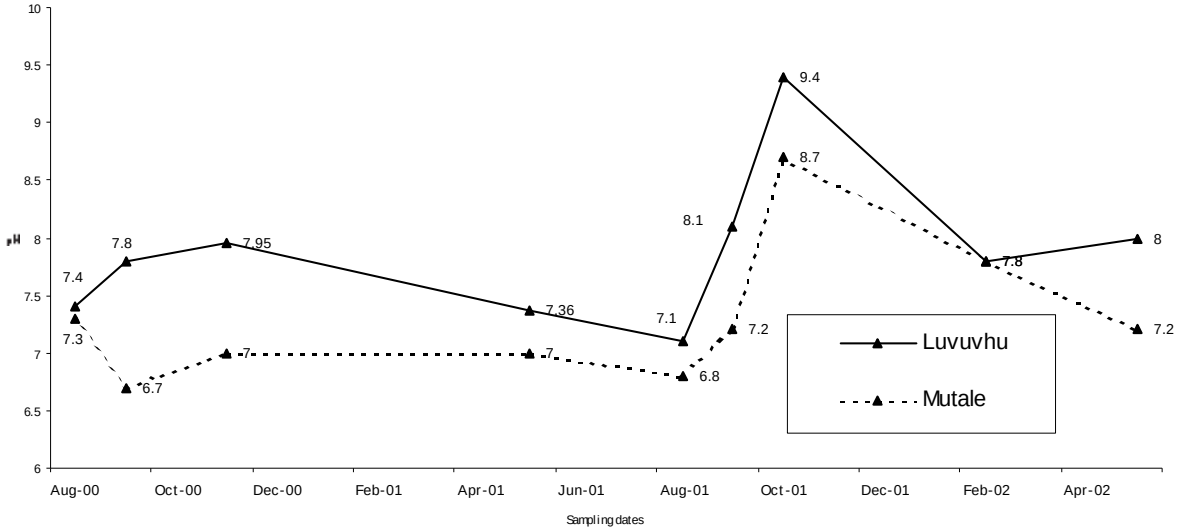

Figure 3.1: A comparison of the pH values measured in the Luvuvhu and Mutale rivers during the period from August 2000 to May 2002

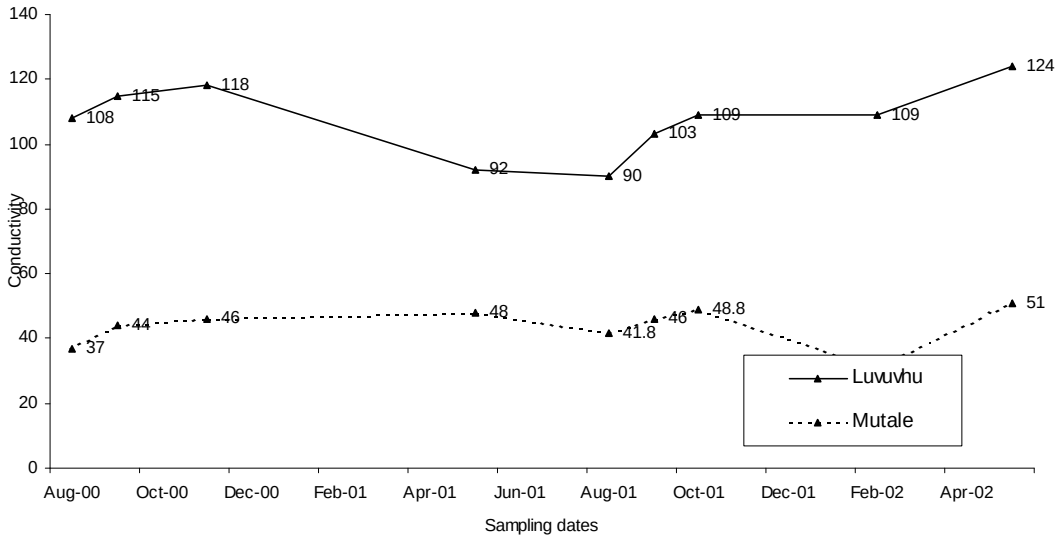

Figure 3.2: A comparison of the electrical conductivity (micro Siemens / cm) measured in the Luvuvhu and Mutale rivers during the period from August 2000 to May 2002

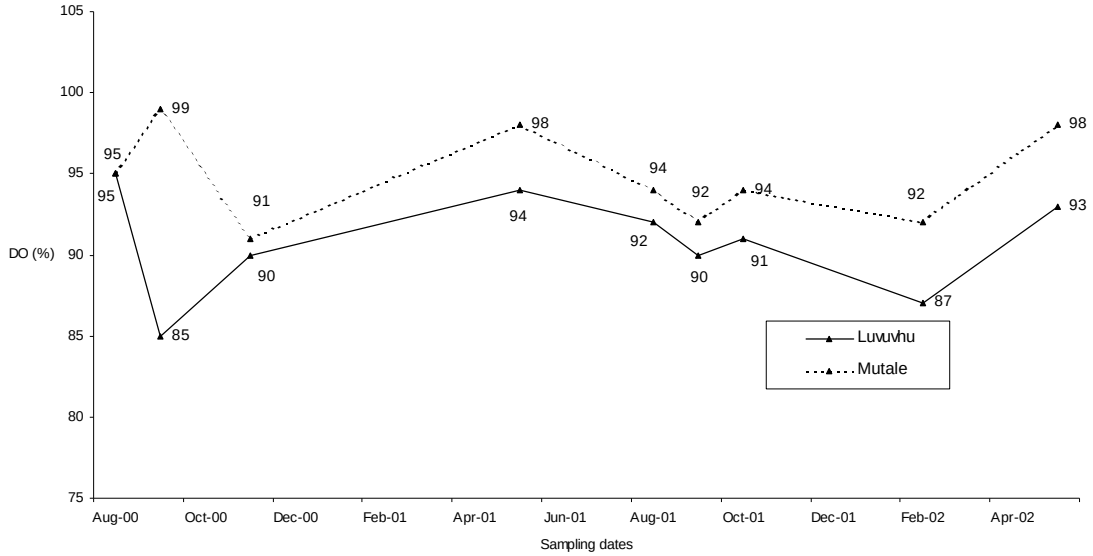

Figure 3.3: A comparison of the Dissolved oxygen values (%) measured in the Luvuvhu and Mutale rivers during the period from August 2000 to May 2002

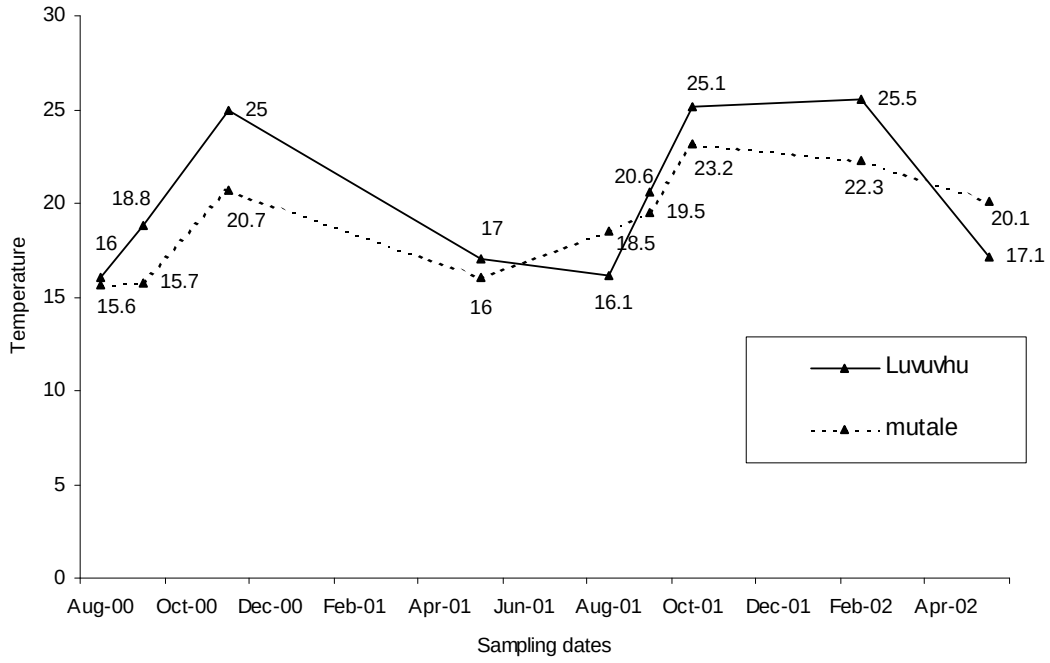

Figure 3.4: A comparison of the water temperatures measured in the Luvuvhu and Mutale rivers in the period August 2000 to May 2002

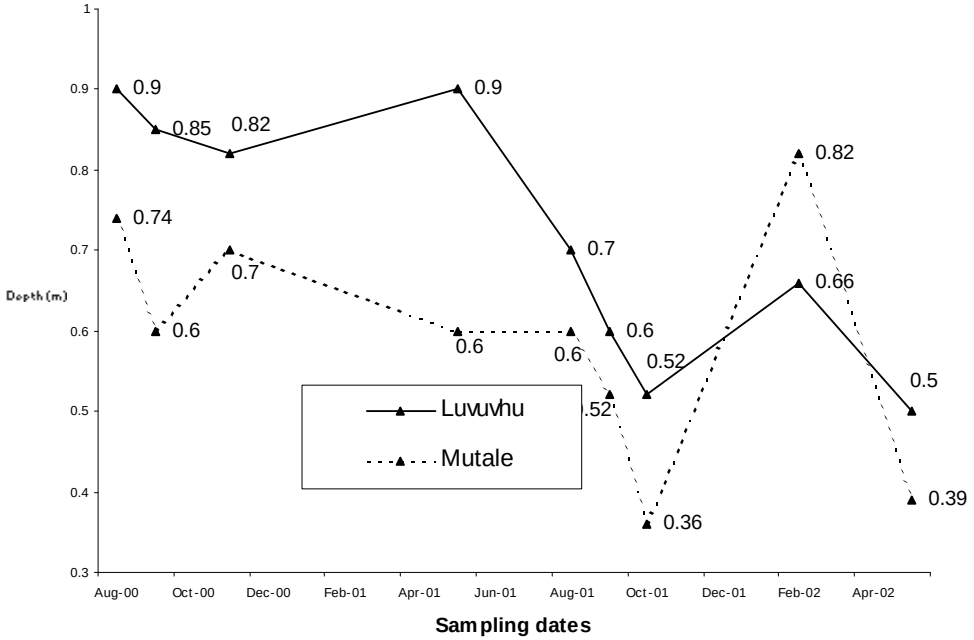

Figure 3.5: A comparison of the maximum depths (m) in the grids of the Luvuvhu and Mutale rivers during the period from August 2000 to May 2002

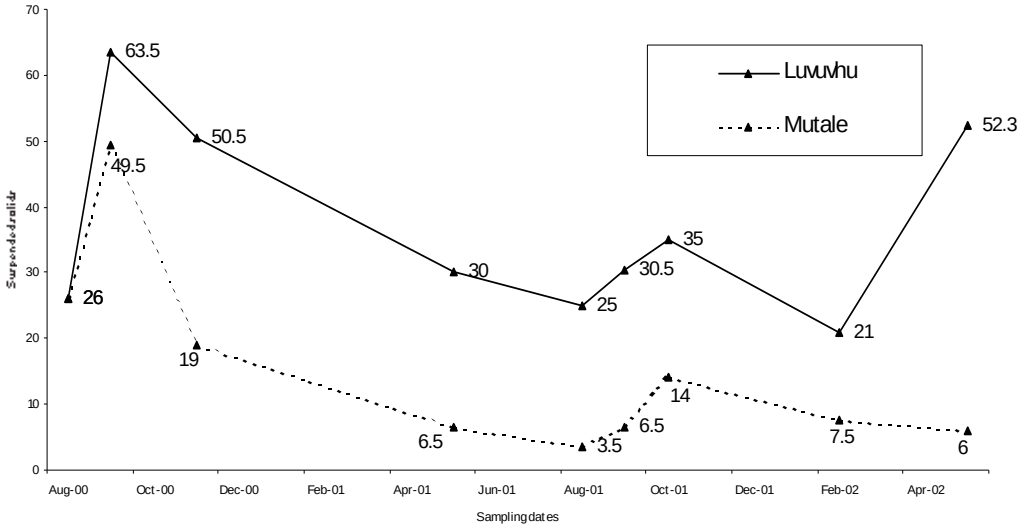

Figure 3.6: A comparison of the Total Suspended Solids (mg/l) in the water collected from the Luvuvhu and Mutale rivers during the period from August 2000 to May 2002

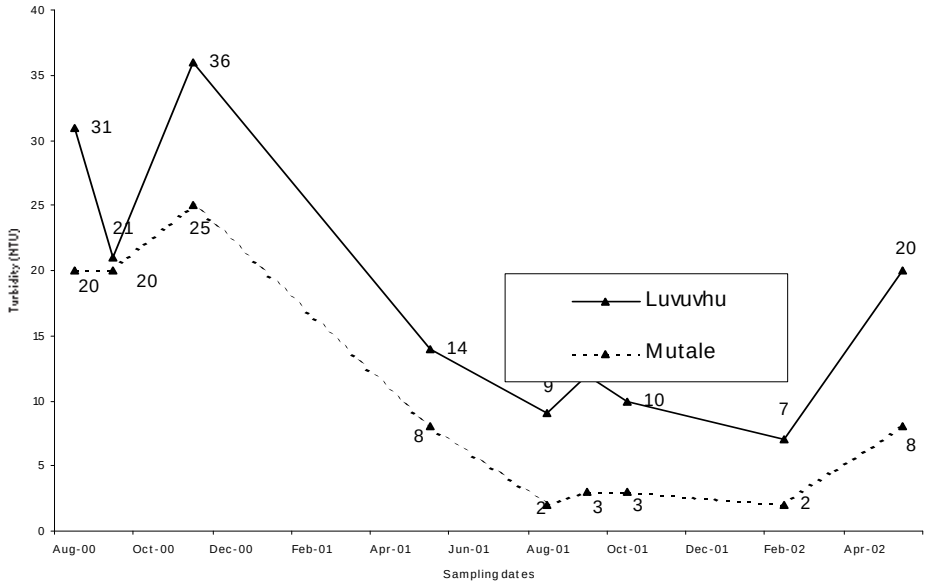

Figure 3.7: A comparison of the turbidity (NTU) of the water collected from the Luvuvhu and Mutale rivers in the period from August 2000 to May 2002

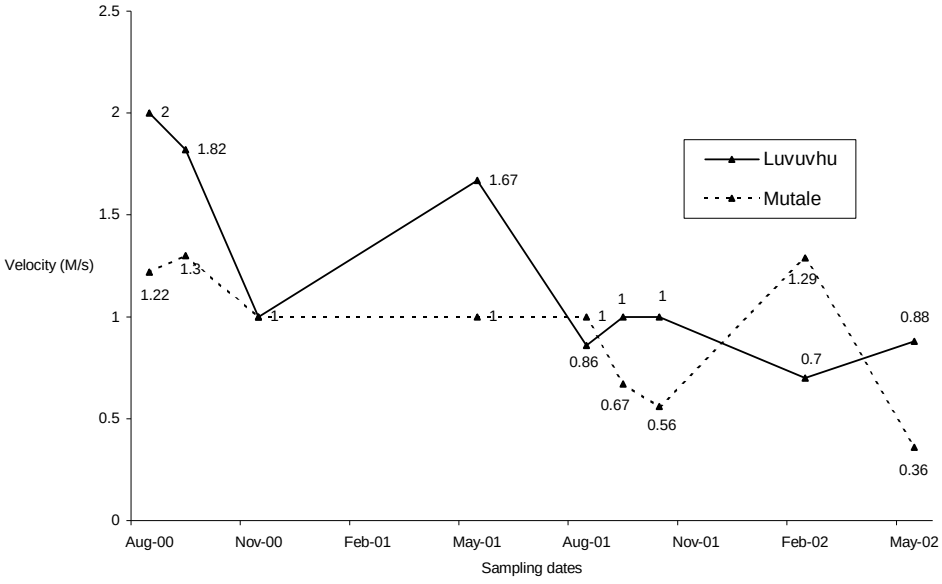

Figure 3.8: A comparison of the maximum water velocity (m/s) measured in the grid of the Luvuvhu and Mutale rivers during the period from August 2000 to May 2002

3.6 Reference list

DEPARTMENT OF WATER AFFAIRS. 1990. *Kruger National Park Rivers Research Programme*. DWAF, Pretoria

GAIGHER, I.G. 1973. Habitat preferences of fishes from the Limpopo River system, Transvaal and Mozambique. *Koedoe* **16** , 103 – 116.

GAIGHER, I.G. 1998. The diversity, distribution, habitat preferences and conservation status of fishes of the Limpopo River system, South Africa. *Verh. Internat. Verein. Limnol*. **26**, 2237 – 2239.

KLEYNHANS, C.J. 1996, A Qualitative procedure for the assessment of the habitat integrity status of the Luvuvhu River (Limpopo system, South Africa). *J. of Aquatic Ecosystem Health* **5**: 41 -54

CHAPTER FOUR

MICROBIOLOGICAL ASPECTS RELATED TO FISH AND DETRITUS

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4.1. General introduction and literature review

Different microorganisms, both pathogenic and non-pathogenic are present in water environments. Pathogenic microorganisms found in water include bacteria (eg. *Salmonella* spp, *Escherichia coli, Clostridium, S. aureus*); enteric viruses (eg. enteroviruses, hepatitis A virus, noroviruses) and parasites (eg. *Cryptosporidium, Giardia*) (Michael, 1993). Fish living in water containing high concentrations of microorganisms, are exposed to the attachment of these pathogens to the skin of the fish, the gall bladder, abdominal pores and eyes is possible under these conditions (Fattal *et al*., 1992). The microorganisms may adhere to the external integument or penetrate into tissue through the gills and other thin epithelia. People living in rural areas use fish as a source of protein due to poor economical status of the area they reside in. Fish may also ingest faeces or detritus containing bacteria. All this are contributing factors that lead to the infection of fish (Fattal *et al.*, 1992). Many fish species found in a river system consume detritus and aquatic insects such as mayfly nymphs, caddisfly and blackfly larvae (Gambie, 1965; De Villiers 1991; Skelton, 1993). Detritus is the main habitat of microorganisms in water and consists of an assemblage of living and dead material that includes fungi, bacteria, microinvertebrates, algal cells, and the substrate itself. Detritus in streams may be either allochthonous, which means that it comes from the surrounding catchment (e.g leaves, twigs, and branches), or autochthonous which means that it is produced by the system itself (e.g. algae, dead aquatic animals, and plants) (Cranford, 1987).

4.1.1. Pathogenic organisms found in detritus and fish

4.1.1.1. *Salmonella* spp

The genus *Salmonella* comprises the causative organisms of salmonellosis. This species was named for Daniel Elmer Salmon, American veterinary pathologist who first isolated the organisms in 1885, (Smith, 1985). Several species of salmonella are infectious, most notably *Salmonella typhi, Salmonella typhimunium, Salmonella enteritidis* and *Salmonella choleraesuis*, (Buffaloe and Ferguson, 1981). Since only a few species, such as *Salmonella typhi*, are indigenous to humans, most infections originate from other animals. In nature,

Salmonella is found in the intestinal tract of humans and animals and they spread by feces either directly or indirectly from one host to another. The principal vehicles are water, milk, or food handler who is a carrier. Flies are mechanical vectors. If the food ingested contains only a small number of *Salmonella*, acid in the stomach of the host destroys them readily. If food is heavily contaminated, some of the bacilli escape the effects of the gastric acid to enter and injure the small bowel, (Smith, 1985). Symptoms of acute salmonellosis include nausea, fever, vomiting, abdominal pain, diarrhea, and headache. Persons of all ages are susceptible to salmonella infections but symptoms are more severe and prolonged among the elderly, infant, and people with underlying illnesses (Buffaloe and Ferguson, 1981).

4.1.1.2. *Escherichia coli*

Immunologic subdivision of *Escherichia coli* is made on the basis of O antigens, H antigens, and K antigens. Most strains of *E. coli* are harmless, but some are pathogenic. Although *E. coli* is part of the normal flora of the intestinal tract, it was suspected for years of being able to cause a moderate to severe diarrhoea in humans and animals, (Smith, 1985). These are the so-called ´faecal coli` that occur normally in the human and animal intestine and it is natural to assume that their presence in food indicate recent contamination with faeces. *E. coli* is however, widespread in nature and although most strain probably had their origin in faeces, it is the commonest cause of urinary tract infections in humans and is also found in suppurative lesions, neonatal septicaemias and meningitis. In animals it causes mastitis, pyometriain bitches, coli granulomata in fowls, and white scours in calves, (Collins and Lyne, 1995). Enteropathogenic (EPEC), enterotoxigenic, (ETEC), enteroinvasive (EIEC), and verotoxigenic (VTEC), are the four types of *E.coli* causes gastrointestinal disease in humans. EPEC has been associated outbreaks of infantile diarrhoea. ETEC strains are thought to cause gastroenteritis in both adult and children. The vehicles are contaminated water and food. EIEC strains cause diarrhoea similar to that in shigellosis. The strains associated with invasive dysenteric infections are less reactive than typical *E. coli*. They may be lysine negative, anaerogenic and resemble shigellas. VTEC is a name derived from cytotoxic effects on vivo cells in tissue culture. The alternative name is enterohaemorhagic *E. coli* (EHEC). They have been associated with haemolytic uraemic syndrome and haemorrhagic colitis and are known to be responsible for food-borne disease (Collins and Lyne, 1995).

4.1.1.3. *Staphylococcus aureus*

Staphylococcus areus occur typically in grape-like clusters. Individual cocci, approximately 1μm in diameter, tend to cluster because their cell division occurs in three planes, and daughter cells so formed tend to remain close together. They are Gram-negative, non-motile, and non-spore forming and grow luxuriantly on all culture media, generally forming opaque smooth, and glistering colonies. Since staphylococci bacteria produce catalase, the hydrogen peroxides that is formed as a metabolite under aerobic conditions, is not toxic to them, and most *Staphylococcus* bacteria grow best in the presence of oxygen. They grow best with temperature between 25°C and 35°C. *S. aureus* produce severe infections. The best known staphylococcal infections are those of the skin and superficial tissue of the body, where staphylococci cause boils, pustules, pimples, furuncles, abscesses, carbuncles and infections of accidental or surgical wounds. Features of skin infections may be influenced by the age of the patients, (Smith, 1985). Staphylococci are normal inhabitant of the humans skin, mouth, throat, and nose. They live in these areas without effect, but once past the barrier of the skin and mucous membrane, they penetrate the unbroken skin via the hair follicles and ducts of the sweats glands. Crucial to the spread of staphylococcal infection are direct, person to person contacts. Virulent organisms may also be passed to humans from livestock and household pets.

4.1.1.4. *Listeria monocytogenes*

Listeria monocytogenes is a small gram-negative, aerobic to microaerophilic, non-spore forming, and motile coccobacillus. The genus named after Lord Joseph Lister (1827-1912). The organisms are facultative anaerobes that produce a narrow band of β hemolysis when growing on blood agar plates, (Smith, 1985). It appears that *L. monocytogens* is primarily an animal pathogen, and it is likely that many human infections are acquired through contact with domestic animals and birds. *L. monocytogens*, however, has been isolated also from soil, water, sewage, and from the feces of a number of asymptomatic humans, rodents, swine, and poultry. Large numbers of *L.monocytogens* have also been found in raw milk obtained from infected cows, and when they are present in such high numbers, normal pasteurization procedures will not eliminate all of them, (Wesley, 1992). As a pathogen, *L. monocytogens* has two principal characteristics. It mainly affects adults who are immunosuppressed or pregnant or have cancer, and it has a special affinity for growth in the central nervous system and the placenta supplying the fetus with nutrients. Growth in the central nervous system is usually expressed as meningitis. When it infects a pregnant woman, the growth on the

placenta leads to a high rate of spontaneous abortion or stillbirth. A surviving newborn may be acutely ill with septicemia and meningitis, (Gerald *et al*., 1992).

4.1.1.5. *Clostridium*

Is a large genus of gram-positive, spore-bearing, anaerobes that are catalase negative. They are normally present in soil, some responsible for human and animal diseases, others are associated with food spoilage. Clostridia are classified according to the shape and position of the spores and by their physiological characteristics. There are different species of clostridium, *C. tetani, C. botulinum, C. perfrigens, C. novyi* and *C. septicum*, (Collins and Lyne, 1995). *C. prefrigens, C. novyi,* and *C. septicum* are the clostridia of gas gangrene. Gas gangrene is a highly fatal disease caused by the contamination of wounds with one or any combination of certain anaerobic, toxin-producing, spore-forming, gram-positive bacteria. Aerobes in the wound facilitate the germination of clostridia spores and their vegetative growth by reducing the oxidation-reduction potential.

4.2 Research objectives

The objectives were to:

- isolate and identify the microbial flora in the gut and the skin of fishes caught from the Mutale and the Luvuvhu rivers, Limpopo Province, South Africa. Special attention were given to detecting bacteria that can cause food borne illness in humans such as *Salmonella* spp*, Escherichia coli, Listeria* spp*, Clostridium perfringens* and *Staphylococcus areus*.
- identify bacterial organisms from detritus obtained from different sites in two rivers of the Limpopo Province, South Africa.

4.3 MATERIALS AND METHODS

4.3.1. Sampling sites

The Luvuvhu and Mutale rivers in the Limpopo Province of South Africa were used as sampling sites. The Luvuvhu River was sampled at Tshino in the Vuwani district of the Limpopo Province and the Mutale River was sampled at Mutshenzheni in the Mutale district of the Limpopo Province. Both rivers serve the rural communities as a primary source for food (fishing), drinking water, irrigation water, washing clothes and recreational activities.

4.3.2. Collection of samples

4.3.2.1. Detritus samples

The sampling site in each river was divided into a grid consisting of 2m x 2m blocks. Each block was assigned a number and a two litre water sample, containing detritus were collected in sterile plastic containers was collected from each block. This was placed on ice in a cooler bag, taken to the laboratory and analysed within six hours of collection.

4.3.2.2. Fish samples

Several *Chiloglanis pretoriae* fish samples were collected in the blocks, where the water samples were taken, by electro-narcotising tcollecting them downstream with scoop nets. The fish were placed in sterile plastic bags containing PBS (Phosphate Buffered Saline, pH 7) (Oxoid, SA) and kept on ice in a cooler bag.

4.3.3. Analysis of samples

4.3.3.1. Detritus

Under sterile conditions, a volume of 0.1ml of each detritus sample was transferred onto different types of media and streaked out to cover the entire surface of the agar plates. The plates were incubated at different temperatures depending on the type of media used and according to the manufacturer's specification. After the incubation period, colonies from each type of media were streaked onto Nutrient agar in order to confirm the isolate using standard identification tests.

4.3.3.2. Fish

All tests were carried out under sterile conditions in a laminar flow cabinet. The fish were shaken in the container with the PBS in order to get all the organisms from the skin of the fish. A volume of 0.1 ml of this PBS solution was then spread on different media and incubated according to manufacture's specifications. After incubation colonies from each of the media plates were transferred to Nutrient agar for identification. The fish was removed from the PBS solution and dissected. The gut of the fish and its contents was transferred to a 1 ml PBS solution, shaken and 0.1 ml transferred to different types of media.

4.3.4. Isolation of pathogenic microorganisms on different media

All media were made according to the manufacturer's instructions, and obtained from Merck (SA) and Oxoid (SA). All Petri plates (90 mm) were obtained from Sterilab (SA).

4.3.4.1. EMB agar for the isolation of *Escherichia coli*

In brief, 42 g of media were suspended in one liter of deionised water and dissolve by heating in a water bath. It was sterilized at 121°C for 15 minutes and poured into 90 mm Petri plates. All plates were kept at 4°C. For the isolation of *Eschericia coli*, 0,1 ml of either the detritus or the fish samples were spread onto the plates and incubated at 37°C for 24 h. Presumptive positive colonies were selected for further identification tests.

4.2.4.2. Deoxycholate citrate agar for the isolation of *Salmonella* spp

In brief, 52g of media were suspended in one liter of distilled water, boiled with constant stirring until dissolved, and poured into 90 mm Petri plates. All plates were kept at 4°C. For the isolation of *Salmonella*, 0,1 ml of either the detritus or the fish samples were spread onto the plates and incubated at 37°C for 24 h. Presumptive positive colonies were selected for further identification tests.

4.3.4.3. DNAse test agar for the isolation of *Staphylococcus areus*

In brief, 42g of media were suspended in one liter distilled water, boiled to dissolve completely, sterilized at 121°c for 15 minutes, and poured into 90 mm Petri plates. All plates were kept at 4°C. For the isolation of *Staphylococcus areus*, 0,1 ml of either the detritus or the fish samples were spread onto the plates and incubated at 37°C for 24 h. Presumptive positive colonies were selected for further identification tests.

4.3.4.4. *Listeria* selective media for the isolation of *Listeria* spp

In brief, 34.4g of media were suspended in 500 ml distilled water, sterilized by autoclaving at 121 °C for 15 minutes, and adding 1 vial Palean Listeria selective supplement. This was mixed and poured into 90 mm Petri plates. All plates were kept at 4°C. For the isolation of *Listeria*, 0,1 ml of either the detritus or the fish samples were spread onto the plates and incubated at 37°C for 24 h. Presumptive positive colonies were selected for further identification tests.

4.3.4.5. *Clostridium perfringens* selective media (OPSP) for the isolation of *Clostridium perfringens*

In brief, 22.8g of media were suspended in 500 ml distilled water and boiled in order to dissolve completely. It was sterilized at 121°c for 15 minutes, allowed to cool to 50 °C where after the contents of 1 vial each of Perfringens agar supplement A and B which have been rehydrated by the addition of 2 ml of sterile distilled water. The media was mix and poured into sterile 90 mm Petri dishes. All plates were kept at 4°C. For the isolation of *Clostridium perfringens*, 0,1 ml of either the detritus or the fish samples were spread onto the plates and incubated at 37°C for 24 h. Presumptive positive colonies were selected for further identification tests.

4.3.5. Identification of isolates

4.3.5.1. Gram Stain test

The method described by Collins and Lyne (1995) where used for all the isolates. Smears of isolates were prepared on the slides, air-dried and heat fixed. They were also labelled and placed on the staining rack. Slides were flooded with crystal violet and allowed to react for one minute. They were then handled with a forceps, tilted to a 45^{o} angle to drain the dye of the slides. While holding the slide at 45^{o} angle, it was rinsed immediately with a gently stream of water from wash bottle. The slides were then replaced on the staining rack and flooded with iodine, allowing iodine to react for one minute. With the forceps, the slides were tilted and allowed to drain and rinsed with water immediately. With the slides still held at a 45^{o} angle it was then decolorized quickly by allowing the acetone alcohol to run over and off the smear. Care was taken not to decolorize the slides too much. The slides were then rinsed with water to stop the decolorizing process and placed on the staining rack where it was flooded with safranin counter stain and allowed to react for 30 to 60 seconds. The slides were drained and rinsed thoroughly with water. The slides were then blotted carefully with paper towel and examined microscopically to distinguish the characteristic blue or pink- red colour (Collins and Lyne, 1995).

4.3.5.2. Oxidase test

Oxidase production test according to the method described by Brewer (1995) was performed. In brief, 24 hours culture of each isolate was made on Nutrient agar (Merck, SA). Few drops of Kovac's oxidase reagent were placed on a filter paper disk. A heavy mass of the isolates was streaked over the moist area using a sterile applicator. A change in colour from a deep

purple to almost black was taken as an oxidase positive reaction and no colour change was indicative of a negative oxidase test results.

4.3.5.3. Citrate test

The media was prepared according to the instructions of the manufacturer (Difco, SA). In brief, 58g of Simmons Citrate Agar was suspended in a litre of distilled water. The media was autoclaved and poured into test tubes to make a slant. Separate tubes of Simmons Citrate Agar slant was inoculated with each of the isolates. The tubes were incubated at 37^{O}C for 48 hours. After incubation, the tubes were observed for growth. A change from green to a royal blue colour indicated a positive reaction for the utilization of Citrate as a carbon source by the organism.

4.3.5.4. H_2S test

Kligler iron agar was prepared according to the manufacturers instructions (Merck, S.A). Aseptic stab inoculations of the iron agar were made using a sterile inoculation needle. Separate tubes for each isolate were used and marked clearly. One uninoculated tube was kept as a comparative control tube. The tubes were incubated at 37^{o}C for 48 hours (Cowan and Steel, 1993).

4.3.5.5. Indole test

Tryptophan broth (Merck, SA) was prepared according to the instruction of the manufacturer, poured into test tubes and autoclaved. One loopful of each of the isolates was inoculated into a separate tube of Tryptophan broth. One uninoculated Tryptophan broth tube was left as the control tube. The tubes where incubated at 37^{o}C for 48 hours. After incubation, a dropper full of Kovac's reagent was added to each tube, shaken gently from side to side and in the presence of indole, a deep red colour developed if the reaction was positive (Cowan and Steel, 1993).

4.3.5.6 Motility

The Motility agar test tubes were prepared according to the manufacture's instruction (Merck, S.A). The motility test shows the activity or movement of bacteria in the media. Each isolates were inoculated by stab method in a motility agar deep tube. The tubes were labelled clearly and incubated at 30^{o}C for 48 hours (Farmer, 1995).

4.4. RESULTS AND DISCUSSION

4.4.1. Microorganisms isolated from fish samples

A total of 95 fish were collected , of which 58 were collected in the Mutale River and 37 in the Luvuvhu River. Tables 4.1 and 4.2 indicate the amount of organisms isolated from the skins and the guts of the fish samples.

All bacteria, except *Listeria* spp., were isolated frequently from the skin of the fish caught in both sites. No *Listeria* isolates were cultured from any of the skin or gut samples of the fish from both rivers. Studies have shown that Gram negative bacteria can migrate from the intestine of fish to the edible portions in living fish within two hours after ingestion (Burras *et al.*, 1985). If people catch these fish in order to eat it, they could transmit these bacteria from the intestine to the edible fish parts through accidental cutting of the intestines (Depaola *et al*., 1994).

Table 4.1: Microorganisms isolated from *Chiloglanis pretoriae* fish samples in the Luvuvhu River (n = 37)

Microorganism	Number of skin samples positive (%)	Number of gut content samples positive (%)
E. coli	15 (41%)	14 (38%)
***Salmonella* spp**	10 (27%)	18 (49%)
C. perfringens	12 (32%)	14 (38%)
S. aureus	20 (54%)	13 (35%)
***Listeria* spp**	0 (0%)	0 (0%)

Table 4.2: Microorganisms isolated from *Chiloglanis pretoriae* fish samples in the Mutale River (n = 58)

Microorganism	Number of skin samples positive (%)	Number of gut content samples positive (%)
E. coli	47 (81%)	29 (50%)
***Salmonella* spp**	38 (66%)	36 (62%)
C. perfringens	36 (62%)	25 (43%)
S. aureus	36 (62%)	26 (45%)
***Listeria* spp**	0 (0%)	0 (0%)

4.4.2. Microorganisms isolated from detritus samples

The microbiological analysis of the detritus samples taken from the two rivers is shown in Figures 4.1 to 4.8. *Escherichia coli, Salmonella* spp, *Staphylococcus areus* and *Clostridium*

perfringens were found at both rivers during the study period. No *Listeria* spp were isolated during the study at any of the sites.

Several parameters measured in the two sites could assist in the survival of these potential pathogenic microorganisms in the detritus. Turbidity is a measure of the light scattering ability of water and indicated the concentration of suspended matter in water (DWAF, 1996). The turbidy levels in the river water samples exceeded the recommended South African guideline value of 0.1 NTU for domestic use (DWAF, 1996). The turbidity varied between 7 and 31 NTU for Tshino and 2 to 25 NTU for Mutale (Chapter 3). High turbidities affected the aesthetic (odour, colour, taste) quality of water and indicated potential disease transmission due to association of microorganisms with particulate matter in the water (DWAF, 1996). High turbidity in the river water could also assist in the survival and growth of potentially pathogenic microorganisms (DWAF, 1996).

Other parameters which could assist in the survival of the microorganisms in these two river sites included the type of substrate in which the detritus were found. Chapter 3 gives a clear indication of the substrates found in each of the grids used to collect detritus and included silt/clay, boulder, bed rock, cobble, gravel and sand. Unfortunately no relationship could be found with the substrate type and the presence of specific microorganisms. The only possible association that could be established is that oxygen could influence the prevalence of these organisms in the water sites.

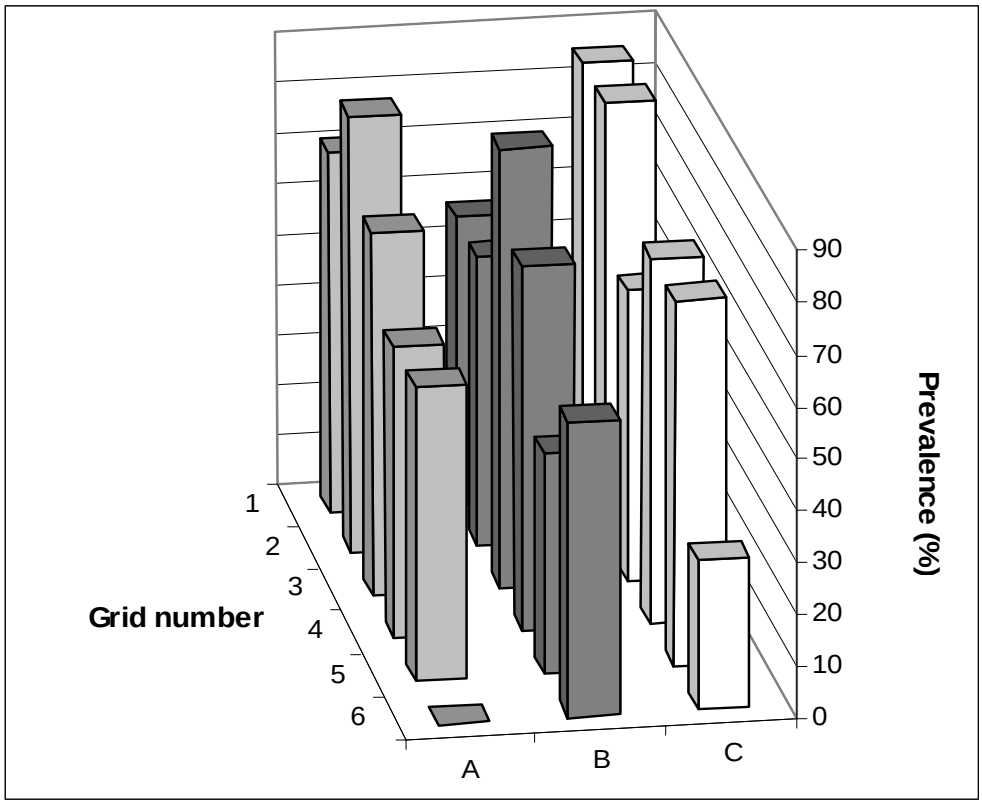

Figure 4.1: Prevalence of *Escherichia coli* in the Luvuvhu River.

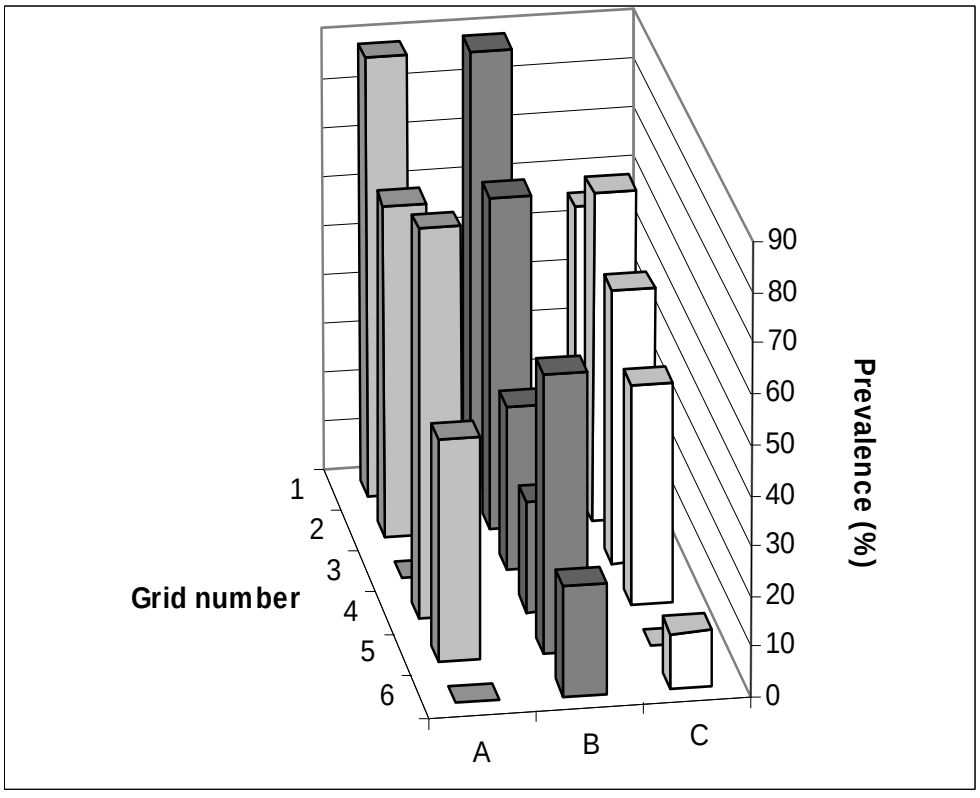

Figure 4.2: Prevalence of *Escherichia coli* in the Mutale River.

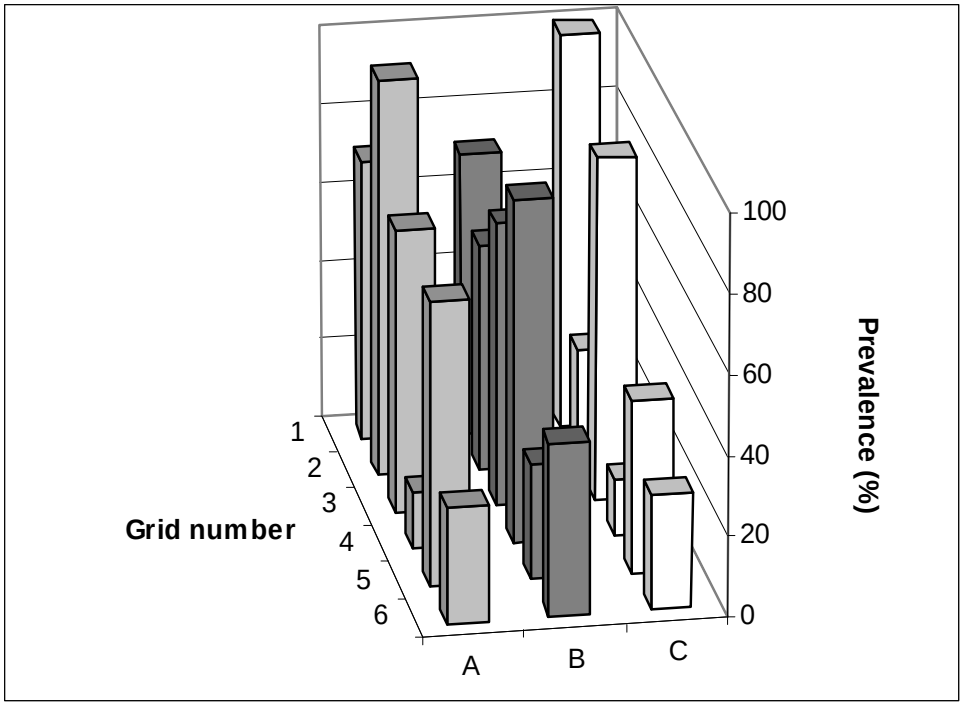

Figure 4.3: Prevalence of *Salmonella* spp in the Luvuvhu River.

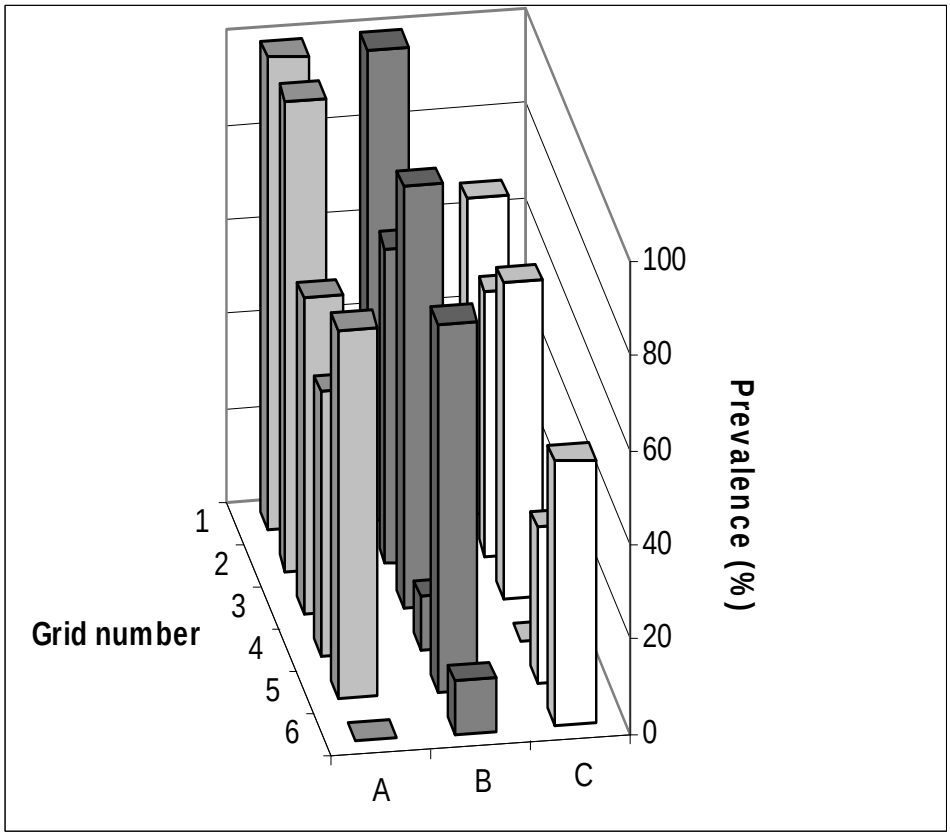

Figure 4.4: Prevalence of *Salmonella* spp in the Mutale River.

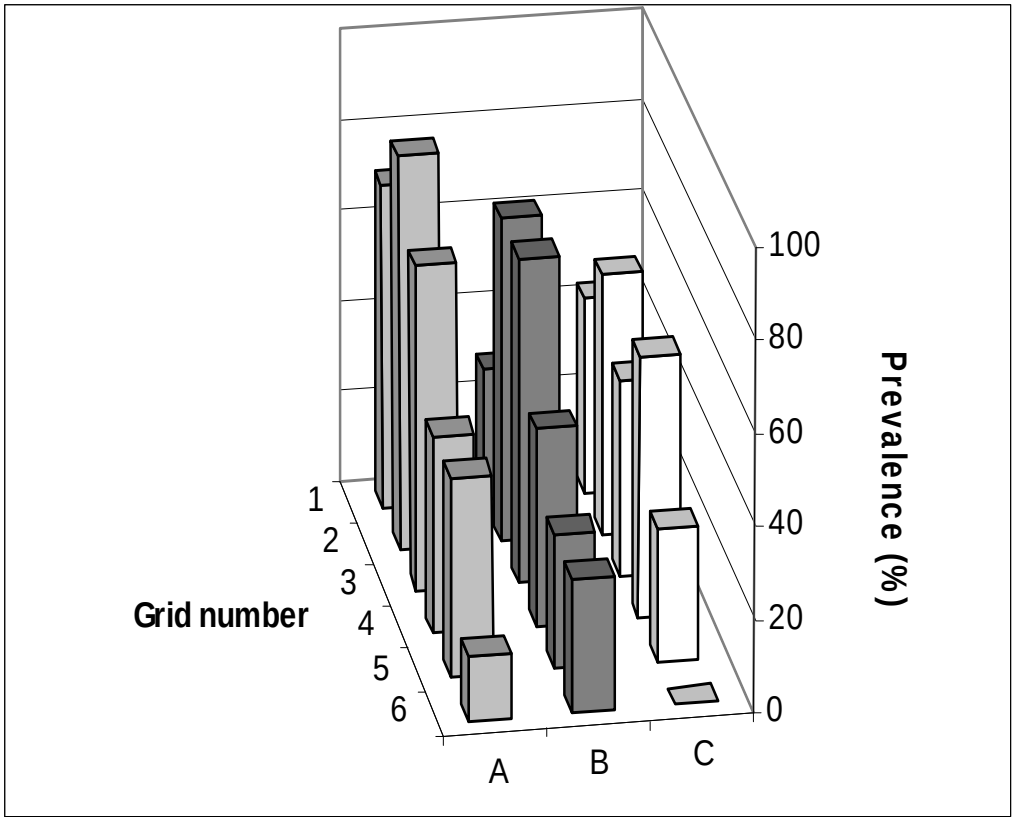

Figure 4.5: Prevalence of *Clostridium perfringens* in the Luvuvhu River.

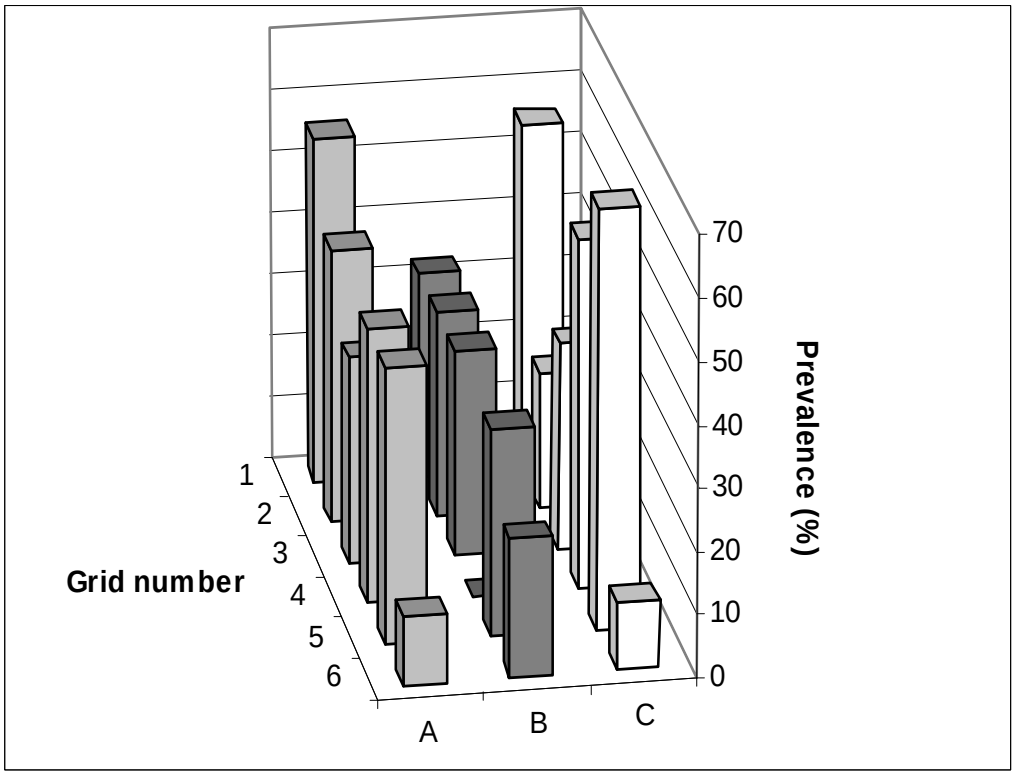

Figure 4.6: Prevalence of *Clostridium perfringens* in the Mutale River.

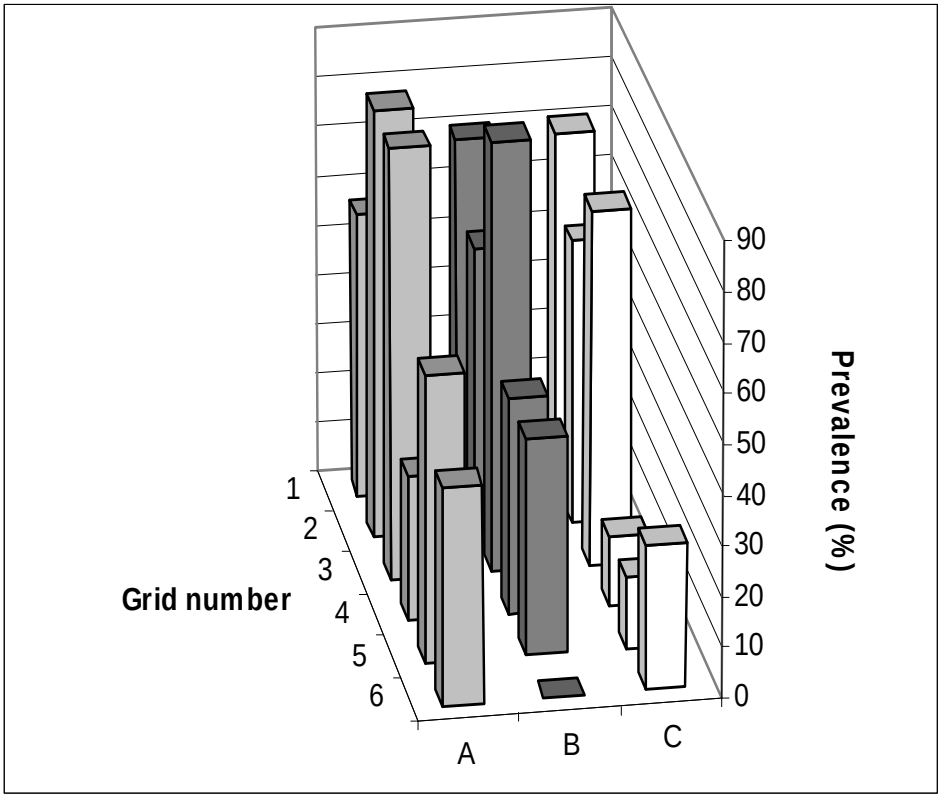

Figure 4.7: Prevalence of *Staphylococcus areus* in the Luvuvhu River

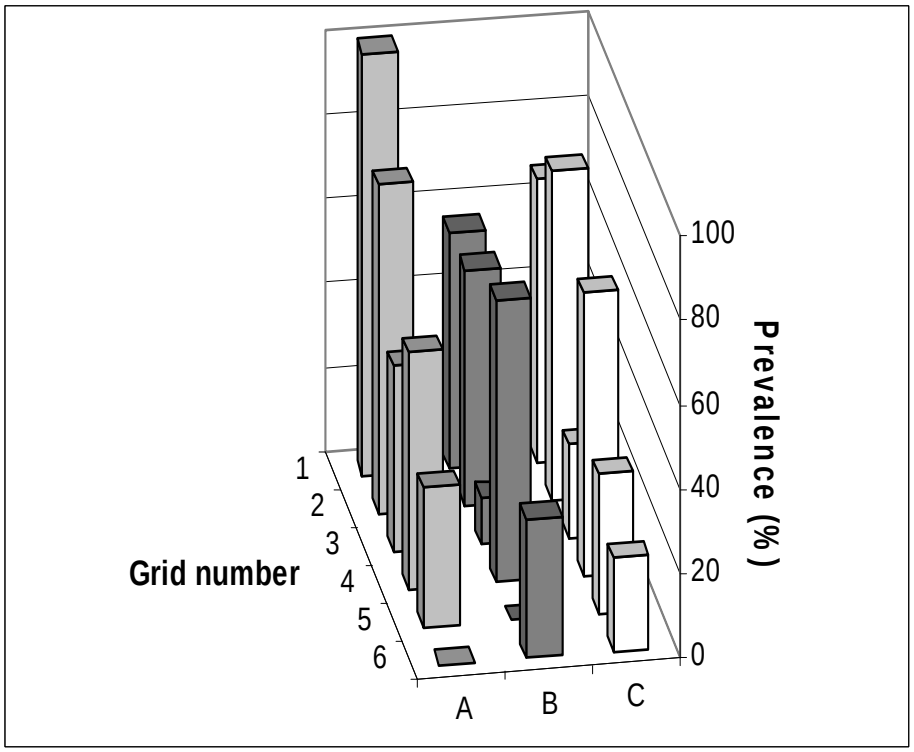

Figure 4.8: Prevalence of *Staphylococcus areus* in the Mutale River.

4.5. CONCLUSION

The distribution of *E. coli*, *Salmonella* spp, *S. areus*, *Clostridium* and *Listeria* spp was studied in fish and detritus samples from two rivers in the Limpopo Province of South Africa. Detritus was present in the gut content of *C. pretoriae* and although this species is not regarded as a detritivore (Fouche and Gaigher, 2001; Skelton 2001) detritus is probably ingested during the foraging process. The results showed a similar tendency in the prevalence of the organisms in both the gut content and the detritus samples of both the study groups and sites. The presence of high numbers of these pathogenic bacteria in the gut content of fish observed in this study has important ecological and public health implications. Consequently, the rural communities using these rivers should be educated on the risks involved with regards to the quality of the fish and the water they use. Another risk factor is the possibility of open wounds on the hands of the fishermen who come in contact with the skin and gut content of the fish they caught. The local rural communities use both these rivers as a source for domestic purposes and recreational activities and the presence of these potentially pathogenic microorganisms have serious implications for high risk individuals such as the very young, the very old and HIV and AIDS infected persons.

4.5. REFERENCES

BUFFALOE, N.D. and FERGUSON, D.V. 1981. *Microbiology*. 2nd edition, Houghton Mifflin Company, Boston.

BURRAS, N., DUEK, L. and NIV, S. 1985. Reactions of fish to microorganisms in waste water. *Applied and Environmental Microbiology* **50,** 989–995.

COLLINS and LYNES. 1995. *Microbiological methods.* 7th edition, British Library Cataloging Publisher.

DEPAOLA, A., CAPERS, G.M. and ALEXANDER, D. 1994. Densities of *Vibrio vulnificus* in the intestines of fish from the U.S. Gulf Coast. *Applied and Environmental Microbiology* **60,** 984–988.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY. 1996. *South African Water Quality Guidelines. Volume 2. Domestic use.* The Goverment Printer, Pretoria, South Africa.

FATTAL, B., DOTAN, A. and TCHORSH, Y. 1992. Rates of experimental microbiological contamination of fish exposed to polluted water. *Water Research* **26** 1621-1627.

FOUCHé, P.S.O. & GAIGHER, I.G. 2001.Niche differentiation in the rheophilic fishes of the Mutshindudi. In Gaigher, I.G. (Ed) "A Socio- biological study of the aquatic resources and their utilization in an underdeveloped rural region, the Mutshindudi River Catchment." *WRC project report 714/3/01,* 52 - 69.

GAIGHER, I.G. 1973. The habitat preferences of fishes from the Limpopo River system, Transvaal, Mozambique. *Koedoe* **16** 103-116.

GAIGHER, I.G. AND FOUCHé, P.S.O. 2001. An index of biotic integrity based on rheophilic fish in Gaigher, I.G. (Ed) "A Sociobiological study of the aquatic resources and their utilization in an underdeveloped rural region, the Mutshindudi River Catchment. *WRC project report 714/3/01,* 70 - 76.

GERALD, J.T., BERDELL, R.F. and CHRISTINE, L.C. 1992. *Microbiology*, 4th edition. The Benjamin\Cummimgs Publishing Company.

NEDOLULA, P.C. and WESTHOFF, D. 1993 Microbiological flora of aquatic Hybrid striped Bass. *Journal of Food Protection.* **56,** 1054-1060.

SKELTON, P.H. 2001. *A complete guide to the freshwater fishes of southern Africa.* Southern Book Publishers, South Africa.

SMITH, A.L. 1985. *Principles of Microbiology*, 10th edition. Mosby College Publishing, New York.

WESLEY, A.V. 1992. *Basic Microbiology,* 7th edition. Harpers Collins Publishers.

CHAPTER 5

MACROINVERTEBRATES

Macroinvertebrate assemblage structure and functional feeding group classification at two rheophilic biotopes in the Luvuvhu River catchment, Limpopo province South Africa.

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5.1 Introduction

The catchment of the Luvuvhu River is characterized by rapidly growing rural communities in its middle reaches and commercial farming in the upper reaches (WRC 2001). The rise in human population numbers will in future place increasing demands on resources of the riverine ecosystem through various subsistence activities such as washing, ploughing and wood collecting in the riparian zone. Pesticide usage and water extraction by upstream commercial farmers will further add to the degradation of this ecosystem's integrity.

When dealing with the deterioration of rivers, the organisms inhabiting these ecosystems could act as integrative measures of system stress. Macro-invertebrates are such a group of organisms that can be used to assess river perturbation. They are considered good indicators because they are relatively sedentary, viz. they enable detection of localized perturbations. They also have life histories which allow for the integration of pollution effects, qualitative sampling is easy, and since the communities are heterogeneous and several phyla are represented, the chances are high that at least some groups will respond to environmental impacts (Berkman *et al.*, 1986).

Effective ecosystem management can only be achieved if effects of various factors, either anthropogenic or natural, on ecosystem structure and functioning can be predicted. The search for causal mechanisms responsible for the decline in the integrity of an ecosystem often starts by searching for correlates of biological response to changes in environmental

variables. Biological assemblage structure and function have been useful in this regard, as they are sensitive to small disturbances and integrate the effects of these changes over the medium to long term (Chandler, 1970).

One model of lotic functioning, the River Continuum Concept (RCC), was proposed by Vannote *et al.* (1980). A prediction from the RCC, with specific relevance to the macroinvertebrates, is that longitudinal changes in the relative abundance of functional feeding groups (FFG's), food resources and stream size will occur (Vannote *et al*., 1980). FFG's represent the way in which lotic heterotrophs consume their food, be it living or dead. The RCC also predicts that as a stream widens in the middle reaches more light irradiates the surface, autochtonous production increases, resulting in an increase of grazers and decrease of shredders (Thorp & Covich, 1991).

The RCC concept has been criticized by Winterbourn *et al.* (1981) as not being applicable to the stochastic nature of Southern Hemisphere conditions. Palmer *et al*. (1991), did however find, in a study of various biotopes of the Buffalo River in the Eastern Cape, that shredders appear to be abundant in headwater and collectors of various types dominated the lower reaches. FFG classification can also vary significantly with life history stage, season and other ecological features complicating its application. Both King *et al.* (1988) and Palmer *et al.* (1991) stated that functional classification should be done with caution and include gut contents, behaviour, and morphological studies.

A three year study between 1999 and 2002 of the macroinvertebrate structure and function at two sites in the Luvuvhu River catchment afforded several opportunities *viz*. a) to study the effects and response of invertebrate taxa to a major flood in 2000 (Fig. 5.1), b) to investigate the effects of micro- and meso-scale environmental variables on macroinvertebrate assemblage structure and species populations, c) to determine which of these environmental variables best explain changes in biological assemblage structure and propose indicator taxa that best reflect changes in environmental variables and finally, d) to test some of the predictions of the RCC about ecosystem function.

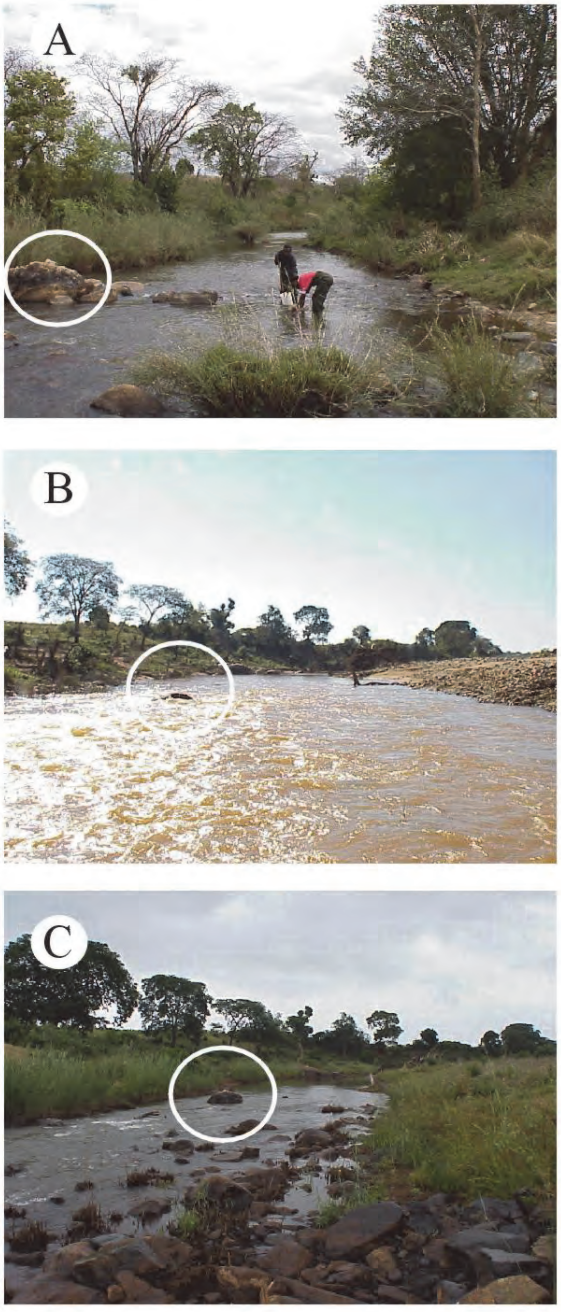

Figure 5.1. Impact of floods of February 2000 on riverine habitat at Tshino. A. 7 October 1999; B. 14 June 2000; C. 17 January 2001. Circle represents a reference point.

5.2. Materials and Methods.

Sampling protocol and substrate classification are detailed in Chapter 2. Physico-chemical data, as reported in chapter three, was determined and recorded. Within each of the $4m^2$ block a $0.1m^2$ box sampler with 100µm mesh was placed on the substratum, and the substratum disturbed 5cm into the substrate. In addition, all the cobbles and small boulders within the area of the box sampler were systematically brushed. The organisms were washed downstream by the current and collected into a jar at the closed end of the box sampler. After removal, the organisms were preserved in alcohol and sorted at a later stage. Specimens were identified up to family level and then classified into functional feeding groups (*sensu* Merrit & Cummins 1984) (Table 5.1).

Table 5.1. Explanation of functional feeding groups (FFG's).

FFG	Description
"shredders"	Organisms observed to shred leaves and which also have a preponderance of leaf fragment in their foregut and thus breaks up coarse particulate matter (CPOM)
"collectors"	Gather or filter CPOM and fine particulate matter (FPOM)
"scrapers"	Remove algae or aufwuchs growing on substrata
"predators"	

From Palmer *et al.* 1991

The multivariate method of non-metric multidimensional scaling (nMDS) (Kruskal & Wish (1978) in Primer v.5.2 (Plymouth Routines In Multivariate Ecological Research) was used to construct ordinations of matrices of the Bray Curtis similarities between each sampling occasion, and between samples in each $4m^2$ quadrat. Differences between factors such as sites were tested with ANOSIM (Clarke & Green 1988), a simple non-parametric permutation procedure applied to the rank similarity matrix of all sites. Evidence for successional changes in macroinvertebrate community structure was statistically tested with the procedure RELATE in Primer v.5.2 that test whether an index of multivariate seriation, defined as a Spearman correlation coefficient (ρ) is significantly

different from zero. A Spearman correlation of zero represents the null hypothesis of no discernable pattern of seriation.

For the purposes of the analysis two kinds of environmental variables were identified (1) meso-scale variables i.e. variables that varied between the sites but not within them (2) micro-scale variables i.e. variables that varied within sites. The effect of these two kinds of environmental variables on biological assemblages were analysed with the BIO ENV (Clarke & Ainsworth 1993) procedure in Primer v.5.2. This procedure is based on the principle that if the environmental variables structuring an assemblage were known, predicting species composition of assemblages, based on these variables, would be possible, i.e. key environmental variables would group sites similar to that of the biotic ordinations.

The ability of environmental variables to predict abundances of individual species were analysed with linear multiple regression in Statistica v. 5.1. As sampling efforts at each site were different, a random sample of twelve sampling units was selected for each of the sampling bouts. Total abundances were then calculated based on this revised data set. Only species that comprised more than 5% of the total abundance in each of the assemblages were analysed. Abundances were square root transformed to correct for heteroscedasticity.

5.3 Results

A total of 30 families and 4551 individuals were collected, three families were singletons and two families were doubletons (Appendix 1). ANOSIM suggested that there was no real difference between macroinvertebrate assemblage structure of Tshino and Mutale (Table 5.2). This is also evident when examining the ordination of this group (Fig. 5.2). The index of multivariate seriation (ρ) was significantly larger than zero ($\rho = 0.432$; $p<0.01$).

Table 5.2. One-way analysis of similarity (ANOSIM) testing for differences in macroinvertebrate assemblage structure between Mutale and Tshino.

	Global R	P	Significance level
Macroinvertebrates	0.135	0.1	ns

* = p<0.05

** = p < 0.01

Agreement between environmental variables measured at small scales and micro-geographic variation in biotic assemblages were very low, the highest correlation was 0.18 (Table 5.3). Combinations of environmental variables at this scale that were the best predictors of assemblage structure suggest that substrate played a larger role in macroinvertebrate assemblage structuring than water quality variables. Meso-scale environmental variables had a larger predictive power than micro-scale variables (Table 5.4) and assemblage composition at this level was best explained by pH, dissolved oxygen, minimum velocity, minimum depth and maximum depth.

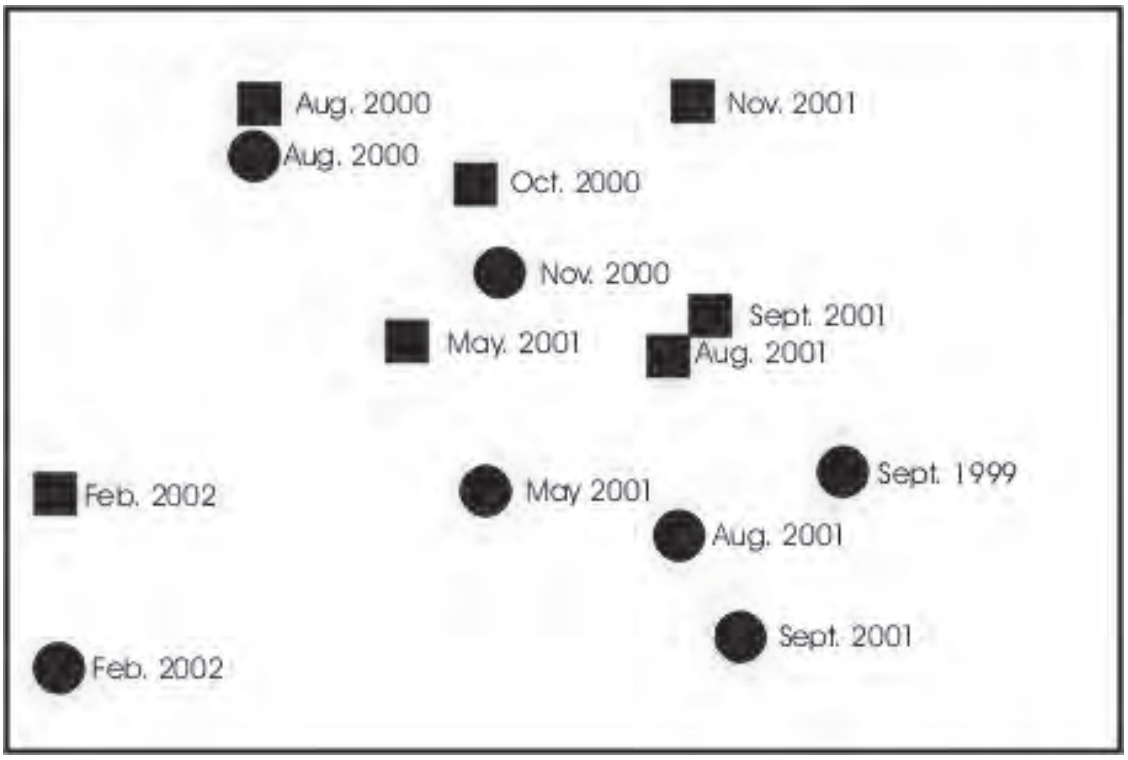

Figure 5.2. MDS of macroinvertebrate assemblages caught at Tshino and Mutale between 2000 and 2002, macroinvertebrate species data were totalled and root square transformed for each site and date; M = Mutale, T = Tshino, Dates: Day/Month/Year. Stress = 0.08.

Multiple regression of species abundances onto environmental variables resulted in only one significant regression, that of the macroinvertebrate family Leptophlebiidae. All environmental variables except temperature, turbidity and maximum depth were significant predictors of leptophlebid abundance (Table 5.5).

Table 5.3. BIO ENV of micro-scale environmental variables and biological assemblages; variables: 1 = velocity, 2 = depth, 3 = bedrock, 4 = boulders, 5 = cobbles, 6 = gravel, 7 = sand, 8 = mud.

MACROINVERTEBRATE ASSEMBLAGES		
Number of Variables	**Correlation**	**Selections of variables**
2	0.18	3,4
3	0.179	3,4,8
4	0.168	2-4,8
5	0.168	2-5,8
4	0.163	3-5,8
1	0.16	4
2	0.159	4,8
4	0.152	2-5
3	0.151	2-4
5	0.149	1,3-5,8

Table 5.4. BIO ENV of meso-scale environmental variables and biological assemblages; variables: 1 = pH, 2 = conductivity (μS), 3 = DO (%), 4 = DO mg/l, 5 = temperature, 6 = TSS mg/l, 7 = turbidity (NTU), 8 = minimum velocity, 9 = maximum velocity, 10 = minimum depth, 11 = maximum depth.

MACROINVERTEBRATE ASSEMBLAGES		
Number of Variables	**Correlation**	**Selections of variables**
4	0.422	1,4,8,10
4	0.419	4,8,10,11
3	0.419	4,8,10
3	0.403	4,8,11
3	0.384	1,4,8
2	0.38	4,8
5	0.376	1,4,8,10,11
4	0.372	1,4,8,11
4	0.348	4,8-10
3	0.337	4,8,9

Table 5.5. Summary of multiple regression analysis. Only those independent variables (environmental variables) that had a significant relationship with a dependant variable are listed.

Baetidae						
R= 0.98 R^2= 0.968 Adjusted R^2= 0.53						
F(11,1)=2.18 p<0.49 Std.Error of estimate: 0.4						
Chironomidae						
R= 0.93 R^2= 0.87 Adjusted R^2= -----						
F(11,1)=0.58 p<0.78 Std.Error of estimate: 0.79						
Heptageniidae						
R= 0.99 R^2= 0.97 Adjusted R^2= 0.69						
F(11,1)=3.44 p<0.4 Std.Error of estimate: 0.31						
Hydropsychidae						
R= 0.97 R^2= 0.94 Adjusted R^2= 0.23						
F(11,1)=1.33 p<0.6 Std. Error of estimate: 0.34						
Leptophlebiidae						
R= 0.99 R^2= 0.99 Adjusted R^2= 0.99						
F(11,1)=2001.3 p<0.02 Std. Error of estimate: 0.02						
		St. rr.		St. Err.		
	BETA	of BETA	B	of B	t(1)	p-level
Intercept			17.69	0.30	58.77	0.01
pH	-0.23	0.02	-0.30	0.02	-14.43	0.04
Conductivity (μS)	0.97	0.02	0.02	0.00	47.55	0.01
DO (%)	-1.24	0.02	-0.25	0.00	-56.73	0.01
DO (mg/l)	0.92	0.03	0.99	0.03	29.65	0.02
TSS (mg/l)	-1.12	0.03	-0.04	0.00	-34.52	0.02
Velocity (minimum)	0.26	0.01	1.28	0.06	21.09	0.03
Velocity (maximum)	0.53	0.01	1.33	0.03	38.24	0.02
Depth (minimum)	-0.39	0.02	-4.19	0.17	-24.59	0.03

Functionally both sites were dominated by collectors, while predators and scrapers comprised a very small percentage of the total individuals caught. Shredders were completely absent and there was a moderate but probably not significant, increase in scrapers at Tshino after the 2000 flood (Figure 5.3).

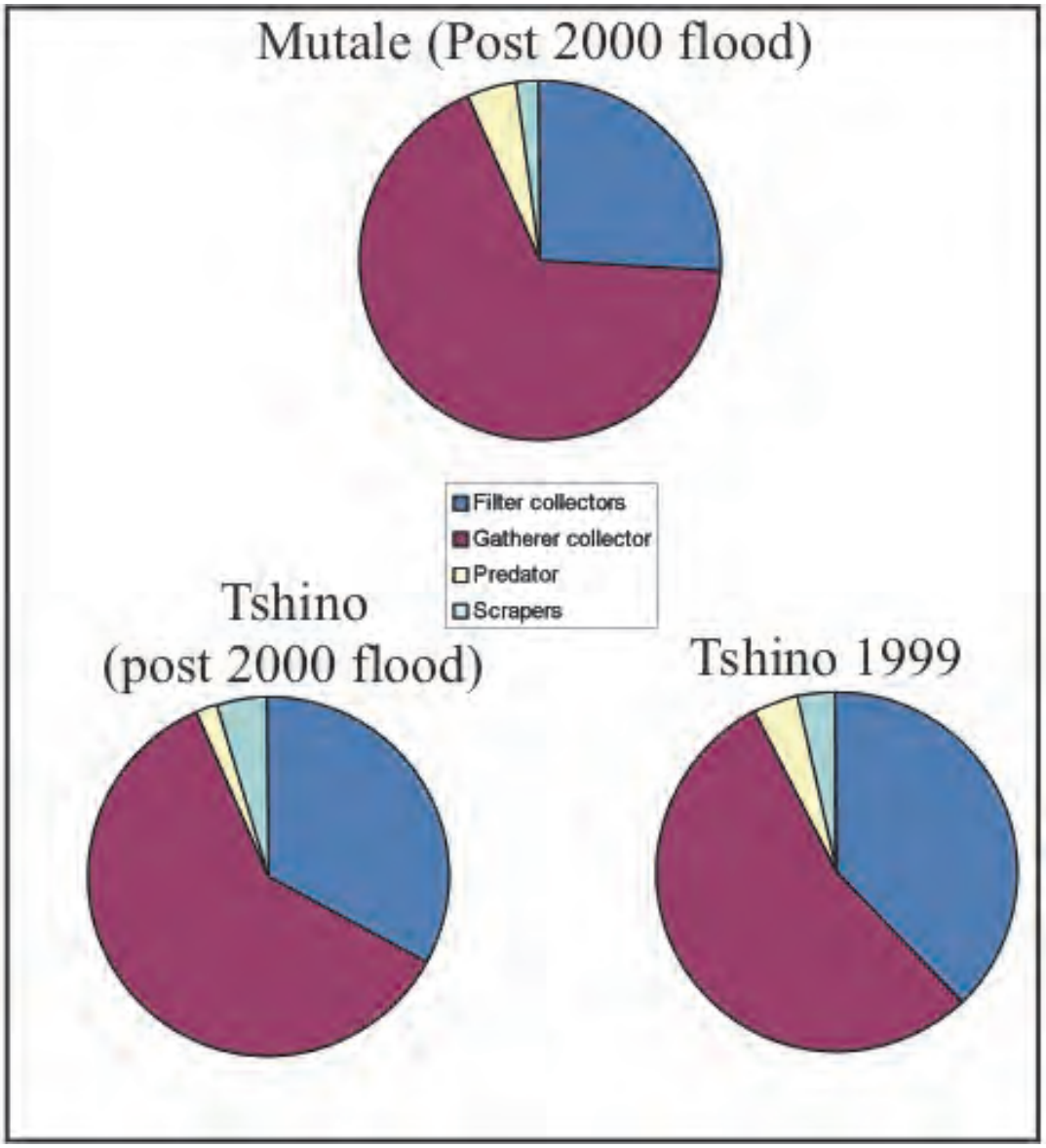

Figure 5.3. Relative abundance of FFG's at the two sites. Separate charts are drawn for Tshino before the floods (1999) and after the 2000 floods.

5.4 Discussion

Macroinvertebrate assemblages were not significantly different between the two sites (Table 5.2). Significant temporal seriation in the assemblage structure suggest a successional recovery in macroinvertebrate communities after the floods of 2000 (Fig. 5.2). Seasonal variation is also evidenced in samples that were taken in February 2002 at both sites (Fig. 5.3). Absence of structural differences between the two sites could be the result of taxanomic resolution.

Identification was only done up to family level, resulting in a loss of information. This coarse taxonomic analysis was however warranted, as this is the level at which water quality assessments (SASS5) are done. Habitat substrate i.e. the presence of boulders, cobbles, bedrock and mud played the most important role in invertebrate assemblage structuring at the small scale.

From the results obtained one macroinvertebrate family, Leptophlebiidae, was identified as a potentially good indicator of a whole suite of environmental variables (Table 5.5). This also corroborates the relatively high SASS5 score this family receives for its sensitivity to water quality. Functionally, the two sites agree with the predictions of the RCC, with collectors dominating the rheophilic sites in these middle reaches of the Luvuvhu and Mutale Rivers. The moderate increase in scrapers at Tshino could suggest the increase of algae or “aufwuchs” on the substrate (Fig. 5.3).

In conclusion, macroinvertebrate assemblages reflect a successional recovery after the major floods of 2000. Environmental variables detailing habitat structure were better predictors of assemblage structure of this group than water quality variables although one family, Leptophlebiidae, responded significantly to most environmental variables measured.

REFERENCES

BERKMAN, H.E., RABENI, C.F., and BOYLE, T.P. 1986. Biomonitors of stream quality in agricultural areas: fish versus invertebrates. *Environonmental Management* **10** 413-419.

CHANDLER, J.R. 1970. A biological approach to water quality management. *Water Pollution Control* **4** 415-422.

CLARKE, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal Ecology* **18** 117-143.

CLARKE, K.R. and AINSWORTH, M. 1993. A method of linking multivariate community structure to environmental variables. *Marine Ecological Progress Se*ries **92** 205-219.

CLARKE, K.R. and GREEN, R.H. 1988. Statistical design and analysis for 'biological effects' study. *Marine Ecological Progress Se*ries **46** 213-226.

KING, J.M., DAY, J.A., HURLEY, P.R., HENSHALL-HOWARD, M.P. and DAVIES B.R. 1988. Macroinvertebrate communities and environment in a Southern African mountain stream. *Canadian Journal of Fisheries and Aquatic Science* **45** 2168-2188.

KRUSKAL, J.B. and WISH, M. 1978. *Multidimensional Scaling*. Sage Publications, Beverly Hills, California.

MERRIT, R.W. and CUMMINS, K.W. 1984. *An Introdutcion to the Aquatic Insects of North America*. Kendall/Hunt Publishing Company, Dubuque, Iowa..

PALMER, C.G., O' KEEFFE, J.H. and PALMER, R.W. 1991. Are macroinvertebrate functional feeding groups in the middle and lower reaches of the Buffalo River, Eastern Cape, South Africa. I. Dietary variability. *Freshwater Biology* **29**.

STATSOFT, INC. 1998 *STATISTICA for Windows (Computer program manual)*. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK74104.

THORP, J.H. and COVICH, A.P. 1991. *North American Freshwater Invertebrates.* Academic Press Inc., San Diego.

VANNOTE, R.L., MINSHALL, G.W., CUMMINS, K.W., SEDELL, J.R. and CUSHING, C.E. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Science*, **37** 130-137.

WINTERBOURN, M.J., ROUNICK, J.S. and COWIE, B. 1981 Are New Zealand stream ecosystems really different*? New Zealand Journal of Marine and Freshwater Research* **15** 321-328.

Appendix 1. Abundances of macroinvertebrates caught in each quadrat. Quadrats with no records are excluded.

	M8/22/00A1	M8/22/00A2	M8/22/00A3	M8/22/00B1	M8/22/00B2	M8/22/00B3	M8/22/00B4	M8/22/00C1	M8/22/00C3	M8/22/00C4	M8/22/00D1	M8/22/00D2	M8/22/00E1	M10/17/00A1	M10/17/00A2	M10/17/00A3	M10/17/00A4	M10/17/00A5	M10/17/00B1	M10/27/00B2	M10/27/00B3	M10/27/00B4	M10/27/00B5	M5/24/01A1	M5/24/01A3	M5/24/01A6	M5/24/01B1	M5/24/01B2	M5/24/01B3	M5/24/01B4
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Baetidae	1	1	3	0	0	0	0	0	2	0	3	0	1	0	0	2	4	7	3	1	0	2	5	0	4	1	12	6	3	2
Caenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	5	2	5	1	4	5	0	7	3	2	0	1	0	14	4	7	8	4	3	2	13	7	9	2	3	1	3	1	0	0
Chlorocyphidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamonautidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Culicidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Gomphidae	1	0	0	0	0	0	1	0	0	0	3	0	1	3	0	0	0	2	0	0	0	2	0	3	0	0	3	0	0	0
Gyrinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Heptageniidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Hydrachnelli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae	2	1	0	0	0	0	1	0	0	0	3	3	11	0	1	4	3	8	2	0	0	0	4	0	19	0	1	9	1	9
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2	0	0	0	2	0	2
Libellulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligoneuridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	4	7	3	2	5	9	10	0	6	8	0	2	0	0	1	2	0
Sphaeridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	1	0	0
Trichorythidae	0	0	0	0	0	0	1	0	0	0	2	0	2	0	0	1	0	0	0	0	2	0	0	0	0	0	2	0	0	0
Veliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1. (cont.)

	M5/24/01B5	M5/24/01B6	M5/24/01C1	M5/24/01C2	M8/16/01A1	M8/16/01A3	M8/16/01A4	M8/16/01A5	M8/16/01B1	M8/16/01B2	M8/16/01B3	M9/20/01A2	M9/20/01A3	M9/20/01A4	M9/20/01A5	M9/20/01B2	M9/20/01B3	M9/20/01B4	M9/20/01B5	M11/1/01A4	M11/1/01A5	M11/1/01B2	M11/1/01B3	M11/1/01B4	M11/1/01B5	M11/1/01C2	M11/1/01C3	M2/7/02A1	M2/7/02A2	M2/7/02A4
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
Baetidae	2	1	6	10	13	11	10	11	7	0	4	3	20	23	2	11	3	21	15	1	10	4	13	3	2	1	0	0	0	1
Caenidae	0	0	1	0	0	0	0	0	1	0	0	2	1	0	0	1	0	2	0	0	0	0	1	1	2	2	1	0	0	0
Ceratopogonidae	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	4	3	0	0	3	0	0	0	0
Chironomidae	0	0	6	5	69	4	9	4	17	4	2	9	20	88	20	83	9	16	2	5	1	9	60	64	13	8	13	0	1	0
Chlorocyphidae	0	0	0	0	2	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamonautidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Culicidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	2	0	0	3	0	0	0	0
Gomphidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	2	1	0	1
Gyrinidae	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Heptageniidae	0	0	2	0	0	0	6	3	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	3	2	0
Hydrachnelli	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Hydropsychidae	0	0	9	6	18	20	0	17	5	11	14	17	2	4	0	5	0	2	28	5	1	2	10	5	1	0	15	3	4	4
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Leptoceridae	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Leptophlebiidae	0	0	0	0	2	4	2	8	1	0	2	1	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	1	0	0
Libellulidae	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Oligoneuridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	1	0	0	0	1	0	1	1	0	1	1	0	4	12	5	1	0	4	0	1	1	0	1	0	1	0	0	0	0	0
Sphaeridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipulidae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Trichorythidae	0	0	0	0	5	0	0	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Veliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

Appendix 1 (cont.)

	M2/7/02A5	M2/7/02B1	T8/29/00C1	T8/29/00C2	T8/29/00C3	T8/29/00C4	T8/29/00D1	T8/29/00D2	T8/29/00D3	T8/29/00D4	T11/17/00A1	T11/17/00A2	T11/17/00A3	T11/17/00A4	T11/17/00A5	T11/17/00A6	T11/17/00B1	T11/17/00B2	T11/17/00B3	T11/17/00B4	T11/17/00B5	T11/17/00B6	T5/10/01A1	T5/10/01A2	T5/10/01A3	T5/10/01A7	T5/10/01A8	T5/10/01B1	T5/10/01B2	T5/10/01B4
Aeshnidae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	1	0	2	3	3	5	4	1	0	1	8	10	9	4	2	0	1	3	4	12	1	0	1	4	0	14	2	0
Caenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	1	13	10	3	4	3	3	3	12	4	3	9	2	8	11	10	7	6	4	5	1	0	1	4	0	0	4	5
Chlorocyphidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamonautidae	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Culicidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphidae	0	1	0	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Gyrinidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heptageniidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	11	0	3	0	0
Hydrachnelli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae	1	2	0	2	2	0	0	7	3	2	5	9	7	6	0	0	0	6	3	2	1	0	1	0	3	9	1	16	2	2
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	2	3	0	9	0	0
Libuldae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Oligoneuridae	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	0	1	0	0	0	0	0	0	0	0	3	9	4	2	5	6	0	1	0	2	8	9	0	0	0	0	0	0	0	0
Sphaeridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Tipulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichorythidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2	0	0	0	1	0	0	0	1	0	5	0	0
Veliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 (cont.)

	T5/10/01B8	T5/10/01C1	T5/10/01C2	T5/10/01C4	T8/2/01A1	T8/2/01A2	T8/2/01A3	T8/2/01A4	T8/2/01A5	T8/2/01A6	T8/2/01A7	T8/2/01B1	T8/2/01B2	T8/2/01B3	T8/2/01B4	T8/2/01B5	T8/2/01B6	T9/27/01A2	T9/27/01A3	T9/27/01A4	T9/27/01A5	T9/27/01A6	T9/27/01B2	T9/27/01B3	T9/27/01B4	T9/27/01B5	T9/27/01B6	T9/27/01B7	T2/13/02A1	T2/13/02A2
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	2	0	0	0	0	0	0	1	0	2	2	0	0	0	0	0	0	0	0	1	0	2	0	2	1	0	0	0
Baetidae	2	16	6	6	8	4	2	3	0	8	3	7	12	23	1	8	5	1	8	5	15	8	7	26	11	14	26	4	0	0
Caenidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	1	0	2	1	1	5	6	0	5	7	2	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	1	2	1	0	16	2	4	0	0	1	1	33	2	3	3	5	7	5	6	1	0	0	26	12	5	5	4	1	0	0
Chlorocyphidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamonautidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Culicidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	12	0	0	0	0
Gomphidae	0	0	1	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Heptageniidae	0	1	3	1	1	3	3	0	0	0	3	6	14	1	0	4	0	1	0	6	0	0	3	4	1	1	4	1	1	3
Hydrachnelli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae	1	5	51	0	2	5	10	3	9	17	0	82	11	23	3	21	3	0	0	2	5	2	3	24	2	88	18	17	0	1
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae	1	3	2	4	7	22	3	0	0	19	2	43	22	14	0	0	2	1	18	18	15	0	6	69	6	37	64	44	0	0
Libellulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligoneuridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planaria	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	0	2	0	0	0	0
Simulidae	0	0	4	0	0	0	1	0	0	0	0	0	0	0	1	2	0	0	2	0	0	0	0	7	0	4	1	1	0	0
Sphaeridae	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	2	1	6	0	3	4	2	0	6	0	0	0
Tabanidae	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichorythidae	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Veliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 (cont.)

	T2/13/02A3	T2/13/02A7	T2/13/02B1	T2/13/02B2	T2/13/02B4
Aeshnidae	0	0	0	0	0
Athericidae	0	0	0	0	0
Baetidae	0	1	0	0	0
Caenidae	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0
Chironomidae	0	0	1	0	1
Chlorocyphidae	0	0	0	0	0
Potamonautidae	0	0	1	0	0
Culicidae	0	0	0	0	0
Elmidae	1	0	0	0	0
Gomphidae	0	0	0	0	0
Gyrinidae	0	0	0	0	0
Heptageniidae	1	0	0	0	0
Hydrachnelli	0	0	0	0	0
Hydropsychidae	5	4	0	0	1
Hydroptilidae	0	0	0	0	0
Leptoceridae	0	0	0	0	0
Leptophlebiidae	2	2	1	1	1
Libellulidae	0	0	0	0	0
Muscidae	0	0	0	0	0
Naucoridae	0	0	0	0	0
Oligoneuridae	0	0	0	0	0
Perlidae	0	0	0	0	0
Planaria	0	0	0	0	0
Simulidae	0	0	0	0	0
Sphaeridae	0	8	0	1	1
Tabanidae	0	1	0	0	0
Tipulidae	0	0	0	0	0
Trichorythidae	1	0	0	0	0
Veliidae	0	0	0	0	0

CHAPTER SIX

FISH

6.1 Subproject: The diversity, community structure and habitat preferences of the fish at the Mutale and Luvuvhu River sites.

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6.1.1 Introduction

Given the appropriate water quality, the number and distribution of fish in a river is primarily regarded to be controlled by the flow regime and associated factors such as timing, velocity, depth, substrate and cover. According to the microhabitat approach to riverine ecology, the longitudinal changes in the community composition could be a function of variables such as mean depth, mean velocity, water quality or the other characteristics exhibiting gradational changes. The species composition and structure of fish assemblages are also the culmination of a number of environmental influences that can be negatively affected by human activities in the catchment. Rheophilic sections of rivers are regarded as the most sensitive habitat and are affected by changes in flow long before any other habitat. Consequently the fish inhabiting these regions are regarded as flow sensitive and play an important role in indices such as the Fish Assemblage Integrity Index or FAII. Determination of habitat preference can be based on presence or absence of fish species in each habitat type without considering density (Gaigher, 1973). Johnson (1967) came to the same conclusion but found that species that had the highest frequency of occurrence for a certain habitat type also tended to be the most abundant in that specific habitat type.

This subproject intended to determine the relationship between habitat characteristics, pH, oxygen, turbidity, total suspended solids and conductivity, and aspects such as fish biodiversity, community structure and fish distribution. Furthermore it is intended to establish whether a relationship exists between microhabitat characteristics such as substrate, depth of water column, velocity and fish distribution and if this could be related

to the habitat preferences of the fish species. For long term survival of the fish stocks, sufficient recruitment is also essential and this project aimed to determine if sufficient recruitment was taking place.

6.1.2 Materials and methods

a) Field work

At both sites the river was divided into a grid consisting of $4m^2$ blocks. A rope was used to mark lines, two metres apart, across the width of the active channel. At two metre intervals along the rope metal stakes were driven in to demarcate the blocks. The lines were labeled alphabetically starting with A at the most downstream line. The blocks, along each line, were numbered starting with "1" on the right hand bank. In each of these blocks the fish were collected using electro-narcosis and scoop nets. The scoop nets were placed at the downstream boundary of each block and at completion of the shocking period the fish in the nets were identified and recorded as originating from block A1, A2, A3 etc.

The fish collected in each block were then placed on ice in a container, marked accordingly and transported to the laboratory.

For each block the physico-chemical data, as reported in Chapter Three, was determined and recorded. The dominant substrate types in each block were determined using the classification of the riparian substrate as suggested by Rowntree and Wadeson (2000) and recorded. In this classification: Boulders: > 256mm (larger than an adult head), Cobble: 64 – 256mm (larger than a fist), Gravel: 2 – 64 mm (small pea to size of fist), Sand: < 2mm (but individual grains still visible) and finally silt and clay. The maximum and minimum depths in each block were measured with a wooden metre rule and the minimum velocity in each block was determined with a Pasco Scientific velocity meter.
After all the blocks had been sampled a rapid, a riffle and pools were identified outside the grid and fish collected in each again using electro-narcosis. The fish collected in each biotope were then recorded separately and the sample fish placed in separate containers.

b) Temporal changes in fish biodiversity at the two sites.

To investigate whether changes in the biodiversity had occurred over the years the data collected during this survey will be compared with historical data such as the records of the Transvaal Provincial Administration and more recent data gathered by the Department of Environmental Affairs of the Limpopo Province.

c) Fish assemblage and population structure determination

In the laboratory the fish species were confirmed using the key provided by Skelton (2001) and the length and mass of each specimen determined. This recorded data was used to establish the assemblage composition and population structure of the fish.

Frequency of occurrence was determined for each survey. To characterize the fish populations of the two rivers, the data of all the surveys was pooled. With the exception of *Anguilla mossambica*, the fish were grouped into 5mm length classes and the frequency in each length class determined. The frequency was then plotted against the length classes. It was assumed that the collecting intensity of each survey was similar and that the samples were representative of the actual fish populations. No compensation was made for possible differences in the number of grids sampled or the number of grids that had suitable habitats for the various fish species during the surveys.

To determine the size structure of the populations of the more common species, the data for each survey was treated independently. The fish of a species were again grouped into 5mm length classes and the numbers recorded were then plotted against that length.

The multivariate method of non-metric multidimensional scaling (nMDS) (Kruskal & Wish (1978) in Primer v.5.2 (Plymouth Routines In Multivariate Ecological Research) as used to construct matrices summarising Bray-Curtis similarities between each survey, and between samples in each $4m^2$ quadrat. Differences between factors, such as sites, were tested with ANOSIM (Clarke & Green 1988) a simple non-parametric permutation procedure applied to the rank similarity matrix of all sites. Pearson product moment correlations (Shannon, Simpson, Pielou's evenness, species richness and total abundance

caught) were used to determine if there was a relationship between diversity indices and time.

d) Habitat preference determination and the environmental variables.

To illustrate habitat preference the frequency of occurrence of each species per habitat category was calculated. The habitat in each block was first classified into flow depth classes. Because of the variation in depth and velocity, it was decided to use the categories described for the Fish Assemblage Integrity Index (FAII) suggested by Kleynhans (1999). In these categories water velocity below 0,3 m/s is regarded as slow and 3m/s and above is regarded as fast. Water depth below 0,5 m is regarded as shallow whereas 0,5 meters and above is regarded as deep. On this basis the blocks were then classified into slow/shallow, slow/deep, fast/shallow and fast/deep. The number of each species collected per block was then recorded and frequency of occurrence per block expressed as percentage.

For the purposes of the statistical analysis two kinds of environmental variables were identified (1) meso-scale variables i.e. variables that differed between the sites but not within them and (2) micro-scale variables i.e. variables that vary within sites. The meso-scale variables would be the aspects that are referred to in the introduction as habitat characteristics whereas the micro-scale characteristics are in line with the microhabitat characteristics. The effects of these two kinds of environmental variables on biological assemblages were analysed with the BIO ENV (Clarke & Ainsworth, 1993) procedure in Primer v.5.2. This procedure is based on the principle that if the environmental variables structuring an assemblage were known, predicting the species composition of assemblages based on these variables would be possible i.e. key environmental variables would group sites similar to that of the biotic ordinations. The ability of environmental variables to predict abundance of individual species were analysed with linear multiple regression in Statistica v. 5.1. As sampling efforts at each site were different, a random sample of twelve sampling units was selected for each of the sampling bouts. Total abundance were then calculated based on this revised data set. Only species that

comprised more than 5% of the total abundance in each of the assemblages were analysed. Abundance was square root transformed to correct for heteroscedacity.

6.1.3 Results

a) General fieldwork

The results obtained in the blocks of the grid are shown in Tables 6.1.1 to 6.1.18. These tables not only show the species and numbers collected in each block but also show the habitat category, velocity and depth, and the substrate observed in the block. The results of the fish collection in the selected habitats are shown in Tables 6.1.19 to 6.1.24 and here the same parameters are shown with the exception that velocity was not measured in all instances and cover, other than substrate, is also included. The species and their applied abbreviations, or codes, are listed in Table 6.1.25. The variation and differences in habitat characteristics, depth, velocity and substrate, between and within sites are clearly illustrated in the tables. As stated in the methods this will be statistically analysed to determine relationships between habitat variables and fish distribution.

b) Temporal changes in fish biodiversity at the two sites.

The historic data displayed in Table 6.1.25 originates from the records of the Transvaal Provincial Administration, more recent data gathered by the directorate of Environmental Affairs of the Department of Finance and Economic Development, Limpopo Province and data collected during the 1999 River Health Programme (RHP) monitoring (WRC, 2001).

Table 6.1.1: Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 15/8/2000. The velocity is the maximum velocity and is expressed in m/s, the depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt and clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,30		0,99		1,06		0,9		0,76							
	Max depth	0,22		0,40		0,50		0,70		0,70							
	Substrate	BCGS		BGS		BC		BC		CGS							
	Fish	BNEE	1	AURA	1	No fish		AURA	2	CPRE	1						
		BVIV	1	BTRI	1			CPRE	1	LMOL	1						
				CPRE	2												
Row B	Velocity	0,48		0,90		0,99		1,22		0,61							
	Max depth	0,25		0,33		0,55		0,74		0,70							
	Substrate	BCGS		BCGS		BC		BC		BCGS							
	Fish	AURA	1	AURA	2	AURA	1	LMOL	1	AURA	1						
		CPRE	1	CPRE	2					CPRE	2						
		MMAC	1	LCYL	1												
Row C	Velocity	0,46		0,69		1,06		0,90		0,61							
	Max depth	0,28		0,52		0,46		0,70		0,78							
	Substrate	BCS		BCS		B		B		BS							
	Fish	BTRI	1	PCAT	1	No fish		CPRE	2	CPRE	1						
		MMAC	1	LCYL	1			LMOL	2	LMOL	1						
Row D	Velocity	0,45		0,60		0,90		0,95		0,60							
	Max depth	0,36		0,63		0,45		0,70		0,75							
	Substrate	BS		BGS		B		B		BS							
	Fish	BEUT	1	CPRE	2	BTRI	1	No fish		AURA	1						
		BNEE	1	LCYL	3					BEUT	1						
										LMOL	1						

Table 6.1.2: Habitat parameters and fish collected in the blocks at the Mutale (B) site in the Mutale River on 22/8/2000. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt and clay (M). The names of the fish are abbreviated as per accepted national code.

Row		1		2		3		4	
Row A	Velocity	1,20		0,60		0,45		0,00	
	Max depth	0,38		0,36		0,27		0,00	
	Substrate	BG		BCG		BG			
	Fish	AURA	1	CPRE	2	AURA	2	No water	
		LCYL	1			CPRE	1		
						PCAT	1		
Row B	Velocity	1,50		0,61		0,45		0,15	
	Max depth	0,41		0,27		0,33		0,09	
	Substrate	RC		RBCG		BCGS		CGS	
	Fish	LCYL	1	CPRE	2	AURA	1	BLIN	1
				LCYL	1	BTRI	2	BNEE	1
						CPRE	2	BVIV	1
						LCYL	2		
Row C	Velocity	1,36		0,61		0,30		0,30	
	Max depth	0,42		0,31		0,23		0,12	
	Substrate	RBCGS		BC		BCGS		CGS	
	Fish	CPRE	1	AURA	1	BTRI	2	AURA	2
				CPRE	1				
Row D	Velocity	1,06		0,75		1,2		0,30	
	Max depth	0,36		0,39		0,25		0,12	
	Substrate	BCGS		BC		BCGS		CS	
	Fish	AMOS	1	No fish		AMOS	1	LCYL	1
						BTRI	1		
Row E	Velocity	0,61		1,06		1,06		0,45	
	Max depth	0,40		0,44		0,30		0,12	
	Substrate	RBC		RBC		BCG		CS	
	Fish	AURA	1	CPRE	2	BTRI	1	AURA	1
				LCYL	2	CPRE	1		

Table 6.1.3: Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 17/10/2000. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt ant clay. The names of the fish are abbreviated as per accepted national code.

		Blocks							
Row		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Row A	Velocity	0,10	0,4	0,16	1,0	1,0			
	Max depth	0,15	0,32	0,50	0,59	0,55			
	Substrate	BCGS	BCG	BG	BC	BCG			
	Fish	No fish	AURA 2	No fish	CPRE 2	No fish			
			LCYL 1						
Row B	Velocity	0,15	0,50	0,67	0,67	0,67	0,5		
	Max depth	0,24	0,39	0,46	0,57	0,71	0,39		
	Substrate	BG	BCG	BG	BG	BG	BCG		
	Fish	No fish	AURA 1	No fish	CPRE 1	No fish	BEUT 1		
			CPRE 1				CPRE 2		
Row C	Velocity	0,25	0,29	0,67	0,67	0,4			
	Max depth	0,12	0,40	0,36	0,77	0,59			
	Substrate	BCGS	BCGS	BCGS	B	B			
	Fish	CPRE 1	LCYL 1	AURA 1	CPRE 1	PCAT			
				BTRI 1					
Row D	Velocity	0,40	0,50	0,50	1,0	0,67			
	Max depth	0,19	0,40	0,45	0,61	0,65			
	Substrate	BCGS	BCS	BCG	BCG	B			
	Fish	No fish	AURA 1	No fish	CPRE 1	No fish			
			LCYL 1						

Table 6.1.4: Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 24/5/2001. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt and cay (M) The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,33		0,44		0,80		1,0		0,47		0,59					
	Max depth	0,28		0,37		0,42		0,57		0,69		0,50					
	Substrate	BCS		BCS		BS		B		BC		BCS					
	Fish	BNEE	1	AURA	2	CPRE	1	CPRE	4	No fish		No fish					
				CPRE	2												
				OPER	1												
Row B	Velocity	0,37		0,48		0,50		0,80		0,67		0,57					
	Max depth	0,14		0,30		0,43		0,65		0,60		0,48					
	Substrate	BC		BS		BC		BGS		BS		BSM					
	Fish	AURA	3	AURA	1	CPRE	8	CPRE	1	CPRE	1	No fish					
		BNEE	1	CPRE	1												
		CPRE	1	LMAR	1												
Row C	Velocity	0,57		0,69		0,51		0,80		0,50		0,38					
	Max depth	0,18		0,25		0,50		0,52		0,78		0,40					
	Substrate	BCS		B		BCS		BC		BCS		BCSM					
	Fish	AURA	1	AURA	1	CPRE	3	CPRE	2	CPRE	1	AURA	1				
Row D	Velocity	0,37		0,83		0,34		1,.0		0,71		0,56					
	Max depth	0,36		0,48		0,44		0,58		0,40		0,64					
	Substrate	BS		BCS		BS		BC		BC		BCSM					
	Fish	AURA	1	AURA	1	No fish		CPRE	5	CPRE	1	No fish					
								LMOL	1								

Table 6.1.5: Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 16/8/2001. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt and clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,20		0,55		0,70		0,67		0,40							
	Max depth	0,15		0,35		0,42		0,6		0,55							
	Substrate	BCM		BCS		BC		CGS		BCGS							
	Fish	No fish		BNEE	1	CPRE	1	CPRE	2	No fish							
						LCYL	1										
Row B	Velocity	0,40		0,74		0,90		1,00		0,50							
	Max depth	0,15		0,35		0,51		0,35		0,44							
	Substrate	BCGS		BCGS		BCG		BCG		BCGS							
	Fish	No fish		AURA	2	AURA	1	AURA	1	CPRE	1						
				CPRE	4	CPRE	2										
				LCYL	1	LCYL	1										
				LMAR	2	LMAR	2										
Row C	Velocity	0,40		0,60		1,00		0,80		0,60							
	Max depth	0,30		0,40		0,28		0,45		0,44							
	Substrate	BCS		BCGS		BCG		BCG		BCG							
	Fish	No fish		LMOL	1	CPRE	4	AURA	1	CPRE	1						
Row D	Velocity	0,40		0,60		0,90		0,80		0,55							
	Max depth	0,36		0,35		0,31		0,4		0,27							
	Substrate	BCS		BCGS		BCG		BCG		B							
	Fish	BNEE	2	LCYL	1	CPRE	3	AURA	1	AMOS	1						
				LMAR	1			BNEE	1	BNEE	2						
								CPRE	3	PCAT	1						

Table 6.1.6 : Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 20/9/2001. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G) and Sand (S). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0		0,40		0,69		0,67		0,67							
	Max depth	0,15		0,30		0,40		0,52		0,46							
	Substrate	CGM		BCGSM		BCG		BCG		BCG							
	Fish	No fish		LMAR	2	CPRE	1	AURA	1	LCYL	1						
								CPRE	1								
Row B	Velocity	0,13		0,29		0,50		0,50		0,33							
	Max depth	0,12		0,32		0,27		0,41		0,45							
	Substrate	BCGS		BCG		BCG		BG		BGC							
	Fish	LMAR	1	AURA	1	AURA	1	AURA	1	AURA	1						
				CPRE	1	CPRE	2										
Row C	Velocity	0,15		0,29		0,50		0,50		0,33							
	Max depth	0,25		0,27		0,33		0,36		0,40							
	Substrate	BCGS		BCG		BC		BCGS		BCGS							
	Fish	AURA	2	LMAR	1	No fish		No fish		AURA	1						
		CPRE	4														
Row D	Velocity	0,15		0,30		0,50		0,52		0,30							
	Max depth	0,30		0,31		0,24		0,33		0,30							
	Substrate	BCGSM		BCG		BC		BCG		BCGS							
	Fish	No fish		CPRE	1	CPRE	2	AURA	1	No fish							
				LCYL	1	LCYL	1	LCYL	1								
								LMAR	1								

Table 6.1.7: Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 1/11/2001. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national convention.

		Blocks															
Row		**1**	**2**		**3**		**4**		**5**		**6**		**7**		**8**		
Row A	Velocity	0	0,30		0,36		0,50		0,30								
	Max depth	0	0,18		0,20		0,36		0,32								
	Substrate		CG		BCGS		BCGS		BCGS								
	Fish	No flow	AURA	1	CPRE	1	CPRE	1	No fish								
			BNEE	1													
			LCYL	1													
			LMAR	1													
Row B	Velocity	0	0,35		0,36		0,45		0,31								
	Max depth	0	0,14		0,25		0,29		0,38								
	Substrate		BCGS		BCGS		BCGS		BCGS								
	Fish	No fish	AURA	1	CPRE	3	AURA	1	LCYL	1							
			LCYL	1			CPRE	1									
			LMAR	1													
Row C	Velocity	0,10	0,24		0,25		0,67		0,23								
	Max depth	0,16	0,15		0,19		0,30		0,27								
	Substrate	CGS	BCS		CGS		BCGS		BCGS								
	Fish	No fish	No fish		BNEE	1	AURA	1	No fish								
					CPRE	1	CPRE	1									
Row D	Velocity	0,20	0,29		0,35		0,50		0,30								
	Max depth	0,25	0,23		0,30		0,20		0,25								
	Substrate	CGS	BCG		BCG		BCG		CGS								
	Fish	No fish	LMAR	1	CPRE	1	No fish		No fish								

Table 6.1.8: Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 7/2/2002. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national convention.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,80		1,00		1,33		1,00		0,80		0,67					
	Max depth	0,29		0,55		0,50		0,82		0,62		0,45					
	Substrate	CGS		BC		BCG		BCG		BCG		CGS					
	Fish	BNEE	1	CPRE	1	CPRE	6	CPRE	2	CPRE	1	AURA	1				
		CPRE	1														
Row B	Velocity	0,75		0,90		1,20		1,00		0,90		0,65					
	Max depth	0,31		0,54		0,50		0,70		0,65		0,37					
	Substrate	CGSM		BCG		CG		BCG		BCGS		GSM					
	Fish	AURA	3	CPRE	1	AURA	1	CPRE	2	No fish		No fish					
		BLIN	1			CPRE	2										
		CPRE	3														
Row C	Velocity	0,65		0,95		1,0		1,0		0,90		0,65					
	Max depth	0,43		0,55		0,43		0,56		0,59		0,37					
	Substrate	CGS		BCG		BCGS		BCGS		BCG		CS					
	Fish	CPRE	3	AURA	1	No fish		CPRE	4	CPRE	1	No fish					
				CPRE	3												

Table 6.1.9: Habitat parameters and fish collected in the blocks at the Mutale (A) site in the Mutale River on 14/5/2002. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**	**2**		**3**		**4**		**5**		**6**		**7**		**8**		
Row A	Velocity	0	0,15		0,33		0,36		0,33								
	Max depth	0	0,20		0,28		0,29		0,28								
	Substrate		BCG		BCS		BC		BCS								
	Fish	No water	AURA	1	AURA	1	AURA	1	LMAR	1							
			LMAR	2	CPRE	5	BTRI	1									
					LMAR	1	LMOL	1									
Row B	Velocity	0	0,10		0,30		0,40		0,33								
	Max depth	0,02	0,15		0,26		0,30		0,34								
	Substrate	S	BCS		BCGS		BCS		BCS								
	Fish	No fish	BLIN	1	CPRE	3	AURA	1	CPRE	1							
			BNEE	1	LMAR	1	CPRE	3	LMAR	1							
			LMAR	1	LCYL	1	LMAR	1									
Row C	Velocity	0	0,10		0,33		0,42		0,33								
	Max depth	0,09	0,25		0,18		0,39		0,23								
	Substrate	BC	BCS		BCS		BCGS		B								
	Fish	No fish	AURA	2	AURA	2	AURA	2	AURA	1							
			CPRE	4	BNEE	1	CPRE	13	CPRE	9							
			LMAR	3	CPRE	8			LMAR	3							
			LMOL	1	LMAR	2			LMOL	1							

Table 6.1.10 : Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 8/8/2000. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks							
Row		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Row A	Velocity	0,34	0,57	0,57	0,57	0,80	1,00	1,50	0,10
	Max depth	0,20	0,45	0,61	0,75	0,90	0,90	0,80	0,15
	Substrate	BCGSM	BCGS	BCG	BC	BC	BC	BC	BCM
	Fish	No fish	No fish	LCYL 1	No fish	Current too strong /deep to collect fish			AURA 2
									CPRE 4
Row B	Velocity	0,40	0,40	0,40	0,50	0,67	0,85	1,50	0,1
	Max depth	0,20	0,41	0,65	0,80	0,92	0,80	0,65	0,20
	Substrate	BCGSM	BCGS	BCG	BC	BC	BC	BC	BCGM
	Fish	No fish	No fish	No fish	AURA 2	Current too strong /deep to collect fish			No fish
Row C	Velocity	0,40	0,40	0,40	0,50	0,67	1,00	1,80	0,33
	Max depth	0,15	0,45	0,49	0,88	0,80	0,80	0,66	0,05
	Substrate	BC	BC	BCS	BCG	BC	BC	BC	BCGSM
	Fish	CPRE 1	No fish	No fish	No fish	Current too strong /deep to collect fish			No fish
Row D	Velocity	0,33	0,40	0,40	0,67	1,00	1,00	2,00	0,50
	Max depth	0,14	0,33	0,55	0,60	0,80	0,77	0,32	0,26
	Substrate	BCGSM	BCS	BC	BCG	BCG	BC	BCG	BCGSM
	Fish	CPRE 1	CPRE 1	CPRE 2	No fish	Current too strong /deep to collect fish			No fish
Row E	Velocity	0,33	0,5	0,5	0,67	0,67	1,00	1,80	0,40
	Max depth	0,15	0,30	0,62	0,57	0,77	1,00	0,55	0,15
	Substrate	BCGSM	BCG	BCG	BC	BC	BC	BC	BCG
	Fish	No fish	No fish	No fish	No fish	Current too strong /deep to collect fish			No fish
Row F	Velocity	0,30	0,40	0,40	0,50	1,00	1,00	1,8	0,67
	Max depth	0,25	0,30	0,52	0,59	0,80	0,80	0,60	0,15
	Substrate	BCGS	BCGS	BCG	BC	BC	BC	BC	BCGSM
	Fish	CPRE 1	No fish	No fish	No fish	Current too strong /deep to collect fish			AURA 2
									CPRE 4

Table 6.1.11: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 29/8/2000. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks							
Row		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Row A	Velocity	0,30	0,50	0,55	0,55	0,80	1,00	0,50	
	Max depth	0,15	0,40	0,56	0,70	0,85	0,85	0,58	
	Substrate	BCGSM	BCGS	BCG	BC	BC	BC	BC	
	Fish	CPRE 1	No fish	No fish	CPRE 2	Current too strong/deep		CPRE 1	
					LMOL 2				
Row B	Velocity	0,35	0,35	0,35	0,44	0,67	1,30	1,30	
	Max depth	0,20	0,40	O,60	0,75	0,88	0,75	0,60	
	Substrate	BCGSM	BCGS	BCG	BC	BC	BC	BC	
	Fish	CPRE 2	No fish	No fish	No fish	Current too strong/deep		No fish	
Row C	Velocity	0,33	0,36	0,40	0,60	1,59	1,00	1,00	
	Max depth	0,10	0,40	0,45	0,83	0,75	0,75	0,62	
	Substrate	BC	BC	BCS	BCG	BC	BC	BC	
	Fish	No fish	CPRE 2	No fish	LMOL 1	Current too strong/deep		No fish	
Row D	Velocity	0,45	0,45	0,33	0,76	1,82	1,00	0,50	
	Max depth	0,10	0,28	0,50	0,55	0,75	0,72	0,20	
	Substrate	BCGSM	BCS	BC	BCG	BCG	BC	BCG	
	Fish	No fish	No fish	LMOL 2	CPRE 4	Current too strong/deep		No fish	
Row E	Velocity	0,31	0,76	0,15	0,91	1,36	1,00	0,50	
	Max depth	0,10	0,25	0,57	0,52	0,72	0,95	0,51	
	Substrate	BCGSM	BCG	BCG	BC	BC	BC	BC	
	Fish	CPRE 1	LMOL 1	No fish	No fish	Current too strong/deep		No fish	
Row F	Velocity	0,36	0,50	0,36	0,76	1,35	1.20	0,40	
	Max depth	0,20	0,20	0,45	0,54	0,75	0,74	0,55	
	Substrate	BCGS	BCGS	BCG	BC	BC	BC	BC	
	Fish	CPRE 1	CPRE 1	CPRE 1	CPRE 1	Current too strong/deep		No fish	
					LMOL 1				

Table 6.1.12.: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 7/11/2000. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,17		0,35		0,33		0,70		1,00		0,70		0,20			
	Max depth	0,20		0,36		0,56		0,56		0,69		0,68		0,45			
	Substrate	CGS		CGS		BCGS		BCGS		BCG		BC		BG			
	Fish	CPRE	1	LMAR	1	AURA	1	CPRE	1	AURA	2	CPRE	2	AURA	1		
		MBRE	1			CPRE	2			CPRE	1			CPRE	6		
Row B	Velocity	0,20		0,30		0,33		0,70		1,00		0,70		0,22			
	Max depth	0,27		0,53		0,57		0,74		0,78		0,82		0,52			
	Substrate	BCGS		BCGS		BC		BCGS		BCGS		BCGS		BSM			
	Fish	CPRE	1	CPRE	5	CPRE	3	CPRE	5	AURA	1	AURA	1	No fish			
								MACU	1	CPRE	10	CPRE	4				
												MACU	1				
Row C	Velocity	0,17		0,33		0,40		0,67		1,00		0,67		0,22			
	Max depth	0,23		0,50		0,55		0,74		0,77		0,80		0,50			
	Substrate	BCGS		BCGS		BCGS		BCG		BCS		BCS		BCS			
	Fish	AURA	1	No fish		AURA	1	CPRE	3	CPRE	3	CPRE	2	No fish			
						BTRI	1					LCYL	1				
						CPRE	1										
						MBRE	1										

Table 6.1.13: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 10/5/2001. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,52		0,67		0,83		0,71		1,05		1,67		1,60		0,65	
	Max depth	0,30		0,52		0,61		0,75		0,77		0,68		0,90		0,57	
	Substrate	BGS		BG		BCG		BC		BC		BGS		BCG		CGSM	
	Fish	BTRI	1	CPRE	3	No fish		CPRE	3	CPRE	5	No fish		CPRE	2	CPRE	1
										LMOL	1					LMAR	1
Row B	Velocity	0,30		0,50		0,80		0,70		1,00		1,70		1,50		0,60	
	Max depth	0,25		0,33		0,65		0,68		0,55		0,63		0,53		0,35	
	Substrate	CG		BCG		BC		BC		BCG		BG		BCGS		CGSM	
	Fish	CPRE	1	No fish		No fish		CPRE	5	CPRE	8	CPRE	1	No fish		No fish	
Row C	Velocity	0,50		0,55		0,70		0,70		1,00		1,67		1,60		0,63	
	Max depth	0,32		0,28		0,65		0,60		0,77		0,80		0,68		0,30	
	Substrate	CGS		BCG		BC		BCG		BC		BCG		CG		SM	
	Fish	CPRE	1	CPRE	2	AURA	1	No fish						CPRE	3	No fish	
						CPRE	4										

Table 6.1.14: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 2/8/2001. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,42		0,42		0,67		0,75		0,75		0,86		0,75			
	Max depth	0,25		0,35		0,55		0,62		0,49		0,54		0,54			
	Substrate	BCGS		BCG		BC		BCG		BCG		BCG		CGS			
	Fish	LMAR	1	No fish		CPRE	3	CPRE	5	CPRE	2	CPRE	1	CPRE	3		
								LCYL	1					LCYL	1		
Row B	Velocity	0,40		0,45		0,70		0,72		0,75		0,90		0,70			
	Max depth	0,12		0,40		0,55		0,55		0,53		0,60		0,52			
	Substrate	BCGS		BCGS		BCG		BC		BC		BC		BC			
	Fish	No fish		CPRE	1	CPRE	3	CPRE	3	AURA	1	CPRE	7	CPRE	9		
										CPRE	7						
Row C	Velocity	0,35		0,42		0,67		0,72		0,72		0,86		0,70			
	Max depth	0,15		0,38		0,40		0,51		O,70		0,69		0,55			
	Substrate	BCGS		BCGS		BCGS		BCS		BCG		BC		CGS			
	Fish	CPRE	1	LMAR	1	CPRE	2	CPRE	1	AURA	1	No fish		No fish			
								LCYL	1								

Table 6.1.15: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 27/9/2001. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0		0,42		0,80		0,60		1,00		1.00		0,60		0,30	
	Max depth	0		0,29		0,39		0,50		0,47		0,50		0,46		0,22	
	Substrate	C		BC		BC		BC		BCGS		BCGS		BCG		BCGS	
	Fish	No fish		BTRI	1	CPRE	2	No fish		CPRE	4	LMAR	1	CPRE	10	CPRE	1
				LCYL	2					LMAR	1						
Row B	Velocity	0		0,20		0,83		0,51		1,00		0,57		0,80		O,20	
	Max depth	0,O		O,15		0,43		0,47		0,36		0,46		0,36		0,27	
	Substrate	BCGS		BCGS		BCG		BCS		BCG		BCS		BGS		GS	
	Fish	No fish		AURA	1	CPRE	13	CPRE	3	AURA	1	CPRE	9	CPRE	7	No fish	
				CPRE	1	LMAR	1	LMAR	1	CPRE	11	LMAR	1	LCYL	1		
Row C	Velocity	0,20		0,27		0,52		0,80		1,00		0,67		0,50		0	
	Max depth	0,05		0,02		0,31		0,32		0,49		0,34		0.43		0	
	Substrate	BCGSM		BCGSM		BCG		BCG		BCS		BCG		BC		M	
	Fish	CGAR	1	BVIV	1	AURA	1	CPRE	9	CPRE	10	CPRE	18	AURA	1		
				CPRE	2	CPRE	6	LMOL	1					CPRE	32		
						LMAR	1							LMAR	1		
Row D	Velocity	0		0,10		0,49		0,80		0,76		0,60		0,52		0	
	Max depth	0,07		0,16		0,28		0,47		0,56		0,43		0,60		0	
	Substrate	BCGSM		BCGM		GS		BCG		BCG		BS		GS		M	
	Fish	No fish		No fish		CPRE	8	CPRE	6	AURA	1	No fish		CPRE	1		
								LMOL	3					LMAR	1		
														LMOL	2		

Table 6.1.16: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 31/10/200. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

Row		1		2		3		4		5		6		7	
Row A	Velocity	0,28		0,67		0,50		0,57		0,70		0,71		0,71	
	Max depth	0,20		0,36		0,46		0,45		0,42		0,43		0,31	
	Substrate	CGS		CG		BC		BCG		BCGS		BCG		BGSM	
	Fish	BLIN	1	CPRE	5	BLIN	1	CPRE	3	No fish		CPRE	11	CPRE	6
		LMAR	1			CPRE	2								
Row B	Velocity	0		0,67		0,50		1,00		0,70		0,87		1,00	
	Max depth	0,04		0,31		0,33		0,42		0,35		0,42		0,38	
	Substrate	BGS		BCGS		BCGS		BC		BCG		BCG		GSM	
	Fish	CPRE	1	CPRE	8	AURA	1	CPRE	8	LMAR	1	CPRE	10	AURA	1
						CPRE	9	LMAR	1					CPRE	3
Row C	Velocity	0		0,67		0,50		0,87		0,50		0,67		0,33	
	Max depth	0,15		0,36		0,32		0,46		0,46		0,35		0,21	
	Substrate	GSM		BCG		BCG		BCG		BC		BCG		GS	
	Fish	No fish		CPRE	3	CPRE	4	CPRE	12	CPRE	6	CPRE	13	No fish	
				LMAR	1	MBRE	1	LMAR	3			LMOL	1		
Row D	Velocity	0,29		0,67		0,50		0,70		0,74		1,00		0	
	Max depth	0,24		0,31		0,41		0,52		0,50		0,50		0	
	Substrate	BCGS		BCG		BCG		BCG		BCS		BCSM		0	
	Fish	CPRE	1	CPRE	6	CPRE	4	CPRE	7	CPRE	5	CPRE	3	No water	
				LMAR	1	LMAR	1			LMAR	1				

Table 6.1.17: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 13/02/2002. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
Row A	Velocity	0,50		0,53		0,69		0,53		0,63		0,57		0,53			
	Max depth	0,39		0,55		0,60		0,65		0,66		0,62		0.30			
	Substrate	BCG		BCGS		BCG		BC		BC		BCG		SM			
	Fish	No fish		BTRI	1	No fish		CPRE	2	LMAR	1	CPRE	4	LMAR	1		
				LMAR	1			LMAR	1			LMAR	1				
Row B	Velocity	0,28		0,55		0,50		0,80		0,70		0,74		0,80			
	Max depth	0,25		0,42		0,47		0,47		0,54		0,57		0,49			
	Substrate	CGS		BCG		BCG		BC		BCG		BCG		GS			
	Fish	LMAR	1	CPRE	3	CPRE	5	AURA	1	No fish		CPRE	2	CPRE	2		
								CPRE	2			LCYL	1	LCYL	1		
														LMAR	1		
Row C	Velocity	0,20		0,67		0,50		0,70		0,74		1,00		0,50			
	Max depth	0,17		0,46		0,51		0,54		0,69		0,70		0,39			
	Substrate	GS		BCG		BCG		BCG		BC		BCS		GS			
	Fish	CGAR	1	CPRE	1	CPRE	5	CPRE	6	CPRE	2	AURA	1	CPRE	1		
		CPRE	3	LMAR	1	LMAR	2	LMAR	1			CPRE	6	MBRE	1		
														LCYL			

Table 6.1.18: Habitat parameters and fish collected in the blocks at the Tshino site in the Luvuvhu River on 14/5/2002. The velocity is the maximum velocity and is expressed in m/s, the maximum depth in meter and the substrate classified as Bedrock (R), Boulder (B), Cobble (C), Gravel (G), Sand (S) and silt/clay (M). The names of the fish are abbreviated as per accepted national code.

		Blocks															
Row		**1**	**2**		**3**		**4**		**5**		**6**		**7**		**8**		
Row A	Velocity	0,10	0,40		0,67		0,80		0,80		0,67		0,57				
	Max depth	0,25	0,36		0,45		0,46		0,50		0,40		0,32				
	Substrate	BCG	BC		BC		BC		BC		BC		GS				
	Fish	No fish	CPRE	1	LMAR	1	CPRE	3	CPRE	5	LCYL	1	CPRE	5			
													LMOL	2			
Row B	Velocity	0,10	0,50		0,67		0,67		1,00		1,00		0,40				
	Max depth	0,11	0,31		0,39		0,42		0,41		0,31		0,21				
	Substrate	B	BCG		BCG		BCG		BCGS		BCG		GS				
	Fish	No fish	CPRE	11	No fish		CPRE	7	LMAR	1	AURA	1	No fish				
			LMAR	2			LMAR	1			CPRE	7					
							LMOL	1									
Row C	Velocity	0,15	0.45		0.70		0.74		0.90		0.80		0.10				
	Max depth	0,10	0,37		0,34		0,42		0,34		0,32		0,02				
	Substrate	B	BC		BC		BC		BCG		BCGS		GSM				
	Fish	No fish	CPRE	11	CPRE	13	AURA	1	CPRE	12	CPRE	16	No fish				
							CPRE	27	LCYL	1							
									LMAR	1							
									LMOL	1							

Table 6.1.19 : Habitat parameters and fish collected in selected habitats at the site in the Mutale River on 15 August 2000. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 1	Point 2	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8
Position & description	On side of stream	Around boulder	Midstream boulder	Side of stream	Side of stream along bank	Along bank	Along bank	Away from side	Along bank
Depth (m)	0.2 - 0.3	Deep	0.5	Deep	0.2		0.2	0,5	0.6
Velocity	Fast	Fast	Fast	Fast	Slow/pool	Slow	Slow	Fast	Slow
Cover in the water column		Boulder		Roots of Reeds	Reeds Roots	roots	Overhang Roots Boulders	Boulders	Roots
Substrate	S	B C	BCS	BCS	CS		Silt	CGS	
Fish collected	LMAR 3	CPRE 1	CPRE	CPRE 1 AURA 1 BTRI 2 BEUT 1	BTRI 15 BLIN 1 LCYL 1 AURA 1	LCYL 1 AURA 1 BLIN 1 BTRI 1 PCAT 1	BEUT 5 BTRI 8 BVIV 3 PCAT 1 AURA 1 MMAC 1	LMOL 1 CPRE 2 BTRI 2	AURA 1 LCYL 1

Table 6.1.19 (cont.) : Habitat parameters and fish collected in selected habitats at the site in the Mutale River on 15 August 2000. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 8	Point 8	Point 9	Point 10	Point 11	Point 12	Point 13
Position & description	Deeper to midstream	Isolated side pool	Midstream Rapid	Towards middle	Side	Side	Midstream
Depth (m)	> 0.6	0.5	0.6	Shallow	0.2 – 0.4	Shallow	0.3
Velocity	Fast	Slow	Fast	Fast	Fast	Fast	Fast riffle
Cover in the water column	Boulders		Tree stump Boulders	Boulders	Boulders Roots	Roots Overhang	BC
Substrate	BC	Mud/d sediment	CG	RB	B	SM	BC
Fish collected	CPRE 1	BNEE 20 BLIN 2	BVIV 1 CPRE 1	LCYL 1	PCAT 1 BTRI 1 LCYL 2 AURA 1	BTRI 2	AURA 3

Table 6.1.20: Habitat parameters and fish collected in selected habitats at the site in the Mutale River on 22 August 2000. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Point 8	Point 9
Position & Description	Rapid	Around big boulder (Rapid)	Riffle	Against side	Against side	Rapid towards mid stream	Rapid around boulder	At side	At side
Depth (m)	0.25	0,45	0.4	0.30	0.22	0.35	0.48	0.38	0.2
Velocity	Fast	Fast	Fast	Slow	Slow	Fast	Fast	Fast	Slow
Cover	CB	B	B	B	B	B	B	BC	BC
Substrate	CB	B	RB	BC	BGS	BC	RB	BC	BCG
Fish collected	CPRE 3	CPRE 3	CPRE 1 LCYL 1	PCAT 1 BLIN 1 CPRE 1	LCYL 1	AURA 1	LCYL 2 CPRE 2	LCYL 1	AURA 3

Table 6.1.20 (continued): Habitat parameters and fish collected in selected habitats at the site in the Mutale River on 22 August 2000. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 10	Point 11	Point 12	Point 13	Point 14	Point 15
Position & Description	At side	At side	Against side	Towards middle	In middle (Riffle)	Against side
Depth	0.3	0.25	0.35	0.3	0,40	0.35
Velocity	S-F	Mod	Slow	Fast	Fast	Fast
Cover	Overhang veg	Fallen tree	Undercut Bank Ov veg	BC	BC	BC
Substrate	BC	BCS	SM	BC	BC	BC
Fish collected	LCYL 3 BTRI 5 PCAT 1 BVIV 1 BLIN 3	PCAT 1 LCYL 1 BVIV 1 BEUT 1	BTRI 2 BEUT 1	LCYL 1 CPRE 1	AURA 1 LCYL 2 CPRE 2	BVIV 1 LCYL 1 BTRI 1

Table 6.1.21: Habitat parameters and fish collected in selected habitats at the site in the Mutale River on 17 October 2000. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 1	Point 2	Point 3	Point 4	Point 5
Position & description	Side of stream (Pool)	(Rapid)	(Riffle)	At side	At side
Depth (m)	0.48	0.6	0.5	0.5	0.25
Velocity (m/s)	0.33	0.67	0.60	0.67	0.5
Cover in the water column	BC	BC	B	Undercut banks, Overhanging vegetation, Root wads	Undercut banks, Roots
Substrate	BCS	BCG	B	BCGS	BCS
Fish	CPRE 1 BVIV 1	AURA 2 BTRI 1 LCYL 1	AURA 3 CPRE 1 LCYL 1	BTRI 1 CPRE 2 AURA 1 LCYL 2	BTRI 9 PCAT 3 MMAC 1 BEUT 2 AURA 2 LCYL 1 BVIV 2 BUNI 13

Table 6.1.22: Habitat parameters and fish collected in selected habitats at the site in the Luvuvhu River at Tshino on 28 August 2000. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 1	**Point 2**
Position & Description	Against side	Against side
Depth	0.2	0.40
Velocity	0,1	0.5
Cover	B Dead reeds	B
Substrate	BCGSM	BC
Fish	LMOL 2 BTRI 1 CPRE 1	CPRE 24 AMOS 1 LCYL 2 LMOL 3 AURA 2

Table 6.1.23: Habitat parameters and fish collected in selected habitats at the site in the Luvuvhu River at Tshino on 7 November 2000. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 1	**Point 2**	**Point 3**
Position & Description	Marginal habitat (Shallow riffle)	At side (Rapid)	Towards middle (Rapid)
Depth (m)	0.20	0,30	0.40
Velocity	Fast	Fast	Fast
Cover	Dead reeds C	B	B
Substrate	CS	BCS	RB
Fish	MBRE 1 LCYL 1 CPRE 5 LMAR 10 BVIV 1	AURA 4 CPRE 22 LCYL 4 BTRI 1	AURA 2 CPRE 6

Table 6.1.24: Habitat parameters and fish collected in selected habitats at the site in the Luvuvhu River at Tshino on 2 August 2001. (The numbers next the abbreviation for the fish species indicates the number collected).

	Point 1	**Point 2**
Position & Description	Against side (Shallow riffle)	Towards middle, but still close to side (Rapid)
Depth (m)	0.2	0.40
Velocity (m/s)	0.5	0.60
Cover	Marg veg Roots of reeds	BC
Substrate	CG	BCG
Fish	CPRE 2 LMAR 13	LCYL 1 LMAR 4 CPRE 25

Table 6.1.25: Current and historical data of fish collected in the same river reaches as the Mutale and Tshino sites. (P indicates that fish is present and A that it is absent).

		Luvuvhu River		Mutale River	
Species	Abbre-viation	Current	Historical	Current	Historical
Anguilla mossambica	AMOS	P	P	P	P
Amphilius uranoscopus	AURA	P	P	P	P
Barbus eutaenia	BEUT	P	P	P	P
Barbus lineomaculatus	BLIN	P	P	P	P
Barbus neefi	BNEE	A	P	P	P
Barbus paludinosus	BPAU	-	-	A	P
Barbus trimaculatus	BTRI	P	P	P	P
Barbus unitaeniatus	BUNI	A	P	P	P
Barbus viviparus	BVIV	A	P	P	P
Clarias gariepinus	CGAR	A	P	A	P
Chiloglanis pretoriae	CPRE	P	P	P	P
Labeo cylindricus	LCYL	P	P	P	P
Labeobarbus marequensis	LMAR	P	P	P	P
Labeo molybdinus	LMOL	P	P	P	P
Micralestes acutidens	MACU	P	P	P	P
Mesobola brevianalis	MBRE	P	P	-	-
Marcusenius macrolepidotus	MMAC	-	-	P	P
Opsaridium peringueyi	OPER	A	P	P	P
Oreochromis mossambicus	OMOS	A	P	-	-
Petrocephalus wesselsii	PWES	-	-	P	P
Pseudocrenilabrus philander	PPHI	A	P	-	-
Tilapia rendalii	TREN	A	P	-	-
Tilapia sparrmanii	TSPA	-	-	A	P

Historical data suggest that only 58% of the fish species previously recorded in the Luvuvhu reaches are still found in the system, whereas 84% of the species complement previously known for the Mutale reach are still present (Table 6.1.25). Species richness shows more variation at the Mutale site with a steady decline over the study period whilst at the Tshino site there is no decline, but rather some variation between seasons with fewer species recorded in winter (Figure 6.1.1). The total number of fish collected per survey showed an increase at Tshino caused mainly by the steady increase of *Chiloglanis pretoriae* (Figure 6.1.2). At Mutale the number of fish collected per survey had an opposite trend with lower fish numbers in the collections towards the end of the study

period. The anomalies in numbers at Mutale seem to be the result of the presence of *Barbus trimaculatus* with peaks in the August 2000 and May 2001 surveys (Figure 6.1.2).

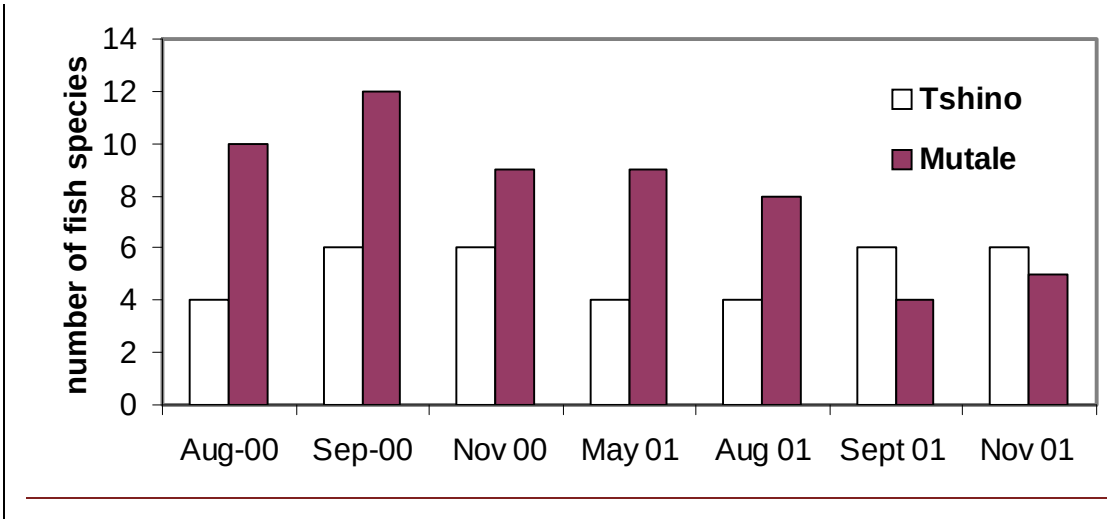


Figure 6.1.1: The number of fish species collected at the Tshino and Mutale sites in the period from August 2000 to November 2001.

c) Fish assemblage structure

Species that are commonly regarded as rheophilic, dominated both sites *viz C. pretoriae, Amphilius uranoscopus, Labeo cylindricus* and *Labeobarbus marequensis* (Skelton, 2001; Gaigher, 1973; Kleynhans, 1991). Species that are not generally considered to be rheophilic were also collected and included *Barbus trimaculatus, B. eutaenia B. neefi B. lineomaculatus, B. unitaeniatus, B. viviparus, Petrocephalus wesselsii* and *Marcusenius macrolepidotus* (Table 6.1.26).

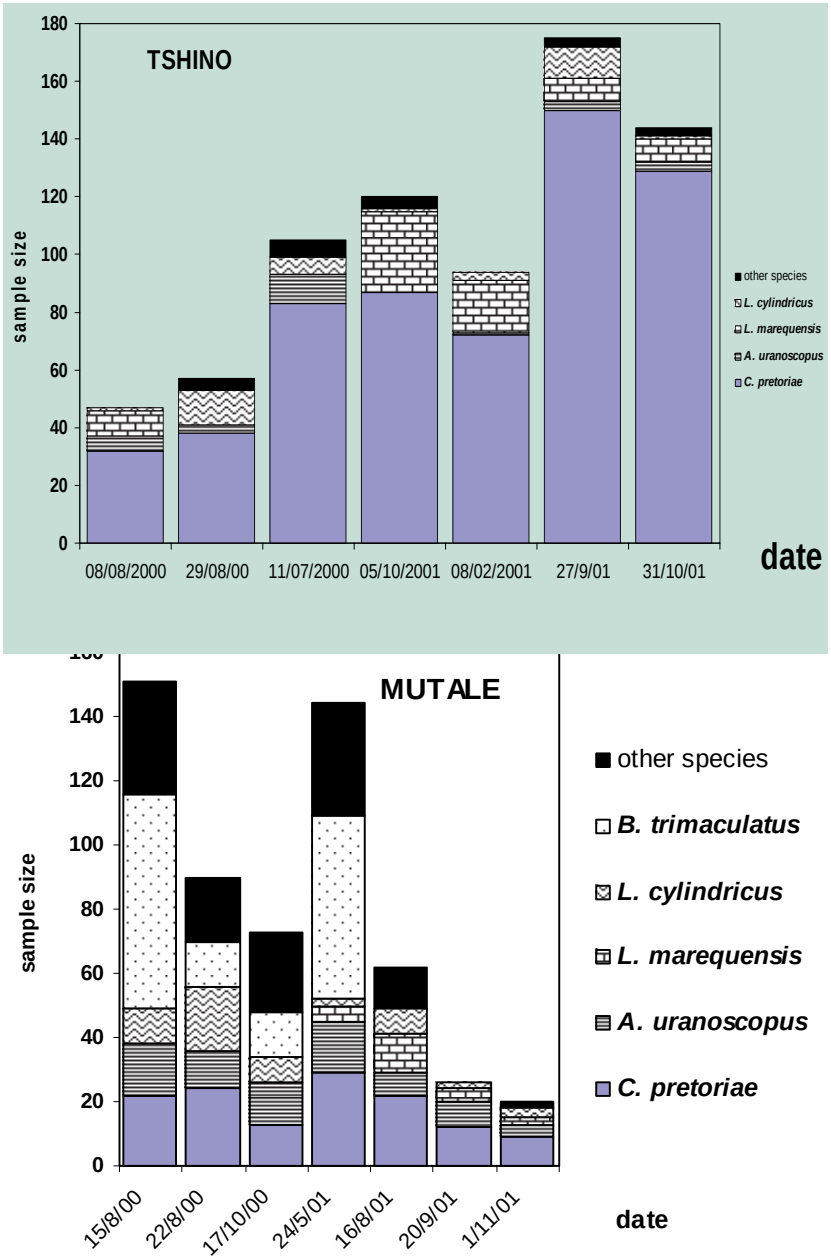


Figure 6.1.2: Temporal changes in fish assemblage composition at the Tshino and Mutale sites for the period from August 2000 to November 2001.

Table 6.1.26: Number of fish specimens collected at the two sites in the Luvuvhu and Mutale rivers in period August 2000 to November 2001.

Fish species	Tshino	Mutale
A. mossambicus	1	2
A. uranoscopus	25	76
B. eutaenia	1	11
B. lineomaculatus	3	27
B. neefi	-	24
B. trimaculatus	5	152
B. unitaeniatus	-	14
B. viviparus	-	19
C. pretoriae	591	131
L. cylindricus	34	54
L. marequensis	71	23
L. molybdinus	4	9
M. acutidens	2	2
M. brevianalis	4	0
M. macrolepidotus	-	7
O. peringueyi	-	2
P. wesselsii	-	13
Total	**741**	**566**

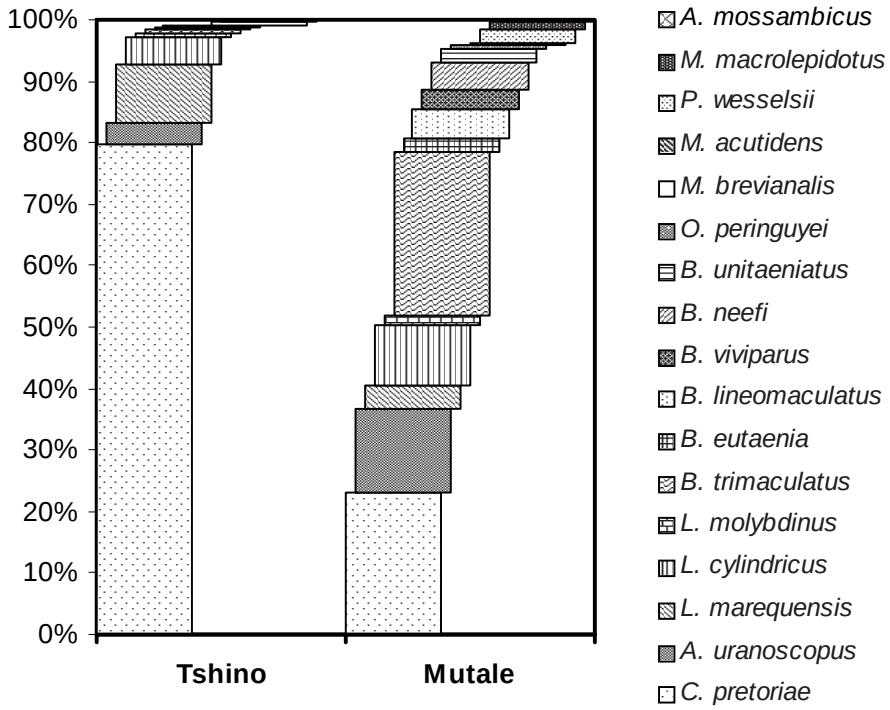


Figure 6.1.3: Accumulative composition of collected fish samples collected at the Tshino and Mutale sites in the period from August 2000 to November 2001.

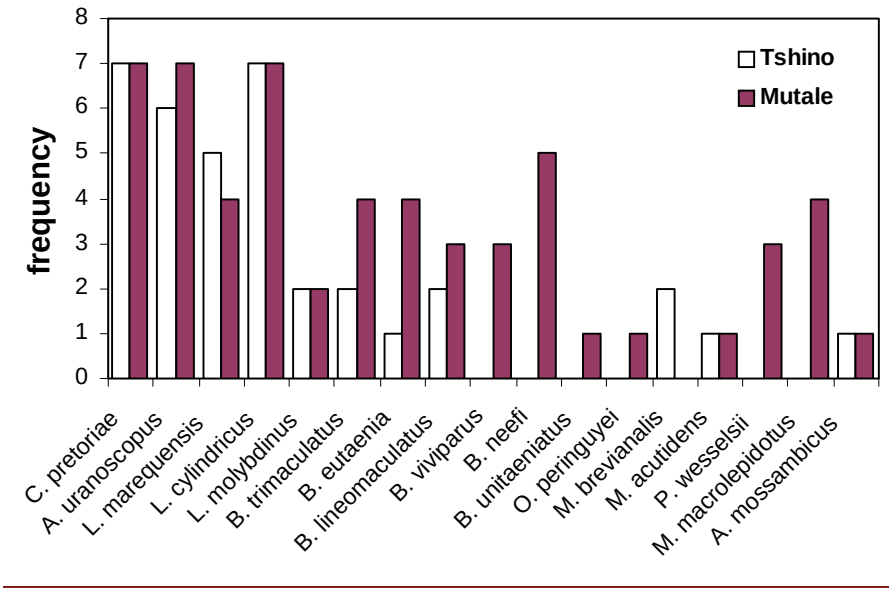


Figure 6.1.4: Frequency of occurrence of fish species collected at the Tshino and Mutale sites in the period from August 2000 to November 2001.

More specimens were caught at Tshino, which was dominated by *Chiloglanis pretoriae*, (Table 6.1. 26 and Figure 6.1.3) resulting in a less equitable community composition than that of Mutale (Figures.6.1.4 and 6.1.5). There were significant decreases in evenness of the fish assemblages over the period of this study and although there was no decrease in species richness, other diversity measures show decreases (Table 6.1.27). At both sites there seems to be a general decrease in equitability over the period August 2000 to May 2002. MDS ordination of fish assemblages shows observable differences between the two sites (Figure 6.1.7). The larger variation in assemblage composition at Mutale is also evident in the more dispersed distribution of Mutale surveys in the ordination. ANOSIM statistically confirmed the difference in overall fish assemblage structure of the two sites (Table 6.1.28).

Table 6.1.27: Pearson product moment correlation of diversity indices over time.

	Species richness	Total no of individuals	Simpsons diversity	Equitability	Shannon diversity
Time	0.04	0.57*	-0.19	-0.51*	-0.34

* $p<0.05$

Table 6.1.28: One-way analysis of similarity (ANOSIM) testing for differences in fish and assemblage structure between Mutale and Tshino.

	Global R	p	Significance level
Mutale vs Tshino	0.463	0.001	**

* = p<0.05

**** = p < 0.01**

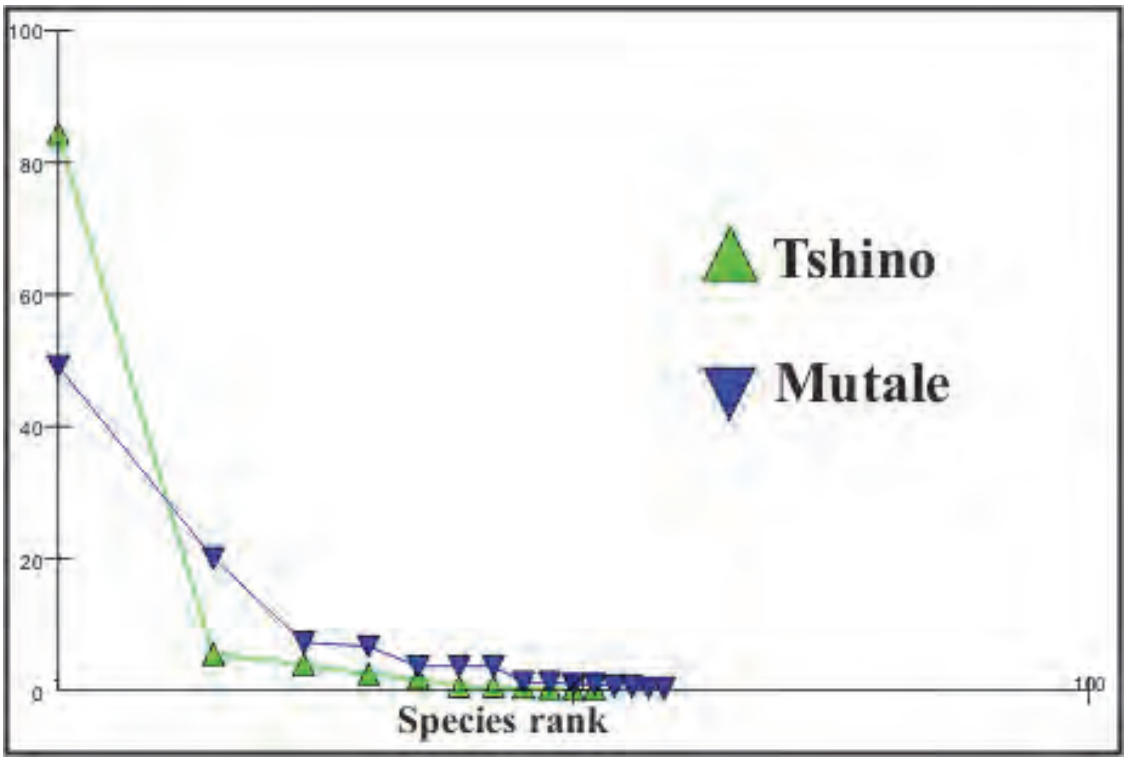


Figure 6.1.5: Rank abundance plot of fish assemblages collected at Tshino and Mutale.

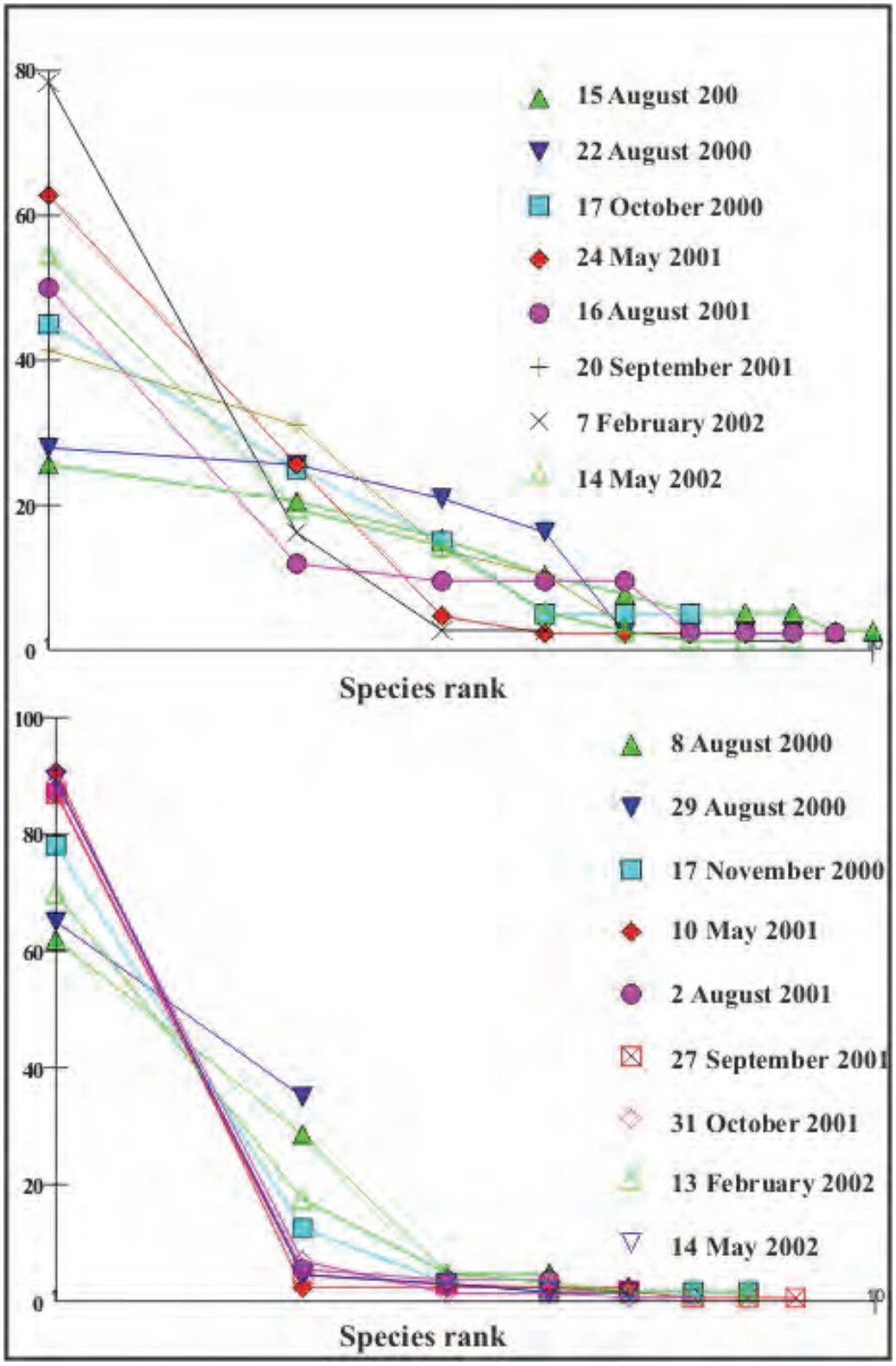


Figure 6.1.6: Rank abundance plots for individual surveys at Mutale and Tshino.

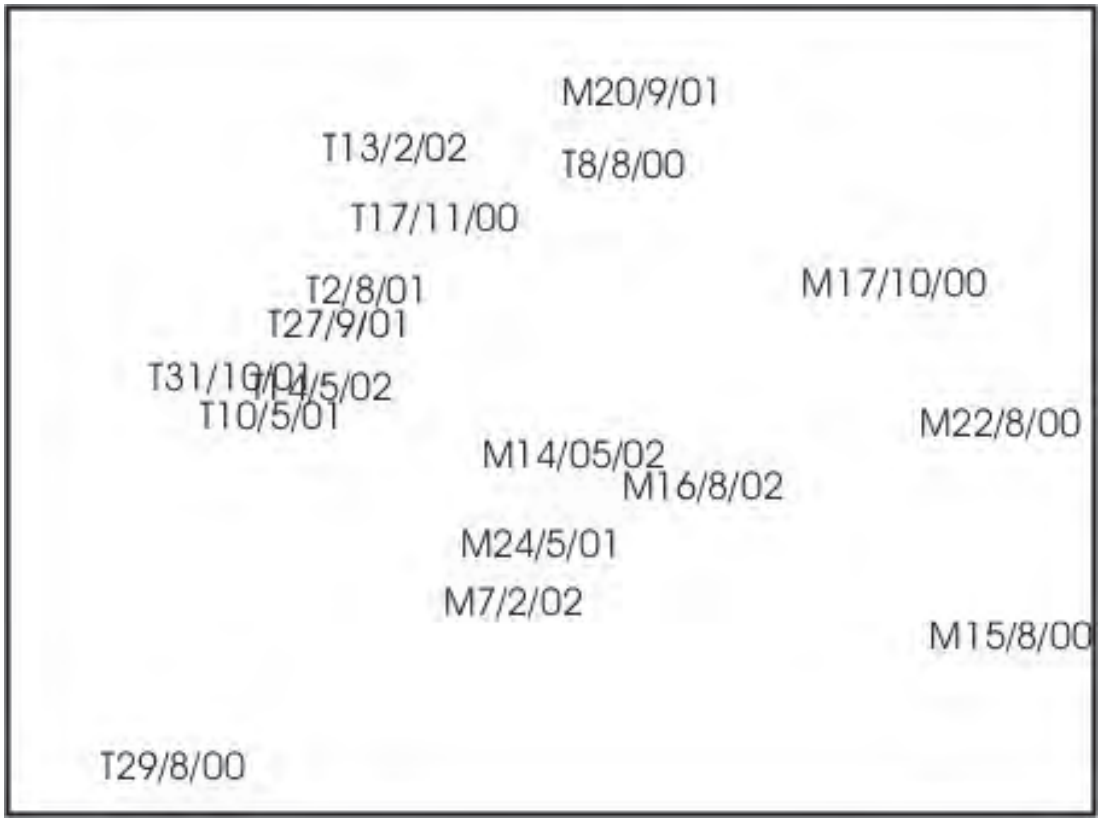
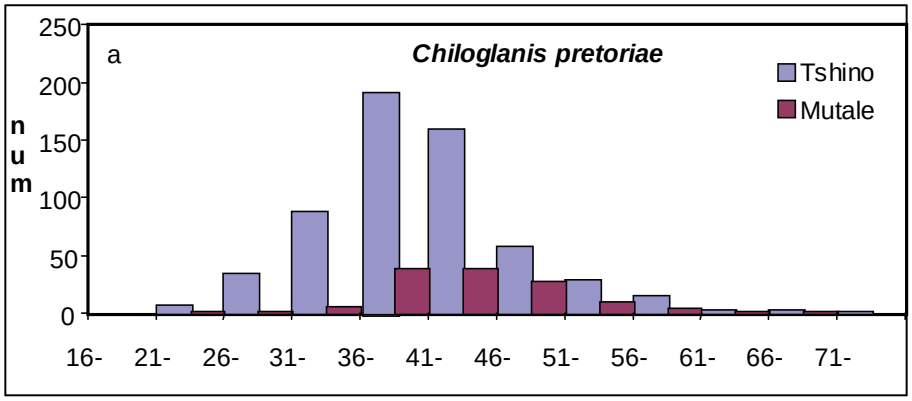


Figure 6.1.7: MDS ordination of the individual surveys at Mutale (M) and Tshino (T), date: D/M/JJ)

d) The composition and structure of fish populations

Species population structure is based on length structure or number of fish per size class and comprises the pooled data of all surveys (Figure 6.1.8 [a – q]).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Chiloglanis pretoriae
Tshino
Mutale
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200
150
100
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16-
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46-
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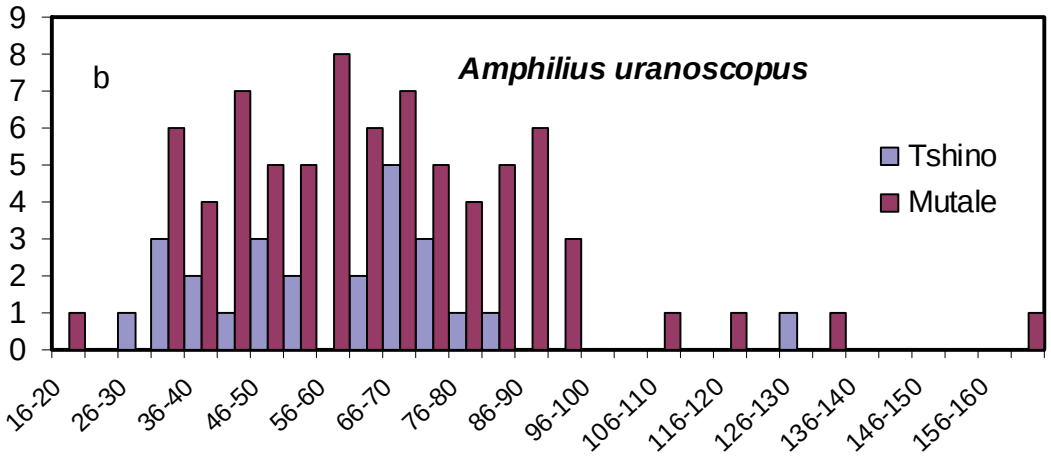
71-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Tshino
Mutale
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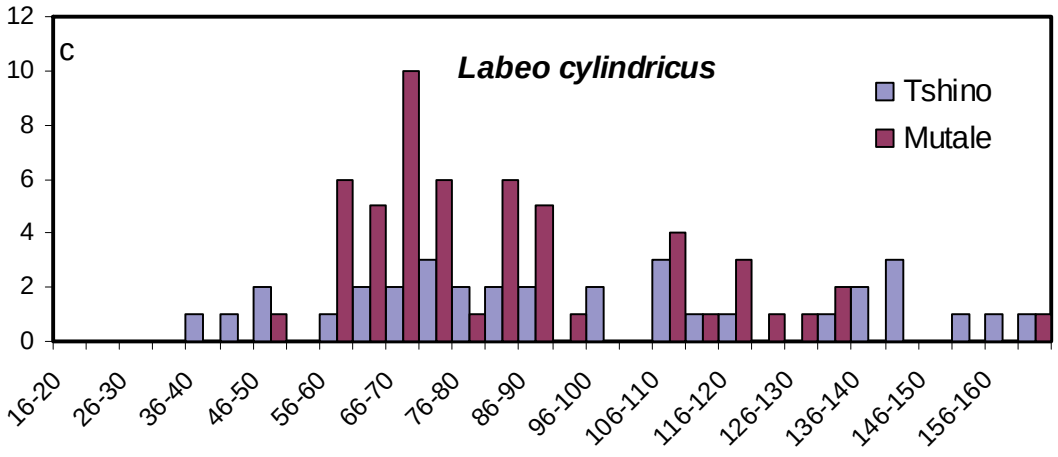
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Tshino
Mutale
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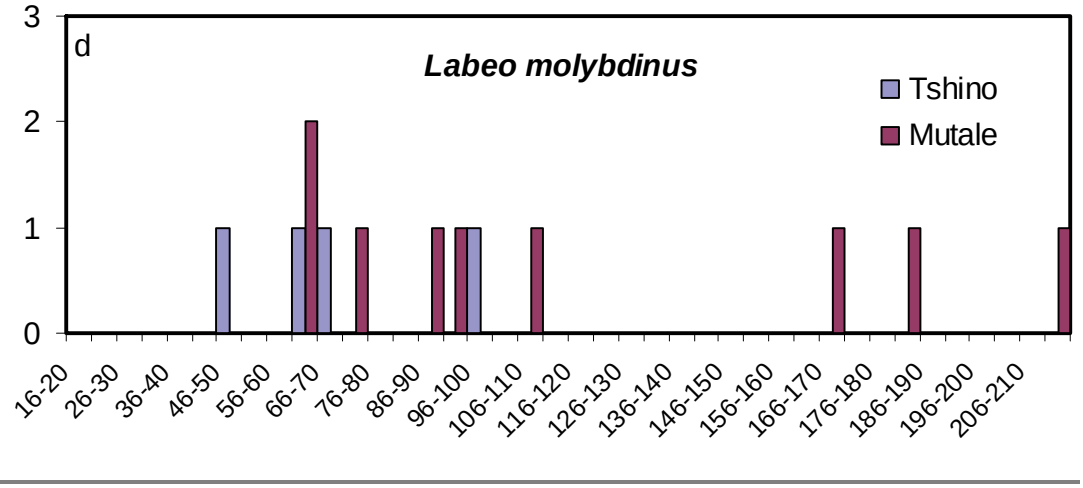
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Labeo molybdinus
Tshino
Mutale
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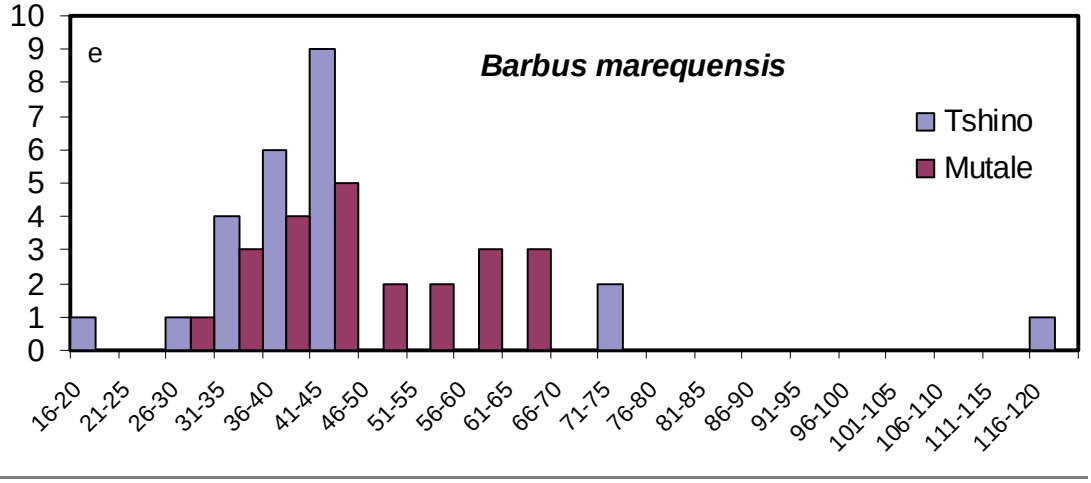
206-210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Tshino
Mutale
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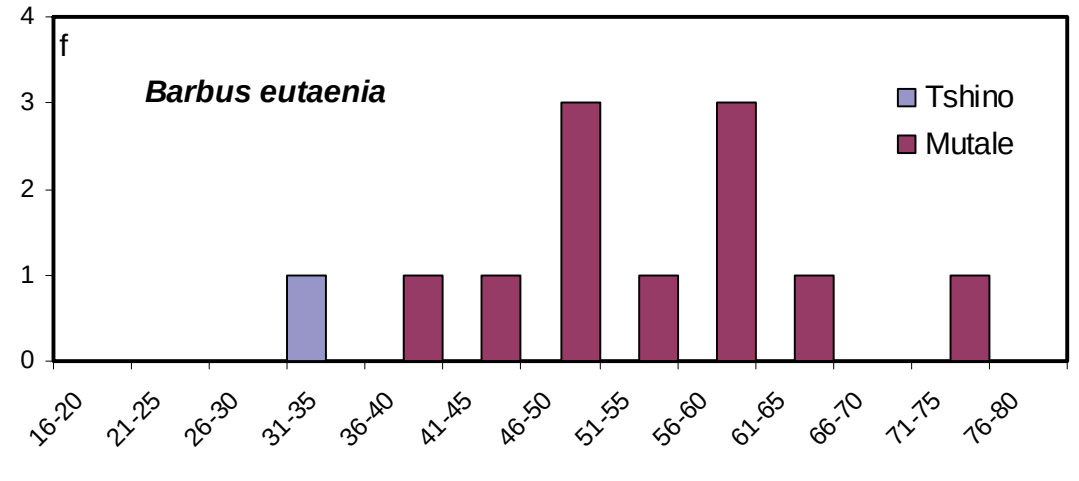
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Tshino
Mutale
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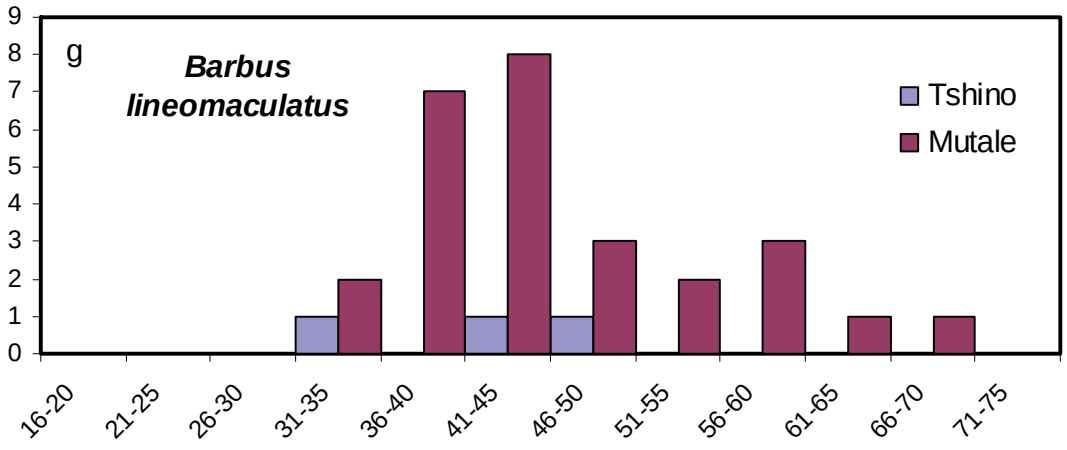
76-80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Tshino
Mutale
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56-60
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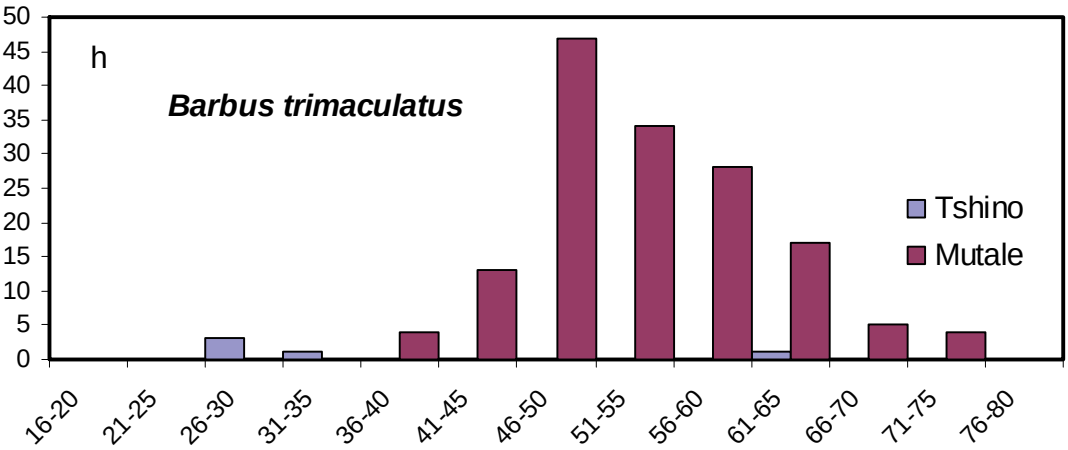
66-70
71-75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Tshino
Mutale
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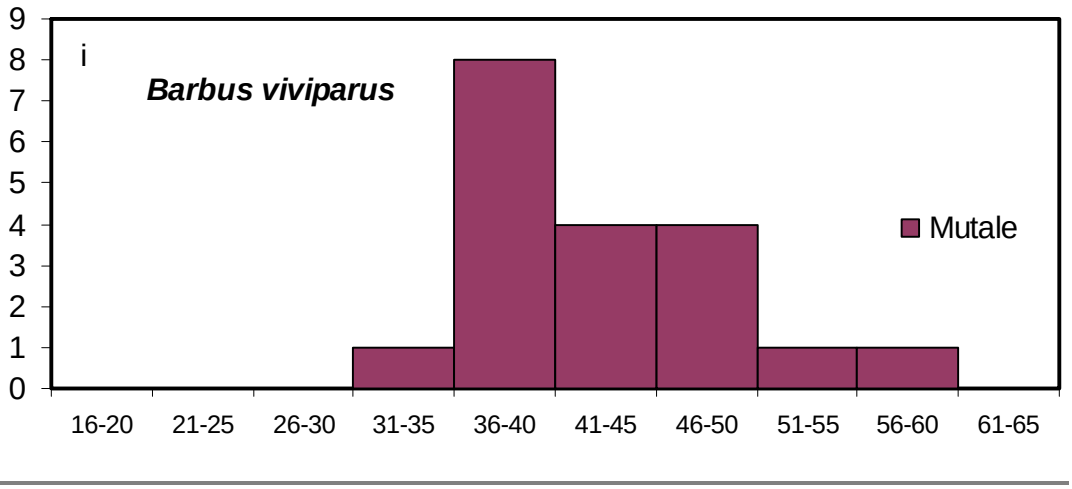
71-75
76-80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Mutale
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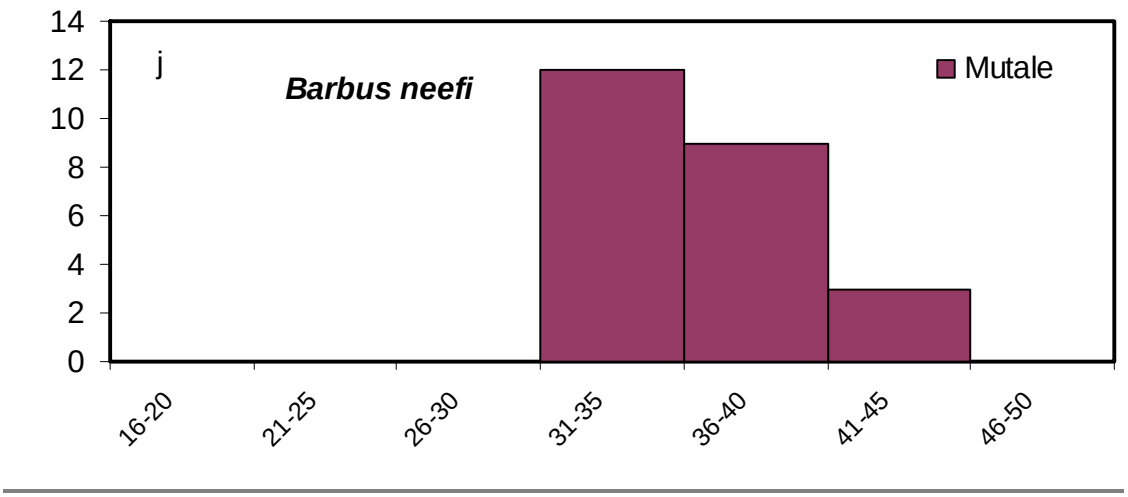
56-60
61-65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Mutale
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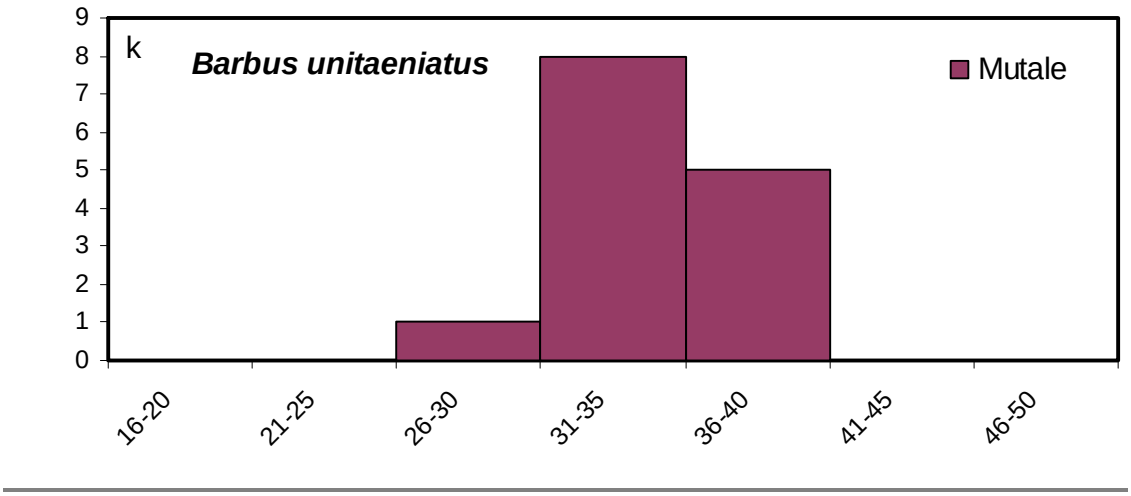
46-50


k
Barbus unitaeniatus
Mutale
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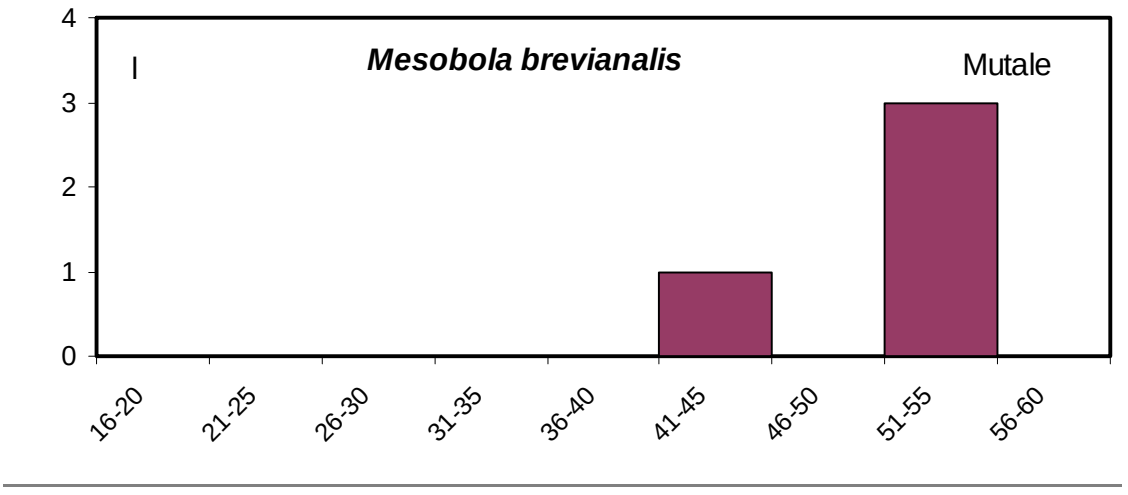
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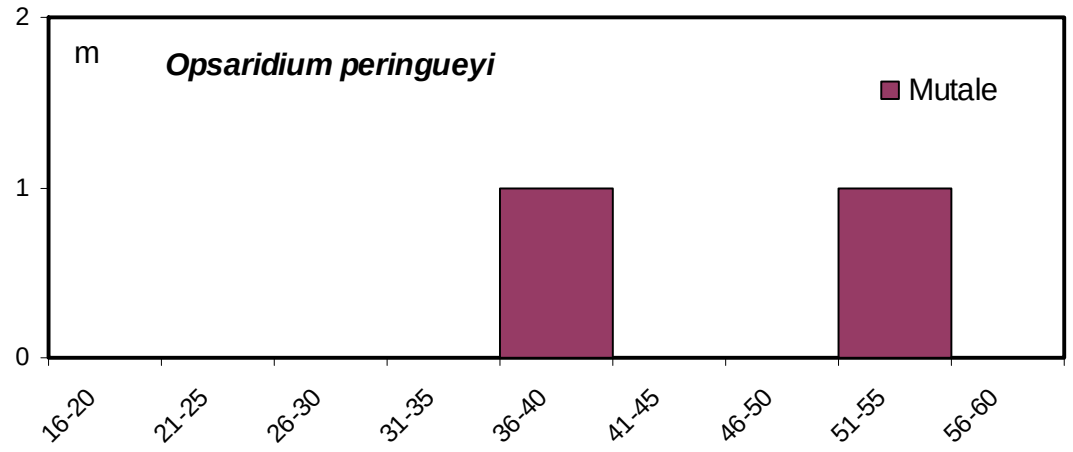
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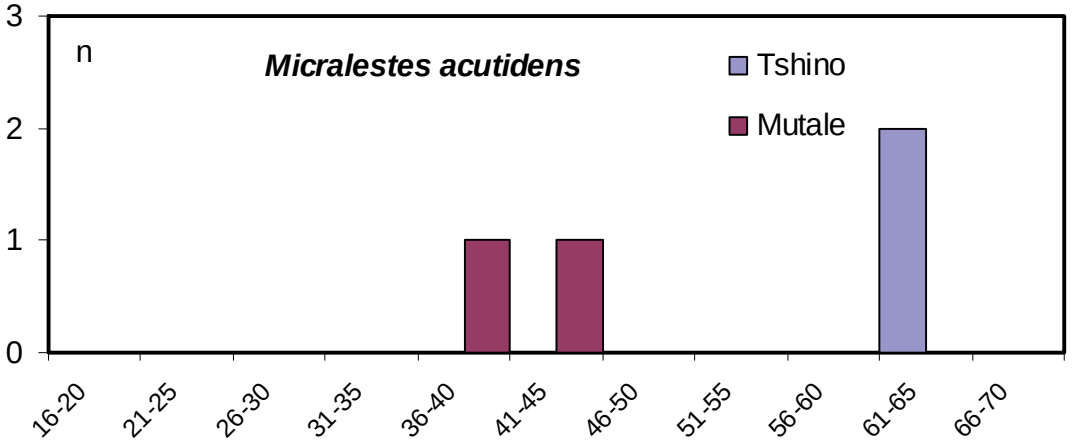
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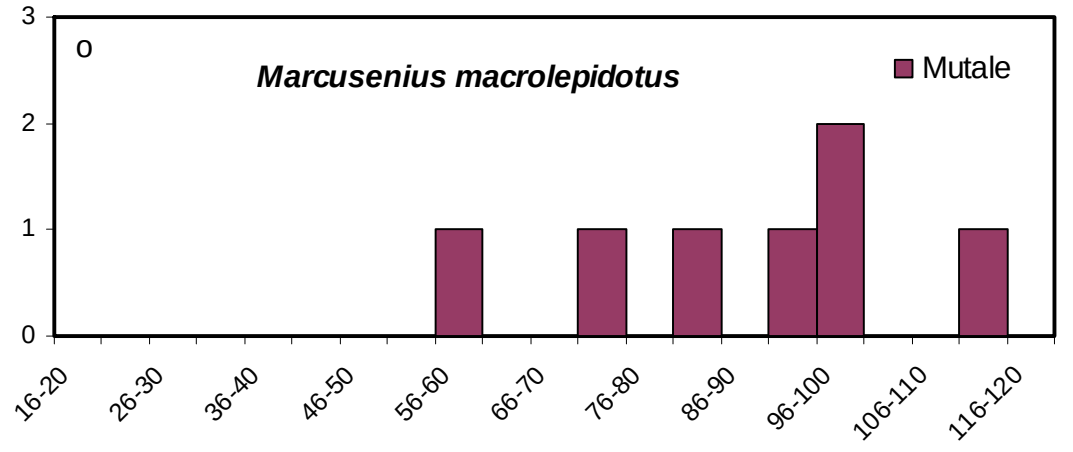
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Mutale
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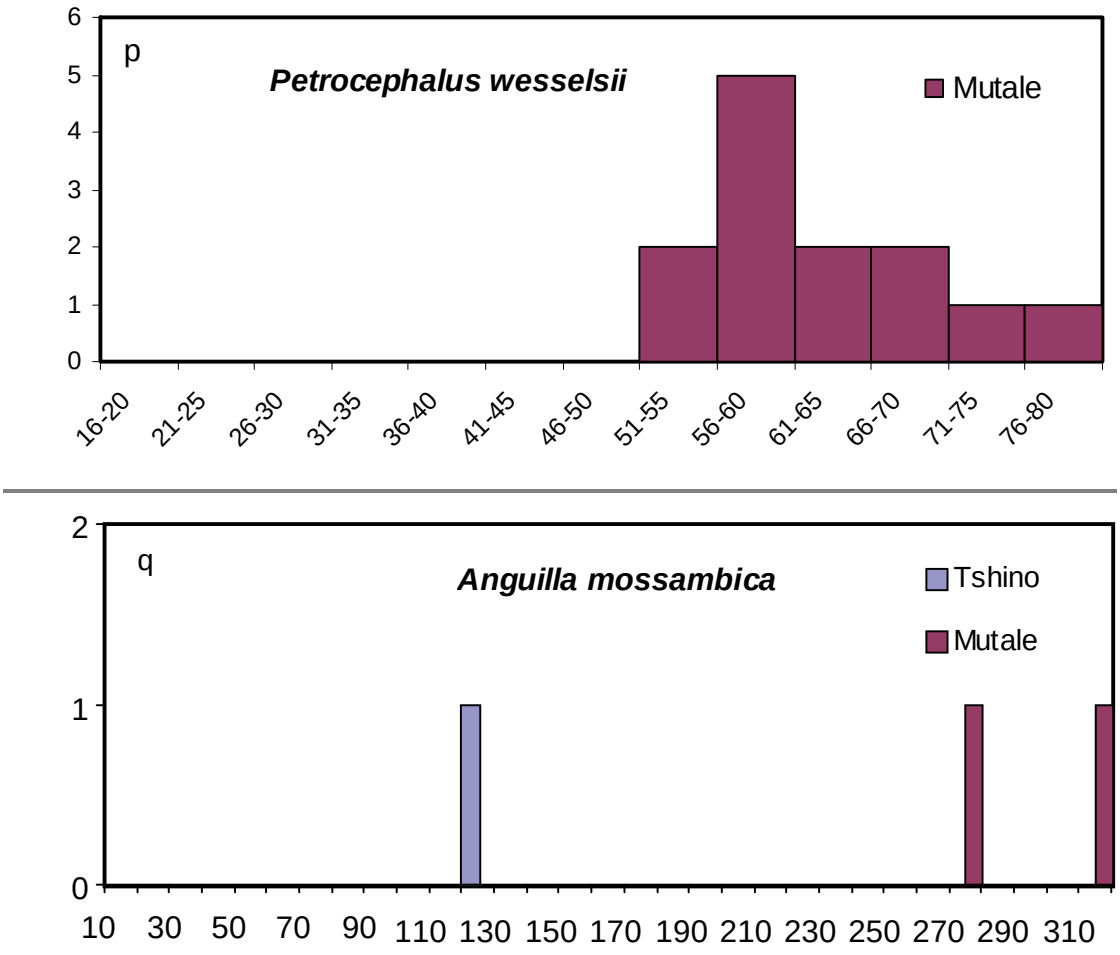

Figure 6.1.8 (a-q): Length frequency distribution of the fish populations, based on pooled data, of the collections at Tshino and Mutale during the period from August 2000 to November 2001.

Although the fish populations of both sites were dominated by juvenile and sub-adult specimens, adult specimens were collected of some species. This included adults of *C. pretoriae, A uranoscopus*, *Labeo* spp. and the smaller *Barbus* species. A prominent difference in the population structures of Mutale and Tshino was that more small specimens were collected at the latter.

e) Size structure of the populations of the more common species.

A sufficient number of specimens per survey were collected of *C. pretoriae, A. uranoscopus, B. neefi* and *L. marequensis* to warrant further analysis of the length distribution of the individual surveys. The length frequencies of the four species are shown in Figures 6.1.9 to 6.1.12 and the population structure and change in the structure over time are discussed. A wider range in length, from 21 to 70mm, was observed in the *C. pretoriae* samples collected at Tshino than the range of 36 to 61mm that was observed at Mutale. In addition, many more specimens were collected at Tshino. Figure 6.1.9 shows a strong cohort of juvenile fish added to the population in August 2000 with a peak at 30mm.

This peak is maintained in the other surveys and offers an opportunity to follow one age cohort over the study period. It has grown from a mean total length of 27 to 41 mm over a 12 month period, indicating the growth increment over a one year period of 14mm. The data of the population sampled at Mutale do not offer a similar clear picture but there is recruitment of 21mm fish in October 2001.

Low numbers of *A. uranoscopus* were collected at both sites but Figure 6.1.10 shows that recruitment of 16mm fish occurred in May 2001 in the Mutale population. At both sites few large fish were collected but at least two separate size classes were present with a spread from 16 to 180 mm in length. At Tshino a single cohort of young *L. marequensis* of 36 to 65mm entered the population in May 2001, and increased in length to between 46-70mm. A next cohort of young of the year seems to have just entered the population in September 2001 with some fish of only 16-20mm length (Figure 6.1.11). This confirms an early spawning season reported by Skelton (2001).

The situation at Mutale is not clear and few specimens were collected, however they were also young fish, possibly only one year old. The absence of larger fish in both collections may be due to the method of collection, involving disturbance that would allow larger fish to move away from the electrofishing equipment, or else the collection sites might be unsuitable habitat for large specimens.

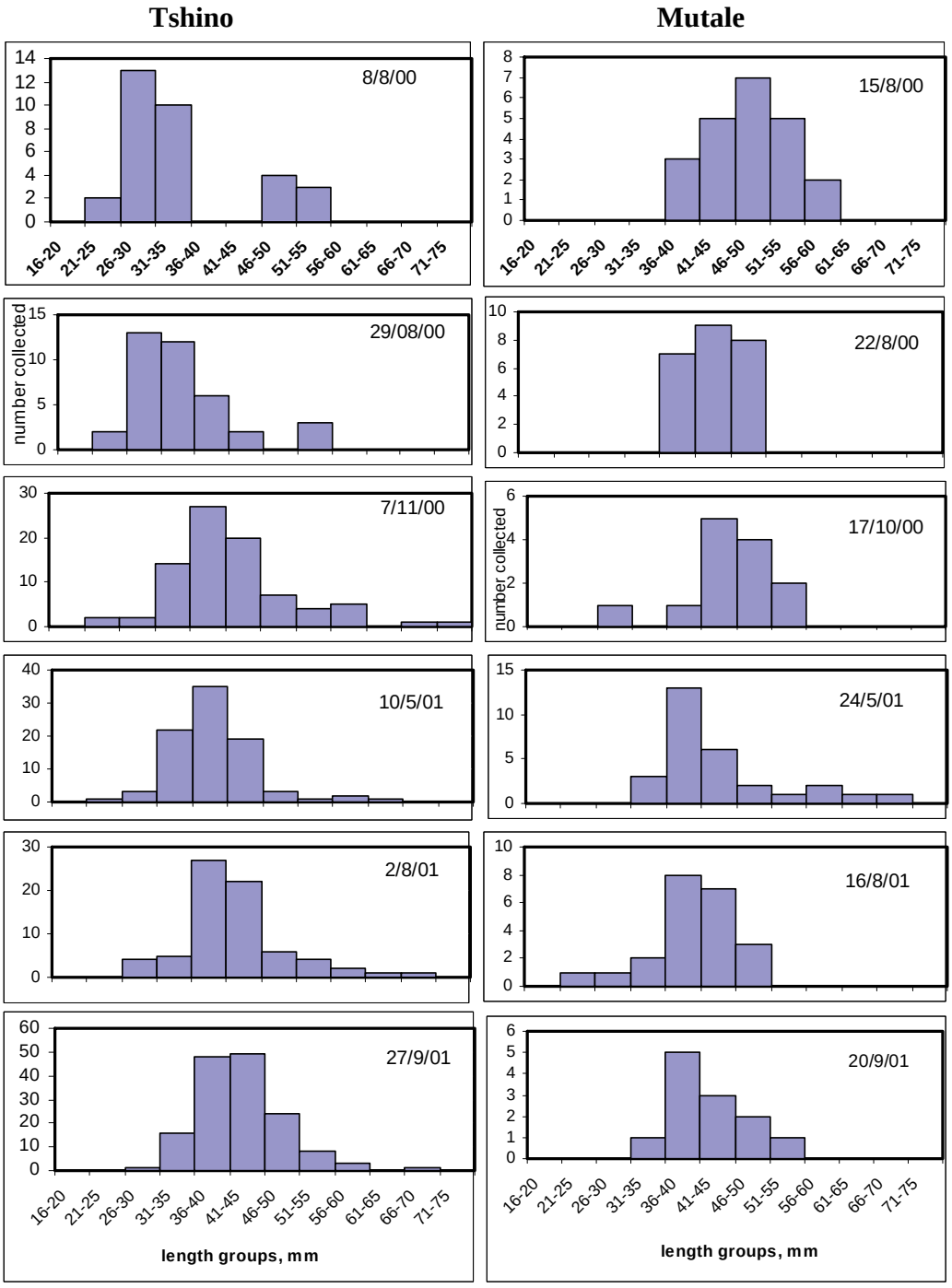

Figure 6.1.9: Length frequencies of *C. pretoriae* collected during the surveys at Tshino and Mutale in the period from August 2000 to September 2001.

TSHINO **MUTALE**

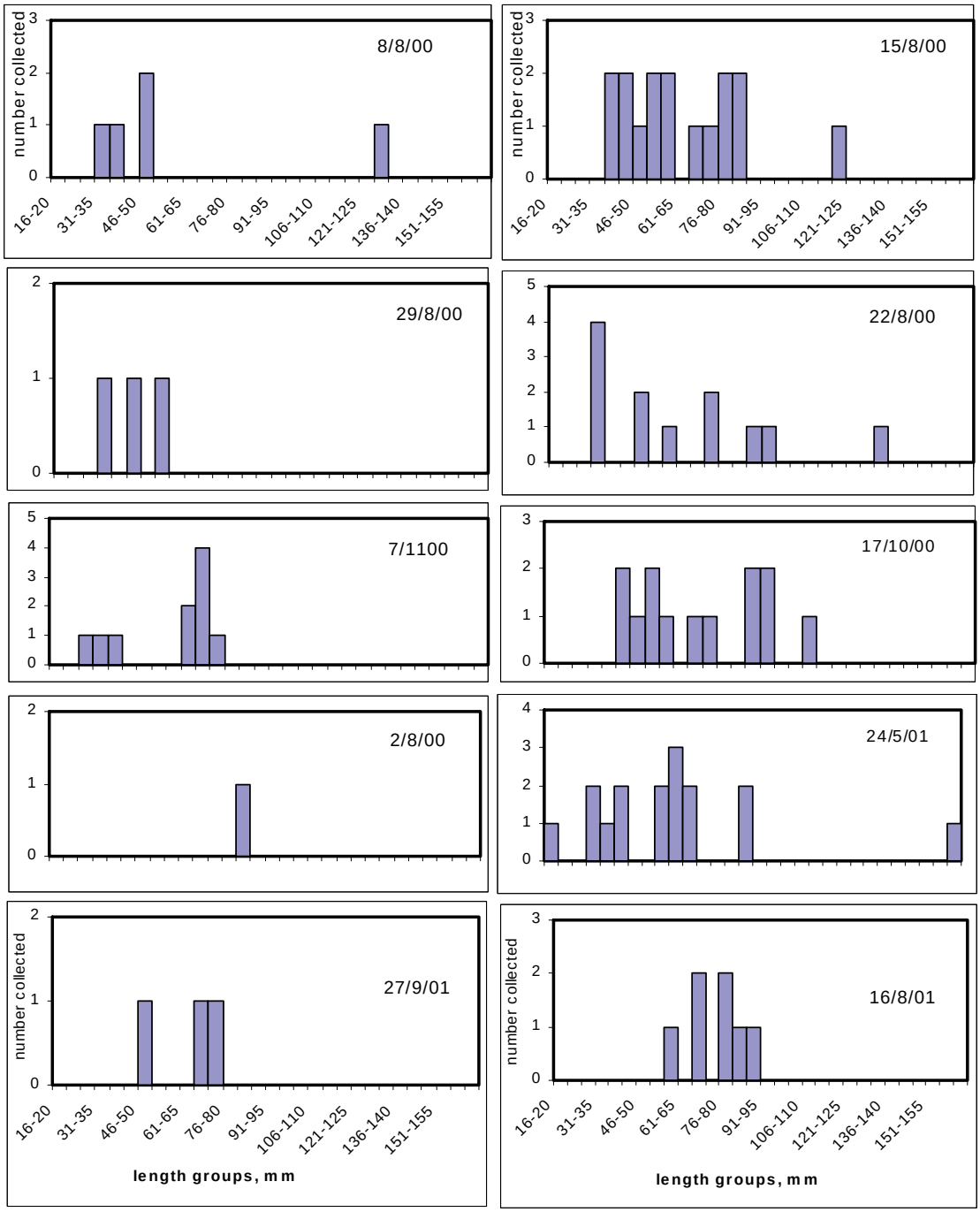

Figure 6.1.10: Length frequencies of *A. uranoscopus* collected during the surveys at Tshino and Mutale in the period from August 2000 to September 2001.

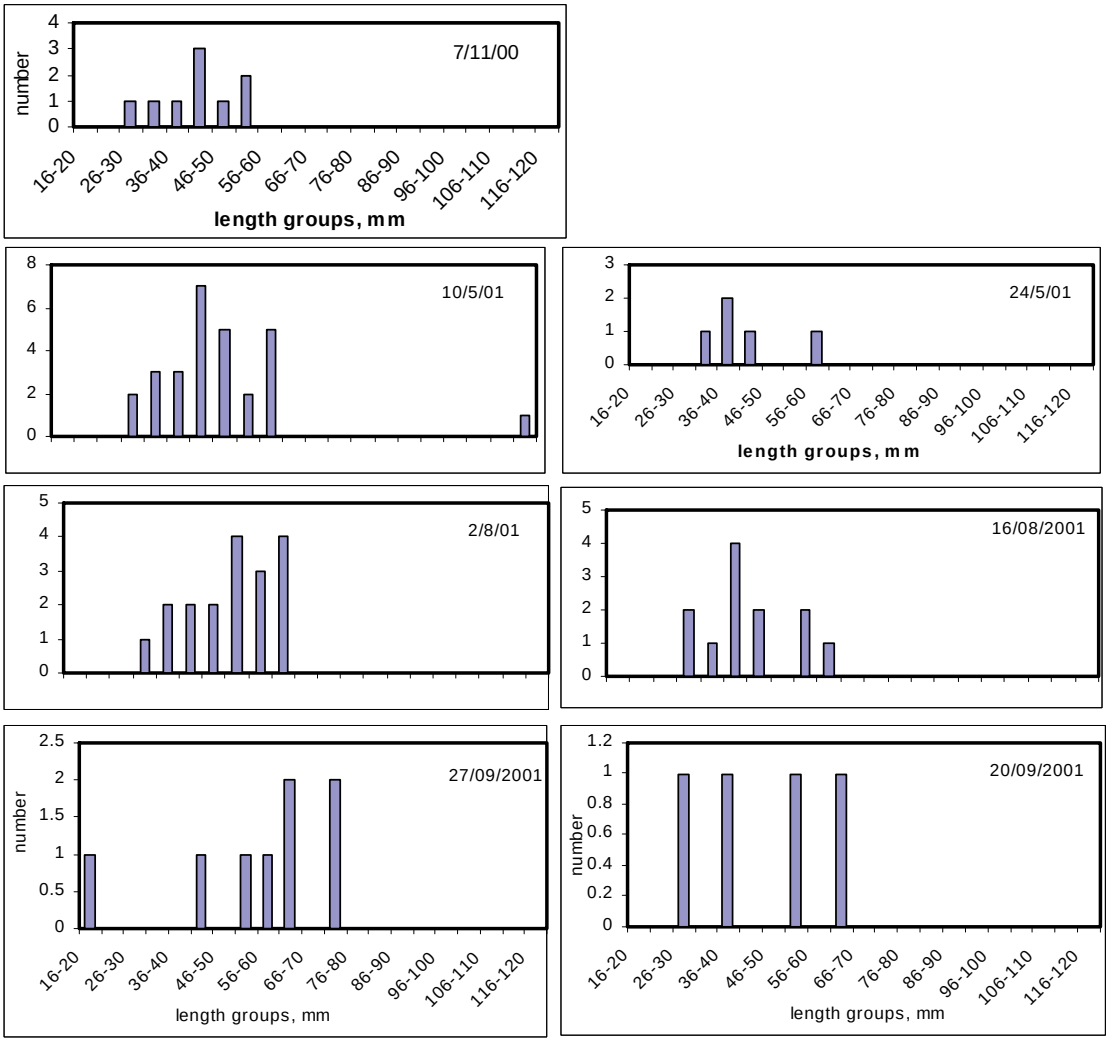

Figure 6.1.11: Length frequencies of *L. marequensis* collected during the surveys at Tshino and Mutale in the period from August 2000 to September 2001.

The size frequency of *B. neefi* ranged between 31 and 45mm, representing sub-adult and adult fishes (Figure 6.1.12). No signs of recruitment of a new cohort are evident but there is an increase in length of the fish after the winter, possibly reflecting the growth of this one cohort. Polling (1999) reports *B. neefi* of up to 55mm SL, (about 60mm TL) in the Selati River, a river of similar size as the Mutale, indicating that larger *B. neefi* were not collected in the habitat sampled.

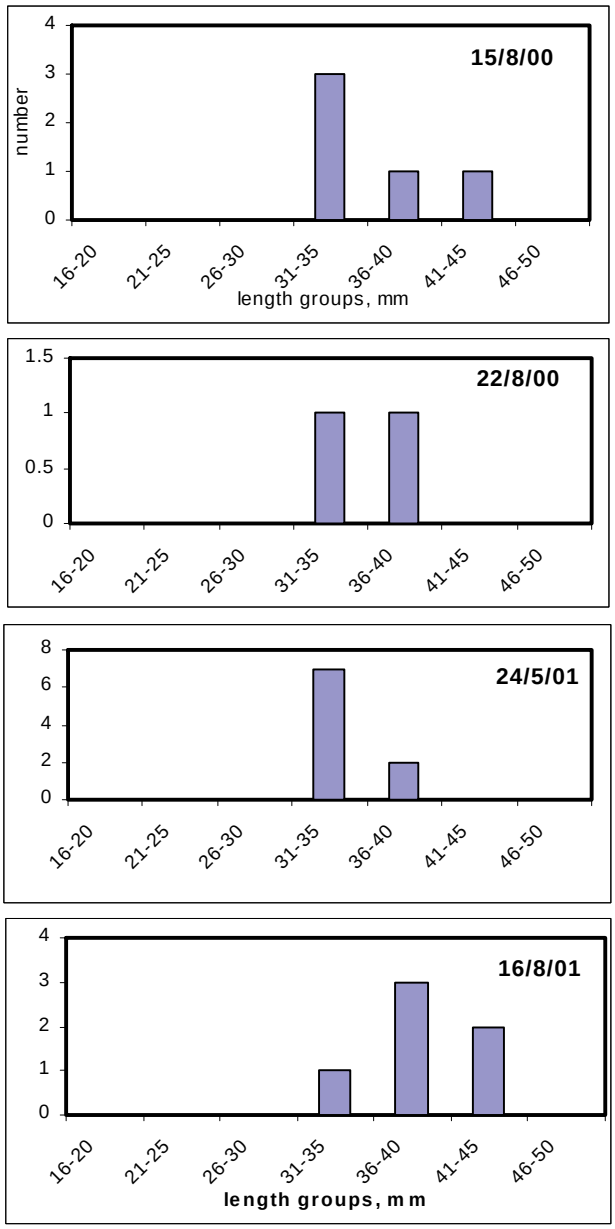

Figure 6.1.12: Length frequencies of *B. neefi* collected during the surveys at Mutale in the period from August 2000 to August 2001.

f) Habitat preference determination and environmental variables

The percentage frequency of occurrence per block, classified into a flow depth class, is shown in the tables below. Where Table 6.1.29 shows the results of four species that

occurred at both sites, Table 6.1.30 shows the results of four of the smaller barbs that were collected at both or one of the sites.

The results indicate that both *C. pretoriae* and *L. cylindricus* are distinctly rheophilic. *C. pretoriae* was more frequently observed in shallow water while *L. cylindricus* in water deeper than 0,5 m. This was found to be similar for both rivers. As far as *L. marequensis* is concerned there was no clear preference observed and the situation was different for the two rivers. This would confirm the decision by Kleynhans (1999) to regard this species as semi-rheophilic. While the majority of *A. uranoscopus* were collected in fast flowing habitats at both sites their preference for shallow or deep water differed between the two sites. The species can however be classified as a true rheophilic species.

Table 6.1.29: The percentage frequency occurrence of four fish species per flow depth category that was collected from both the Luvuvhu and Mutale River sites in the period August 2000 to May 2002. (AURA = *Amphilius uranoscopus*; LMAR= *Labeobarbus marequensis*; CPRE = *Chiloglanis pretoriae*; LCYL = *Labeo cylindricus*).

Species	Site	Slow/ shallow	Slow/ Deep	Fast/ shallow	Fast/ Deep
A. uranoscopus	Mutale	10	0	78	12
	Luvuvhu	0	0	31	68
L. marequensis	Mutale	67	0	33	0
	Luvuvhu	4	0	85	11
C. pretoriae	Mutale	8	0	60	32
	Luvuvhu	4	0	57	39
L. cylindricus	Mutale	0	0	17	83
	Luvuvhu	0	0	20	80

Table 6.1.30: The percentage frequency occurrence of the four barb species per flow depth category collected from the Luvuvhu and Mutale rivers. (BNEE= *Barbus neefi*; BLIN = *Barbus lineomaculatus*; BTRI = *Barbus trimaculatus* ; BVIV = *Barbus viviparus*) for the period August 2000 to May 2002.

Species	Site	Slow/ shallow	Slow/ Deep	Fast/ shallow	Fast/ Deep
B. neefi	Mutale	29	0	42	29
B. lineomaculatus	Mutale	57	0	43	0
B. trimaculatus	Mutale	63	0	37	0
	Luvuvhu	100	0	0	0
B. viviparus	Mutale	78	0	12	0

g) Statistical analyses of the results.

Agreement between environmental variables measured at small scales and micro-geographic variation in biotic assemblages were very low, the highest correlation was 0.18 (Table 6.1.31). Combinations of these environmental variables suggest that velocity and depth were important variables structuring fish communities. Meso-scale environmental variables had a larger predictive power than micro-scale variables. The highest correlation was that for fish assemblages, 0.43 (Table 6.1.32), where combinations of pH, conductivity, dissolved oxygen, temperature and maximum velocity explained the most variability in the fish assemblages.

Multiple regression of species abundances onto environmental variables resulted in only two significant regressions, that of the two fish species, A. *uranoscopus* and *L. marequensis*. Conductivity was a significant predictor of abundance of these two fish species (Table 6.1.33).

Table 6.1.31: BIO ENV of micro-scale environmental variables and fish assemblages; variables: 1 = velocity, 2 = depth, 3 = bedrock, 4 = boulders, 5 = cobbles, 6 = gravel, 7 = sand, 8 = mud.

Tshino		
Number of Variables	Correlation	Selections of variables
3	0.137	1,2,4
4	0.137	1-4
2	0.128	1,2
3	0.128	1-3
2	0.122	2,4
3	0.122	2-4
2	0.118	1,4
3	0.118	1,3,4
4	0.117	1,2,4,5
5	0.117	1-5
Mutale		
Number of Variables	Correlation	Selections of variables
3	0.176	1,2,8
4	0.16	1-3,8
2	0.159	1,8
4	0.147	1,2,5,8
4	0.146	1,2,6,8
5	0.143	1,2,5,6,8
5	0.142	1,2,4,6,8
5	0.142	1-3,6,8
3	0.142	1,6,8
3	0.142	1,3,8

Table 6.1.32: BIO ENV of meso-scale environmental variables and fish assemblages; variables: 1 = pH, 2 = conductivity (μS), 3 = DO (%), 4 = DO mg/l, 5 = temperature, 6 = TSS mg/l, 7 = turbidity (NTU), 8 = minimum velocity, 9 = maximum velocity, 10 = minimum depth, 11 = maximum depth.

Number of Variables	Correlation	Selections
5	0.432	1-3,5,9
4	0.431	2,3,5,9
5	0.43	2-5,9
5	0.429	2,3,5,9,11
3	0.429	2,3,9
4	0.427	1-3,9
4	0.427	2,3,9,11
4	0.426	2,4,5,9
4	0.426	2-4,9
5	0.425	1-3,9,11

The fish communities of Tshino and Mutale therefore differ significantly and analysis of environmental variables suggest that this is due to differences in water quality variables between the two sites (Fig. 6.1.13). More specifically the pH, conductivity, dissolved oxygen, temperature and maximum velocity. Micro-scale variables were very weak predictors of fish assemblage structure. Some explanations may include the disturbance of fish out of microhabitats because of the sampling protocol or that fish do not perceive their environment at these fine scales.

Table 6.1.33: Summary of the multiple regression analysis. Only those independent variables (environmental variables) that had a significant relationship with a dependant variable are listed.

Amphilius uranoscopus						
R= 0.97 R^2= 0.95 Adjusted R^2= 0.83						
F(11,5)=8.29 p<.02 Std.Error of estimate: 0.13						
		St. Err.		St. Err.		
	BETA	of BETA	B	of B	t(5)	p-level
Conductivity (us)	-0.62	0.22	-0.01	0.00	-2.82	0.04
TSS (mg/l)	-1.06	0.30	-0.02	0.00	-3.54	0.02
Turbidity (NTU)	1.25	0.30	0.04	0.01	4.24	0.01
Chiloglanis pretoriae						
R= 0.93 R^2= 0.86 Adjusted R^2= 0.56						
F(11,5)=2.86 p<0.13 Std.Error of estimate: 0.25						
		St. Err.		St. Err.		
	BETA	of BETA	B	of B	t(5)	p-level
Conductivity (us)	1.03	0.36	0.01	0.00	2.87	0.04
Labeo cylindricus						
R= 0.78 R^2= 0.61 Adjusted R^2= -----						
F(11,5)=0.72 p<0.7 Std.Error of estimate: 0.35						
Labeo marequensis						
R= 0.98 R^2= 0.96 Adjusted R^2= 0.85						
F(11,5)=8.86 p<0.01 Std.Error of estimate: 0.17						
		St. Err.		St. Err.		
	BETA	of BETA	B	of B	t(5)	p-level
Conductivity (us)	0.70	0.21	0.01	0.00	3.27	0.02
Labeo molybdinus						
R= 0.82 R^2= 0.68 Adjusted R^2= -----						
F(11,5)=0.96 p<0.56 Std.Error of estimate: 0.35						

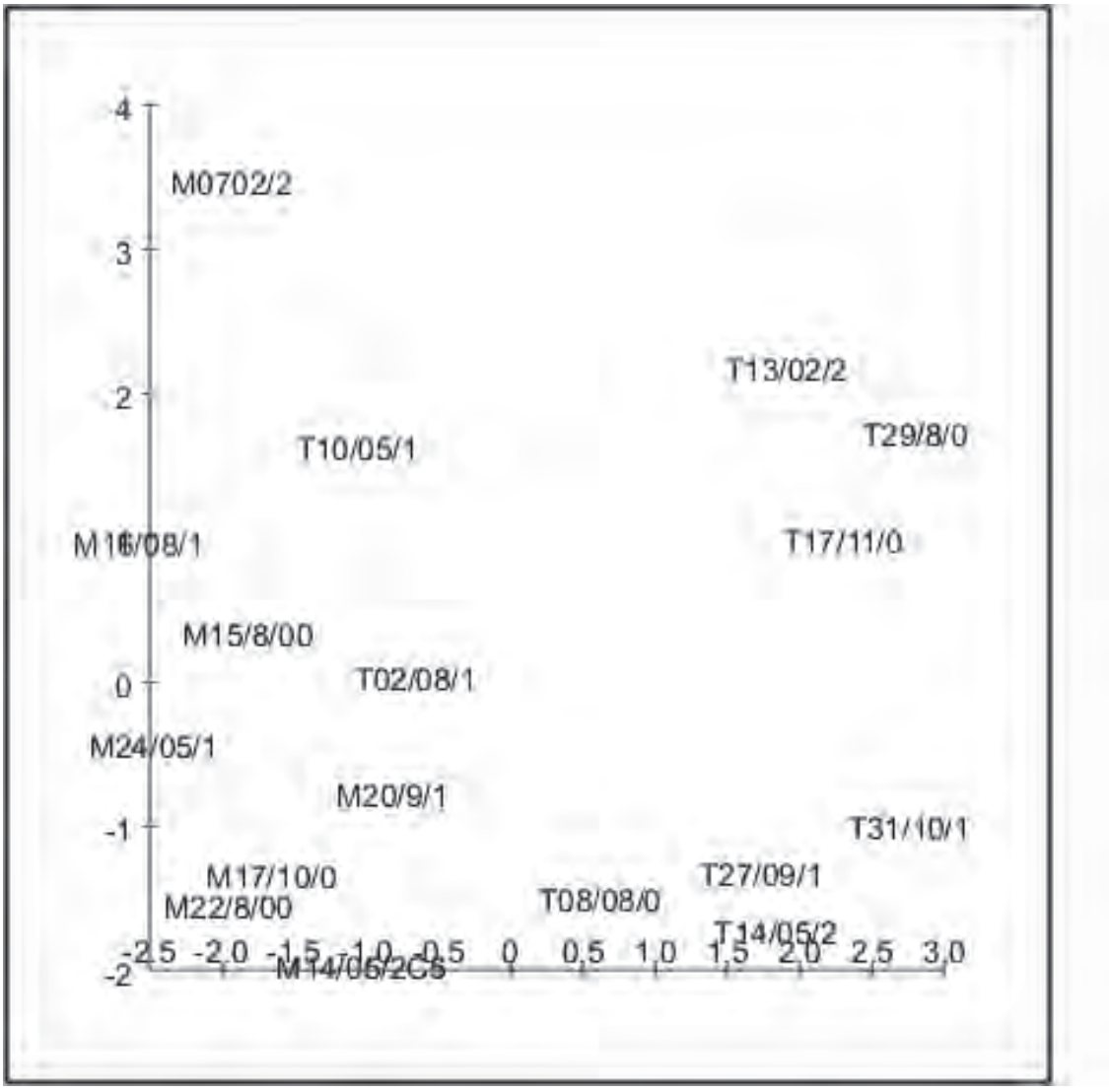

Figure 6.1.13: PCA of normalized meso-scale environmental variables. PC 1 and PC2 explain 57% of the observed variation.

6.1.4 Discussion

The expected rheophilic fish species (Skelton, 2001) dominated the fast flowing habitats but a number of species that are generally not considered to inhabit such habitats, were also found. This may be an indication that these species have a wider adaptation than generally assumed and make regular use of rheophilic habitats or that these fish were migrating. The Mutale had more specialist and sensitive species than Tshino. Included in this group are *B. eutaenia*, *B. neefi* and *O. peringuyei* (Kleynhans, 1991). The general

impression is that the Mutale River is in better condition than the Luvuvhu at Tshino. This supports the findings of the River Health Programme (WRC, 2001). This higher biodiversity can possibly be linked to the better habitat integrity of the Mutale River where human impact on the catchment is lower than that for the Tshino site in the Luvuvhu River below the intensely occupied Levubu commercial agricultural area. The decline in fish biodiversity at Mutale is however of great concern and clearly indicates that although the site is in better condition than Tshino habitat deterioration is already occurring.

A seasonal change in species composition at the sites is expected if the community is affected by seasonal influences. The data presented do not seem to indicate clear seasonal changes, especially not at Tshino. Prominent seasonal effects on the fish abundance or composition were not observed. Instead a steady change in the abundance at both sites, which indicates dynamic fish communities that are possibly not stable and still adjusting from the effects of the February 2000 flood, were observed.

The sudden absence of some species over the study period is evident for some species. In the Luvuvhu *B. lineomaculatus* was collected only in early summer and the warm water species *M. acutidens* and *M. brevianalis* only in early summer, but the opposite trend was recorded for *L. marequensis* that was more common in winter.

At Mutale a similar general tendency of fewer fish species towards the last surveys was observed. *B. trimaculatus, B. eutaenia* and *B. viviparus* were collected regularly in the first four surveys from May to October 2000, but not thereafter, Another anomaly was the relative scarcity of *L. marequensis* that was not collected during the 2000 surveys in the Mutale but only in 2001. *L. marequensis* was found once at Tshino in 2000 and then more regularly in 2001.

The fish community at Tshino is not stable and is changing over time, with an increase in numbers of especially *C. pretoriae* whereas the Mutale community is more stable and showed some seasonal variation but with an overall impression of a chnge in the

community size and composition. This may also be an artifact of lowered catch effectiveness during periods of higher stream flow when it became difficult to operate in the rivers when water levels were high and velocities increased.

Not all the species that had been historically recorded at the sites, and specifically in the river reaches where the sites are situated, were collected in the current 2000 – 2002 survey. At the Mutale River site three of the nineteen species that were historically collected were not observed. At the Tshino site the situation was worse where eight of the original nineteen species were not collected during the current survey. A similar trend of change was also observed during the period that the sites were surveyed.

An investigation of the habitat preferences of the fish showed that in general the findings of this study is in agreement with what is accepted (Fouche and Gaigher 2001, Fouche *et al*, 2003). As expected *L. cylindricus, A. uranoscopus* and *C. pretoriae* are classified as rheophilic and *L. marequensis* as semi-rheophilic. The fact that such a large percentage of *L. marequensis* in the Mutale River were collected in shallow slow habitats could be ascribed to the fact that they were small specimens, possibly juveniles, which were utilizing this habitat for survival. The observations concerning the smaller barbs, *B. neefi, B. lineomaculatus* and *B. trimaculatus* is of notable importance and could play a role when these species are selected as possible indicators in flow-stress-response studies and other indices of biotic integrity.

Statistically the fish communities of Tshino and Mutale differ significantly and analysis of environmental variables suggest that this is due to differences in water quality variables between the two sites. More specifically the pH, conductivity, dissolved oxygen, temperature and maximum velocity. Micro-scale variables were very weak predictors of fish assemblage structure. Some explanations may include the disturbance of fish out of microhabitats because of the sampling protocol or that fish do not perceive their environment at these fine scales. Habitat substrate i.e. the presence of boulders, cobbles, bedrock and mud are statistically not an important part in composition of fish assemblages at the small scale while velocity and depth influenced fish assemblages.

Analysis of the data sets does suggest that fish are better predictors of water quality than the macro-invertebrates. No real seasonality was evident in these communities and water quality variables were the best predictors of their composition. Multiple regression analyses suggests that two fish species are potentially good indicators of conductivity. Fish assemblages as a whole respond to water quality variables to a large extent.

References

CLARKE, K.R. and AINSWORTH, M. 1993. A method of linking multivariate community structure to environmental variables. *Marine Ecological Progress Ser*ies **92** 205-219.

CLARKE, K.R. and GREEN, R.H. 1988. Statistical design and analysis for 'biological effects' study. *Marine Ecological Progress Ser*ies **46** 213-226.

FOUCHé, P.S.O. & GAIGHER, I.G. 2001.Niche differentiation in the rheophilic fishes of the Mutshindudi. In Gaigher, I.G. (Ed) "A Socio- biological study of the aquatic resources and their utilization in an underdeveloped rural region, the Mutshindudi River Catchment." *WRC project report 714/3/01,* 52 - 69.

FOUCHE, P.S.O., VLOK, W. and ANGLISS, M.K. 2003. The habitat preferences and food selection of *Labeobarbus marequensis* in the Luvuvhu and Mutale rivers. *Proceedings of the 7th Yellowfish Working Group Conference*, Elgro Lodge, Potchefstroom, 22 – 24 May 2004.

GAIGHER, I.G. 1973. Habitat preferences of fishes from the Limpopo River system, Transvaal and Mocambique. *Koedoe* **16** , 103 – 116.

GAIGHER, I.G. and FOUCHé, P.S.O. 2001. An index of biotic integrity based on rheophilic fish in Gaigher, I.G. (Ed) "A Sociobiological study of the aquatic resources and their utilization in an underdeveloped rural region, the Mutshindudi River Catchment. *WRC project report 714/3/01,* 70 - 76.

GAIGHER, I.G., VAN DER WAAL, B.C.W. and FOUCHé, P.S.O. 2001. Fish distribution in the Mutshindudi River system. In Gaigher, I.G. (Ed) "A Sociobiological study of the aquatic resources and their utilization in an underdeveloped rural region, the Mutshindudi River Catchment." *WRC project report 714/3/0*1, 45 - 51.

JOHNSON, D.S. 1967. Distributional patterns in Malayan freshwater fish. *Ecology* **48** (5): 722 – 730.

KLEYNHANS, C.J. 1991. Voorlopige riglyne vir die klassifisering van die Transvaalse vissoorte in sensitiwiteitsklasse. Transvaalse Hoofdirektoraat Natuur en Omgewingsbewaring, Werkswinkel, Skukuza.

KLEYNHANS, C.J. 1999. The development of a fish index to assess the biological integrity of South African rivers. *Water SA* **25** (3) 265 – 278.

KRUSKAL, J.B. and WISH, M. 1978. *Multidimensional Scaling*. Sage Publications, Beverly Hills, California.

POLLING, L. 1999 Ecological aspects of the Ga-Selati River, Olifants System, Northern Province, Republic of South Africa. DPhil thesis, University of the North, unpublished, 312pp.

ROWNTREE, K. and WADESON, R. 2000. *Field manual for channel classification and condition assessment.*

SKELTON, P.H. 2001. *A Complete Guide to the Freshwater Fishes of Southern Africa*. (2nd Edition). Southern Book Publishers, Halfway House.

WILLIAMS, D.D. and FELTIMATE, B.W. 1992. *Aquatic insects*. CAB International, Oxford.

WRC. 2001.State of the Rivers report: Letaba and Luvuvhu Rivers.

6.2 Subproject : Food habits and intestinal morphology of the fish at the Mutale and Luvuvhu rivers.

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6.2.1 Introduction

It is hypothesized that differences in the dimensions, layering, topography and general structure of the intestines of fish can be related to their diet. Such differences in the structure could also be related to changes in the diet of fish of various ages. It would imply that niche differentiation could be inferred from the dimensions of the intestines and *vice versa*. In South Africa a number of studies have been carried out on fish intestinal structure (Göldner, 1964; Mulder, 1971; Bowen, 1976; Eccles, 1986; Fouche and Gaigher, 2001) but very few studies have been dedicated to stomach structure as such. It is also postulated that these differences in stomachs would be more pronounced in fish that tend towards carnivory or where complex stomachs are observed.

The aim of this subproject was

a) To determine what the fish eat through microscopical investigation of the stomach contents.
b) To compare the intestines of the fish to determine the types of stomachs observed in the fish.
c) To compare the structures of the “true” stomachs.
d) To compare the muscle layers of these stomachs in c).

6.2.2 Materials and methods

The fish that were collected at the two study sites in the Mutale and Luvuvhu rivers were placed on ice in bottles and transported to the laboratory.

In the laboratory, the fish species were again identified using the key proposed by Skelton (2001). The length of each specimen was measured to the nearest millimeter on a measuring board and mass was determined to the second decimal on a Sartorius balance.

The abdominal cavity was then dissected open using the method described by Willers (1991) to expose the viscera. The stomach type was identified and recorded after which it was removed and the diameter and length of the sections measured with calipers to the nearest millimeter. In the more complex stomachs, such as the Type I siphon or U-shaped stomachs (Fouche and Gaigher, 2001), the measurements were done on each subsection. In order to compare various size groups of fish, these dimensions were expressed as a percentage of the fork length. The average of each class size was calculated to differentiate whether differences related to fish age and size could be detected. The stomach structure of nine *Micralestes acutidens* and ten *Opsaridium peringueyi* specimens, collected lower down in the Luvuvhu River during the same period as this study, were also investigated for comparison.

The stomach contents were removed by slitting open the entire stomach and rinsing out the stomach contents with a small volume of water into a petri dish. The content was then examined under a dissecting microscope at 30 times magnification. Individual food items were manually separated and identified to the lowest possible taxon (Davies and Day, 1998; Williams and Feltimate, 1999). The volumes of the food types were estimated with the aid of a grid that was placed under the petri dish (Gaigher, 1967; Windell, 1971) and then expressed as a percentage of the stomach content. Frequency of occurrence of food types was determined by tabulating the food types identified. To determine the percentage frequency of occurrence the individual food items were sorted out and the number of stomachs in which it occurred was expressed as a percentage of the total number of stomach of the fish species in question (Gaigher, 1967).

The emptied stomachs were then placed in formol saline for 24 hours as a preliminary fixative. This was followed by a secondary fixation period of 6 hours in Helly's fluid (Baker and Silverton, 1980). After fixation, the tissues were dehydrated in alcohol solutions of different concentrations ranging from 30% to absolute alcohol. They were then impregnated in paraffin wax for at least 6 hours and finally moulded into blocks. Thin, 8μm, sections were cut from the moulded blocks using a Reichert-Jung rotary microtome and fixed to the glass slide. The slides were then dewaxed, hydrated and

stained with methylene blue and eosin yellow, (Baker and Silverton, 1980) after which they were provided with cover slips, fixed with Canada Balsam. After curing for 24 hours in an oven at 60^{o} C, they were viewed with a light microscope at 40X, 100X and 400X magnification.

The thickness of the muscle layers of the stomach wall was measured using an optical lens with a built-in micrometer scale, and expressed as a percentage of the total thickness of the stomach wall.

6.2.3 Results

6.2.3.1 Stomach contents

Tables 6.2.1 to 6.2.9 summarize the percentage volume and percentage frequency of occurrence of the three major categories of stomach contents. The three categories are: *filamentous algae*, *detritus* (which also includes diatoms and sediments) and material that could be identified as of *invertebrate* origin. As far as percentage frequency of occurrence of the latter is concerned only the values of the most common food of invertebrate origin is reflected. This kind of categorization was employed mainly because of difficulties encountered in identifying the invertebrate material. It also facilitated the division of the fish into different trophic types namely: herbivores (or algivores), detritivores and insectivores (carnivores). In Tables 6.2.1 to 6.2.9 the insect families that could be identified, as well as other invertebrate material that was observed, are listed in one line below the three major categories.

Sufficient amounts of stomach contents were collected from nine species to enable trophic categorization. Tables 6.2.1 to 6.2.9 also show that in some of the species diets was not the same for all the size classes. *Labeobarbus marequensis* and *Barbus viviparus* seemed to tend more to insectivory as size increased while in *C. pretoriae* the insect component became less.

Table 6.2.1 : Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *L. marequensis* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**		**90 - 99**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae	57,1	39,0	78,6	56,8	62,4	43,8	85,7	51,3	45,5	52,1	100	77,8	66,7	42,5
b) Detritus (including diatoms)	14,3	15,0	28,6	12,7	30,3	16,8	28,6	16,5	1,9	3,3	0	0	16,7	6,1
c) Invertebrate material:	28,5	46,0	28,5	30,5	33,3	39,4	28,6	32,2	27,3	44,6	28,6	22,2	32,3	51,4
Baetidae, Ceratopogonidae, Chironomidae, Chlorolestidae, Hydrophilidae, Leptoceridae and Lestidae Cladocera														

Table 6.2.2 : Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *B. trimaculatus* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**		**90 - 99**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae			75,0	67,8	77,8	62,8	72,2	68,3	57,1	62,5				
b) Detritus (including diatoms)			12,5	1,5	3,8	1,9	22,2	3,6						
c) Invertebrate material:			25,0	30,7	18,4	35,3	33,0	28,1						
Ceratopogonidae, Chironomidae, Coenagrionidae, Elmidae, Gerridae and Pleidae.														

Table 6.2.3: Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *L. cylindricus* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**50 – 69**		**70 - 79**		**90 – 139**		**140 - 219**							
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol						
a) Filamentous algae	46.5	4,3	67,8	8,1	67,3	7,3	66,7	5,8						
b) Detritus (including diatoms)	71,3	76,5	82,8	69,4	86,3	79,4	80,0	77,4						
c) Invertebrate material:	15,7	19,2	21,4	22,5	14,3	13,3	13,6	16,8						
Caenidae, Ceratopogonidae, Chironomidae, Elmidae, Pleidae and Tipulidae.														

Table 6.2.4: Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *A. uranoscopus* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**		**90 - 99**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae	0	0	29,8	13,3	27,3	10,3	48,5	11,7	18,3	21,6	41,7	15,1		
b) Detritus (including diatoms)	0	0	13,5	19,2	27,2	17,6	10,0	21,1	17,5	15,9	23,0	21,8		
c) Invertebrate material:	71,4	100	66,7	67,5	45,5	72,1	54,5	67,2	50,0	62,5	25,0	63,1		
Ancylidae, Ceratopogonidae, Chironomidae, Coenagrionidae, Elmidae, Gerridae, Heptageniidae, Hydrometridae, Hydrophilidae, Libellulidae, Hydropsychidae and Veliidae. Unionidae														

Table 6.2.5: Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *B. viviparus* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**		**90 - 99**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae	70,0	68,7	61,8	71,3	83,3	74,6								
b) Detritus (including diatoms)	25,0	16,4	20,0	7,8	0	0								
c) Invertebrate material:	14,3	14,9	22,2	20,9	33,3	25,4								
Ceratopogonidae, Chironomidae, Coenagrionidae, Hydrometridae, Hydropsychidae, Pleidae and Simulidae														

Table 6.2.6 : Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *B. lineomaculatus* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**		**90 - 99**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae	72,4	67,8	69,0	58,8	62,5	58,8								
b) Detritus (including diatoms)	27,8	12,1	4,2	10,8	12,7	20,0								
c) Invertebrate material:	30,8	20,1	57,1	31,2	25,0	21,2								
Ceratopogonidae, Chironomidae, Elmidae, Gerridae, Hydropsychidae, Tipulidae and Veliidae														

Table 6.2.7: Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *B. eutaenia* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**		**90 - 99**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae			67,5	53,8	63,4	43,3	100	61,0						
b) Detritus (including diatoms)			9,6	20,8	20,0	27,7	10,0	14,0						
c) Invertebrate material:			20,0	25,4	40,0	29,0	20,0	25,5						
Aeshnidae, , Chironomidae, Chlorolestidae, Coenagrionidae, Gerridae and Veliidae.														

Table 6.2.8: Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *C. pretoriae* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes (mm)													
Food items	**20 - 29**		**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae	28,6	13,3	11,7	14,9	22,5	12,5	58,9	18,2						
b) Detritus (including diatoms)	0	0	5,0	4,9	20	18,2	5,0	13,4						
c) Invertebrate material:	100	86,7	100	80,2	91,7	69,3	100	68,4						
Aeshnidae, Baetidae, Ceratopogonidae, Chironomidae, Corduliidae, Elmidae, Heptageniidae, Hydrophilidae Libellulidae, Naucoridae, Pleidae and Tipulidae.														

Table 6.2.9: Percentage frequency of occurrence (FOO) and percentage volume (% vol.) of the food types in the digestive tracts of *B. neefi* collected in the Luvuvhu and Mutale rivers from August 2000 - May 2002.

	Length classes													
Food items	**30 - 39**		**40 - 49**		**50 – 59**		**60 - 69**		**70 - 79**		**80 - 89**		**90 - 99**	
	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol	% FOO	% vol
a) Filamentous algae	76,2	62,2	63,2	58,7										
b) Detritus (including diatoms)	3,8	13,7	7,7	19,7										
c) Invertebrate material:	20,5	24,1	42,9	21,6										
Ceratopogonidae, Chironomidae, Elmidae, Hydrophilidae, Hydropsychidae and Pleidae.														

6.2.3.2 Stomach morphology.

Because of the variability and differences that exist between the stomachs of carnivores it was decided to concentrate on the stomach morphology of this trophic category. Sufficient numbers of *A. uranoscopus, M. macrolepidotus* and *P. wesselsii,* which are all classified as carnivores and specifically insectivores, (Skelton, 2001; Fouche and Gaigher, 2001) were collected during the study. But since very few *M. acutidens* and *O. peringueyi* were collected, it was decided to include specimens that were collected at Nandoni in the Luvuvhu River and upstream of the present Mutale River site. *L. marequensis* is included because of its tendency towards carnivory later in life as illustrated in this study.

L. marequensis and *O. peringueyi* were shown to have monogastric stomachs. It is a straight tubular expansion of the intestines that widens rapidly at the oesophageal or anterior end and then narrows towards the intestine. The regions of transition between the oesophagus and stomach and stomach and intestine could easily be determined. These stomachs were regarded as type II stomachs and their lengths and widths were measured and then calculated as a percentage of the average fork length (Table 6.3.10). *A. uranoscopus, M. acutidens* and *P. catostoma* have curved or U-shaped stomachs that are referred to as type I stomachs. Here there is a clear distinction between the stomach and the intestines due to the curvature at the posterior portion of the stomach. The distinction between the oesophagus and the stomach was also easy to detect and was characterized by a sudden narrowing of the digestive tract. To determine the physical dimensions of these stomachs, length and width of the sections before and after the stomach curvature were measured separately. The stomach length was then calculated as the percentage of the average fork length (Table 6.3.10).

Pyloric caecae were observed in *P. catostoma*, *M. macrolepidotus* and *M. acutidens* as finger-like projections that appeared in pairs and are blind sacs of the intestine, just beyond the stomach. Pyloric caecae may be highly branched or simple and are better developed in carnivores but in the abovementioned species they are simple.

Table 6.2.10. The stomach dimension and the ratios of the insectivorous fish collected in the Mutale and Luvuvhu rivers. (SD: standard deviation)

Species	N	Class size	Average length (SD)	Average stomach length	Stomach length as a % of fork length
A. uranoscopus	3	30-39	34.3 (2.08)	7.03 (2.92)	30.5
	5	40-49	43.6 (3.21)	12.2 (5.95)	37.8
	2	50-59	51.0 (1.40)	9.65 (1.06)	25.1
	9	60-69	65.0 (3.64)	16.26 (3.72)	32.6
	2	70-79	73.5 (2.12)	16.2 (5.38)	30.2
	7	80-89	85.2 (3.08)	15.62 (4.25)	25.5
	2	90-99	92.5 (2.12)	15.4 (1.41)	23.4
	3	100-109	103.7 (4.73)	18.62 (1.79)	25.3
	2	110-129	124.0 (5.70)	19.5 (1.41)	24.6
L. marequensis	6	20-39	34.8 (6.40)	10.4 (3.66)	29.2
	7	40-49	42.7 (2.75)	13.3 (3.81)	34.5
	5	50-59	52.4 (3.58)	14.1 (4.0)	32.6
	5	60-69	65.2 (3.11)	18.9 (5.06)	34.1
	5	70-79	74.2 (3.27)	25.7 (3.95)	40.0
	4	80-129	93.5 (17.71)	32.9 (13.9)	42.5
M. macrolepidotus	3	80-119	97.3 (15.57)	16.8 (2.86)	26.2
M. acutidens	2	30-49	42.0 (4.25)	14.05 (4.31)	41.3
	2	60-69	67.0 (0.00)	30.95 (1.63)	33.7
	5	70-79	70.8 (1.09)	19.82 (3.15)	37.0
O. peringueyi	3	40-49	46.7 (1.53)	10.27 (0.21)	27.0
	7	50-59	54.3 (3.86)	12.36 (2.54)	28.0
P. wesselsii	2	50-59	55.5 (2.12)	10.2 (1.55)	27.2
	3	60-69	63.0 (3.30)	14.97 (5.08)	26.8

Table 6.2.11 The micro-anatomy and histology of the stomachs of insectivorous fish collected in the Mutale and Luvuvhu rivers.

Species	N	Length class (mm)	Thickness of stomach wall (mm)	Thickness of muscle layer (mm)	Muscle layer as a % of the wall thickness
A. uranoscopus	3	30-39	0.22	0.05	22.7
	5	40-49	0.25	0.05	20.0
	2	50-59	0.2	0.046	23.0
	9	60-69	0.36	0.13	36.1
	2	70-79	0.30	0.10	33.3
	7	80-89	0.33	0.10	30.3
	2	90-99	0.42	0.16	38.1
	3	100-109	0.58	0.15	25.9
	2	110-129	0.60	0.14	23.3
L. marequensis	6	20-39	0.31	0.09	29.0
	7	40-49	0.29	0.08	27.6
	5	50-59	0.23	0.085	37.0
	5	60-69	0.25	0.08	32.0
	5	70-79	0.23	0.09	39.1
	4	80-129	0.23	0.09	39.1
M. macrolepidotus	3	80-119	1.25	0.5	40.0
M. acutidens	2	30-49	0.41	0.12	29.3
	2	60-69	0.32	0.10	31.3
	5	70-79	0.25	0.11	40.0
O. peringueyi	3	40-49	0.28	0.11	39.3
	7	50-59	0.26	0.07	26.9
P. wesselsii	2	50-59	0.41	0.22	53.7
	3	60-69	0.43	0.23	53.5

6.2.4 Discussion

Stomach content analyses (Tables 6.2.1 to 6.2.9) show that *A. uranoscopus* and *C. pretoriae* are carnivorous, feeding mostly on invertebrate material. In the case of the latter species the invertebrate content becomes less as the fish grows but still remains more than 68 %. The change in diet composition of this species could be ascribed to an increase in the detritus component and could be the result of the scraping action that

forms part of the feeding habit of the fish. *L. marequensis* seems to be more omnivorous, with a tendency towards herbivory in the smaller fish. This changes in the 70 – 79 mm size class when it becomes more carnivorous.

The small barbs investigated, *B. viviparus, B. trimaculatus, B. lineomaculatus, B. neefi* and *B. eutaenia* are herbivores as the diet consists of plant or algal material more inclined towards a diet of filamentous algae. The plant component of the diet of *B. viviparus* was found to increase with age while in the case of the other species it decreased.

The stomach content of *L. cylindricus* on the other hand clearly illustrates its detritivorous lifestyle.

The findings concerning *B. viviparus, B. neefi, B. lineomaculatus* and *B. eutaenia,* namely that they are all four herbi – or algivores is supported by previous studies (Fouche and Gaigher, 2001) where similar stomach contents were observed.

The change in the diet composition of *L. marequensis* is also reflected by the increase in stomach length (Table 6.2.10) and by the increase in muscularity (Table 6.2.11) that accompanies growth in length. It is of note that these changes occur at the same length size classes as where the change in diet was observed. The fact that no such changes occur in *A. uranoscopus* is in line with its unchanging diet composition.

From the findings concerning the stomach dimensions and musculature of *P. wesselsii, M. acutidens* and *M. macrolepidotus* it can be inferred they are carnivores, and specifically insectivores, and it is expected that their stomach contents will underscore the assumption.

Fouche and Gaigher (2001) found that the stomach contents of *O. peringueyi* consisted only of animal material, but in contradiction to the other carnivores they found a type I stomach. These fish like the true carnivores such as *A. uranoscopus* and *M. acutidens* do

however have relatively short intestines. This study found that this fish had small stomachs and that the muscular component seems to lessen with age.

Although the findings of this study are not conclusive, because of small sample sizes, it does indicate tendencies and these tendencies need to be investigated more closely as they would supply invaluable information regarding the sensitivity of the species.

6.2.5 Reference list

BAKER, F.J. and SILVERTON, R.E. 1980. *Introduction to Medical Laboratory Technology*. Butterworth Scientific, London.

BOWEN, S.H. 1976. Feeding ecology of the cichlid fish, *Sarotherodon mossambicus*, in Lake Sibaya, Kwazulu. Unpublished Ph.D. Thesis, Rhodes University.

DAVIES, B. R.. and DAY, J. 1998. *Vanishing Waters*. UCT Press, Cape Town.

ECCLES, D.H. 1986. Development of the gut in the South African cyprinid fish *Barbus aeneus* (Burchell). *S. Afr. Tydskr. Dierk.* **21** (2) 165 – 169.

FOUCHé, P.S.O. & GAIGHER, I.G. 2001.Niche differentiation in the rheophilic fishes of the Mutshindudi. In Gaigher, I.G. (Ed) "A Socio- biological study of the aquatic resources and their utilization in an underdeveloped rural region, the Mutshindudi River Catchment." *WRC project report 714/3/01,* 52 - 69.

FOUCHE, P.S.O., VLOK, W. and ANGLISS, M.K. 2003. The habitat preferences and food selection of *Labeobarbus marequensis* in the Luvuvhu and Mutale rivers. *Proceedings of the 7th Yellowfish Working Group Conference*, Elgro Lodge, Potchefstroom, 22 – 24 May 2004.

GAIGHER, I.G. 1967. Aspects of the ecology of the tigerfish, *Hydrocynus vittatus* Castelnau, in the Incomati River system. Unpublished M.Sc. Thesis, Pretoria University.

GöLDNER, H.J. 1964. Maaginhoudanalise van *Clarias*, *Tilapia* en *Micropterus* soorte. *Progress Report*, *Provincial Fisheries* Institute, Lydenburg.

SKELTON, P.H. 2001. *A Complete Guide to the Freshwater Fishes of Southern Africa*. (2nd Edition). Southern Book Publishers, Halfway House.

MULDER, P.F.S. 1971. 'n Ekologiese studie van die hengelvisfauna in die Vaalriviersisteem met spesiale verwysing na *Barbus kimberleyensis* (Gilchrist & Thompson). Unpublished Ph.D. Thesis, Randse Afrikaanse Universiteit.

WILLERS, B. 1991. *Trout Biology*. Lyons and Burford, New York.

WILLIAMS, D.D. and FELTIMATE, B.W. 1992. *Aquatic insects*. CAB International, Oxford.

WINDELL, J.T. 1971. Food analysis and rate of digestion. In: *Methods for assessment of fish production in fresh waters.* (ed.) Ricker, W.E. Blackwell Scientific Publications, Oxford.

WRC. 2001.State of the Rivers report: Letaba and Luvuvhu Rivers.

CHAPTER 7

A PREDICTIVE MODEL OF ANTHROPOGENIC INFLUENCES IN THE LUVUVHU RIVER

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7.1 Objectives

The objective of this study was to develop a predictive system that would capture the complex causal relationships between various measurable factors and specific problems in an *associative net* (Figure 7.1). A system was envisaged that would train itself when given well-defined data inputs and classifications. It would then provide a reliable diagnosis of the current state of the river and also provide a prognosis of trends in the river quality. Ideally, a complete model would suggest control actions to return the river to its desired state. Thus, it was hoped to contribute to the maintenance of biotic integrity of the Luvuvhu River by the provision of a reach-scale tool that can be applied to catchment-scale actions.

7.2 General methodology

Initially, a simplified conceptual model was developed in an attempt to abstract important attributes at increasing levels of detail (Fig.7.2). This model provides the essential framework for the understanding of the various influences on the river, as it captures those concepts that are important for the maintenance of biodiversity.

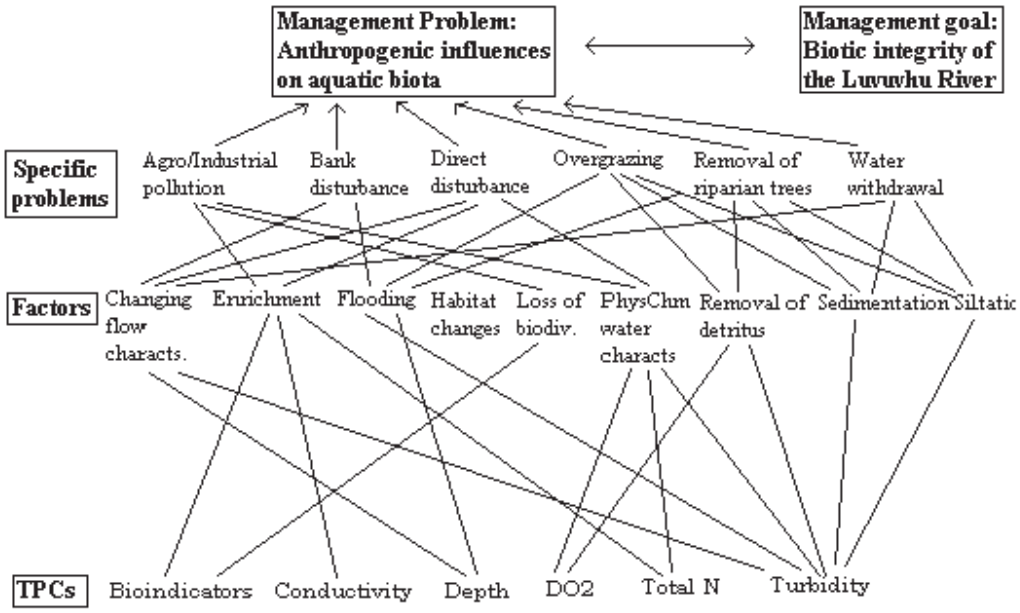


Figure 7.1: Network model of relationships between various environmental factors and specific problems.

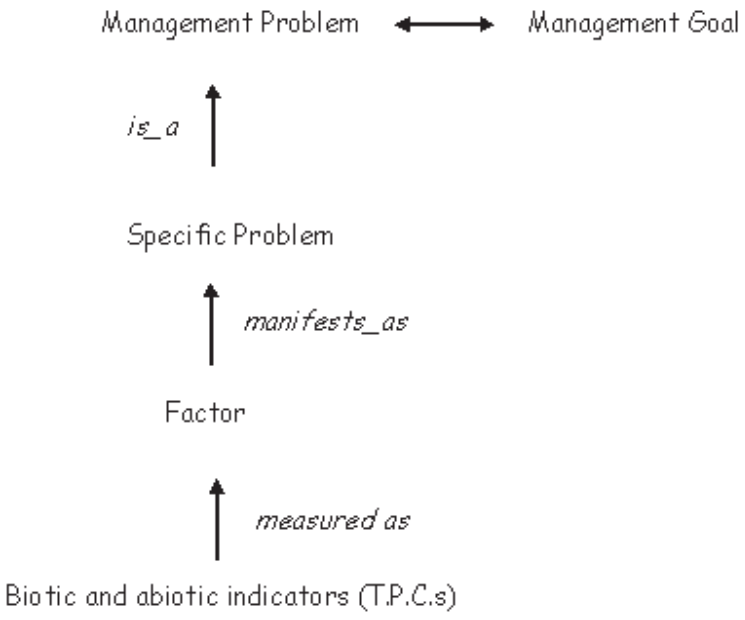


Figure 7.2: Overall conceptual model.

At the highest level of river management, the *management objective* is assumed to be the maintenance of biodiversity, with the most important *management problem* being the anthropogenic influences on aquatic biota. These influences can be categorized further in several *specific problems* that are manifested, *inter alia*, in the form of contributing *factors* that lead to several measurable responses for which specific criteria can be laid down.

Thus, measurable bio/physico/chemical parameters were needed that would enable not only a categorical classification of river health, but also indicate the sources of concern (*i.e*., which of the above Factors or Specific problems predominate).

7.3 Factors contributing to specific management problems

In the case of the Luvuvhu River, these factors may be summarized as:

Agro/Industrial pollution
Bank disturbance
Direct disturbance
Overgrazing
Removal of riparian trees
Water withdrawal

Each of these problems can be addressed through management action.

Problems with river management manifest, *inter alia*, in the form of contributing *factors*, summarized as chemical variables, biotic factors, energy sources, habitat structure and flow regime (Karr *et al.*, 1986). We were especially interested in the following eight.

7.3.1 Changing flow characteristics

Variations in rainfall from year to year impact on river flow. Generally, the drier the catchment and the lower the base flow, the greater the variation in flow regime between years. Anthropogenic disturbances to river banks and the river itself, and water withdrawal, contribute to channel modification and changing flow characteristics.

In turn changes in flow regimes affect human populations living near the river that depend on water from the river. Although dams provide flood attenuation they also lead to the sediment loss in the standing water body. The latter aspect leads to enhanced downstream scouring during flood releases and consequently sediment loads higher than normal are experienced downstream. Climate change may lead increasingly to flood events. Unfortunately, changing river flow is one of the most difficult disturbances to detect, needing extensive flow records and mathematical testing of non-stationarity (Radziejewski *et al.*, 2000).

7.3.2 Enrichment

This seems to be a global problem, usually caused by sewage effluent and agricultural and household fertilizer runoff, and is measurable as nitrate and phosphate. Nutrient enrichment causes increased growth of some algae, resulting in habitat changes for aquatic animals. While moderate quantities of nutrients stimulate the production of healthy levels of phytoplankton, excessive algae may deplete oxygen, block sunlight needed by submerged aquatic vegetation, and cause the overall health and aesthetic quality of the river to decline.

7.3.3 Flooding

An increased runoff rate in a compromised riparian zone may cause increased sediment delivery, in-channel habitat degradation and riparian zone degradation.

7.3.4 Habitat changes

River width and depth, bank stability, channel morphology, gradient, instream cover, the canopy, substrate, current, siltation and riparian vegetation contribute to habitat structure. Any changes or impacts on these aspects would then lead to habitat change.

7.3.5 Loss of biodiversity: microbes, fish, insects

Here disease, reproduction, competition, predation, feeding and parasitism are all contributing factors. Biota respond to these natural factors, which act cumulatively. As these factors are integrated over time, a characteristic community structure emerges.

When human actions affect a system, the biological population and consequently the biodiversity will change and possibly result in decreased resilience of the biotic community. Sensitive taxa disappear, food webs are disturbed, diversity usually decreases, and pest species may dominate.

7.3.6 Physicochemical water characteristics

These include solubility and adsorption of pollutants (organics and nutrients), alkalinity, hardness and pH, dissolved oxygen, turbidity and temperature.

7.3.7 Removal of detritus and sedimentation

Sedimentation is the action or process by which matter settles to the bottom of a body of water. It is the result of soil erosion, causing major on-site degradation of a non-renewable natural resource, and off-site sediment deposition in fields, floodplains and water bodies. Erosion and the resulting sediment deposition are natural processes forming the landscape, but they are easily accelerated by human intervention. Conversion of land to urban and agricultural uses by clearing of the natural forest buffers on riverbanks, associated with overgrazing and poor farming practices, accelerate sedimentation caused by storm water runoff. In turn sedimentation and excessive algal growth lead to a decline in submerged aquatic vegetation, which serves as a habitat for juvenile fish. . Sedimentation can also lead to an increased embedding of substrate such as cobbles. This implies that interstitial spaces amongst cobbles are filled with sediment and the “hatching areas” of the eggs of fish destroyed.

7.3.8 Siltation

Silt is loose sedimentary material with rock particles. Siltation is caused mainly by cropping and grazing, urban runoff and construction, and has several water quality impacts. Siltation decreases water clarity, causing a decrease in aquatic plant production, obscuring sources of food, habitats, refuges and nesting sites for fish. It also fills gravel spaces in stream bottoms, smothering fish eggs and juvenile fish. Pesticides become attached to soil particles and so enter the water. Finally, siltation decreases the recreational and aesthetic value of streams and decreases the quality of drinking water.

Each of these factors leads to a measurable response. However, the fact that each factor results in several responses, which may overlap, complicates the model. An effect involving any one factor can set off a chain of events, which results in cumulative changes reflected by most, or even all, of the interacting factors. Biological integrity is the combined result of chemical, physical and biological processes. The interaction of these three factors is especially apparent where diffuse pollution is concerned. It will therefore be useful to measure both the interacting processes and the integrated result of chemical and biological processes.

7.4 Measurable indicators of river health

The principal problem in river management is to predict and monitor the biodiversity responses of rivers and river reaches to changes in the abovementioned factors. Firstly, it is necessary to find a series of reliable, easily determined indicators (as their critical values or *Thresholds of Probable Concern - TPC*) of the state of the factors. Secondly, a predictive model is needed which relates these TPC's to factors and specific management problems. For regulatory purposes a Total Maximum Daily Load (TMDL) may also be considered.

TPC's are lower level management goals that are 'scientifically based, spatially and temporally bounded targets of ecosystem condition' which 'act as amber lights to warn managers of possible unacceptable environmental change' (Mackenzie, van Coller and Rogers, 1999). The values of particular TPC's may vary depending on several factors, and must therefore be periodically revised (Bestbier, Jacoby and Rogers, 2000), which may lead to controversy. For our present purposes, the following measurable indicators and indices (with upper and lower bounds shown, where relevant) were selected:

Measurable indicators

Bioindicators:

- Algae
- Diatoms
- Invertebrates
- Vertebrates

Electrical Conductivity	*85 $mS.m^{-1}$
Depth	
Detritus load -Total suspended solids	*15 $mg.dm^{-3}$
Dissolved oxygen	*6 $mg.dm^{-3}$
Total dissolved solids	*200 $mg.dm^{-3}$
Flow rate	
Non-nutrient chemicals:	
Ammonium ion	*0,02 $mg.dm^{-3}$
Calcium	**9 $mg.dm^{-3}$
Magnesium	**6 $mg.dm^{-3}$
Sulphate	**110 $mg.dm^{-3}$
Nutrient chemicals:	
Total phosphorus	**0,02 $mg.dm^{-3}$
Nitrite + nitrate	**0,2 $mg.dm^{-3}$
Poisonous chemicals	
pH	**6,5-8,1
Temperature	**8-25°C
Turbidity	*8 NTU

Note: * TPC's determined for the Luvuvhu River (Rogers and Bestbier, 1997: 85).

** Proposed TPC's based on criteria for the Sabie, Olifants, Letaba and Crocodile Rivers (Rogers and Bestbier, 1997: 83-88.)

Indices

SASS5

FAII (Fish Assemblage Integrity Index)

FIOBI (Fish Index of Biotic Integrity)

7.5 Methods

The model to be developed will be highly specialized in use and application, and will attempt to model the complex causal relationships between TPC's and factors and

specific problems (Fig. 7.1). The system should be able to supply a reliable diagnosis of the current state of the river, and also be able to provide a prognosis concerning trends in the river quality. Ideally, a complete model would suggest control actions to return the river to its desired state. Artificial Intelligence (AI) approaches are becoming recognized as the most suitable for the modelling of these highly complex, (mostly) nonlinear relationships.

An expert system is a computer program that represents and reasons with specialist knowledge, and can emulate human cognitive skills such as problem solving or visual perception. This technology developed from the computer science research discipline of Artificial Intelligence (AI) and has been successfully applied to a diverse range of domains, such as chemistry, engineering and internal medicine (Jackson, 1990). Typically, an expert system has to perform a *classification task*, where the emphasis is on finding an approximate but meaningful mapping between input data and solutions at some level of abstraction. All available evidence has to be considered and combined in such a way that one of a range of previously defined categories (the *solution space*) is chosen as the solution.

Two of the most popular classes of expert systems are *rule-based systems* and *artificial neural networks*. Rule-based systems depend on deriving, evaluating and tuning sets of decision rules with the help of human experts, whereas artificial neural networks are designed as 'self-improving' systems that can learn concepts from examples.

7.5.1 Rule-based systems

Artificial intelligence research is based on the premise that intelligent behaviour is rule-governed. Intelligence is generally associated with regularities in behaviour, even if the precise rules are not known. Production rules are a formalism used in various fields before being used in psychological modelling and expert systems. They are also called *condition-action* rules or *situation-action* rules because they can be used to encode empirical links between data presented to the system and actions the system should perform as a consequence. In other words, given some set of inputs, the rules generate

some form of behaviour (Buchanan and Shortliffe, 1984). The rules generally have the form (Jackson, 1990):

if *conditions* P_1 and .... and P_m are true

then perform *actions* Q_1 and .... and Q_n

Rule-based expert systems rely on in-depth expert knowledge, which is incorporated in the system through relationships. The better the knowledge of these relationships, the better the performance of the system. The expert system keeps track of the relations and inferences invoked, and can therefore explain why certain information is needed and how certain conclusions are reached. Rule-based systems are modular, so that reasoning mechanisms (the *inference engine*) and knowledge (the *knowledge base*) can be developed separately (Fig. 7.3). The knowledge base contains as much available knowledge about the problem as possible. This knowledge is acquired (often through a separate knowledge acquisition system) from one or more human experts, and encoded in a form that the expert system can interpret. The expert system makes recommendations based on rules provided by the human expert to the inference engine. These rules are a formal rendition of the ways in which the experts would take their decisions based on the available evidence. Adding fuzzy logic to the inference engine (Carrasco *et al.*, 2002) enables the system to use incomplete data and to provide non-exact solutions. In the processor the rules, the knowledge base, and the user's inputs are used to make deductions and to decide on questions to be asked of the user. The processor combines loose pieces of information to build up a final solution.

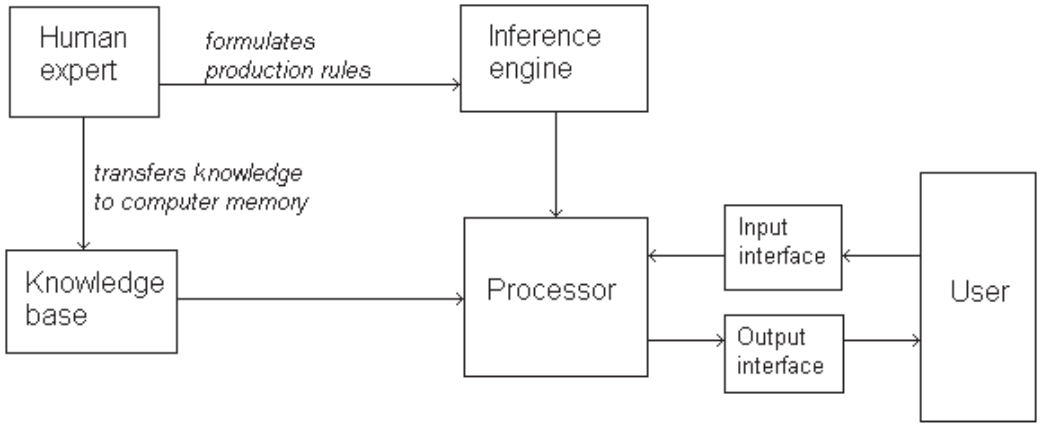


Figure 7.3: Block diagram of a rule-based expert system.

Yet, there are also several disadvantages associated with rule-based systems. The expert system can be wrong, and its reliability is totally dependent on the knowledge previously incorporated. Where a human expert uses much information from a variety of knowledge areas, much of it of a generic nature, and some of it intuitive, the expert system is limited in knowledge to the contents of its knowledge base, and in reasoning power to the reasoning possibilities of its inference engine.

Nevertheless, rule-based expert systems have been applied successfully in such diverse areas as plant identification (Gouws, 2000) and wastewater treatment (Carrasco *et al*., 2002).

7.5.2 Artificial neural networks

The capability of neural networks in learning complex systems has led to their use in data classification. The first attempts in machine learning (McCulloch and Pitts, 1943; Minsky and Selfridge, 1961) tried to mimic the learning process of animals at the neural level. In computer simulation of brain function, sets of idealized neurons are stimulated and prompted to adapt their behaviour via digital forms of reward and punishment. More recently the discovery of new neural network architectures and powerful new learning algorithms have led to renewed interest in neural network learning.

For example, Wei *et al.* (2001) were able to quantify the interaction between abiotic factors and algal blooms,

Laberge *et al.* (2000) predicted metal bioleaching in continuous processing of municipal sewage, Ballester *et al.* (2002) predicted hourly surface ozone concentrations, Choi and Park (2001) trained a neural network as a software sensor for wastewater quality, Brion and Lingireddy (1999) were able to identify non-point sources of microbial contamination in a drinking water reservoir, and Aguilera *et al.* (2001) applied a Kohonen neural network as a decision-making tool in coastal water quality management.

Neural networks comprise a set of highly interconnected but simple processing units, referred to as nodes, that are responsible for carrying out a few rudimentary computations (Rich and Knight, 1991). The nodes are arranged in several layers that are interconnected through a large number of weighted functional linkages encoding the knowledge in the network. Figure 7.4 shows a layout for a typical three-layered feed-forward network, made up of an input layer, a hidden layer, and an output layer. A superficial relationship to Figure 7.1 can be immediately recognized.

Neural networks (such as Hopfield's, 1982) are characterized by:

- Distributed representation: Memory is represented by activation of a number of processing elements;
- Distributed, asynchronous control: Each processing element takes decisions based only on its own local situation, that contributes to a global solution;
- Content-addressable memory: A number of patterns can be stored in a network. Such a pattern can be retrieved by specifying only part of it. The network automatically finds the best mapping/fit;
- Fault tolerance: The network functions correctly even when some processing elements fail.

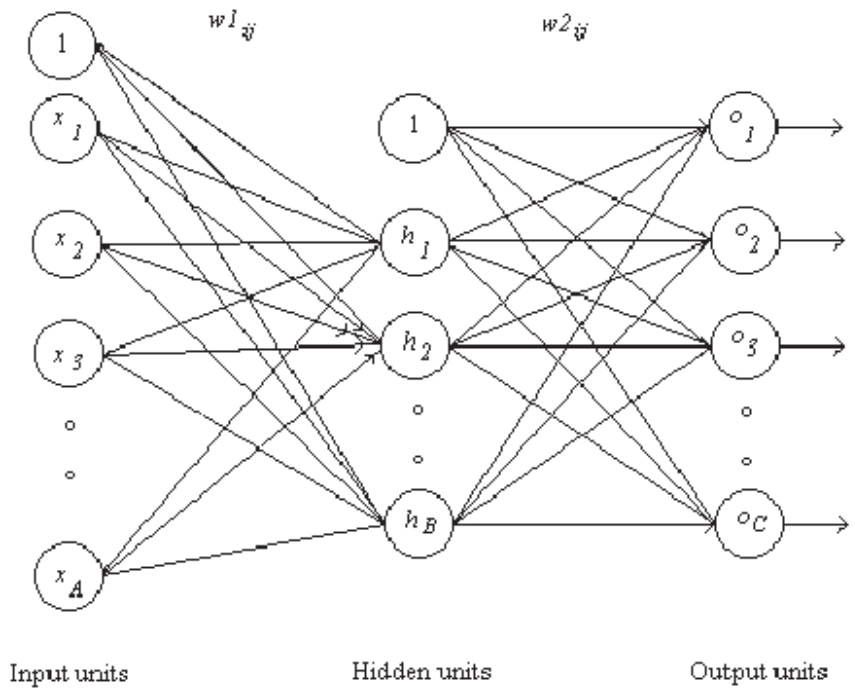


Figure 7.4: A typical feed-forward neural network.

These characteristics enable a neural network to learn complex systems. If provided with a sequential set of input and known output for a given system, the network organizes itself internally so that it closely predicts the expected output for any other given output. The nodes in the network take a weighted (w) sum of its inputs (x), and send the output 1 if the sum is greater than a certain adaptable threshold value, or else 0. The inputs and outputs typically are real values. The weight is positive if the presence of a feature (input) activates the node, and negative if the presence of the feature inhibits the node.

The learning process consists of a continuing modification of the weights and the threshold. The threshold is implemented conveniently as just another weight (w_0), and can be regarded as the tendency for a node to fire independent of its inputs. The node fires if the weighted sum of its inputs is greater than 0, and calculates a binary function of its input. This internal process of self-organization or developing a generalized representation of the system is referred to as “training” and is crucial for an efficient prediction phase of the neural network.

A network is trained on input-output pairs until the correct function is calculated. Being independent of each other, nodes can be trained independently.

A neural network is said to be well-trained if the deviation between output, predicted by the neural network, and the targeted output is within a tolerable limit. Two of the factors that affect the training process are the number of hidden layers and the number of nodes employed in the hidden layers. Multiple hidden layers in neural networks provide additional learning power to separate non-linearly separable classes, but according to the Hornik-Stinchcombe-White theorem a layered artificial neural network with only two node layers is sufficient as long as there are enough nodes in the single hidden layer. If there are few hidden layer nodes, there may not be enough opportunity for the network to capture the intricate relationships between inputs and output. On the other hand, too many hidden layer nodes require a large computation time for accurate training and may also result in "over-training." A neural network is over-trained when the network focuses on the characteristics of individual data points rather than just capturing the general patterns present in the entire training dataset. Having more than two layers increases the computational complexity and instability in training, and impairs its use because the extra layers (acting sequentially) slow down the processing. On the other hand additional layers may circumvent the use of a very large number of nodes in a single hidden layer. In this study, hidden layer nodes that are twice the number of input and output layer nodes were used.

In a feed-forward network, each node in one layer is connected in the forward direction with each node in the next layer. They are most commonly trained using a back-propagation algorithm. In a backpropagation network a slightly modified activation function is chosen for the units, *viz*. a sigmoid function which is continuous and differentiable, as required by the backpropagation algorithm. The weighted inputs are summated but the output (for a unipolar function) is a real value between 0 and 1, given by

$$output = (1 + e^{-sum})^{-1}$$, where *sum* = the weighted sum of the inputs to a unit.

The backpropagation network starts with a random selection of weights that are modified every time the network perceives an input-output pair. Each pair requires two stages: a forward and a backward pass. With the forward pass the network is presented with an input and the nodes are allowed to fire (or 'activations flow') until the output layer is reached. With the backward pass the real output of the network is compared with the calculated output and error estimates of the output nodes are calculated. The weights linked to the output nodes are then adjusted to decrease these errors. Next, error estimates for the hidden layers are calculated, layer by layer, until errors are propagated back to the input layer. The weights are therefore adjusted incrementally every time after an input-output pair has been inspected. One iteration of the algorithm can be written as

$$w_{k+1} = w_k - \alpha_k g_k$$

where w_k is the vector of current weights and biases, g_k is the current gradient, and α_k is the learning rate. After all the input-output pairs have been seen and all the weights have been adjusted, one *epoch* has been completed. Training of a backpropagation network usually requires many epochs, although modern algorithms exist which dramatically reduce the number of epochs and therefore calculation requirements.

In this study, the Neural Network Toolbox in the **MATLAB** (Version 7, Mathworks, Inc., 2004) was used to create a two-layer feedforward backpropagation network. During the training the weights and biases were iteratively adjusted to optimize the network performance, and evaluated with the mean square error (MSE) between the network outputs and the target outputs. The default value of the training goal (MSE 1e-10) was used.

By assembling a database of input and known output values, the network can be trained and then used to classify new data sets. As an example of how this output can be used for river health classification, this study has considered altitude, pH, conductivity, DO_2, temperature, TSS, turbidity, velocity and depth, as well as the presence (1) or absence (0) of bedrock, boulders, cobbles, gravel, sand, and mud.

7.6 Results and discussion

A synthetic dataset (n = 20) was used to train a feed-forward backpropagation neural network consisting of one intermediate layer of 10 neurons. The following measures were used: Ammonium ion, Biocides, DO_2, EC, Fluoride, heavy metals, TDS, TSS, Turbidity, and pH. These 11 measures were successful in classifying the dataset into six distinct groups according to which Factor(s) predominate. These preliminary results demonstrated that neural network modelling can be used as a diagnostic or classification tool.

7.6.1 Classification model training and prediction

Six data attributes were used to classify the training set (Class1), using a binary code, as follows:

- If pH is less than 6.0 or above 8.0, add 1.
- If conductivity is above 85 mS/m, add 2.
- If dissolved oxygen is below 6.0 mg/l, add 4.
- If total suspended solids is above 15 mg/l, add 8.
- If turbidity is above 8 NTU, add 16.

Theoretically, 32 classes are possible, but in our present collection only six were found:

- Class1 = 0: Pristine.
- Class1 = 1: pH exceeds threshold.
- Class1 = 10: Conductivity and total suspended solids exceed threshold.
- Class1 = 24: Total suspended solids and turbidity exceed threshold.
- Class1 = 26: Conductivity, total suspended solids, and turbidity exceed threshold.
- Class1 = 27: pH, conductivity, total suspended solids, and turbidity exceed threshold.

As it was thought that the wide range of values might influence the training characteristics, the classes were transformed to a new series of values from 0 to 5 (Class2).

To use the model for classification purposes it first had to be self-trained. To accomplish this, a subset of data points from the 307 available (see Tables 6.1.1 to 6.1.28 in Chapter

6) for the two years of sampling, was randomly selected for use in training. Table 7.1 shows an example of the randomly selected data points as they were input into a feed-forward backpropagation neural network model during training. A single hidden layer consisting of 31 neurons was used, using batch gradient steepest descent optimization. The transfer functions used were *logsig* for the hidden layer (a function of the type defined above, which accepts any input between minus and plus infinity and transforms it to sigmoidal output between 0 and 1) and *purelin* for the output layer (which transforms the sigmoidal output of the hidden layer linearly to any value between minus and plus infinity). This strategy has been shown to be highly successful in modelling complex nonlinear relationships.

The training function used was TRAINLM, and the learning function LEARNGDM. TRAINLM, using the Levenberg-Marquardt backpropagation algorithm (Hagan and Menhaj: 1994), appears to be the fastest method for training moderate-sized feed-forward neural networks (up to several hundred weights) and is implemented very efficiently in MATLAB. LEARNGDM adds gradient descent with momentum to the algorithm (Hagan *et al*., 1996:12-9). The default values of the learning rate (lr = 0.01), and momentum constant (mc = 0.9) were used.

The neural network model was trained and used for prediction of data withheld from the training to fulfil two different objectives: evaluation of the overall applicability of the neural network model for site classification and portability of the model to sites other than those used for training.

7.6.2 Overall applicability of the classification model to all sites for prediction after training

First, to test if the model would accurately classify all 307 sites, a subset of data representing all of the sites was used to train the model.

A training set of 206 data points was selected and used to train the neural network model. The remaining data points (the validation set) were used to verify the efficacy of the

trained neural network model in classifying the sites. No attempt was made to optimize the selection process and all data points were randomly selected. After 100 error back-propagation iterations the training process already produced acceptable results. Figure 7.5 shows the convergence characteristics of the training process in a typical run. This required a computational time of 75 seconds on a personal computer (Pentium 1.8 GHz). Typical output on the points predicted by the model is shown in Table 7.2.

The neural network predictions presented in Table 7.2 are close to the actual numerical values obtained, which can be attributed to the fact that a sufficiently large training set could be compiled (Brion and Lingireddy, 1999).

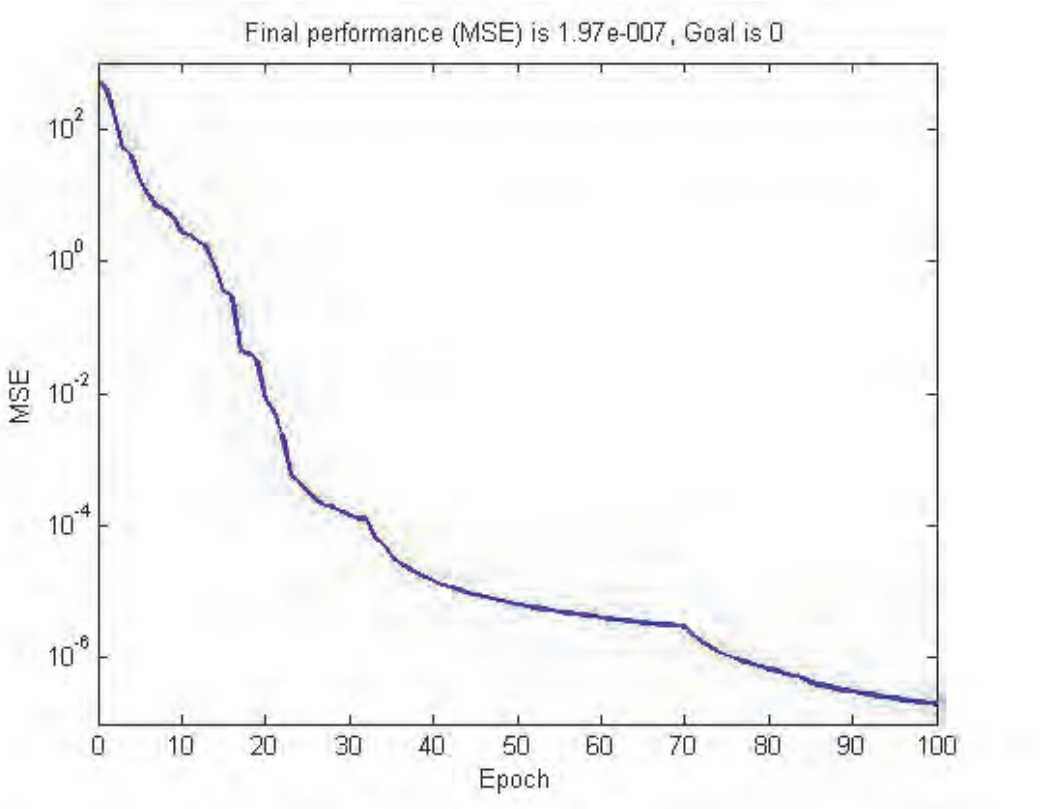


Figure 7.5: Training convergence of initial classification data (Class1).

Table 7.1: Example of actual training data input to the neural network model.

	M17/10/0D4	M24/05/1A1	M20/09/1D3	M01/11/1A2	T02/08/1C5	T27/09/1A2	T31/10/1D6	T13/02/2A2	T13/02/2C7	T14/05/2A2
Altitude	668	668	668	668	576	576	576	576	576	576
pH	7	7	7.2	8.7	7.1	8.1	9.4	7.8	7.8	8
Cond uS	46	48	46	48.8	90	103	109	109	109	124
DO%	91	98	92	94	92	90	91	87	87	93
DO mgl	7.6	8.8	8.3	8.2	8.2	8.5	8.6	6.7	6.7	8.2
Temp	20.7	16	19.5	23.2	16.1	20.6	25.1	25.5	25.5	17.1
TSS mgl	19	6.5	6.5	14	25	30.5	35	21	21	52.3
Turb NTU	25	8	3	3	9	12	10	7	7	20
Velocity	1	0.33	0.5	0.3	0.72	0.42	1	0.53	0.5	0.4
Depth	0.61	0.28	0.24	0.18	0.7	0.29	0.5	0.55	0.39	0.36
Bedrock	0	0	0	0	0	0	0	0	0	0
Boulders	1	1	1	0	1	1	1	1	0	1
Cobbles	1	1	1	1	1	1	1	1	0	1
Gravel	1	0	0	1	1	0	0	1	1	0
Sand	0	1	0	0	0	0	1	1	1	0
Mud	0	0	0	0	0	0	1	0	0	0
Class1	24	0	0	1	26	27	27	10	10	26
Class2	3	0	0	1	4	5	5	2	2	4

Table 7.2: Typical Class1 predicted values compared with actual values.

	M17/10/0D2	M24/05/1A2	M20/9/1D4	M01/11/1A4	M14/05/2C3	T08/08/0A3	T02/08/1C4	T27/09/1A3	T13/02/2A4	T13/02/2A7
Altitude	668	668	668	668	668	576	576	576	576	576
pH	7	7	7.2	8.7	7.2	7.4	7.1	8.1	7.8	7.8
Cond. uS	46	48	46	48.8	51	108	90	103	109	109
DO%	91	98	92	94	98	95	92	90	87	87
DO mgl	7.6	8.8	8.3	8.2	8.7	8.4	8.2	8.5	6.7	6.7
Temp	20.7	16	19.5	23.2	20.1	16	16.1	20.6	25.5	25.5
TSS mgl	19	6.5	6.5	14	6	26	25	30.5	21	21
Turb NTU	25	8	3	3	8	31	9	12	7	7
Velocity	0.5	0.44	0.52	0.5	0.33	0.57	0.72	0.8	0.53	0.53
Depth	0.4	0.37	0.33	0.36	0.18	0.61	0.51	0.39	0.65	0.3
Bedrock	0	0	0	0	0	0	0	0	0	0
Boulders	1	1	1	1	1	1	1	1	1	0
Cobbles	1	1	1	1	1	1	1	1	1	0
Gravel	0	0	1	1	0	1	0	0	0	0
Sand	1	1	0	1	1	0	1	0	0	1
Mud	0	0	0	0	0	0	0	0	0	1
Class1	24	0	0	1	0	26	26	27	10	10
Pred1	24	0.010832	0.000124	0.99955	0.000569	26	25.951	27.002	9.9996	9.9991

Neural networks use their powerful function approximation capability in predicting the outcome for a given set of input parameters. Therefore, even a well-trained network is only expected to predict the outcome close to the actual value and not necessarily the actual value. In a second experiment the original training set of 206 observations was divided in a training set of 103, and validation and testing sets of 51 observations each. Using only 5 hidden neurons, the training stopped after 37 epochs because the validation error increased. The error on the test set remained minimal (Fig. 7.6). The regression graph (Fig. 7.7) strikingly displays the good fit obtained between actual and predicted classifications.

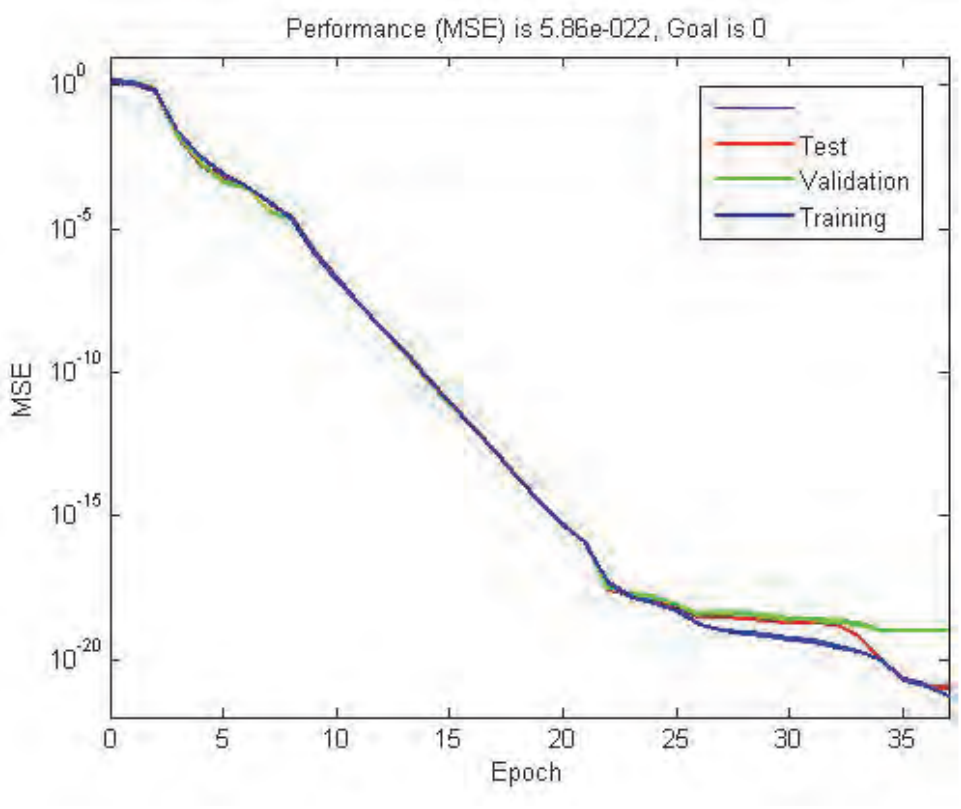


Figure 7.6: Training convergence of training, validation, and test sets for Class1.

As the classification is unevenly spread over only six values (0, 10, 24, 26, and 27 were calculated), these values were converted to six evenly spread integers (0 to 5), and the training repeated. Again, TRAINLM and LEARNGDM converged in less than 100 epochs and effortlessly modelled the test data (Fig. 7.8 and Table 7.3).

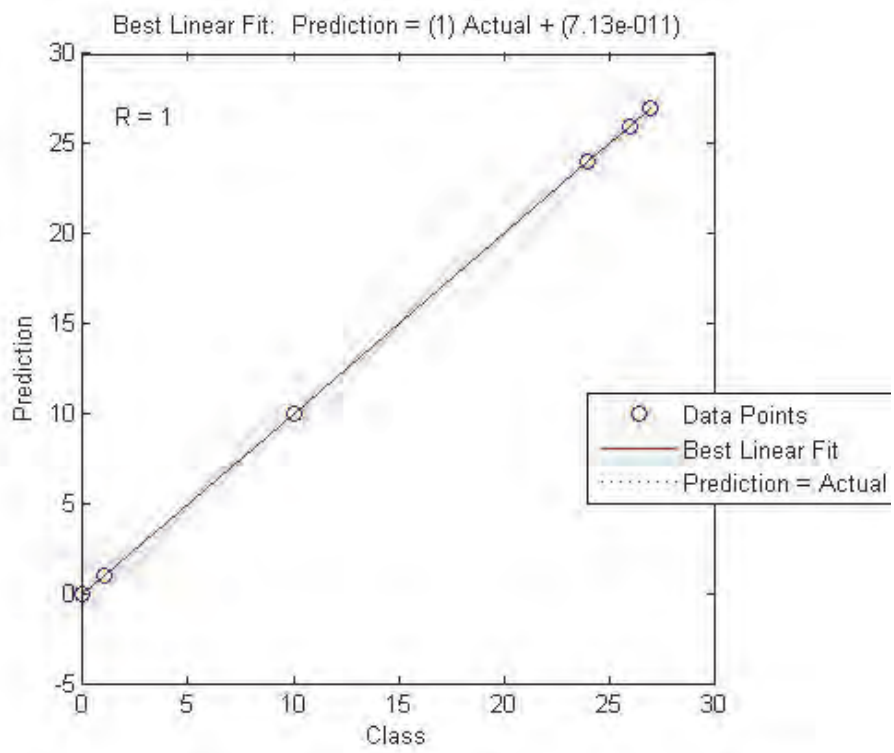


Figure 7.7: Regression of predicted on actual classifications for Class1.

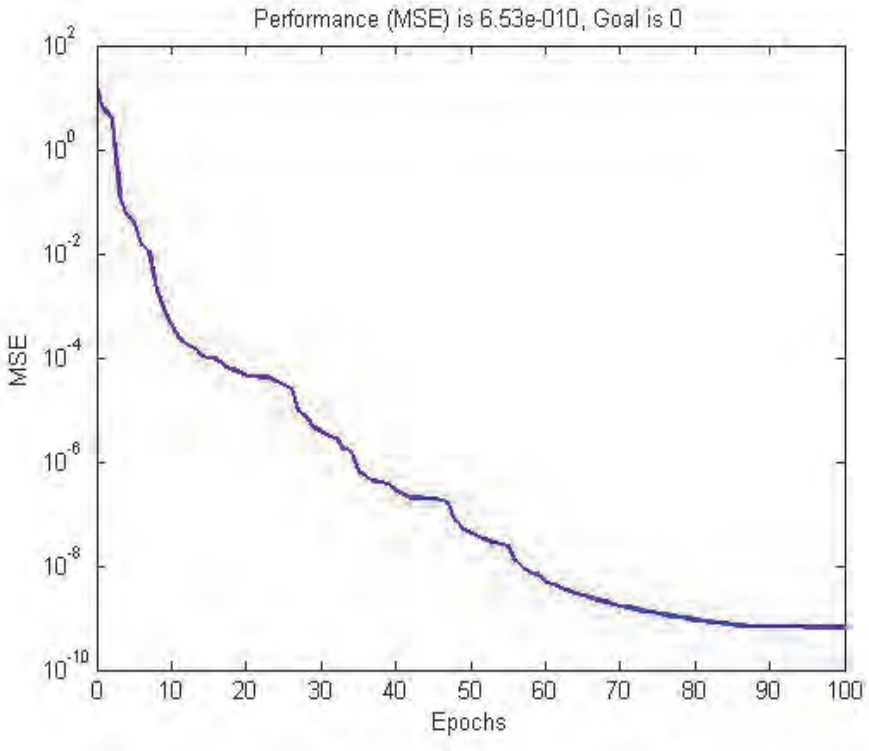


Figure 7.8: Training convergence of revised classification data (Class2).

Table 7.3: Typical Class2 predicted values compared with actual values.

	M17/10/0D2	M24/05/1A2	M20/09/1A4	M01/11/1A4	M14/05/2A5	M14/05/2C3	T08/08/0A3	T10/05/1A8	T27/09/1A6	T13/02/2B3	T14/05/2C4
Altitude	668	668	668	668	668	668	576	576	576	576	576
pH	7	7	7.2	8.7	7.2	7.2	7.4	7.36	8.1	7.8	8
Cond uS	46	48	46	48.8	51	51	108	92	103	109	124
DO%	91	98	92	94	98	98	95	94	90	87	93
DO mgl	7.6	8.8	8.3	8.2	8.7	8.7	8.4	8.3	8.5	6.7	8.2
Temp	20.7	16	19.5	23.2	20.1	20.1	16	17	20.6	25.5	17.1
TSS mgl	19	6.5	6.5	14	6	6	26	30	30.5	21	52.3
Turb NTU	25	8	3	3	8	8	31	14	12	7	20
Velocity	0.5	0.44	0.67	0.5	0.33	0.33	0.57	0.65	1	0.5	0.74
Depth	0.4	0.37	0.52	0.36	0.28	0.18	0.61	0.57	0.5	0.47	0.42
Bedrock	0	0	0	0	0	0	0	0	0	0	0
Boulders	1	1	1	1	1	1	1	0	1	1	1
Cobbles	1	1	1	1	1	1	1	1	1	1	1
Gravel	0	0	1	1	0	0	1	1	1	1	0
Sand	1	1	0	1	1	1	0	1	1	0	0
Mud	0	0	0	0	0	0	0	1	0	0	0
Class2	3	0	0	1	0	0	4	4	5	2	4
Pred2	3	-1.18E-10	2.27E-10	1	3.81E-10	3.15E-10	4	3.9995	5	2	4

7.6.3 Portability of classification model to other sites

The ultimate aim of mathematical models such as that presented here is to be able to classify any site making use of a set of water quality parameters measured at another site. A model should not be site-specific but be portable even between watersheds. Therefore, the same neural network model was retrained on the Mutale site and used for prediction on the Tshino site. Unfortunately, this experiment, as well as the reverse, resulted in failure. This is another indication of the fundamental difference between these sites, as also shown in Chapters 5 and 6.

7.6.4 Prediction of biotic response: fish abundances

This experiment was an attempt to train the neural network model to predict the occurrence of certain fish at the various sites. Again the training set of 206 data points was used to train the neural network model. The remaining data points were used to verify the efficacy of the trained neural network model in predicting fish occurrence. After many unsuccessful attempts to accurately predict numbers of fish of each of the 17 species of interest, it was decided to eliminate seven species that were seldom recorded. Using a hidden layer of 40 neurons, *logsig* and *purelin* for the transfer functions, and the training and learning functions, TRAINLM and LEARNGDM, as before, the network was moderately successful (about 70% correct) at quantitatively predicting the abundances of fish species (Fig. 7.9 and Table 7.5). The regression results for the ten representative fish species using the developed network are shown in Table 7.4. When the results are plotted, however, the success of the model at modelling patterns is clearly demonstrated (Figures 7.10 and 7.11).

Table 7.4 Regression of trained (*Y*) on observed (*X*) fish species.

Fish species	Best linear fit: $Y = mX + b$		Regression coefficient
	m	***b***	
AURA	0.41	-0.0262	0.345
BLIN	0.572	0.0106	0.56
BNEE	0.299	0.0113	0.394
BTRI	0.32	-0.0154	0.349
CGAR	0.244	0.0457	0.251
CPRE	0.472	0.704	0.449
LCYL	0.238	0.068	0.268
LMAR	0.429	0.0729	0.391
LMOL	0.66	0.0529	0.55
MBRE	0.773	0.0535	0.471

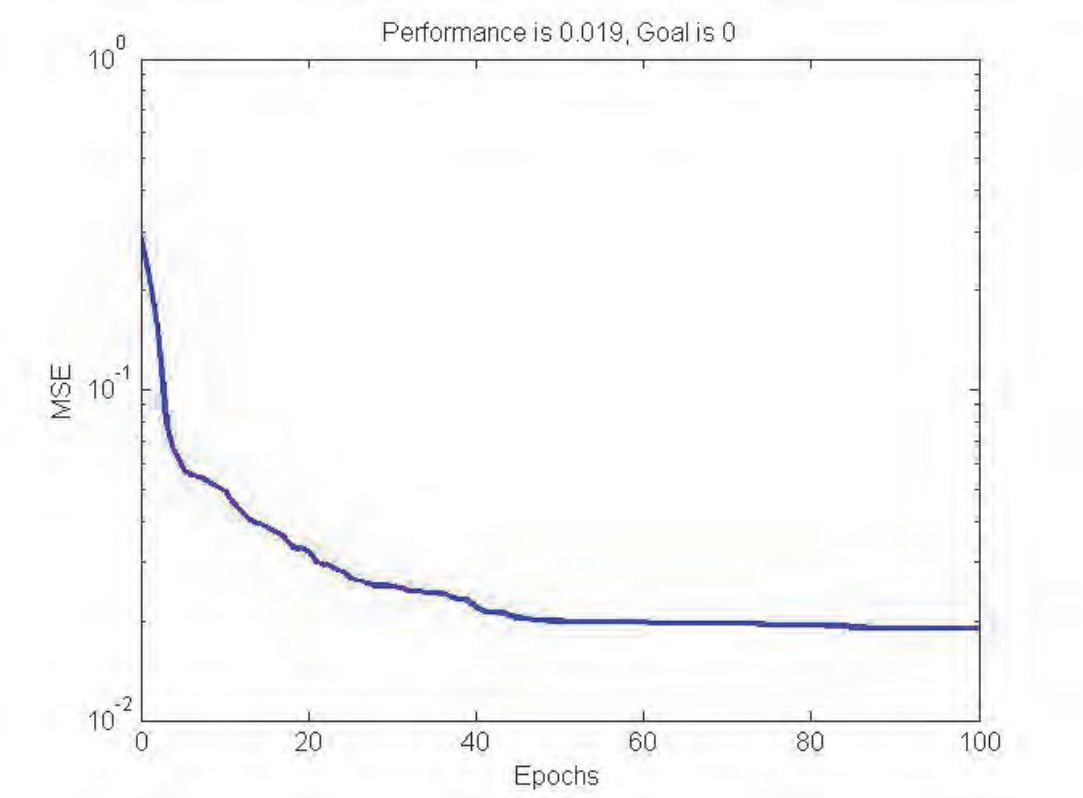


Figure 7.9: Training convergence on fish data.

Table 7.5: Typical predicted fish abundances compared with actual abundances. Values below 0.3 were assumed to be 0.

	M15/8/00A4	M15/8/00B2	M22/8/00E3	M17/10/0A4	T27/09/1C4	M15/8/00A5	M15/8/00B1	M01/11/1A3	M01/11/1B2	T02/08/1A3	T02/08/1A4
Actual Abundances											
AURA	2	2					1		1		
BLIN											
BNEE											
BTRI			1								
CGAR											
CPRE	1	2	1	2	9	1	1	1		3	5
LCYL		2	2						1		1
LMAR									1		
LMOL					1	1					
MBRE											
Predicted Abundances											
AURA	0.4	0.3	0.6	0.5	0.4		0.4				
BLIN											
BNEE											
BTRI			0.4								
CGAR											
CPRE			2.0	0.9	13.6	1.5	0.5	1.3	1.1	5.2	2.6
LCYL											
LMAR					0.6			0.3	0.3		
LMOL	0.4	0.4				0.4	0.4				
MBRE											

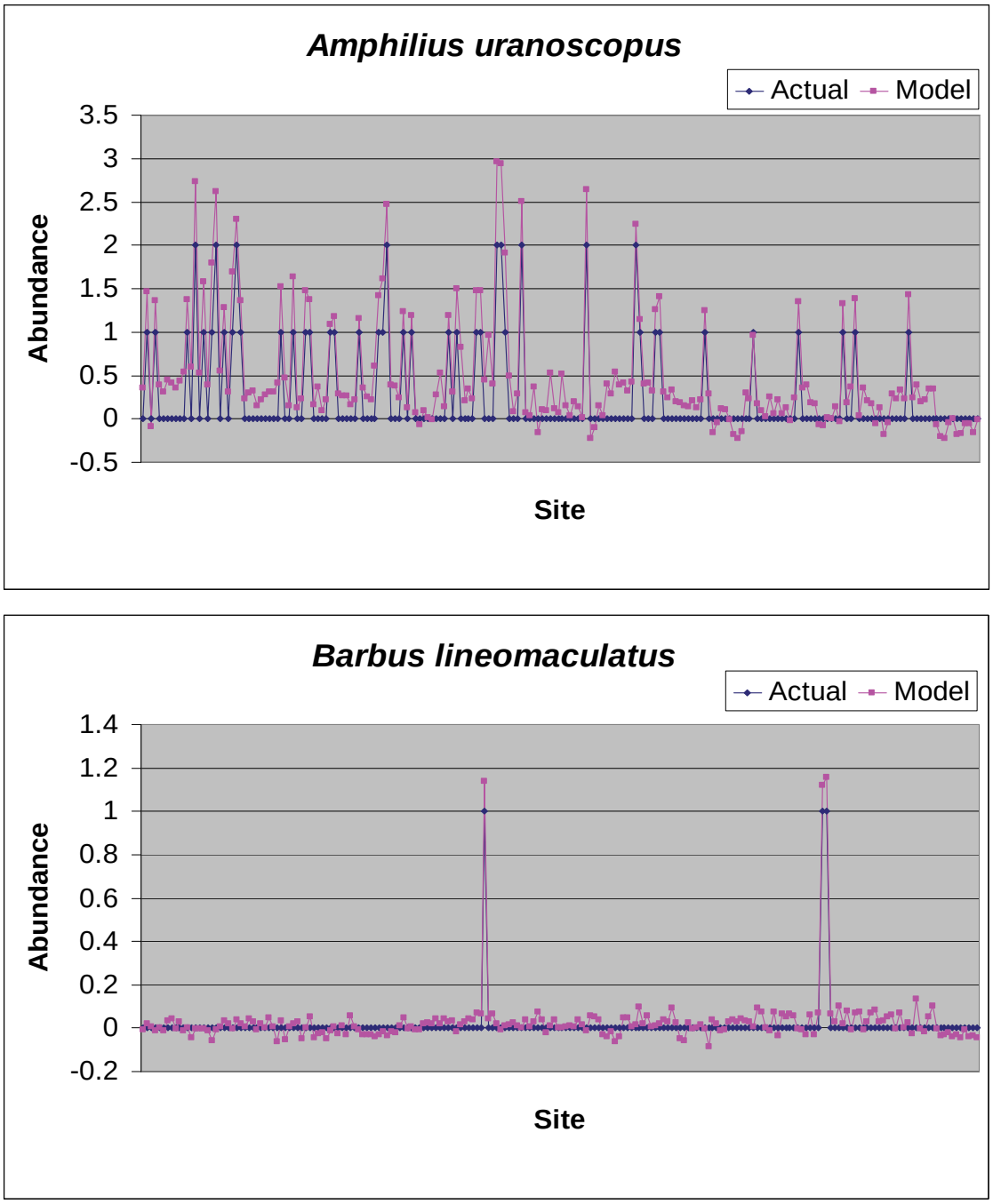
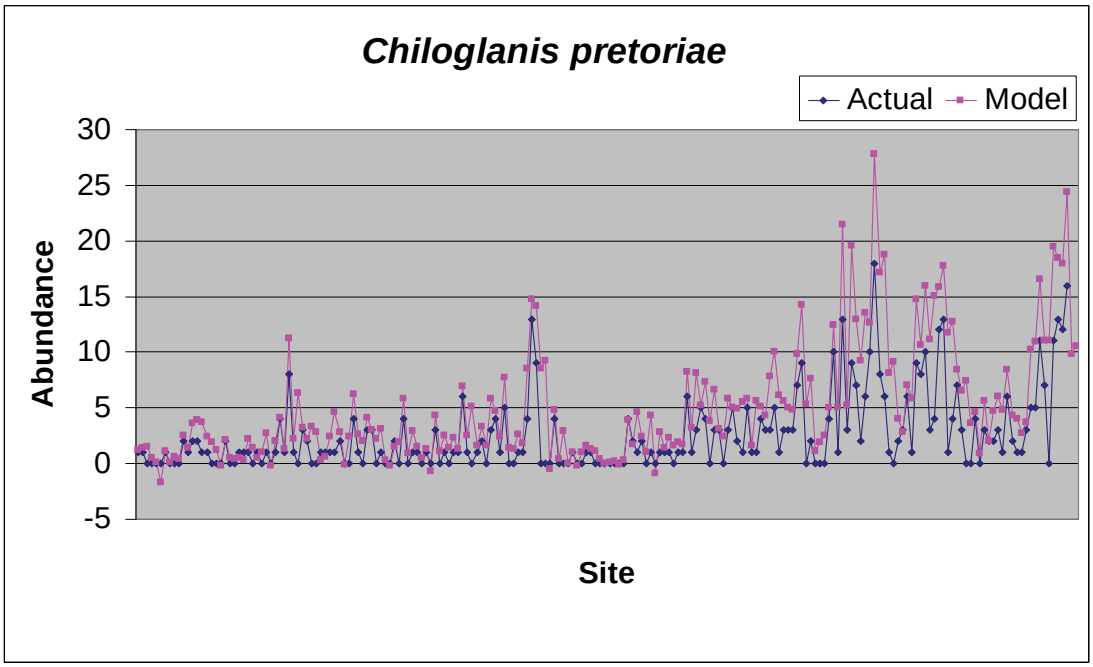


Figure 7.10: Training results of actual and predicted fish abundances.



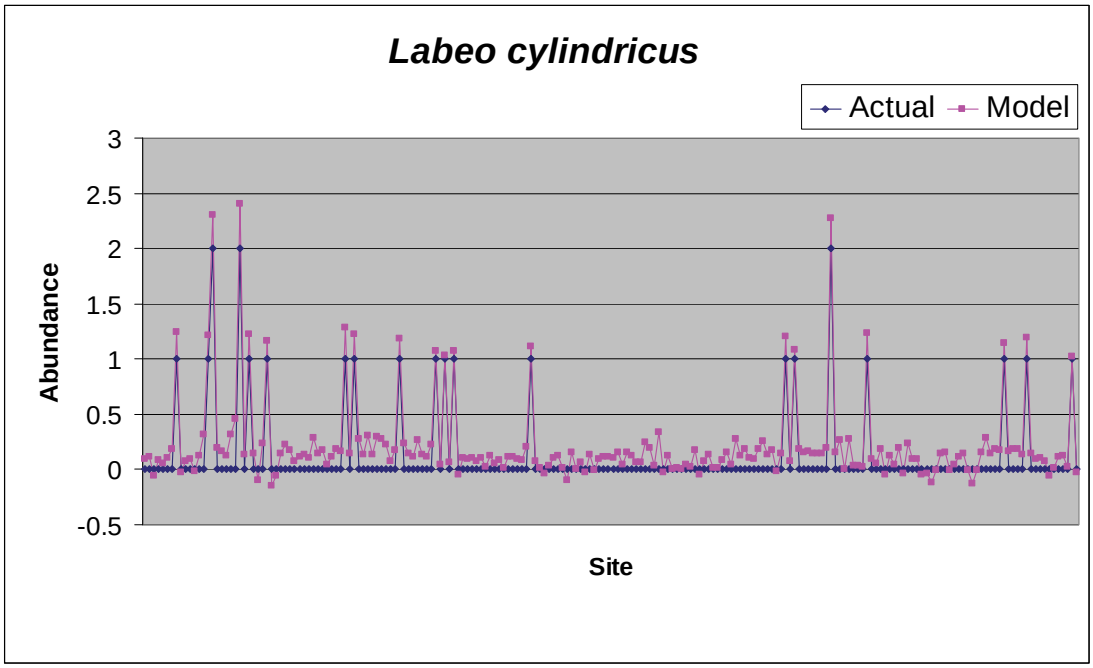
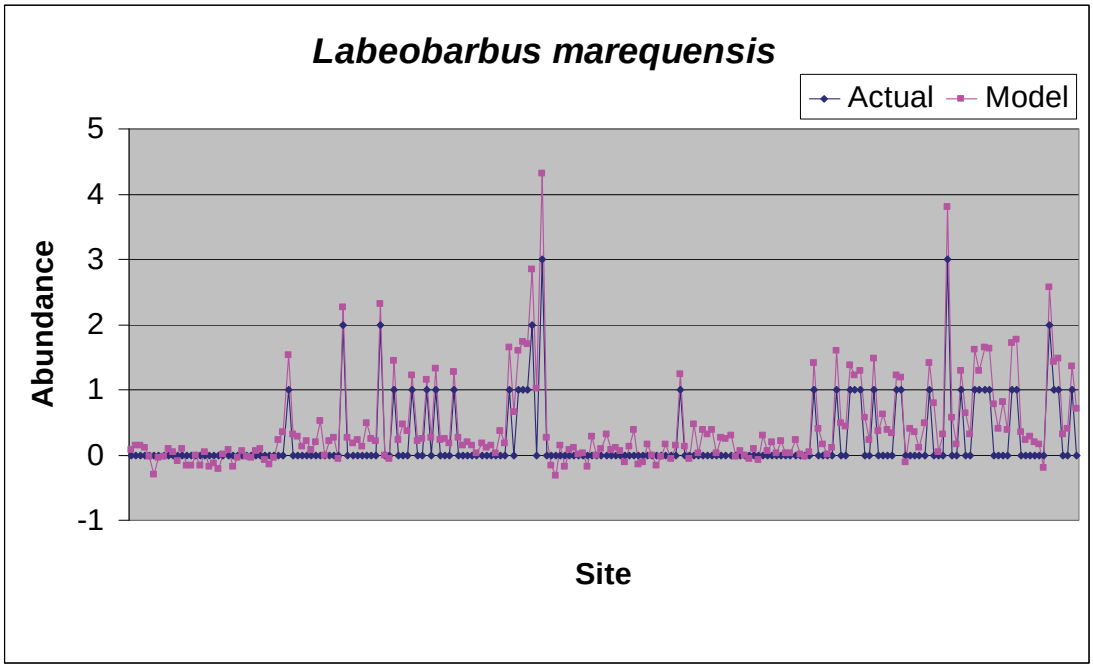


Figure 7.10:*(Continued)***Training results of actual and predicted fish abundances**.



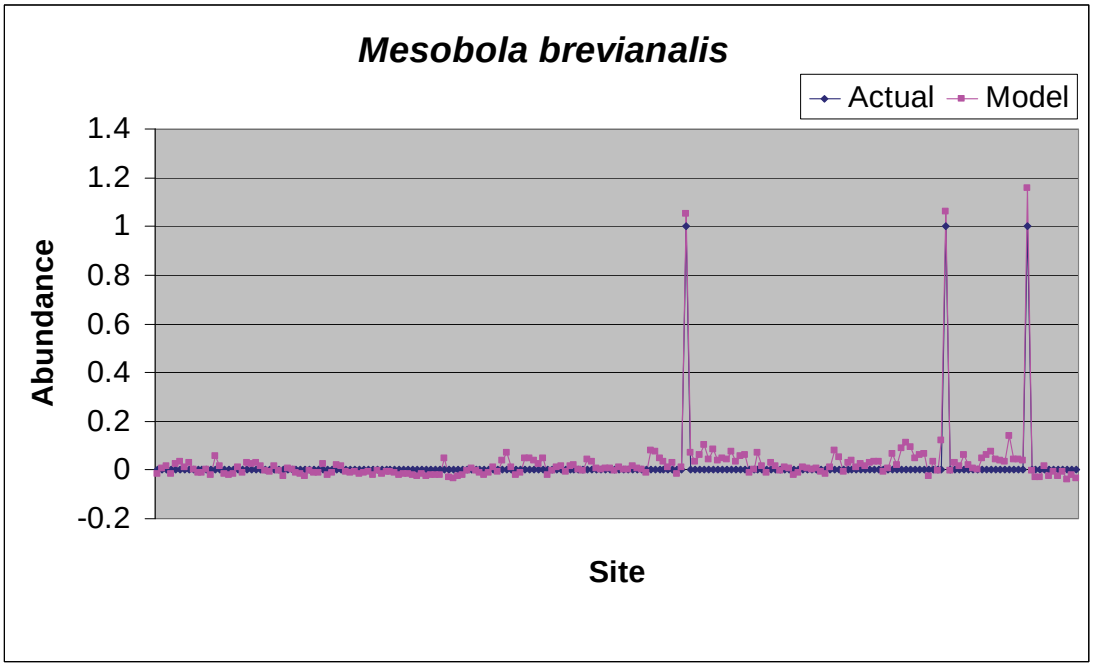


Figure 7.10:*(Continued)* **Training results of actual and predicted fish abundances.**

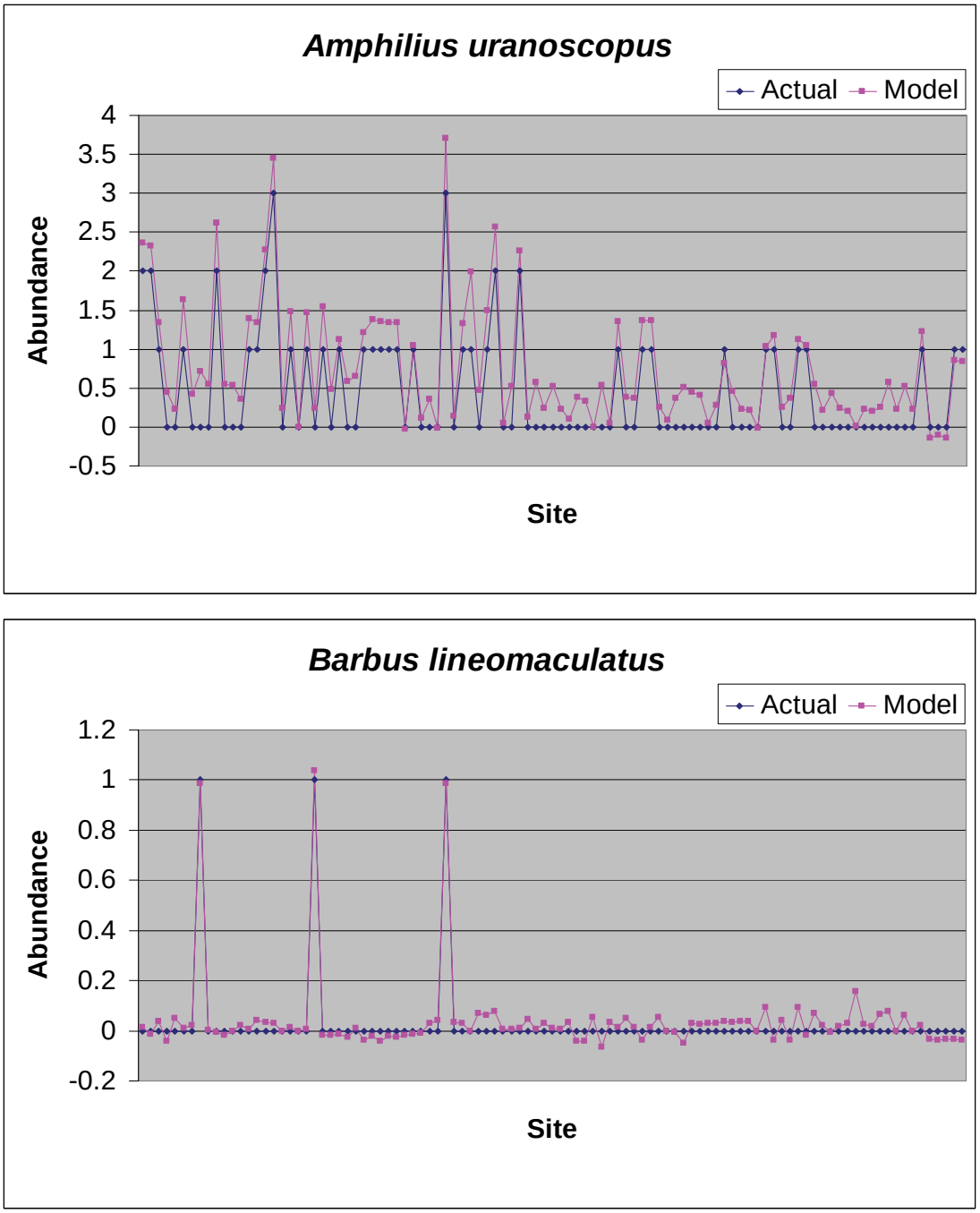
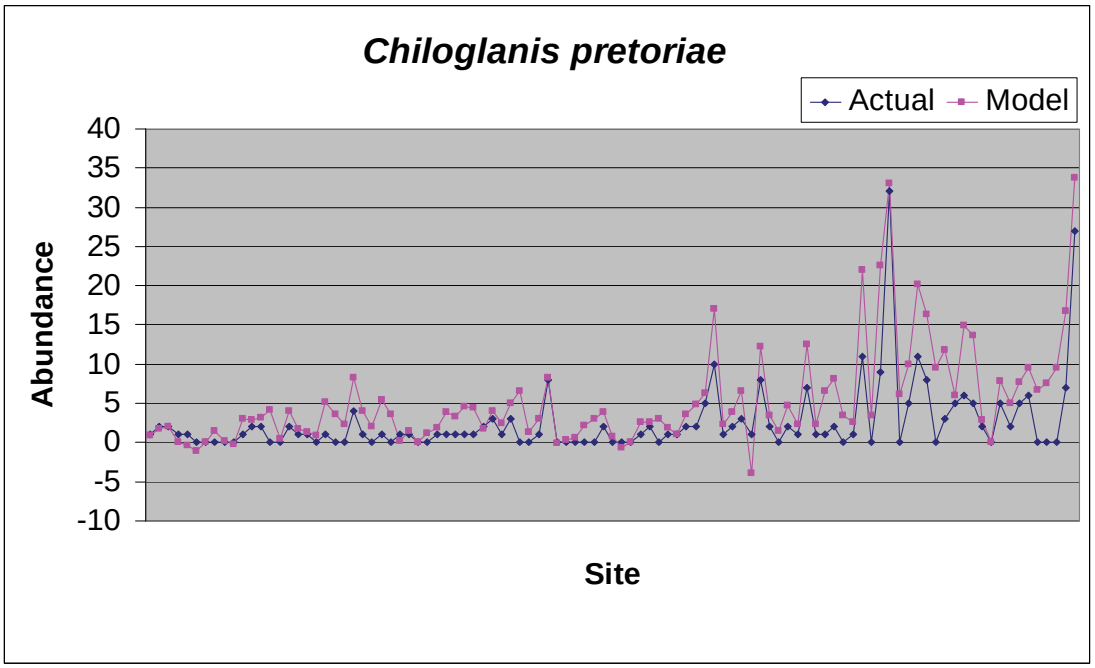


Figure 7.11: Predicted (untrained) fish abundances using the trained model.



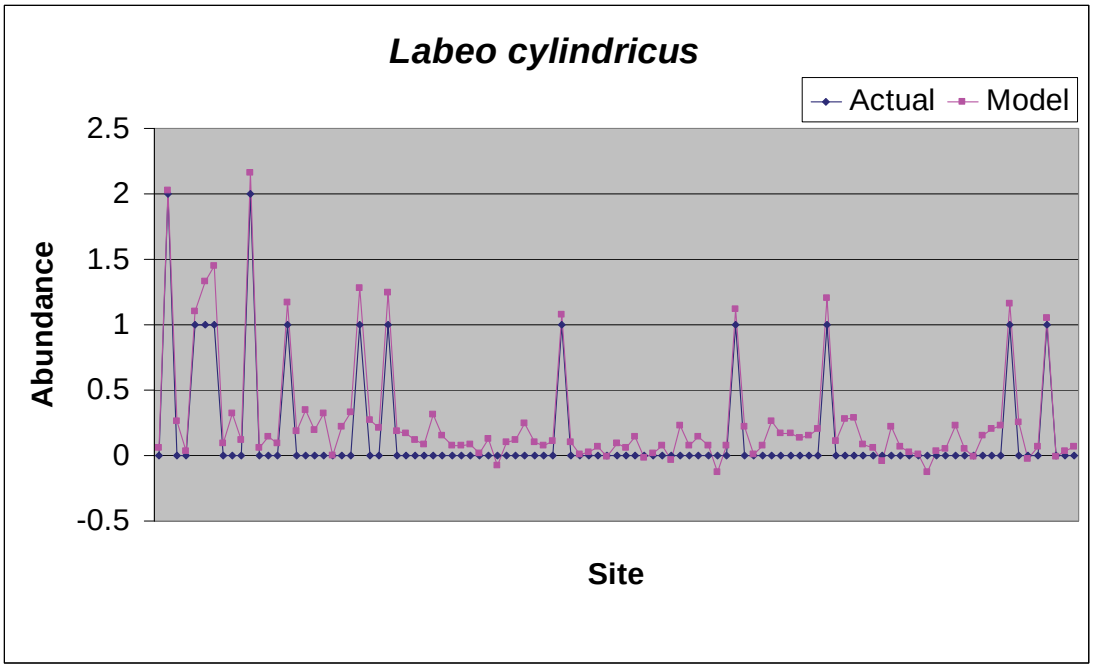


Figure 7.11:*(Continued)***Predicted (untrained) fish abundances using the trained model.**

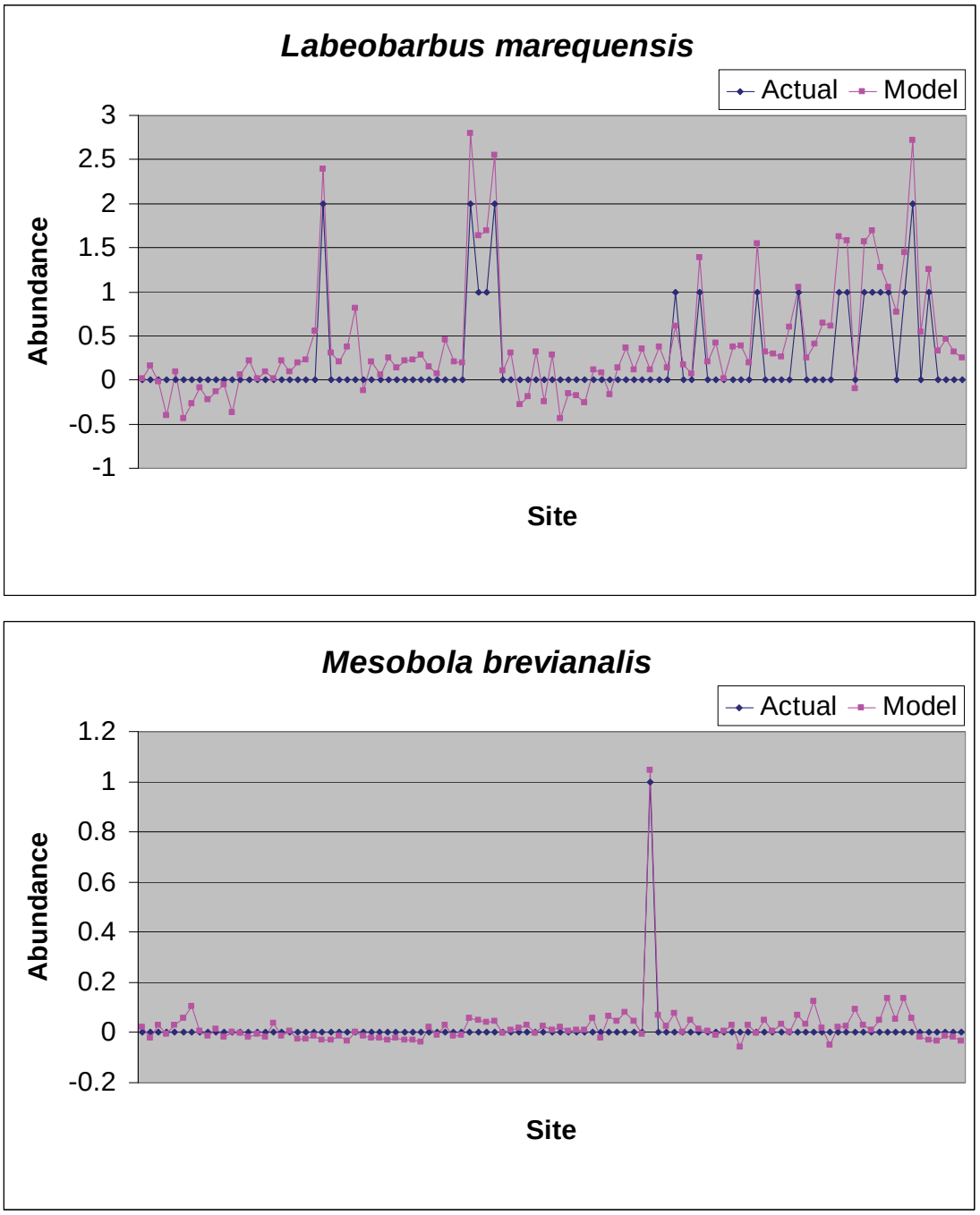


Figure 7.11: *(Continued)* **Predicted (untrained) fish abundances using the trained model.**

7.6.5 Prediction of biotic response: insect abundances

This was an attempt to train a neural network model to predict the occurrence of certain macroinvertebrates at the various sites. A data set of 80 points was used to train and verify the neural network model. Based on the experience with the fishes it was decided to use the ten families that were most abundant. Using a hidden layer of 10 neurons, *logsig* and *purelin* for the transfer functions, and the training and learning functions, TRAINLM and LEARNGDM, as before, the network was moderately successful in modelling the total abundance of macroinvertebrates (Fig. 7.12 and Table 7.7). This also applied to individual families (Fig. 7.13 and Table 7.7). The regression results for the ten representative families using the developed network are shown in Table 7.6. When the results are plotted, the success of the model at modelling patterns of certain family abundances is clearly demonstrated (Fig. 7.13).

Table 7.6: Regression of trained (*Y*) on observed (*X*) insect families.

Fish species	**Best linear fit: $Y = mX + b$**		**Regression coefficient**
	m	***b***	
Baetidae	0.454	3.19	0.454
Caenidae	0.359	0.396	0.53
Chironomidae	0.497	5.21	0.46
Elmidae	0.806	0.365	0.758
Heptageniidae	0.0378	0.914	0.0791
Hydropsychidae	0.674	5.26	0.662
Leptophlebiidae	0.415	2.09	0.51
Simulidae	0.515	0.647	0.426
Sphaeridae	0.174	-0.145	0.18
Trichorytidae	0.381	0.112	0.456

Table 7.7: Typical macroinvertebrate total abundances and family occurrences.

	M22/8/00B2	M22/8/00B3	M24/05/1C1	M24/05/1C2	M0702/2A5	M0702/2B1	T10/05/1A7	T10/05/1A8	T27/09/1B5	T27/09/1B6	T27/09/1B7
Baetidae	0	0	6	10	0	0	4	0	14	26	4
Caenidae	0	0	1	0	0	0	0	0	5	7	2
Chironomidae	4	5	6	5	0	0	4	0	5	4	1
Elmidae	0	0	0	0	0	0	0	0	12	0	0
Heptageniidae	0	0	2	0	0	0	11	0	1	4	1
Hydropsychidae	0	0	9	6	1	2	9	1	88	18	17
Leptophlebiidae	0	0	0	0	0	0	3	0	37	64	44
Simulidae	0	0	0	0	0	1	0	0	4	1	1
Sphaeridae	0	0	0	0	0	0	0	0	0	6	0
Trichorythidae	0	0	0	0	0	0	1	0	0	0	0
Total individuals	4	5	24	21	1	3	32	1	166	130	70
Predicted individuals	37.5	1.3	12.2	17.9	0.8	9.9	28.0	12.7	87.8	18.5	15.6
Total families	1	1	6	3	1	6	8	1	11	10	7
Predicted families	5.4	5.0	6.3	5.9	7.2	4.2	6.0	4.2	5.6	5.2	5.5

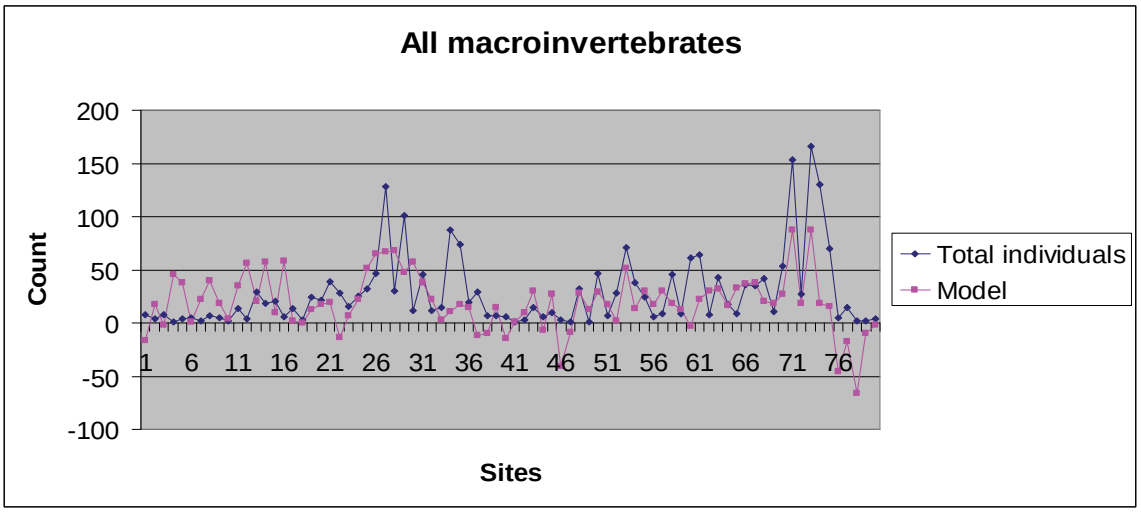


Figure 7.12: Total macroinvertebrate count.

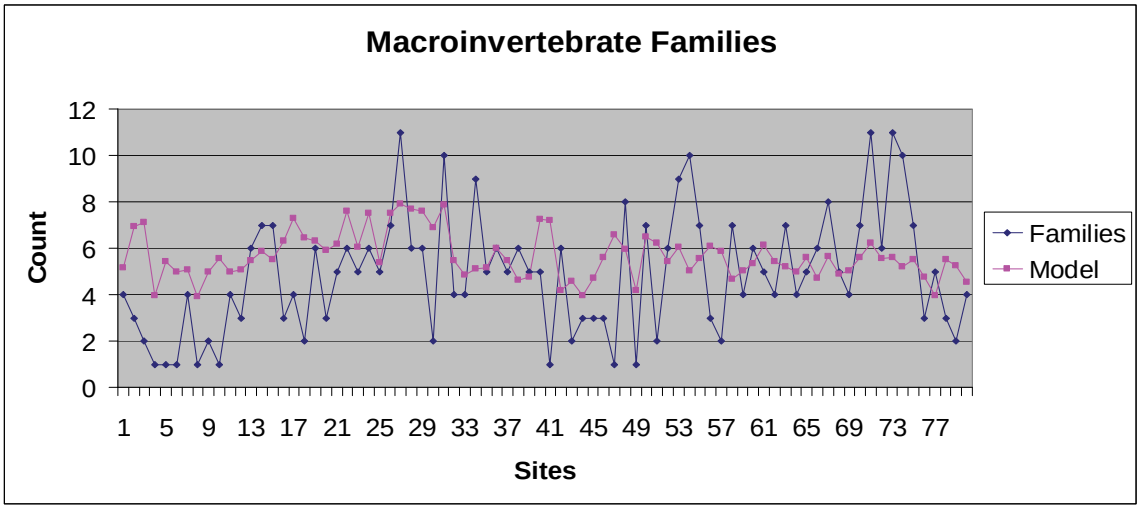


Figure 7.13: Family count.

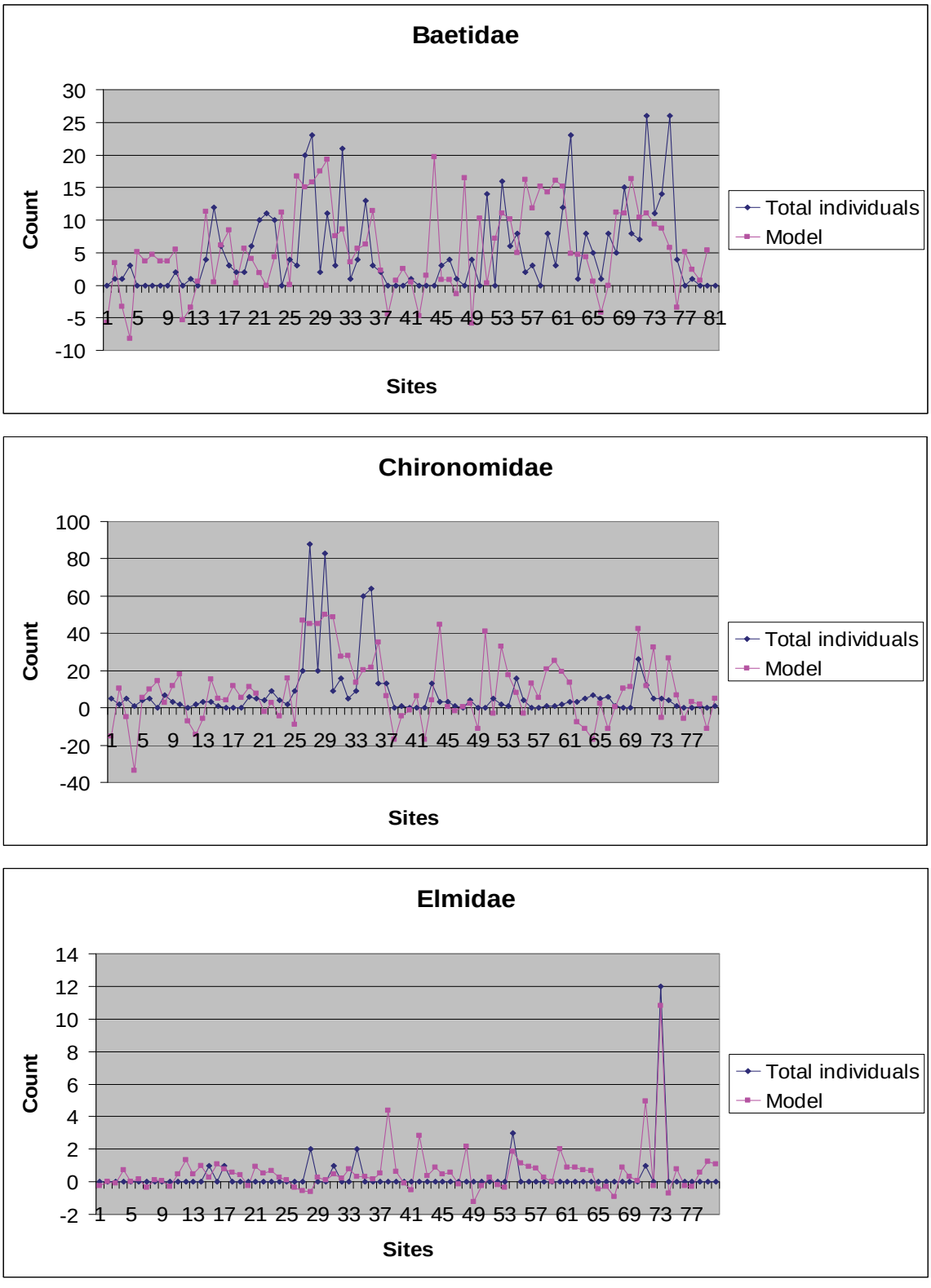


Figure 7.14: Training results of actual and predicted macroinvertebrate abundances.

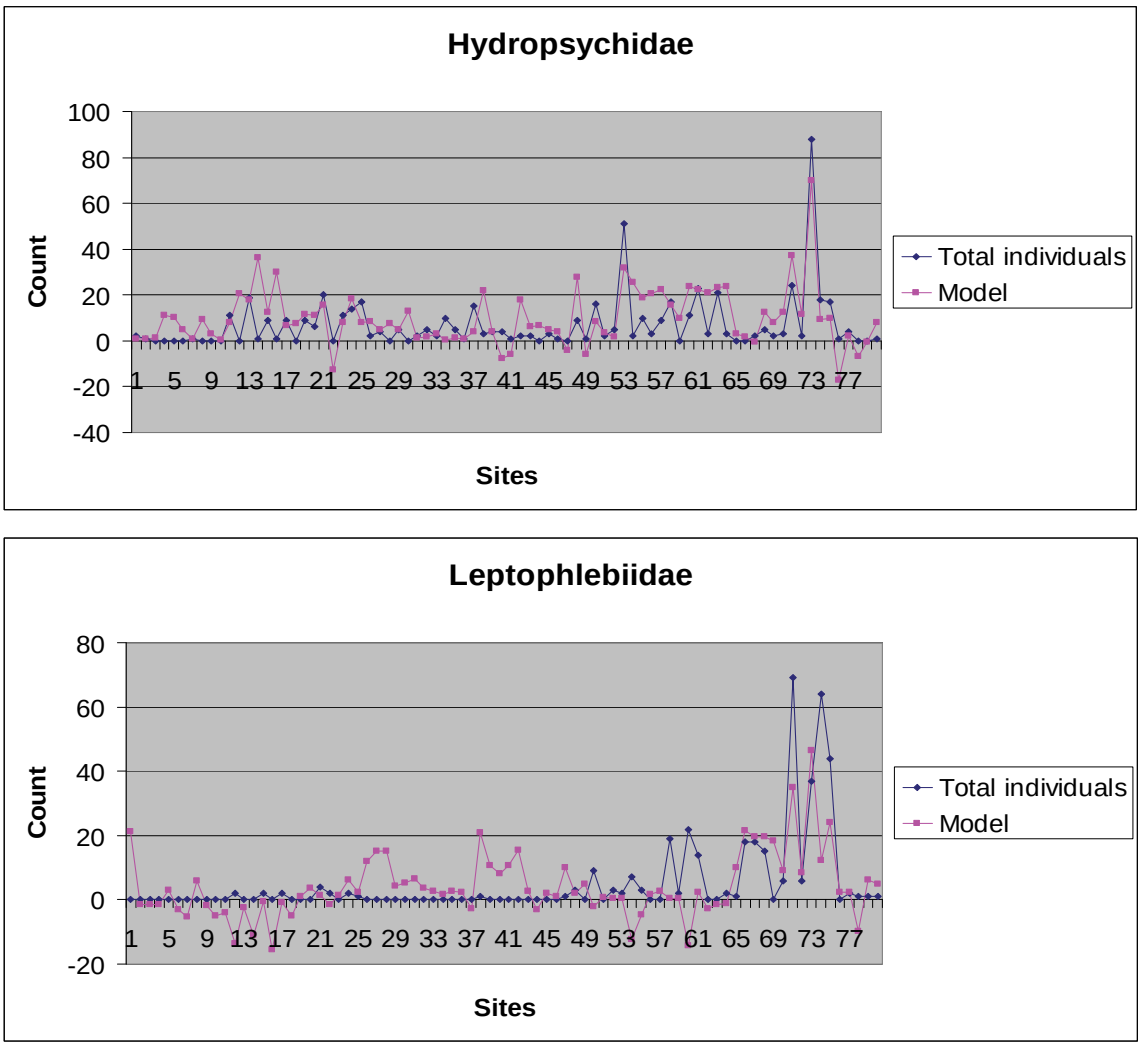


Figure 7.14: ***(Continued)*** **Training results of actual and predicted macroinvertebrate abundances.**

7.7 Conclusions

From this initial investigation it would appear that neural network analysis of river data will shed some light on river health and is an approach well worth pursuing. The preliminary model classified sites correctly and enabled the identification of sites impacted by anthropogenic influences. This kind of model needs to be investigated more fully and used for examination of complex, multivariable water quality systems.

The obvious next step would be application of this technique for prognosis of river health 6 months to 1 year ahead, so that preventive action can be taken in time. It is also important to see if the model will continue to classify sites accurately over time periods separate from those in which the original training data were obtained. Time series of all possible measurements should therefore be compiled. The model should also be tested in other watersheds to support the regionalization of these findings.

7.8 Literature References

AGUILERA, P.A., FRENICH, A. GARRIDO, TORRES, J.A., CASTRO, H., VIDAL, J.L. MARTINEZ, and CANTON, M. 2001. Application of the Kohonen neural network in coastal water management: Methodological development for the assessment and prediction of water quality. *Wat. Res.*, **35**(17), 4053-4062.

BALLESTER, E. BALAGUER, CAMPS I VALLS, G., CARRASCO-RODRIGUEZ, J.L., OLIVAS, E. SORIA and DEL VALLE-TASCON, S. 2002. Effective 1-day ahead prediction of hourly surface ozone concentrations in eastern Spain using linear models and neural networks. *Ecological Modeling*. **156**, 27-41.

BESTBIER, R.X., JACOBY, D.L. and ROGERS, K.H. 2000. *A goal maintenance system for the management of the Kruger National Parks riverine alien vegetation: Developing a protocol and a prototype*, Water Research Commission Report No 813/2/00, Pretoria: WRC.

BRION, G.M. and LINGIREDDY, S. 1999. A neural network approach to identifying non-point sources of microbial contamination. *Wat. Res.*, **33**(14), 3099-3106.

BUCHANAN, B.G. and SHORTLIFFE, E.H., Eds. 1984. *Rule-based expert systems*, Reading MA: Addison-Wesley.

CARRASCO, E.F., RODRIGUEZ, J., PUNAL, A., ROCA, E. and LEMA, J.M. 2002. Rule-based diagnosis and supervision of a pilot-scale wastewater treatment plant using fuzzy logic techniques. *Expert Systems with Applications*, **22**, 11-20.

CHOI, D.J. and PARK, H. 2001. A hybrid artificial neural network as a software sensor for optimal control of a wastewater treatment process. *Wat. Res.*, **35**(16), 3959-3967.

GOUWS, J. 2000. Gebruik van ‘n ekspertstelsel vir die identifikasie van *Lithops*. *S.A. Tydskr. Natuurwet. Tegnol.*, **19**(2), 42-46.

HAGAN, M.T., DEMUTH, H.B. and BEALE, M.H. 1996. *Neural Network Design*, Boston: PWS Publishing.

HAGAN, M.T. and MENHAJ, M. 1994. Training feedforward networks with the Marquardt algorithm. *IEEE Transactions on Neural Networks*, **5**(6), 989--993.

HOPFIELD, J.1982.Neural networks and physical systems with emergent collective computational abilities. *Proceedings of the National Academy of Sciences of the USA*, **9**, 2554.

JACKSON. P. 1990. *Introduction to expert systems*, 2nd Ed, New York: Addison-Wesley.

KARR, J.R., FAUSCH, K.D., ANGERMEIER, P.L., YANT, P.R. and SCHLOSSER, R.J. 1986. *Assessing biological integrity in running waters: A method and its rationale*, Special Publication No. 5, Champaign, IL: Illinois Natural History Survey.

LABERGE, C., CLUIS, D. and MERCIER, G. 2000. Metal bioleaching prediction in continuous processing of municipal sewage with *Thiobacillus ferrooxidans* using neural networks. *Wat. Res.*, **34**(4), 1145-1156.

MACKENZIE, J.A., VAN COLLER, A.L. and ROGERS, K.H. 1999. *Rule based modelling for management of riparian systems*, Water Research Commission Report No 813/1/99, Pretoria: WRC.

MCCULLOCH, W.S. and PITTS, W. 1943. A logical calculus of the ideas immanent in neural nets. *Bull. Mathematical Biophysics*, **5**, 115-137.

MINSKY, M. and SELFRIDGE, O.G. 1961. Learning in neural nets. In *Proceedings of the Fourth London Symposium on Information Theory*. New York: Academic Press.

RADZIEJEWSKI, M., BÁRDOSSY, A. and KUNDZEWICZ, W. 2000. Detection of river flow using phase randomization. *Hydrol. Sci. J*, **45**(4), 547-558.

RICH, E. and KNIGHT, K. 1991. *Artificial Intelligence*, 2nd Ed. New York: McGraw Hill.

ROGERS, K.H. and BESTBIER, R.X. 1997. *Development of a protocol for the definition of the desired state of riverine systems in South Africa*. Pretoria: Department of Environmental Affairs and Tourism

ROSENBLATT, F. 1962. *Principles of neurodynamics: Perceptrons and the theory of brain mechanisms*. Washington, DC: Spartan Books.

WEI, B., SUGIURA, N. and MAEKAWA, T. 2001. Use of artificial neural networks in the prediction of algal blooms. *Wat. Res.*, **35**(8), 2022-2028.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 Introduction

As indicated in chapter one to understand a loss of diversity it is important to examine and understand the fine scale determinants of freshwater rheophilic assemblages. These determinants could include aspects such loss of available habitat and changes in the physico-chemical parameters, which in turn will determine habitat preference, distribution, niche partitioning and assemblage structure as well as nutrient pathways and adaptations to feeding.

As one of its aims this project was to determine the influences of the above-mentioned factors on aquatic biota in the rheophilic biotopes through an understanding of the habitat requirements and niche partitioning. It also aimed to explain loss of diversity through an understanding and modeling of the aspects of niche differentiation and habitat requirements. Furthermore it aimed to use this information to determine thresholds of probable concern.

Based on these aims it was envisaged that the project could deliver the following as products:

a) Thresholds of Probable Concern (TPCs) and
b) A neural network model that describes niche partitioning and habitat requirements in rheophilic biotopes and applies TPCs.

8.2 The results obtained

Macroinvertebrate communities were found not to be significantly different between the two sites and temporal seriation in the assemblage structure suggests a successional recovery in communities after the floods of 2000.

Seasonal variation was evident at both sites. Substrate, rather than water quality variables, played the most important role in assemblage structuring at the small scale. One family, Leptophlebiidae, was identified as a potentially good indicator of a whole suite of environmental variables.

The fact that not all the expected fish species were recorded during this study illustrates that a loss of diversity has occurred. If the decline in fish biodiversity, observed at Mutale during the study period, is taken into consideration it does appear as if this loss is continuing. Statistically the fish communities of the two sites differ significantly and analysis of environmental variables suggest that this be due to differences in pH, conductivity, dissolved oxygen, temperature, and maximum velocity. While substrate was found not to be statistically important, velocity and depth influenced fish assemblages. Although the Mutale River was regarded to be in a better condition, which can be attributed to better habitat integrity and a consequent higher biodiversity, the decline indicates habitat changes, caused by anthropogenic factors, are taking place. The decline in velocity and water depth, observed during the study period in the Mutale River, can be related to water extraction for irrigation upstream of the site.

The neural network predictions were found to be close to the actual numerical values obtained. This is in line with what is internationally accepted, as even a well-trained network is only expected to predict the outcome close to the actual value, or a trend, and not necessarily the actual value.

The attempt to train the neural network model to predict the occurrence of certain fish at the various sites was moderately successful when it was decided to eliminate seven species that were seldom recorded. The network was moderately successful (about 70% correct) at quantitatively predicting the abundances of fish species. It is however important to take cognisance of the fact that predictions were closer to actual observations in the case of the rheophilic (*A. uranoscopus C pretoriae and L cylindricus*) and semi-rheophilic fish species (*L. marequensis*). This is not the case in the limnophilic

species (*M. brevianalis* and *B. lineomaculatus*) and underpins the importance of flow and specifically velocity.

The ultimate aim of models is to be portable in order to classify any site making use of a set of water quality parameters measured at another site. Ideally it should not be site-specific but be portable even between watersheds. This was however not the case as the application between the sites resulted in failure. This is another indication of the fundamental difference between these sites.

The attempt to train a neural network model to predict the occurrence of certain macroinvertebrates at the various sites was also moderately successful in modelling the total abundance of macroinvertebrates and individual families.

8.3 Conclusion

From the results obtained it can be concluded that in general, with exception of the TPCs, the aims of the project had been achieved and the envisaged products delivered. Although the TPCs are as yet not numerically described, the role and importance of water velocity, and consequently depth, has been illustrated and underpinned.

The habitat preferences and niche differentiation illustrated in this study will go a long way in assisting decisions regarding indicator species for the use in biotic indices for river health determination.

The results and development of the predictive model is regarded as encouraging as it is envisaged as a managerial tool to be developed for future use.

8.4 Identified shortcomings in the project and recommendations for future research.

With regard to the macro-invertebrates, the absence of structural differences between the two sites could be the result of taxonomic resolution. Identification was only done up to family level, resulting in a loss of information. This needs to be addressed and the macro-invertebrates identified to species level in studies of a similar nature.

The importance and role of nutrient cycling in rheophilic biotopes needs to be addressed in more detail. Preliminary studies such as the work done on the intestinal morphology and stomach contents of the fish needs to be expanded to more species and should include an in-depth study of the feeding adaptations and trophic niche differentiation amongst the macro-invertebrates. As part of nutrient cycling the role of allochthonous and autochthonous inputs can not be disregarded and the inclusion of investigation of detritus input and the algal communities is of utmost importance.

It was found that neural network analysis did shed light on river health aspects in as far as it classified sites correctly and enabled the identification of sites impacted by anthropogenic influence. This is an approach well worth pursuing and a model of this kind needs to be investigated more fully and used for the examination of complex, multivariable water quality systems. A next step would be predictive model used for timeous preventive action can be taken in time. Research therefore needs to be done to determine if the model will continue to classify sites accurately over time periods separate from those in which the original training data were obtained. Time series of all possible measurements should therefore be compiled. The model should also be tested in other watersheds to establish the portability of the model.