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A guide to the development of conservation plans for threatened southern African fish species

Report to the
Water Research Commission

by

JA Venter, PSO Fouché, W Vlok, NAG Moyo, P Grobler and S Theron
WRP (Pty) Ltd

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The publication of this report emanates from a project entitled The development of a conservation framework for threatened African fish using *Opsaridium peringueyi* as a reference species (WRC Project No. K5/1677)

EXECUTIVE SUMMARY

Introduction

Water Research Commission funding was granted to develop a conservation framework applicable to threatened fish species southern Africa's highly diverse and important freshwater ecosystems.

The World Conservation Union's (IUCN) World Conservation Strategy recognizes six broad categories of threats to the survival of threatened species. These are habitat destruction, over exploitation, impact of introduced species, loss or contamination of food supply, killing to protect crops or livestock, and incidental capture or destruction.

There are a number of factors threatening the ecological functioning of rivers in Southern Africa. The effluent and seepage from mines, industries, agriculture and human settlements has already caused serious, and sometimes irreversible, changes in water quality in river systems. The presence of dams, weirs and alien fish also poses a major threat for freshwater fish species survival. A substantial number of fresh water fish species from South Africa are listed in the IUCN Red List and it is possible that apart from the species listed many other fish species in Southern Africa are also threatened.

The lack of clearly defined methodology for fish species conservation planning and management could be detrimental to fish conservation. Project funding was granted to a group of researchers from the University of Limpopo (lead organization), the University of Venda, Eastern Cape Parks Board and the University of the Free State to develop a conservation framework for African threatened fish species, applicable to South African conditions that will lead to an effective conservation strategy.

Because of its sensitivity rating and conservation status the Southern Barred Minnow, *Opsaridium peringueyi*, was selected as a candidate species to test the framework.

Objectives

The objectives of this research were:

- To determine the current distribution of Opsaridium peringueyi and other species in the genera in its historical distribution range.
- To characterize the habitat and habitat preference of Opsaridium peringueyi.
- To determine the threats to Opsaridium peringueyi survival as a species.
- To determine the effect of biotic, abiotic and human induced factors on the distribution of Opsaridium peringueyi.
- To determine the genetic population status of Opsaridium peringueyi populations.
- To determine the feeding biology of Opsaridium peringueyi.

- To determine growth and fecundity of Opsaridium peringueyi.
- To breed Opsaridium peringueyi in captivity.
- To use the relevant gathered information to develop a conservation framework for Opsaridium peringueyi.
- Develop a generic conservation framework for threatened African fish species.

Methods and Results

The report consists of three different sections. Section one is a generic framework which explains methodology in developing conservation plans for threatened fish species. Section two is the conservation plan (biodiversity management plan for species) for the test species, *Opsaridium peringueyi*. Section three is the background report for the test species.

Section 1

In the project a suitable species was chosen that could serve as a candidate species for developing the methodology. The selection criteria included that the species should be listed as threatened, sensitive to environmental variables and that its perceived population size should be large enough to accommodate research and sampling. *O. peringueyi* proved to be suitable and was subsequently selected as the test species for the study. An attempt was made to use the widest possible range of methodology and procedures. This allowed the team to produce a practical conservation framework with different options, i.e. comprehensive versus rapid planning methodology.

This section contains the generic conservation framework for threatened southern African fish species. The conservation framework describes a comprehensive process that could be followed to develop a conservation plan for a threatened fish species.

Section 2

NEMBA provides the opportunity and legislative support for the development of biodiversity management plans for indigenous species (BMP-S). A project was launched to develop a BMP-S for a fish species, *O.peringueyi.* Throughout the development of the plan it became clear that the plan has the potential, not only to ensure the long term survival of the species, but several other aquatic species as well as ecological processes, river types and ecological goods and services. Aquatic species conservation poses unique challenges mainly because of the uni-directional environment (rivers) they occupy. In most cases sensitive fish species such as *O. peringueyi* is widely distributed (in several river systems) but conversely localised in a few river reaches with suitable habitat quality. The background report was used to inform the participants and identified stakeholders in the development of a BMP-S. An initial stakeholder workshop took place in Skukuza during October 2008 where the BMP-S for *O. peringueyi* was developed. It is necessary for a national institution to play the leading role either directly or indirectly, through an appointed implementing agency and provincial conservation agencies to serve as regional implementing agencies. Because of the large

distribution range of the species the BMP-S makes provision for planning and stakeholder involvement processes on Evolutionary Significant Unit (ESU) or regional level. Management Goals and associated Key Result Areas (KRA) with specified actions to achieve these KRAs were developed with associated timeframes. Provision is made for developing focused subsidiary conservation plans for each ESU.

Section 3

An intensive three year research project was launched focusing on the ecology, biology and distribution of *O. peringueyi* and the results were compiled into a background report. This section reports on the back grounddata collected and deals inter alia with the conservation status, current distribution and the habitat preference of the species in its historical distribution range as well selected biological aspects and the genetic status of the species. The section also contains a general and geomorphological description of the river systems in the distribution range and lists the potential threat, available habitat and the fish diversity. Analyses of historic data and the surveys done during the project was used to construct distribution maps of four distinct survey periods and the results of surveys show that the distribution of *O. peringueyi* had changed over time. The latest data show that the distribution range has shrunk with more than 50%. Results regarding the genetic status of the species were unfortunately inconclusive and despite numerous attempts at full optimization, it was not possible to obtain clear sequences. On a regional scale fish assemblage structure could be significantly explained by four environmental variables. Altitude, as one of these variables, produced a significant response model and it was found that the species showed a uni-modal response to altitude with an optimum at 600 m above sea level. On a local scale four other environmental variables namely sand, maximum velocity, root wads and gravel explained significant amounts of systematic variation. Sand as a predictive variable is the only environmental factor that produced a significant response model and it was found that the species has uni-modal response to sand as a substrate. In fine scale habitat selection it was observed the species prefer slow-deep biotopes where schools form with Barbus *eutaenia* and where they co-occur with *Varichorhinus nelspruitensis*. Seasonal differences in biotope preference were apparent with the abundance in the slow-deep biotopes high during colder months which changed to a preference for fast deep biotopes when the water depth increased and temperatures rose. In both biotopes it was observed that sand was present if not dominant. From the results it could be concluded that the species breed only once per year during spring or early summer. This is supported by the gonadal development and the distribution of the egg sizes with the latter aspect to an extent indicating that the species is a fractional spawner and that the spawning event could extend over a prolonged period. Under experimental conditions in glass tanks it was found that the species prefer to breed in water with strong flow at a temperature of 22°C. Breeding occurs when conductivity is altered to simulate rainwater and occurs on a breeding substrate which is a mixture of gravel and sand. Analyses of the stomach contents showed that O. peringueyi feeds predominantly on invertebrates that are closely associated with riffles and feed both at the water surface and in mid water. No obvious ontogenetic shift in the feeding was observed. Data with regard to the geomorphology, threats and habitat diversity was collected at the historic sites in the Luvuvhu, Shingwedzi, Letaba, Olifants, Sabie, Crocodile, Komati, Usutu and Mphongolo rivers during surveys and are reported as such. During the

surveys the fish collected at each site and within each biotope was recorded and these assemblages are recorded in tabulate form.

The majority of the objectives were met during the project period. However it was anticipated that the genetic data generated during the study would facilitate the identification of *O. peringuyei* populations with unique genetic characteristics. That would have enabled the writers of this report to assign specific a status (level of uniqueness, if any) to each of the populations sampled. This is critical information for conservation planning. Although significant time and effort was spent to determine genetic population status, it was not successful. To compensate for this each population was viewed as an evolutionary significant unit in the planning phase.

Conclusions

The timing of this project was ideal as it coincided with the publication of the norms and standards for the development BMP-S through NEMBA. It also took place in a period when there is severe degradation in aquatic systems which in turn places enormous pressure on species and ecosystems. The *O. peringuyeyi* biodiversity management plan is the first BMP-S that was developed for a fish species. The *O. peringuyeyi* BMP-S, if implemented, will not only contribute to the long term survival of the species, but several other aquatic species as well as ecological processes, river types and ecological goods and services in the species current distribution range. The potential also exists, to use other aquatic species in different systems in a similar way, to conserve entire aquatic ecosystems and the species associated with them. We are aware that there are several other factors (financial, political and social) that could influence the successful implementation of species conservation plans. The generic plan was developed with these factors in mind and should not be considered rigid. Conservation planners and managers will be able to, and should, tailor-fit the generic plan to their unique circumstances, species and aquatic systems.

Recommendations

- The genetic status of the different *O. peringuyeyi* populations is still critical information that is needed for the BMP-S. Further research on this aspect is encouraged.
- The BMP-S process makes provision for the conservation plan to be endorsed by the Minister and thus formally incorporated into legislation. This gives the conservation plan a more formal status which could prove beneficial for the successful implementation of the plan. It is recommended that the *O. peringueyi* BMP-S be taken through this process in order to formalize the plan and give it legal status.

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Section 1

A generic conservation framework for threatened southern African fish species

JA Venter, PSO Fouché, W Vlok, P Grobler and S Theron

1.1 INTRODUCTION

The conservation of biodiversity in southern Africa's highly diverse and important freshwater ecosystems are an important but often neglected conservation management priority. A recent assessment of southern Africa's freshwater biodiversity resources (Darwall *et al.* 2009): concluded that

i. The freshwater aquatic systems of southern Africa support a high diversity of aquatic species with high levels of endemism. Many of these species provide direct and indirect benefits to people.
ii. Predicted future levels of threat to these species are very high, in particular due to development of water resources. The level of threat to species in South Africa is higher than in other countries.
iii. The current protected area network is not designed for protection of freshwater species with many falling outside of any protected area. Future protected areas must be designed for the effective conservation of freshwater species.
iv. Species information remains very limited for many parts of the region.

Dudgeon *et al.* (2006) grouped the main threats to freshwater biodiversity under five categories; these are over-exploitation, water pollution, flow modification, destruction or degradation of habitat, and invasion by exotic species. A substantial number of fresh water fish species from southern Africa are listed in the IUCN Red Data List (Darwall *et al.* 2009) (Table 1.1). Skelton, (2001) indicated that apart from the species in the IUCN Red List many other fish species in Southern Africa may be threatened.

Table 1.1: The number of fish species in each regional Red List Category in the southern African region (Darwall *et al.* 2009).

	Regional Red List Category	Number of Species	Number of regional endemics
Threatened Categories	Critically Endangered	12	12
	Endangered	19	19
	Vulnerable	9	9
	Near Threatened	9	8
	Least concern	235	134
	Data Deficient	71	57
Total		355	239

It is thus important that sufficient tools are available to conservation organizations which should ensure that effective conservation management towards the survival of threatened fish species is achieved.

Water Research Commission (WRC) Project funding was granted to a group of researchers, with the University of Limpopo as lead organization, to develop a conservation framework for threatened fish species. It was envisaged that this framework should be applicable to southern African conditions and should lead to effective conservation management. The conservation framework developed for *Opsaridium peringueyi* during the project could serve as one of the tools for effective conservation management of threatened fish species. In the project a suitable species was chosen that could serve as a candidate species for developing the needed methodology. The selection criteria included that the species should be listed as threatened, sensitive to environmental variables and that its perceived population size should be large enough to accommodate research and sampling. *O. peringueyi* proved to be suitable and was subsequently selected as the test species for the study. An attempt was made to use the widest possible range of methodology and procedures. This allowed the team to produce a practical conservation framework with different options, i.e. comprehensive versus rapid planning methodology.

The approach during the project was:

PHASE 1: COLLECTING BACKGROUND INFORMATION ON THE SPECIES

1. To determine the current distribution of *O. peringueyi* in its historical distribution range.
    a. To characterize the habitat and habitat preference of *O. peringueyi*.
    b. To determine the threats to *O. peringueyi* survival as a species
2. To determine the effect of biotic, abiotic and human induced factors on the distribution of *O. peringueyi*.
3. To determine the genetic status of *O. peringueyi*.
4. To determine the feeding biology of *O. peringueyi*.
5. To investigate the breeding biology of *O. peringueyi*.
6. To breed *O. peringueyi* in captivity.

PHASE 2: DEVELOP A CONSERVATION PLAN FOR THE SPECIES

7. To use the relevant gathered information to develop a conservation plan for *O. peringueyi*.

PHASE 3: DEVELOP A GENERIC PLAN FOR THREATENED SPECIES

8. Develop a generic conservation framework for threatened southern African fish species using lessons learned from phase 1 and 2.

This report contains the generic conservation framework for threatened southern African fish species. The conservation framework describes a comprehensive process that could be followed to develop a conservation plan for a threatened fish species (see diagram in Addendum 1).

1.2 IDENTIFICATION OF THREATENED SPECIES

The need to develop and implement conservation measures to save a species from possible extinction is usually the outcome of observations of a medium to long term decline in population

size of a species. This decline can be the result of cumulative factors (i.e. degradation of habitat by development), or a single disastrous event (i.e. pollutant spill). For cumulative factors the IUCN Red data list is normally a good indication that a species might need special intervention (IUCN, 2009). In the case where a single event causes population decline it might be that a the species is not listed as threatened but need protection measures to be saved from extinction in a certain geographical area (i.e. river system).

Although the need for a conservation plan is initiated when a certain species is threatened by extinction, the plan normally addresses the whole array of threats causing the decline and the protection of one aquatic species could thus be beneficial for a whole aquatic system with associated species.

The IUCN Red Data List is a list providing species risk of extinction (IUCN, 2009; Skelton, 1993 and 2001). Each listed species would have been assessed against the IUCN Red List Criteria as the standard for assessing a species risk of going extinct within a specific time frame. A substantial number of fresh water fish species from South Africa are listed in the IUCN Red List (IUCN, 2009). Skelton (1993) indicated that apart from the species in the IUCN Red List many other fish species in southern Africa. The South African Institute of Aquatic Biodiversity (SAIAB) has recently reviewed the IUCN Red Data List for southern African Fish (IUCN. 2009). Vié *et al*. (2009) regards the Red Data Lists as a "key conservation tool" and stresses the importance of its use as a conservation tool.

The documents produced by Darwall *et al*. (2009), Kotze *et al*. (2006), Roux *et al.* (2008) and Vié *et al*. (2009) are excellent reading material that will assist the team to a great extent and is regarded as essential.

The conservation status and therefore the risk of extinction of the species are obtained from the IUCN Red Data List for Southern African Fish (IUCN, 2009). The process and use of Red Data lists is well described in Vié *et al*. (2009) and is suggested that the publication should be utilized. Information regarding the sensitivity and specifically sensitivity rating as well as the frequency of occurrence of the species can be obtained from Kleynhans *et al*. (2007).

The next step is to assemble a planning team. Before this is done it should be born in mind that in addition to the development of the plan certain aspects of the selected species and the area should be researched. Based on this it is suggested that the team consist of a conservation planner, a GIS specialist and specialist scientists that can deal with the biology and ecology of the fish species as well as with the limnology, geomorphology and physical and chemical instream aspects of the identified river reaches or water bodies. It is also important that one or more of the specialist scientists have working knowledge of a) the currently employed freshwater monitoring, and specifically biomonitoring, techniques and indices and b) a rating of the impacts, both at an instream and river reach level.

1.3 TECHNICAL ADVISORY COMMITTEE

The function of this committee is to get a wide representation of specialists to assist the research project members with the project. The complexity of the water sector and the management of a river system or fish species necessitate a "broad" reference group. It should be noted that the main function of the committee is to guide and assist the researchers and not to manage the project. As the title indicates it should give sound technical advice to guide and ensure that the final product on the table is what was intended at the onset of the project. The advisory committee must share their expertise, experiences and lessons learned with the members of the project team.

1.4 LITERATURE SURVEY

Once the candidate species has been selected a literature survey has to be carried out. This literature survey should cover the historic occurrence data, species ecology and biology as well as information on aquatic systems in the known distribution range. This component of the exercise invaluable and extremely important as it will not only supply the available knowledge but it will also highlight the "gaps" in the knowledge base. This latter aspect then becomes the basis for the in-depth studies on the biology of the species.

The main sources of historic distribution data is the data that are housed within the provincial environmental conservation agencies, the national department of environmental affairs (DWEA), universities, museums and in particular in the data records of the South African Institute of Aquatic Biodiversity (SAIAB). Care should be taken to access the data of historic environmental agencies such as for example the Transvaal Provincial Administration (TPA) or the Cape Provincial Administration (CPA). At the same time the personal data of the regional experts should not be forgotten and it is advised that a list of possible contributors be drawn up by the team and that a specific team member be tasked to access this data. At the same time the large number of published books and guides as well as theses should also be consulted. The following is an example of authors of these books, guides and theses that could be included:

Bell-Cross and Minshull, 1988; Crass, 1964; Gaigher, 1969; Jubb 1967; Le Roux and Steyn, 1978; Russell 1997 and Scott *et a*l. 2004.

With regard to the literature survey of the ecology and biology of the species the focus should be on published scientific articles and theses. However the list authors mentioned above can again contribute greatly to the necessary data.

1.5 CURRENT SPECIES STATUS: SPECIALIST INPUT

This part of the project is a crucial component and is linked to other components within the overall process. Once the literature survey is completed it will be time for the project team to reflect on the extent of the problem and they must identify the way forward.

The best approach would be to have workshops with various regional role players to determine the current status of the species under consideration. The number of workshops to be held will mainly depend on the distribution of the species and can ranges for a single workshop that bring all identified experts where the species distribution rage is limited to the table or regional workshops if the distribution range is extended. If possible, a single workshop is regarded as the best option as it brings all the experts together and allows the discussion of possible problems that might occur further down the line. A typical problem that can occur will be the issue of rivers traversing more than one province or country. This latter situation will not only imply that different provincial legislations apply but also raises the question on who will be responsible for implementation of the strategy once it is completed and what the issues will be with regard to sampling and transport of material.

The main aim of the workshop is to discuss the current distribution of the species and to gather and collate anecdotal information with regard to aspects such as: the best possible sampling sites, who to contact to get access to certain restricted areas, when to sample and any other relevant information. The workshops also offer an opportunity for expert inputs with regard to observed and perceived threats to the species, the habitat and other general aspects regarding the rivers, fish habitat and water quality. The information gathered will guide the research team to ensure that all possible avenues with regard to historic information, and in particular grey data" is exploited. To date access to grey data is one of the most difficult issues and should receive a large deal of attention.

The information gathered during the workshop forms the basis for further research strategies and surveys. The information will also feed into the "species distribution" component which is aimed at surveying historical sites and distribution ranges. The continued links with the experts is vital as the majority of them can also serve on the reference or technical advisory committee.

The data collected also forms a major component of the "Background report" which will serve as the starter document for the "Background report review workshop". While this process is ongoing, the research team can start with the specialist studies regarding *inter alia* the distribution, ecology, biology, breeding and population genetics of the species as well as the aquatic ecosystem can commence.

1.6 RESEARCH AND FIELDWORK

1.6.1 Species distribution

1.6.1.1 Distribution from historic data

An attempt should be made to survey each of the historic sites to determine if the candidate species is still present. In cases where a number of sites are in close proximity in the same river stretch a representative site should be selected and then surveyed as the representative site. Where difficulties are encountered in locating the historic site, such as where the original coordinates in the historic data are not accurate, the site name can be linked with names on topographical maps. If this is not possible a representative site in the general area of the given coordinates can be identified and surveyed.

Surveys at the historic sites also provides the opportunity to collect additional data (See paragraph 6.2)

Over and above the aspects mentioned the data recorded should include the number and fork lengths of the candidate species collected at each site. This will be used to establish the population size. In addition all the species collected should be recorded in order to establish the fish assemblage composition and diversity at the site.

At each site the physico-chemical aspects such as electrical conductivity, total dissolved solids, temperature and dissolved oxygen should be determined and recorded.

The macro-invertebrates at each are to be collected, using SASS5 protocol (Dickens and Graham, 2002) and identified on site. A sample can be preserved for a later, more intensive, identification.

1.6.1.2 Distribution through niche modeling.

GIS based systems like Biomapper and Maxent that uses locality data to predict habitat suitability as well as species distribution can be useful tools. These software packages use only presence data which is useful if no reliable absence data is available. Biomapper uses ecological niche factor analysis to compute habitat suitability maps and define the niche of a species according to a few important habitat variables (Hirzel *et al.* 2002). Maxent, on the other hand, estimates a target probability distribution of maximum entropy (i.e. that is most spread out or closest to uniform), subject to a set of constraints that represent the incomplete information about the target distribution (Phillips *et al.* 2006). The information about the target distribution presents itself as a set of real variables called “features”. When Maxent is applied to presence only species distribution modeling, the pixels of the study area make up the space on which the Maxent probability distribution is defined (Phillips *et al.* 2006). Pixels with known species occurrence data constitute sample points and the features are different variables like vegetation types, slope, aspect, rainfall

etc. The result for both the Biomapper as well as Maxent is a raster map indicating potential suitable habitat for a species.

These models can be useful when some historical locations are known and the possibility exist that the species might occur in other areas where little sampling has been done. The distribution maps generated can then be used to guide sampling/searching effort to find the species in unsampled areas.

1.6.2 Species biology

In any conservation planning exercise knowledge of the reproductive biology, feeding biology, migratory patterns, and habitat selection of the proposed indicator species is imperative.

It should be noted that the aspects listed below are only done if the literature survey shows that data with regard to these aspects is limited or does not exist.

It is also important that a team of experts be put together to do the in-depth studies. This team should consist of accredited specialist scientist in the relevant fields indicated below.

Habitat selection.

During the survey at each of the selected sites the following procedure should followed:

- The general data, e.g. coordinates and macro dimensions of the site must be determined and recorded.
- The site must be investigated and the different velocity- depth classes (biotopes) identified (Kleynhans, 2007).
- A sketch map must be drawn on which these biotopes are indicated
- The fish are then collected in each biotope using collection methods described in Kleynhans (2007).
- The fish must be identified and recorded as the fish data per biotope. Where in the past voucher samples of the species collected had to be submitted to SAIAB the current trend is to rather supply SAIB with a good digital photograph that displays the distinguishing characters described in Skelton (2001). The site data should e submitted on the forms available on the SAIAB website
- In each biotope the following should be determined: substrate composition (Rowntree and Wadeson, 2000), cover (Kleynhans, 2007), depth, velocity.

Breeding ecology and biology

As part of the research on the reproductive biology the habitat preference of the various life stages, the gonadal development as well as the preparatory physiology prior to breeding of the species must be included. To obtain these results it is imperative that specimens be collected monthly at a

selected site. This should be a site where sufficient numbers of the species occur and care should be taken that only single specimens of adult fish are collected. The following are regarded as important aspects and should be included in the research:

a) a Visual observation, and classification, of the condition or classes of fat deposition (Nikolsky, 1963) and gonadal development De Villiers (1991)

b) The condition factor that can be calculated as proposed by Nikolsky (1963) and Hamman (1974).

c) Seasonal reproductive trends that are determined by calculating the monthly Gonadosomatic Index (GSI) values (Glazier and Taber, 1980) and the Maturity Coefficient (MC) (Gaigher: 1969 and 1976). This is to be combined with the size frequency distribution method used by Gaigher (1976) to determine whether the species is a total or multiple spawner. Ova counts and size distribution of the ova will assist to establish the fecundity of the species, while the length at sexual maturity (Gaigher, 1969) supplies the necessary information on the size at which the species starts reproducing . The spawning chronology is determined by a combination of the GSI values and the ova diameters as suggested by Settles and Hoyt (1978).

Feeding biology

The basic functions of an organism, namely growth, development and reproduction, all take place at the expense of energy which enters the body in the form of food. Feeding is therefore one of the most important functions of an organism. The same specimens used in the reproductive study can be utilized for this component.

The specimens should be dissected and the stomachs removed and preserved. The stomach contents are routinely examined and the contents identified (Marriott *et al*., 1997). The frequency of occurrence of the different prey items and index of dominance is determined and goes a long way to establish the preferred food of the species.

Migration patterns

Studies concerning the migratory patterns are time-consuming and the majority of information is based on expert knowledge and anecdotal information. It is therefore imperative that contact should be made with experienced local experts. However a large amount of this data has been compiled through workshops on the migratory behavior of fish and is collated into a report (Bok *et al.* 2007).

1.6.3 Population genetics

This section of the study is specifically designed to determine the current genetic status of the species.

The objectives for a detailed genetic analysis of the species under investigation should be:

• To screen for the possible genetic structure within the species, i.e. to investigate whether different genetic variants exist in geographically isolated populations. This is in line with the Evolutionary Significant Unit (ESU) concept, a concept now widely recognized in international and South African conservation programmes for all taxa.

• To determine the levels of genetic diversity in isolated populations. This data will serve to:

i. Identify the best possible source populations for any future breeding and augmentation programmes, since populations with the best levels of genetic diversity can then be selected for breeding.
ii. Assist in identifying populations, through establishing reduced levels of genetic diversity that has experienced genetic bottlenecks as a result of human influences.

• To elucidate overall systematic relationships within the genus, if more than one species occur in the genus, to which the species belong.

• To use trends and patterns observed in the different populations, if applicable to the specific species, to formulate wider strategies for conservation of southern African freshwater fish species.

1.6.4 Aquatic system information

When the background information regarding the species, based on a literature survey and the research results, have been gathered it is advisable to compile a document that capture all information collected. Apart from serving as a reference document, it can form part of the documents for the review reports and later workshops. It can also form the basis of documents that can be prepared for other stakeholder groups.

Apart from feeding off the literature survey for information, this part will also gain from the "Technical advisory workshop" and the information gathered from the expert panel. This component will supply information to the sub project on "artificial breeding", as this information will assist with regard to water quality needs for breeding, temperature regimes during breeding and the juvenile stages and habitat requirements to a lesser extent.

This section of the project must gather critical data on the current status of the water quality in the total distribution range of the species and this must include aspects causing pollution and habitat modification. The information gathered must give direction to future monitoring needs for species conservation/protection and must guide the relevant authorities to put measures in place that will improve the water quality in general, but specifically to protect the endangered species under investigation.

It is important to look at current data bases to see what historic information is available, but it is also very important to include additional parameters. The fact that certain parameters are excluded doesn't mean it is not important. With new technologies and knowledge available, one can improve the understanding of the species, its needs and the impacts from the environment.

Data generated will on a regular basis feed information for the "background reports". The interpretation must be done on a regular basis, as this will assist all the other components dependent on the water quality information (listed above). Apart from that, it will give the research team material to present to stakeholder and managing authorities to at an early stage implement strategies to improve the water quality.

1.6.5 Artificial breeding

Captive breeding is a conservation strategy that is widely used for the recovery and reintroduction of endangered fish species.

As little is known about the reproduction of many threatened species, research is needed to develop and standardize techniques for captive breeding. This expertise can then be used to help conserve threatened species. A captive breeding program can provide a further measure of protection against extinction and conservation of the gene pool of the species. Captive breeding is becoming accepted as one component of species improvement making the conservation effort more effective (Gipps, 1991).

Captive breeding have been used to help conserve populations of nearly 30% of the North American fish species listed as endangered. Indeed, captive breeding may provide a means to prevent extinction of threatened populations, especially during the early implementation of an environmental recovery and rehabilitation program (Arkush and Siri, 2001) providing a responsive, research-based mechanism for adaptive management.

1.7 BACKGROUND REPORT

1.7.1 Report

From the collected data a background report must be compiled. Over and above serving as a record of the work done the report is intended as reading material for the participants in the: a) the stakeholder workshop where the conservation plan is to be discussed and b) the red listing re-evaluation process, if deemed necessary.

It is suggested that the following aspects form part of the report:

- A summary of the literature survey regarding the selected species. Care should be taken to indicate the gaps in the existing knowledge and in particular its IUCN rating.
- The historic distribution of the species.
- The current distribution of the species based on the results of survey carried out by the team.

- A summary of the status of the rivers within the distribution range. Care should be taken to report on the geomorphology, available fish habitat, impacts and threats and well as outstanding features such as associated wetlands. In each of the rivers the probable/possible conservation areas should be identified and listed.
- Specialist scientific reports on various aspects regarding the selected species.

It is suggested that habitat selection, breeding biology and ecology, feeding biology, genetics as well as migration patterns are included. Although the "knowledge gaps" identified in the literature survey form the major gist of these reports, it is important that the reports should include summaries of the existing and available knowledge on each of the topics.

1.7.2 Background report review workshops

When the first draft of the background report is available the technical advisory committee and project team needs to review the report. This workshop is an important step in the process and its main aim is to evaluate progress during the preceding period and to determine if the set objectives have been achieved.

The review will determine if there are any gaps in information assembled, that needs to be addressed, or if the background report is ready for distribution to stakeholders.

1.7.3 Population viability assessment

Population habitat viability assessment (PHVA) is a procedure that allows managers to simulate extinction processes that act on small populations and therefore assess their long-term viability through the use of computer models (Clark *et al.* 1990). In both real and simulated populations, a number of interacting demographic, genetic, environmental, and catastrophic processes determine the vulnerability of a population to extinction (Clark *et al.* 1990). These four types of extinction processes are simulated in computer models and the effects of both deterministic and stochastic forces can be explored (Clark *et al.* 1990). In turn, the outcome of various management options, such as reducing mortality, supplementing the population, and increasing carrying capacity can also be simulated. The purpose of the Population and Habitat Viability Analyses (PHVAs) is to help managers understand the risks facing small populations, to identify the relative importance of the factors that put a small population at risk, and to evaluate the effectiveness of various management strategies (Lacy *et al.* 1992). PHVA also offers managers a powerful strategic planning and policy tool when faced with limited financial resources. The PHVA modeling procedure does require certain types of data which in some cases is difficult to acquire or not available.

1.8 STAKEHOLDER WORKSHOP

1.8.1 Workshop

Once the background report is complete it must be distributed to all identified stakeholders. The intent is that all stakeholders are informed about the species and its status prior to stakeholder workshop. Stakeholders include interested and effected parties within the species important distribution areas. This would generally include implementing agents, government departments, water use associations, land owners, NGOs, local government and local communities.

The objective of the stakeholder workshop is to develop a vision, key result areas and goals for the conservation plan (BMP-S). During the workshop, actions as well as responsibilities are determined to reach the set goals.

1.8.2 IUCN Red-list

Since the IUCN Red Listing procedure makes provision for the re-assessment of species when new information becomes available, the status of the selected candidate species could re-evaluated if new information indicates a need for it. This decision is based on the findings of the specialists that are collated in the background report. If a re-evaluation is deemed necessary the items described below should be followed.

As part of the BMP-S development the re-evaluation should consist of a stakeholder workshop during which the conservation status of species is re-assessed as a population within the borders where the species occurs. It is also important to include the specialists in the process. These specialists should include local, regional and national freshwater specialists and particularly specialists in freshwater fish. The involvement of person/s with experience in the IUCN Red data listing process is imperative. If possible, the experts who did the most recent IUCN evaluation should be involved. Other stakeholders attending the workshop must include future role players in the conservation exercise such as aquatic experts from the involved provincial conservation agencies and regional representatives of the Department of Water Affairs. The specialist scientist and the other members of the research team should form the other participants attending the workshop.

Prior to the workshop all the identified participants should be supplied with a copy of the “background report.

The findings of the workshop should be collated in report format if the re-assessment shows a change in the current IUCN listing of the species the findings should be incorporated in the current IUCN red list for fish species. Official procedures should then be followed the change the listing in the IUCN Red list.

1.9 BMP-S DEVELOPMENT AND IMPLEMENTATION

South African legislation (National Environmental Management: Biodiversity Act (Act 10 of 2004)) provides the opportunity and legislative support for the development biodiversity management plans for indigenous species (BMP-S). It has been shown that a conservation plan for a fish species has the potential, not only to ensure the long term survival of the species, but several other aquatic species as well as ecological processes, river types and goods and services (Venter *et al.* 2009). Aquatic species conservation poses unique challenges mainly because of the uni-directional environment (rivers) they occupy. In most cases a threatened and sensitive fish species is widely distributed (in several river systems) but conversely localized in a few river reaches with suitable habitat quality.

Because of the large distribution range of the some species a BMP-S can make provision for planning and stakeholder involvement processes on evolutionary significant unit (ESU) or regional level. Management goals and associated key result areas (KRA) with specified actions to achieve these KRAs should be developed with associated timeframes. Provision could be made for developing focused subsidiary conservation plans for each ESU where geographical distance and area uniqueness warrants it.

In South Africa a set of norms and standards were developed which were adopted and approved by the Minister of Environmental Affairs and Tourism in 2008. The norms and standards set clear guidelines to the process and content of a BMP-S (Department of Environmental Affairs and Tourism, 2008). The BMP-S process makes provision for the conservation plan to be endorsed by the Minister and thus formally incorporated into legislation. This gives the conservation plan a more formal status which could prove beneficial for the successful implementation of the plan.

The purpose of the BMP-S is to ensure that implementing agent has clearly defined objectives and activities to direct the protection of the species over a five year time horizon. The BMP-S indicates where the implementing agent and partners should focus its efforts in the five year project period. The BMP-S thus provides the medium-term operational framework for the prioritized allocation of resources and capacity in the implementation of the plan. It must be noted that the BMP-S focuses on strategic priorities rather than detailing all operational and potential reactive courses of action. While planning for some emergencies is part of the BMP-S, it remains possible that unforeseen circumstances could disrupt the prioritization established in a BMP-S. These could be addressed in the annual review and update of the BMP-S.

1.10 IMPLEMENTATION MODELS

Species in general and fish in particular tend to have distributional ranges that transcend National and Provincial boundaries. National and Provincial Government Authorities have specific management mandates within their areas of due restriction. This in association with poor communication between National and Provincial Government Authorities often results in the

mismanagement of species through inconsistent conservation policies and changing conservation priorities depending on the density of the species, available habitat and existing land use practices. Three examples of how species or sector coordinators/managers can assist in the integrated implementation of management plans that transverse provincial and National boundaries will be cited in an attempt to provide the reader with enough information to be able to decide on the way forward for the implementation of species management plans.

Orange Vaal River Yellowfish conservation and management association

In an attempt to ensure a more integrated approach the Orange Vaal River Yellowfish Conservation and Management Association was set up in 1996 at the small farming town of Bothaville in the Free State. A coordinator was elected to drive this Association. The aim was to develop a Yellowfish Conservation Area in both the Orange and Vaal Rivers. The initial site was in the Vaal River between the Vaal Dam and Bloemhof Dam. It was immediately understood by all concerned that the Association had to develop conservation principles that needed to be applied in almost all the Provinces in South Africa. This was done and an inter-Provincial steering committee was set up and managed. The aim was to integrate the management capacity of all the associated Government Departments with the entrepreneurial and technical skills in the private sector. The Association (OVRYCMA) has been in operation since 1996. The same principles are still being applied today. There are over 700 members and in excess of 700 kilometers of riparian zone and its associated river reaches are being managed as part of this Association. Quarterly steering committee meetings take place in order to drive the process.

A critical aspect of this success is the existence of an inter-Provincial Programme manager/coordinator. The existence of the inter-Provincial steering committee has facilitated good communication between the different Provincial Departments, funders and the private sector. This type of management scenario would be the ideal option to test when implementing the Biodiversity Management Plans for threatened fish species.

C.A.P.E. Estuaries program

The World Bank funded Cape Action for People and the Environment (C.A.P.E.) Estuaries Programme was initiated in 2005 with a regional stakeholder workshop aimed at developing priorities for the overall programme. It was once again understood by all concerned that this programme would encompass an area that was being managed by two Provinces as well as several District and Local Authorities. Based on lessons learned in the overall C.A.P.E. Programme the position of a coordinator/programme manager was identified as being essential to the success of the overall programme. Without this focus a new concept or programme will not be developed. In other words the principle of a programme coordinator is accepted at International Best Practice (World Bank funding standards).

A programme coordinator was employed in April 2006. The first task of this coordinator was to set up the C.A.P.E. Estuaries Programme Intergovernmental Task Team. This immediately facilitated good communication between the different Government Departments and Provinces. The co-

coordinator was then able to focus developing a Generic Estuary Management Plan Framework (=Protected Species Management Plan) and a Regional Estuary Conservation Planning Document (= Background report on species) to provide managers with a regional perspective on the value (biodiversity, social and economic) of each estuary. Once these guiding documents were produced and reviewed the first six estuary management plans were developed. The coordinator followed all the necessary procurement processes for both the Local Departments and the World Bank. No Departmental officials have time to do this extra work. It is important that the coordinator is an experienced conservation manager. In addition to this the coordinator was in a position to focus all the attention on the consulting teams developing the plans. Facilitating and the recording the interactions between stakeholders is also a crucial role that is played by the co-coordinator. A co-coordinator does not represent any one Department so can remain unbiased in the evaluation of problems/processes.

In essence the focused implementation process has resulted in 25 estuary management plans at various stages of development. The area being covered is from the Olifants estuary on the West Coast to the Mtentu Estuary on the East Coast. The concept of an inter-Governmental Task Team driving or steering the process is a sound one. The coordinator can always rely on the Government officials for support and the Government officials can achieve a great deal more by working through this co-ordinated process. International funders support this type of co-operative process as there is a stable track record that they can follow and audit.

This type of integrated co-operative approach driven by an experienced programme manager is supported by Government Departments who understand that this process facilitates the implementation of different Government management mandates within a programme that has been prioritized by all participating Government Departments. It is important that the management interventions carried out by different Government Departments in the same ecosystem is co-ordinated.

Endangered Wildlife Trust – Working Groups

The Endangered Wildlife Trust (EWT) is a non-governmental conservation organization which focuses on conservation action through the use of specialist Working groups. These working groups include the Bat Conservation Group, Ground Hornbill Working Group, Riverine Rabbit Working Group and the Crane Working Group. Most of the working groups operate with the use of dedicated field workers. The field workers cover areas which broadly correspond with the focus species distribution range and not necessarily political boundaries. The field workers are able to engage with a broad spectrum of stakeholders which include government, land owners communities and communities. They are not bound by overly burdensome burocratic structures, political boundaries and are able to focus on species or habitat specific issues. The EWT has over the year been able to build a solid conservation track record and are thus recognized and respected in the conservation arena of South Africa.

1.11 CONCLUSION

The process described in this document is a guideline and could be adapted where necessary. Some cases might exist where an urgent need for a plan or financial constraints will necessitate a simpler or less involved process of developing a conservation plan. There are also several other tools available that might prove effective under certain conditions and it is not the intent of the authors to set rigid rules for conservation planning processes. It is however important that any conservation plan should be adaptive, practical and cost efficient. In addition possible planning and implementation should be backed up by good background data. It is also imperative that an effective monitoring system should form part of the conservation plan in order to measure effectiveness of conservation actions implemented.

1.12 REFERENCES

ARKUSH, K.D and SIRI, P.A (2001). Exploring the role of captive broodstock programs in salmon restoration. *Fish Biol.* 179: 319-329.

BELL-CROSS, G and MINSHULL, J.L. (1988). *The fishes of Zimbabwe*. Trustees of the National Museums and Monuments of Zimbabwe, Harare.

BOK, A., KOTZE, P., HEATH, R. & ROSSOUW, J. (2007). Guidelines for the planning, design and operation of fishways in South Africa. *WRC Report No, TT 287/07*. Water Research Commission, Pretoria, South Africa.

CLARK, T.W., BACKHOUSE, G.N. AND LACY, R.C. (1990). The population viability assessment workshop: A tool for threatened species management. Endangered species update 8:1-5.

CRASS, R.S. (1964). *Freshwater Fishes of Natal*. Shuter & Shooter, Pietermaritzburg.

DARWALL, WRT, SMITH, KG, TWEDDLE, D. and SKELTON. P. (eds) (2009). *The status and distribution of freshwater biodiversity in southern Africa.* Gland, Switzerland: IUCN and Grahamstown.

DEPARTMENT OF ENVIRONMENTAL AFFAIRS AND TOURISM, (2008). National norms and standards for the development of the Biodiversity Management Plan for Species (BMP-S). Notice Nr. of 2008, National Environmental Management: Biodiversity Act, Act 10 of 2004.

DE VILLIERS, (1991). *The Ecology and Culture of the rock catlet, Chiloglanis pretoriae* (Pisces: Mochokidae). Unpublished M.Sc. Thesis, Rhodes University

DICKENS CWS and GRAHAM PM (2009). The South African Scoring System (SASS) version 5 Rapid assessment method for rivers. *African Journal of Aquatic Science* **27:** 1-10.

DUDGEON, D., ARTHINGTON, A.H., GESSNER, M.O., KAWABATA, Z-I., KNOWLER, D.J., LÉVÊQUE, C., NAIMAN, R.J., PRIEUR-RICHARD, A-H., SOTO, D., STIASSNY, M.L.J. AND SULLIVAN, C.A. (2006).

Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Review* **81**:163-182.

GAIGHER, I.G. (1969). *Aspekte met betrekking tot die Ekologie, Geografie en Taksonomie van Varswatervisse in die Limpopo- en Incomatiriviersisteem.* Unpublished Ph.D. Thesis, Randse Afrikaanse Universiteit.

GAIGHER, I.G. (1976). The reproduction of *Barbus cf. kimberleyensis* (Pisces, Cyprinidae) in the Hardap Dam, South West Africa. *Zoologica Africana* **11** (1): 97-110.

GLAZIER, J.R. and TABER, C.A. (1980). Reproductive biology and age and growth of the Ozark minnow, *Dionda nubila. Copeia* **3** : 547-550.

GIPPS, J.H.W, editor (1991). Beyond captive breeding: reintroducing endangered species through captive breeding. *Zoo. Soc. London Symp*. **62**. 284 p.

GROSS, M.R, (1998). One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 131-144.

HAMMAN, K.C.D. (1974). '*n Ondersoek na die lengte, massa ouderdom en gonade ontwikkeling van die groter visspesies in die H.F. Verwoerddam*. Unpublished M.Sc. Thesis, Randse Afrikaanse Universiteit.

HIRZEL, A.H., HAUSSER, J. CHESSEL, D. & PERRIN, N. (2002). Ecological niche factor analysis: how to compute habitat suitability maps without absence data. *Ecology* **83**: 2027-2036

HUNTLEY, B.J. (1989). *Biodiversity in southern Africa: Concepts and conservation*.

IUCN (2009). IUCN Red List of Threatened Species. Version 2009.1. <www.iucnredlist.org>.

JUBB, R.A. (1967). *Freshwater Fishes of Southern Africa*. A.A. Balkema, Cape Town.

KLEYNHANS, C.J., (2007). Module D: Fish Response Assessment Index in River EcoClassification: Manual for EcoStatus Determination (version 2) Joint Water Research Commission and Department of Water Affairs and Forestry report. *WRC Report No. TT330/08.*

KLEYNHANS CJ, LOUW MD, MOOLMAN J. (2007). Reference frequency of occurrence of fish species in South Africa. Report produced for the Department of Water Affairs and Forestry (Resource Quality Services) and the Water Research Commission. *WRC Report No TT331/08.*

KOTZE J.M, REYERS B, SCHONEGEVEL L.Y, NEL J.L and ROUX, D. (2006). A Conservation Vision for the Freshwater Biodiversity of the Olifants, Inkomati and Usutu-Mhlathuze Water Management Areas: Final report. *CSIR Report Number CSIR/NRE/ECO/ER/2006/0199/C CSIR,* Stellenbosch.

LACY, R., FOOSE, T., BALLOU, J. AND ELDRIGE, J. (1992). Small population biology and population habitat viability assessment. Unknown source.

LE ROUX, P. and STEYN, L. (1978). *Fishes of the Transvaal.* Cape and Transvaal Printers, Inc. Cape Town.

PHILLIPS, S.J., ANDERSON, R.P. & SCHAPIRE, R.E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modeling* **190**: 231-259.

PIENAAR, U. de V. (1978). *The freshwater fishes of the Kruger National Park*. Sigma Press, Pretoria.

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

ROUX, F. (2006). *Reproduction strategy of the smallscale yellowfish (Labeobarbus polylepis) and breeding behaviour in the Blyde and Spekboom Rivers.* MSc. Thesis. University of Johannesburg, Johannesburg.

ROUX D.J, NEL J.L, ASHTON P.J, DEACON A.R, DE MOOR F.C, HARDWICK D, HILL L. KLEYNHANS C.J, MAREE G.A, MOOLMAN J. and SCHOLES, B. (2008). Designing protected areas to conserve riverine biodiversity: Lessons from a hypothetical redesign of the Kruger National Park, *Biological Conservation* **141 :** 100-117.

MARRIOTT, M.S., BOOTH, A.J. and SKELTON, P.H. (1997). Reproductive and feeding biology of the Natal mountain catfish, *Amphilius natalensis* (Siluriformes:Amphilidae). *Environmental Biology of Fishes* **49**: 461-470.

NIKOLSKY, G.V. (1963). *The Ecology of Fishes.* Academic Press, New York.

RUSSELL, I.A. (1997). Monitoring the conservation status and diversity of fish assemblages in the major rivers of the Kruger National Park. *Unpublished Ph.D. Thesis, University of the Witwatersrand, Johannesburg.*

SCOTT, L.E.P., SKELTON, P.H., BOOTH, A.J., VERHEUST, L. DOLLEY, J. and HARRIS, R. (2004). *Atlas of Southern African Fish*.

SETTLES, W.H. and HOYT, R.D. (1978). The reproductive biology of the Southern Redbelly Dace, *Chrosomus erythrogaster* Rafinesque, in spring-fed stream in Kentucky. *Am. Midl. Nat*. **99** (2) : 290-298.

SKELTON, P.H., (1993). South African red data book – fishes. *South African National Scientific Programs Report no. 137.* Foundation for research and development. CSIR, Pretoria, South Africa.

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

VENTER, J.A., FOUCHÉ, P.S.O., VLOK, W., THERON, S. & MOYO, N.A.G. (2009). Biodiversity management plan for species – Southern Barred Minnow (*Opsaridium peringueyi*). Draft Version 2. Water Research Commission, Pretoria, South Africa.

VLÉ, JC, HILTON-TAYLOR, C and STUART, SN. (eds.) (2009). *Wildlife in a changing world – An analysis of the 2008 IUCN Red List of threatened species.* Gland, Switzerland, IUCN.

Addendum 1: A diagram representing the process and aspects related to the development of a conservation plan for a threatened fish species.

THREATENED SPECIES IDENTIFIED

1) Determine need for a conservation plan
2) Assemble planning team

Technical advisory committee

Constitute technical advisory committee and have initial meeting.

Literature Survey

Collection of available historic occurrence data, literature on species ecology and biology and information on aquatic systems in the known distribution range; also conservation status .

DEPENDING ON TIME CONSTRAINTS AND AVAILABLE INFORMATION, COLLATING INFORMATION ON THE SPECIES MIGHT REQUIRE RESEARCH AND FIELDWORK ON VARIOUS ASPECTS

Species niche modeling

The possible distribution could be predicted using niche modeling software

Current species status specialist input

Workshops and individual consultation with regional aquatic specialists with the aim of collecting current information on: distribution, habitat and threats

Species distribution

Re-surveying of areas where the species was found historically. Critical information needed is species occurrence and population size.

Species biology

In depth studies on the species biology. This include feeding ecology, breeding ecology, migratory patterns, behavior and habitat selection (fine and broad scale)

IUCN Endangered Species List

Determine if re-assessment is needed. Re-asses if new information is available that might influence current assessment

PHVA Process for Species

Determine if PHVA would be useful. If it is the case run PHVA process during a workshop with specialists and include in the background report.

Background report

Compile a background report on information

Aquatic system information

Investigation into water quality, catchment status and impacts, human influence etc. GIS mapping could be an important tool in this investigation

BMP-S Stakeholder workshop

All stakeholders, specialists, implementing agents and interested parties will meet to give inputs, set goals and raise issues which are then included in

Background report distributed to stakeholders

Background report review workshop

Technical advisory committee to review the first draft background report. To advise if more information should be collected/supplied. Project team to finalize the background report.

Artificial breeding

Breeding experiments on species, to develop effective methodology. Allow for controlled experiments on feeding, and breeding biology and behavior.

Population genetics

An investigation in the population genetics to identify ESU's or lack thereof.

BMP-S Development

The first draft BMP-S developed by project team and distributed to stakeholders for comments.

ADDITIONAL PROCESS IN SOUTH AFRICA ACCORDING TO BIODIVERSITY LEGISLATION

Final BMP-S: Submitted for approval

The final BMP-S submitted Minister for approval

BMP-S Development

The BMP-S finalized by project team

BMP-S Approved

The final BMP-S submitted approved Minister

BMP-S Implementation

Plan handed over to implementing agent for implementation.

ELEMENTS OF THE BMP_S

Background report

Medium term subsidiary plans

Regional subsidiary plans developed if need arises

Medium term BMP-S

Implementation

Annual Operational Plan

Plan of annual activities from BMP-S with budget allocations

Section 2

Biodiversity management plan for species – Southern Barred Minnow (*Opsaridium peringueyi)*

J A Venter, PSO Fouché, W Vlok, S Theron and NAG Moyo

2.1 INTRODUCTION

NEMBA provides the opportunity and legislative support for the development biodiversity management plans for indigenous species (BMP-S). A project was launched to develop a BMP-S for a fish species, *O.peringueyi.* Throughout the development of the plan it became clear that the plan has the potential, not only to ensure the long term survival of the species, but several other aquatic species as well as ecological processes, river types and ecological goods and services. Aquatic species conservation poses unique challenges mainly because of the uni-directional environment (rivers) they occupy. In most cases sensitive fish species such as *O. peringueyi* is widely distributed (in several river systems) but conversely localised in a few river reaches with suitable habitat quality. The background report was used to inform the participants and identified stakeholders in the development of a BMP-S. An initial stakeholder workshop took place in Skukuza during October 2008 where the BMP-S for *O. peringueyi* was developed. It is necessary for a national institution to play the leading role either directly or indirectly, trough an appointed implementing agency and provincial conservation agencies to serve as regional implementing agencies. Because of the large distribution range of the species the BMP-S makes provision for planning and stakeholder involvement processes on Evolutionary Significant Unit (ESU) or regional level. Management Goals and associated Key Result Areas (KRA) with specified actions to achieve these KRAs were developed with associated timeframes. Provision is made for developing focused subsidiary conservation plans for each ESU.

2.2 LIST OF ACRONYMS

"BMP-S" – Biodiversity Management Plan for Species

"DWEA" – National Department of Water and Environmental Affairs

"DWAF" – Department of Water Affairs and Forestry

"ESU" – Evolutionary significant unit

"IUCN" – International Union for Conservation of Nature

"KRA" – Key result area

"TPC" – Threshold of potential concern

"WRC" – Water Research Commission

2.3 GLOSSARY

In this BMP-S, unless the context indicates otherwise, a word or expression defined in the Biodiversity Act or Protected Areas Act has the same meaning, and—

"Biological diversity or biodiversity" means the variability among living organisms from all sources including: terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part and also includes diversity within species, between species and of ecosystems.

"Evolutionary significant unit" means a set of populations that is morphologically and genetically distinct from other similar populations or a set of populations with a distinct evolutionary history.

"Indigenous species" means a species that occurs, or has historically occurred, naturally in a free state in nature within the borders of the Republic, but excludes a species that has been introduced in the Republic as a result of human activity.

"IUCN Red Data List" means a global or national list providing information on a species' risk of extinction (usually by taxonomic group) and prepared under the auspices of the International Union for the Conservation of Nature.

"Long-term survival" means to ensure the survival of a species until the next human generation, approximately 30 years.

"Stakeholder" means a natural or juristic person(s) that has an interest in, or may be affected by, a particular obligation or decision or activity, relating to or resulting from a management plan, either as individuals or representatives of a group and include landowners where appropriate.

"Species" means a kind of animal, plant or other organism that does not normally interbreed with individuals of another kind and includes any sub-species, cultivar, variety, geographic race, strain, hybrid or geographically separate population.

"Threat" means any action that causes a decline and compromises the future survival of a species or anything that has a detrimental effect on a species. Threats can be human induced or natural. BMP-S should focus on mitigating human induced threats to species.

"Umbrella species" are species selected for making conservation related decisions, typically because protecting these species indirectly protects the many other species that make up the ecological community of its habitat.

"Viable" in relation to a species or population means the ability to survive or persist and develop or multiply over multiple generations or a long time period.

2.4 INTRODUCTION

2.4.1 The Southern barred minnow (*Opsaridium peringuyei*)

Opsaridium peringueyi, (Gilchrist & Thompson, 1913) forms part of the genus, Opsaridium described by Peters in 1854 as a ray-finned fish in the Cyprinidae family. Cyprinidae is the largest family of freshwater fish, with over 2400 species in about 220 genera. The family belongs to the order Cypriniformes. *O. peringueyi*, *O. Zambezense* and *O. tweddleorum*, are some of the species in the *Opsaridium* genus. These species have a wide distribution across the African continent. They are distinguished from most other small cyprinid fish by a distinctive pattern of vertical stripes and typically inhabit clear flowing streams. Until recently only the Barred Minnow, *Opsaridium zambezense* (Peters, 1854) was recognized from Zimbabwe (Bell-Cross and Minshull, 1988) but a revision of the Southern African forms has revealed that another species, the Southern barred Minnow, *O. peringueyi* (Gilchrist and Thompson, 1913) also occurs in Zimbabwe and South Africa (Skelton, 1996). *O. peringueyi* is an endangered species in Zimbabwe where it may be extinct or close to extinction (Marshall and Gratwicke, 1999; Bills *et al.* 2007). In a previous assessment in South Africa the species was given the status of "intermediate-rare" but were recently reclassified as "least concern" in the 2007 IUCN classification (Bills *et al.* 2007). During the BMP-S workshop for the species in Skukuza (October 2008) a process was followed where the IUCN red listing criteria was used to re-asses the species with new data generated trough this project. From the outcome it would appear as if IUCN rating the species on a regional scale (in South Africa) could be graded from "least concern" to "vulnerable". This re-assessment still needs to be submitted for inclusion in the IUCN listing.

Opsaridium are shoaling species most frequently found in clear waters in strongly flowing rivers and are especially common in rocky stretches and in riffles. They feed on benthic macro-invertebrates such as chironomid and simuliid larvae, as well as adult insects taken from the surface (Marshall and Gratwicke, 1999).

O. peringueyi distribution range within the Save, Limpopo, Incomati, Umbeluzi and Phongola river systems. A relict population of this species existed in the Lephalala River, a tributary of the Limpopo in the Waterberg Mountains in South Africa (Kleynhans and Hoffman, 1992). In South Africa, this species is mostly found in areas below 1200 m altitude. It is found mainly along the lower escarpment and lowveld of Limpopo, Mpumalanga, Swaziland and in KwaZulu-Natal. The species is regionally extinct in Gauteng, South Africa (Bills *et al.* 2007).

2.4.2 Benefits of the plan

The BMP-S for *Opsaridium peringueyi* , if approved by the Minister will, be the first conservation plan for a fish species under the National Environmental Management: Biodiversity Act, 2004 (Act 10 of 2004)(NEMBA).

Throughout the development of the plan it became clear that the plan has the potential, not only to ensure the long term survival of the species, but for several other aquatic species as well as ecological processes, river types and ecological goods and services. Aquatic species conservation poses unique challenges mainly because of the uni-directional environment (rivers) they occupy. In most cases sensitive fish species like *O. peringueyi* is widely distributed (in several river systems) but conversely localized in a few river reaches with suitable habitat quality. This creates the challenge of implementing a conservation action on a national level while using different provincial agencies as implementing agents in association and/or partnership with a number of stakeholders. The BMP-S plan will greatly enhance effective conservation of the species trough national and inter-provincial common goal setting, action planning and communication. Because it is one of the first conservation plans developed according to the Act, it will serve to identify gaps and challenges in the newly developed BMP-S Norms and Standards.

2.5 SPECIES BACKGROUND

2.5.1 Biology, conservation status and legislative context

A comprehensive species background report has been compiled of which the first draft was available for use during the BMP-S stakeholder workshop in October 2008. The background report forms section three of this report.

2.6 PLANNING METHODOLOGY

2.6.1 Role players

Because the distribution of *O.peringueyi* in South Africa spans over three provinces (Limpopo, Mpumalanga and KwaZulu-Natal) it necessitated a national approach with a national institution taking the lead role. The norms and standards for the development of a BMP-S are not prescriptive regarding role players and implementing agents. However the reality is that a provincial conservation department would find it difficult to operate and lead a project across provincial borders. It is thus necessary for a national institution to play the leading role either directly or indirectly, trough an appointed implementing agency. It is recommended that DWEA be appointed as the lead agency and the three provincial conservation departments (Ezemvelo KZN Wildlife, Mpumalanga Parks & Tourism Agency and Limpopo Department of Economic Development, Environment and Tourism) serve as implementing agencies.

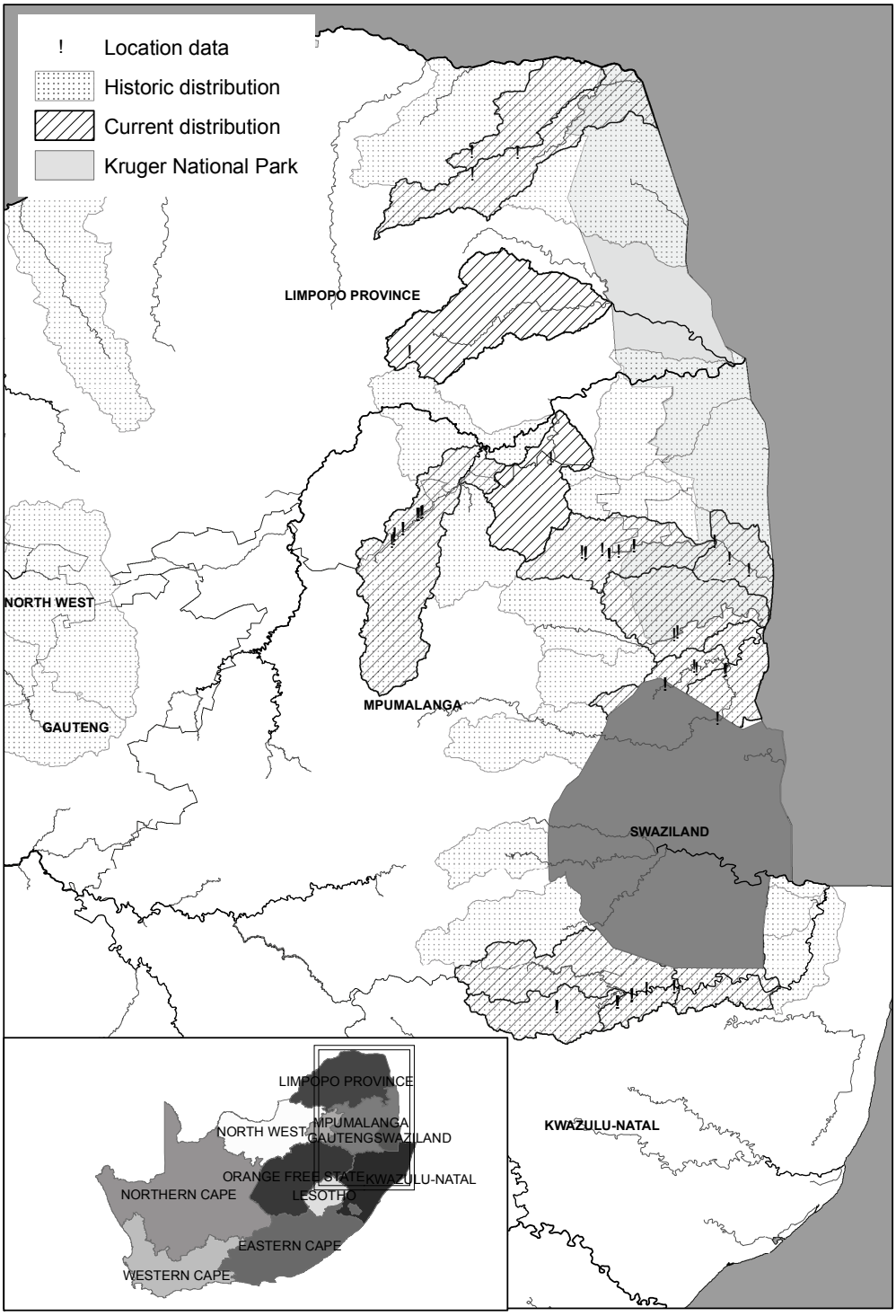


Figure 2.1: The historic and current distribution of *O. peringueyi,* using tertiary catchments as spatial units

2.6.2 Process background and description

The major envisaged outcome of WRC project K5/1677 was the development of a conservation plan for *O. peringueyi.* During recent years more and more emphasis was placed on conservation planning and specifically on ecosystems and species. This shift in emphasis was caused by the events and the stimulated reactions listed below.

a) As a signatory of the Convention on Biological Diversity in 1995 South Africa agreed to implement general measures for conservation. The convention calls for the development of national strategies, plans or programs and the integration of conservation and sustainable use of biological diversity into relevant sectoral or cross-sectoral plans, programmes and policies (Department of Environment and Tourism, 2005).
b) During the 2002 World Summit on Sustainable Development hosted by South Africa, South Africa committed to achieving the goals and targets of the Johannesburg Plan of Implementation. This included the Millennium Development Goals and biodiversity targets of which reducing the rate of loss of biodiversity by 2010 is an example. The National Biodiversity Strategy and Action Plan (NBSAP) sets out a plan to achieve this target (Department of Environment and Tourism, 2005).
c) The NBSAP was driven by a national imperative namely Section 24 of the South African Constitution (Act 108 of 1996) which stipulates the right of humans to have the environment protected in ways that ensure conservation and sustainable use. This principle is put into effect by the recent promulgation of the National Environmental Management: Biodiversity Act, 2004 (Act 10 of 2004)(NEMBA) and the National Environmental Management: Protected Areas Act 2003 (Act 57 of 2003) (NEMPAA) (Department of Environment and Tourism, 2005).
d) NEMBA provides the opportunity and legislative support for the development of norms and standards for the process and the format and scope that should be used to develop biodiversity management plans for indigenous species (BMP-S). Section 43 of NEMBA states 'that any person, organization or organ of state desiring to contribute to biodiversity management may submit to the Minister, for approval, a draft management plan for an indigenous or migratory species warranting special conservation attention'. In October 2006 a stakeholder workshop was called to initiate the development and compilation of norms and standards for the development process and format of biodiversity management plans for indigenous and migratory species under the auspices of the National Environmental Management: Biodiversity Act (Department of Environment and Tourism, 2006). The BMP-S has since been published.
e) Over the last couple of years several provinces went through a systematic conservation planning exercise in order to identify priority biodiversity conservation areas within the boundaries of the province and set targets to meet biodiversity conservation goals.
f) The IUCN Red Data List is a list providing the species at risk of extinction (usually by taxonomic group) with a classification on conservation status. Each listed species would have been assessed against the IUCN Red List Criteria as the standard for assessing a species risk of going extinct within a specific time frame. The South African Institute of Aquatic Biodiversity (SAIAB) has drafted an IUCN Red Data List for Southern African Fish which was published in the latter half of 2007 (Bills *pers comm.* 2007).

It was regarded as important that the conservation plan that was to be an outcome of project should integrate both with current national and provincial biodiversity conservation initiatives. In order to integrate with existing biodiversity conservation initiatives, the following steps were included as part of the development of the conservation plan.

a) An intensive three year research project was launched focusing on the ecology, biology and distribution of *O. peringueyi* and the results were compiled into a background report.
b) The background report was used to inform the participants and identified stakeholders in the development of a BMP-S.
c) Since the IUCN Red Listing procedure makes provision for the re-assessment of species when new information becomes available the status of *O. peringueyi* was re-evaluated. As part of the BMP-S development a process was followed where the conservation status of *O. peringueyi* was re-assessed as a population within South Africa's borders. Some of the specialists involved in the initial assessment for the current IUCN Red data list were involved in this process. The re-assessment took place during the initial stakeholder workshop (October 2008). The intent was that the re-assessment be incorporated in the current IUCN red list for fish species and the latter process is currently underway.
d) An initial stakeholder workshop took place in Skukuza during October 2008 where the BMP-S for *O. peringueyi* was developed.
e) The final BMP-S will be submitted for approval and implementation by implementing agents as described in the BMP-S Norms and Standards.
f) Because of the large distribution range of the species the BMP-S makes provision for planning and stakeholder involvement processes on ESU or regional level.

2.6.3 Stakeholder consultation

To ascertain stakeholder involvement it was decided to implement a two-phased approach:

a) The first phase took place on a national level and involved all the proposed /affected implementing agents, specialists and other interested parties. An initial stakeholder workshop was held in October 2008 where the higher-level goals and objectives for the plan were developed. The participants to the first stakeholder process are listed in table 2.1.

b) The second phase of stakeholder consultation is planned to take place during the implementation phase of the conservation plan. As stated previously the distribution range of *O. peringueyi* spans over three provinces, i.e. Limpopo, Mpumalanga and KwaZulu-Natal and within these geographic areas five ESUs were identified (Figure 1) as an outflow of the data collected during the project. These ESUs were confirmed during the initial workshop. Because of the physical and socio-economic differences within each ESU, it was realised that it would be time consuming and expensive (therefore not be feasible) to conduct one stakeholder consultation process. It is therefore proposed that the second stakeholder consultation process should lead to a subsidiary conservation plan for each ESU. Each subsidiary conservation plan will then address site specific issues and focused

stakeholder involvement within the ESU. The conclusion of the initial workshop was that the above process would be the only viable method of implementation.

Table 2.1: The participants during the initial stakeholder workshop held at Skukuza during October 2008*

Organization	Representative
Department of Water Affairs and Forestry	Mrs. P Maseti
University of Limpopo	Prof. N.A.G. Moyo
University of Limpopo	Mr. S. Theron
University of Venda	Mr. P.S.O Fouché
University of the Free State	Prof. P. Grobler
Eastern Cape Parks	Mr. J.A. Venter
BioAssets	Dr. W. Vlok
South African National Biodiversity Institute	Mr. S. Nkoana
South African Institute of Aquatic Biodiversity	Mr. R. Bills
Limpopo Department of Economic Development Environment and Tourism	Mr. M. Angliss
Limpopo Department of Economic Development Environment and Tourism	Mr. S. Rodgers
Ezemvelu KZN Wildlife	Dr. N. Rivers-Moore
CapeNature	Mr. Pierre de Villiers

* South African National Parks and Mpumalanga Parks and Tourism were invited but could not attend.

2.6.4 Agreements needed for implementation

To enable the successful implementation of the BMP-S it is regarded as essential the following agreements should put in place:

a) An agreement between DWEA and an implementing agent. It is recommended that an implementing agent be appointed to manage the implementation of the BMP-S.
b) An agreement between the implementing agent/DWEA and the three formal conservation authorities. This is intended to secure commitment from the provincial conservation authorities which will have to implement the BMP-S on a provincial level.
c) Other agreements. Any agreement between the implementing agent/DWEA and stakeholders in order to facilitate conservation actions from the BMP-S as well as the ESU subsidiary conservation plans.

2.7 MANAGEMENT OBJECTIVES FRAMEWORK

Vision

To ensure the protection of *Opsaridium peringueyi* as an umbrella species to maintain or improve the diverse population patterns and ecological integrity of associated species assemblages and processes and secure ecological goods and services in the five catchments to the benefit of humankind.

Key result areas and goals

The following management Goals and associated Key Result Areas (KRA) were developed for the BMP-S.

KRA1: CONSERVATION MEASURES FOR THE CONSERVATION OF *OPSARIDIUM PERINGUEYI*

Goal: To ensure the implementation of conservation measures for *O. peringuyei* in its distribution range in order to secure the long term survival of the species.

KRA2: MAINTANANCE OF ECOLOGICAL PATTERNS AND PROCESSES

Goal: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, *O. peringueyi.*

KRA3: SUSTAINABLE ECOLOGICAL GOODS AND SERVICES

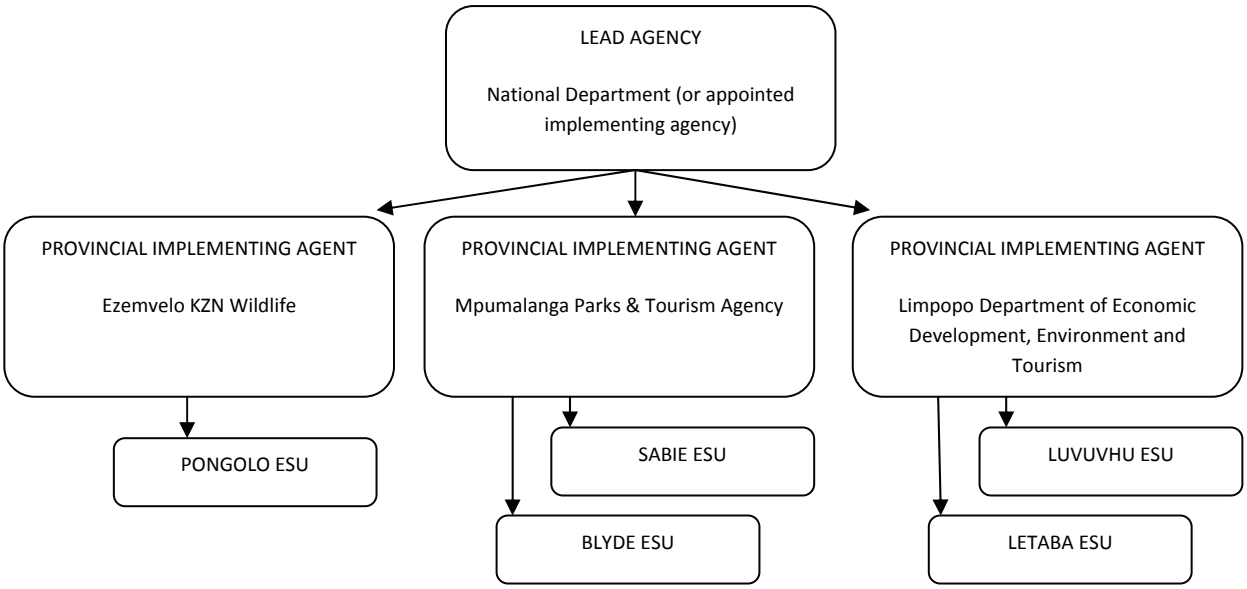
Goal: To ensure that through the conservation of the species associated goods and services are protected for the benefit of humankind

KRA4: EFFECTIVE COMMUNICATION

Goal: To ensure effective stakeholder engagement and communication throughout the implementation of the project.

2.8 ORGANIZATIONAL IMPLEMENTATION STRUCTURE

The organizational implementation structure for implementation of the BMP-S is illustrated in the following diagram.



2.9 STRATEGIC IMPLIMENTATION FRAMEWORK

Five ESUs were identified during the field research component of this project (Figure 1). Because each catchment where these ESUs are present have their own unique set of circumstances and threats they are used as conservation management units with their own prioritized activities.

Each *KRA* is directed by a number of *objectives*. Each objective will be achieved through a set of *activities* and will result in a number of *deliverables* within a defined *time frame*. The prioritized achievement of the objectives will be monitored within a set *time frame*. These objectives, activities, time frames are presented in a log frame format for each KRA.

LUVUVHU RIVER ESU (ESU CODE = LU)

KRA 1 – GOAL: To ensure the implementation of conservation measures for* O. peringueyi *in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 1.1: To identify and address threats to O. peringueyi long term survival							
LU 1.1.1) Water quality: Establish the extent of siltation sources and compile mitigating strategy.						**M**	
LU 1.1.2) Water quality: Communicate best practices to communities involved in association with Dept Agriculture						**H**	Dept. Agriculture Thohoyandou Regional Office
LU 1.1.3) Water quality: Develop a strategy and implement measures to protect the riparian zone						**H**	
LU 1.1.4) Water quality: Develop a strategy and implement measures to establish and maintain a buffer-zone between agriculture areas and the riparian zone						**H**	
LU 1.1.5) Water quality: Ensure that monitoring are conducted according to National Water Act						**M**	
LU 1.1.6) Water quantity: Confirm legality of water abstraction trough communication with water user associations						**H**	
LU 1.1.7) Water quantity: Ensure that monitoring are conducted according to National Water Act						**H**	
LU 1.1.8) Alien impact: Communicate and motivate for upstream alien plant eradication program to ensure prioritization of the catchments and riparian areas						**H**	
LU 1.1.9) Connectivity: Identify and map barriers up and downstream						**H**	
LU 1.1.10) Connectivity: Monitor migration upstream and downstream Mutale, Mukhase/Mbwedi rivers and downstream Luvuvhu River . Re-assessment in five years						**M**	See LU 1.2.2
LU 1.1.11) Utilization: Investigate the possible impact of utilization						**M**	

KRA 1 – GOAL: To ensure the implementation of conservation measures for* O. peringueyi *in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 1.2: To identify, prioritize and conduct research and monitoring on O. peringueyi							
LU 1.2.1) Liaison and integration with existing monitoring programs						**M**	River health, DWAF Water Quality etc.
LU 1.2.2) Design and implement a monitoring program to monitor the up and downstream migration of O. peringueyi.						**H**	
LU 1.2.3) Develop monitoring program to include water quality, alien species, utilization, habitat modification, habitat integrity, population dynamics and fish health						**H**	
LU 1.2.4) Lodge all data in existing national data-bases						**M**	
LU 1.2.5) Identify appropriate TPCs for long term monitoring of conservation effectiveness							
Objective LU 1.3: To establish aquatic reserve for the source area of O. peringueyi							
LU 1.3.1) Identify and map boundaries for the aquatic protected area (river length and buffer zone)						**H**	
LU 1.3.2) Link with existing conservation planning initiatives (i.e. Mphapuli Cycad Reserve Strategic management plan) who's responsible for strategies?						**H**	
LU 1.3.3) Investigate a vehicle for protection in the Mutale River						**M**	Possibilities include PAA or stewardship program
LU 1.3.4) Set up management forum						**H**	

KRA 1 – GOAL: To ensure the implementation of conservation measures for* O. peringueyi *in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 1.4: To secure upstream and downstream processes							
LU 1.4.1) Secure upstream and downstream processes + strategies						**H**	Monitoring program to guide if further action would be needed regarding improving connectivity
LU 1.4.2) Active participation and liaison regarding general environmental management within the catchment and downstream						**M**	To secure upstream and downstream processes

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 2.1: To identify and conserve species assemblages associated with O.peringueyi and river types that the species occurs in.							
LU 2.1.1) Identification and description of species assemblages						**H**	Confirmation of *O. peringueyi* as suitable umbrella species
LU 2.1.2) Identification of interactions within species assemblages						**M**	Confirmation of *O. peringueyi as suitable*

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
							umbrella species
LU 2.1.3) List all species being protected as a result of the conservation of *O. peringueyi*						**M**	
LU 2.1.4) Identification of future research programs						**M**	See LU 1.2
Objective LU 2.2: To identify and conserve the river types, integrity and processes in O. peringueyi distribution range							
LU 2.2.1) List effects of degradation of river + mitigation						**H**	*O. peringueyi* is a key indicator of a A/B-class category in the Mpapuli cycad reserve and B-class in the Mutale river (ecological management class) If the class changes, what would the effect be? Evaluation of goods and services lost going from a class B to a class C river
LU 2.2.2) Identification and description of habitat integrity, processes and river types						**H**	Confirmation of *O. peringueyi as suitable umbrella species*

KRA 3 – GOAL: To ensure that trough the conservation of the species associated goods and services are protected for the benefit of humankind.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 3.1: To identify goods and services from the different river ecological categories and promote benefits to stakeholders							
LU 3.1.1) List the goods and services from a Class A/B river and state the consequence of over utilisation of the resource						**M**	Market value of B-class river type to water users, land owners and conservation organizations (River health reports 2001 and 2003)

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 4.1. To develop a stakeholder database							
LU 4.1.1) Identify local, regional, national and international stakeholders						**H**	
LU 4.1.2) Develop and coordinate a stakeholder database						**M**	
LU 4.1.3) Arrange information transfer to listed stakeholders						**M**	
Objective LU 4.2: To develop a stakeholder engagement strategy and implement it in the ESU							
LU 4.2.1) Appoint public participation manager						**H**	
LU 4.2.2) Background information survey done and presented						**M**	

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
to stakeholder group							
LU 4.2.3) Initial stakeholder meeting						**M**	
LU 4.2.4) Set up steering committee for ESU						**H**	
LU 4.2.5) Feedback to project management						**H**	

LETABA RIVER ESU (ESU CODE = LE)

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LE 1.1: To identify and address threats to O. peringueyi long term survival							
LE 1.1.1) Water quality: Ensure water quality guidelines are met by adjacent land-owners						**H**	
LE 1.1.2) Water quality: Ensure forestry-chemical use on banks of river conforms to required agreed standards						**H**	
LE 1.1.3) Water quality: Develop a strategy and implement measures to protect the riparian zone						**H**	
LE 1.1.4) Water quality: Develop a strategy and implement measures to establish and maintain a buffer-zone between forestry areas and the riparian zone						**H**	
LE 1.1.5) Water quality: Ensure that monitoring are conducted according to National Water Act						**H**	
LE 1.1.6) Water quantity: Promote environmentally friendly						**M**	Roads, sawmill

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
land use practices							effluent etc.
LE 1.1.7) Water quantity: Ensure that monitoring are conducted according to National Water Act						**H**	
LE 1.1.8) Alien impact: Communicate and motivate for upstream alien plant eradication program to ensure prioritization of the catchments and riparian areas						**H**	
LE 1.1.9) Connectivity: Identify and map barriers up and downstream						**H**	
LE 1.1.10) Connectivity: Confirm legality of water abstraction						**H**	
LE 1.1.11) Connectivity: Monitor migration upstream and downstream of river stretch						**M**	See LE 1.2.2
LE 1.1.12) Utilization: Investigate the possible impact of utilization						**M**	
LE 1.1.13) Alien impact: Establish and maintain an alien fish eradication program in the river, dam, off-channel storage dams and conservation area						**H**	Sensitive to confirmed bass-masters dams
Objective LE 1.2: To identify, prioritize and conduct research and monitoring on O. peringueyi							
LE 1.2.1) Liaison and integration with existing monitoring programs						**M**	River health, DWAF Water Quality etc.
LE 1.2.2) Design and implement a monitoring program to monitor the up and downstream migration of *O. peringueyi.*						**h**	
LE 1.2.3) Develop monitoring program to include water quality, alien species, utilization, habitat modification, habitat integrity, population dynamics and fish health						**H**	
LE 1.2.4) Lodge all data in existing national data-bases						**M**	SA-BIF
LE 1.2.5) Identify appropriate TPCs for long term monitoring of conservation effectiveness							

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LE 1.3: To establish aquatic reserve for the source area of O. peringueyi							
LE 1.3.1) Identify and map boundaries for the aquatic protected area (river length and buffer zone)						**H**	
LE 1.3.2) Link with existing conservation planning initiatives						**M**	
LE 1.3.3) Investigate a vehicle for protection						**M**	Possibilities include PAA or stewardship program
LE 1.3.4) Set up management forum						**M**	Establishment should bear in mind the umbrella species concept (e.g. other species)

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LE 1.4: To secure upstream and downstream processes							
LU 1.4.1) Secure upstream and downstream processes						**H**	Monitoring program to guide if further action would be needed regarding improving connectivity. Upstream and downstream migration would be limited because of two large dams
LU 1.4.2) Active participation and liaison regarding general environmental management within the catchment and downstream						**H**	To secure upstream and downstream processes

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LE 2.1: To identify and conserve species assemblages associated with O.peringueyi and river types that the species occurs in.							
LE 2.1.1) Identification and description of species assemblages						**M**	Confirmation of *O.*

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
							peringueyi as suitable umbrella species
LE 2.1.2) Identification of interactions within species assemblages						**M**	Confirmation of *O. peringueyi as suitable umbrella species*
LE 2.1.3) List all species being protected as a result of the conservation of *O. peringueyi*						**M**	
LE 2.1.4) Identification of future research programs						**H**	See LU 1.2
Objective LE 2.2: To identify and conserve the river types, integrity and processes in O. peringueyi distribution range							
LE 2.2.1) List effects of degradation of river + mitigation						**H**	*River stretch was classified as D-class for ecological reserves (Letaba reserve 2004).* If the class changes, what would the effect be? Evaluation of goods and services lost going from a class B to a class C river
LE 2.2.2) Identification and description of habitat integrity, processes and river types						**M**	Confirmation of *O. peringueyi as suitable umbrella species*

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
LE 2.2.3) Identify future research priorities						**H**	
LE 2.2.4) Evaluate the goods and services lost going from a class B to a class C river						**H**	

KRA 3 – GOAL: To ensure that trough the conservation of the species associated goods and services are protected for the benefit of humankind.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LE 3.1: To identify goods and services from the different river ecological categories and promote benefits to stakeholders							
LE 3.1.1) List the goods and services from a Class A/B river and state the consequence of overutilization of the resource						**H**	Market value of B-class river type to water users, land owners and conservation organizations

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 4.1. To develop a stakeholder database							
LU 4.1.1) Identify local, regional, national and international stakeholders						**H**	
LU 4.1.2) Develop and coordinate a stakeholder database						**M**	
LU 4.1.3) Arrange information transfer to listed stakeholders						**M**	
Objective LE 4.2: To develop a stakeholder engagement strategy and implement it in the ESU							
LE 4.2.1) Appoint public participation manager						**H**	
LE 4.2.2) Background information survey done and presented to stakeholder group						**M**	
LE 4.2.3) Initial stakeholder meeting						**M**	
LE 4.2.4) Set up steering committee for ESU						**H**	
LE 4.2.5) Feedback to project management						**M**	

BLYDE RIVER ESU (ESU CODE = BL)

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective BL 1.1: To identify and address threats to O. peringueyi long term survival							

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
BL 1.1.1) Water quality: Ensure that water quality guidelines are met by adjacent land-owners						**H**	
BL 1.1.2) Water quality: Develop a strategy and implement measures to protect the riparian zone						**H**	
BL 1.1.3) Water quality: Develop a strategy and implement measures to establish and maintain a buffer-zone between agriculture areas and the riparian zone						**H**	
BL 1.1.4) Water quality: Ensure that monitoring are conducted according to National Water Act						**H**	
BL 1.1.5) Water quantity: Confirm legality of water abstraction trough communication with water use associations + monitoring						**H**	
BL1.1.6) Water quantity: Ensure that monitoring are conducted according to National Water Act						**M**	
BL 1.1.7) Water quantity: Engage with DWAF to ensure timing and volumes of flows from the Blydepoort dam as declared in the ecological reserve for the river						**H**	
BL 1.1.8) Alien impact: Communicate and motivate for upstream alien plant eradication program to ensure prioritization of the catchments and riparian areas						**H**	
BL 1.1.9) Alien impact: Establish and maintain an alien fish eradication program in the Blydepoort dam, off-channel storage dams and conservation area						**H**	
BL 1.1.10) Connectivity: Identify and map barriers up and downstream						**M**	
BL1.1.11) Connectivity: Monitor migration upstream and downstream Blyde and Olifants rivers and downstream Re-assessment in five years						**M**	See LU 1.2.2
BL 1.1.12) Utilization: Investigate the possible impact of utilization						**M**	

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective BL 1.2: To identify, prioritize and conduct research and monitoring on O. peringueyi							
BL 1.2.1) Liaison and integration with existing monitoring programs						**M**	River health, DWAF Water Quality, University of Limpopo Olifants River Research, National toxicity program etc.
BL 1.2.2) Design and implement a monitoring program to monitor the up and downstream migration of *O. peringueyi.*						**H**	
BL 1.2.3) Develop monitoring program to include water quality, alien species, utilization, habitat modification, habitat integrity, population dynamics and fish health						**M**	
BL 1.2.4) Lodge all data in existing national data-bases						**M**	
BL 1.2.5) Identify appropriate TPCs for long term monitoring of conservation effectiveness							
Objective BL 1.3: To establish aquatic reserve for the source area of O. peringueyi							
BL 1.3.1) Identify and map boundaries for the aquatic protected area (river length and buffer zone)						**H**	
BL 1.3.2) Link with existing conservation planning initiatives (i.e. Mpapuli Cycad Reserve Strategic management plan)						**H**	Kruger to canyon biosphere reserve, possible RAMSAR site declaration
BL 1.3.3) Investigate a vehicle for protection in the Blyde River						**H**	Possibilities include PAA or stewardship program. Should bear in mind

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
							umbrella species concept
BL 1.3.4) Set up management forum						**M**	
Objective BL 1.4: To secure upstream and downstream processes							
BL 1.4.1) Secure upstream and downstream processes						**M**	Monitoring program to guide if further action would be needed regarding improving connectivity
BL 1.4.2) Active participation and liaison regarding general environmental management within the catchment and downstream						**M**	To secure upstream and downstream processes

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective BL 2.1: To identify and conserve species assemblages associated with O.peringueyi and river types that the species occurs in.							
BL 2.1.1) Identification and description of species assemblages						**M**	Confirmation of *O. peringueyi as suitable umbrella*

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
							species
BL 2.1.2) Identification of interactions within species assemblages						**M**	Confirmation of *O. peringueyi as suitable umbrella species*
BL 2.1.3) List all species being protected as a result of the conservation of *O. peringueyi*						**M**	
BL 2.1.4) Identification of future research programs						**H**	See BL 1.2
Objective BL 2.2: To identify and conserve the river types, integrity and processes in O. peringueyi distribution range							
BL 2.2.1) List effects of degradation of river + mitigation						**H**	*O. peringueyi* is a key indicator of a B-class category in the If the class changes, what would the effect be? Evaluation of goods and services lost going from a class B to a class C river
BL 2.2.2) Identification and description of habitat integrity, processes and river types						**H**	Confirmation of *O. peringueyi as suitable umbrella species*

KRA 3 – GOAL: To ensure that trough the conservation of the species associated goods and services are protected for the benefit of humankind.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective BL 3.1: To identify goods and services from the different river ecological categories and promote benefits to stakeholders							
BL 3.1.1) List the goods and services from a Class B river and state the consequence of overutilization of the resource						**M**	Market value of B-class river type to water users, land owners and conservation organizations (River health reports 2001 and 2003)

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective LU 4.1. To develop a stakeholder database							
BL 4.1.1) Identify local, regional, national and international stakeholders						**H**	
BL 4.1.2) Develop and coordinate a stakeholder database						**M**	
BL 4.1.3) Arrange information transfer to listed stakeholders						**M**	
Objective BL 4.2: To develop a stakeholder engagement strategy and implement it in the ESU							
BL 4.2.1) Appoint public participation manager						**H**	
BL 4.2.2) Background information survey done and presented to stakeholder group						**m**	

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
BL 4.2.3) Initial stakeholder meeting						**m**	
BL 4.2.4) Set up steering committee for ESU						**H**	
BL 4.2.5) Feedback to project management						**M**	

SABIE RIVER ESU (ESU CODE = SA)

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective SA 1.1: To identify and address threats to O. peringueyi long term survival							
SA 1.1.1) Water quality: List impacts						**H**	Point source and diffuse, possible gold mining activities upstream
SA 1.1.2) Water quality: Develop action plans to address impacts + implementation						**M**	See SA 1.1.1
SA 1.1.3) Water quality: Communicate best practices to communities and land owners in association with government departments						**M**	
SA 1.1.4) Water quality: Develop a strategy and implement measures to establish and maintain a buffer-zone between leisure, agriculture and forestry areas and the riparian zone						**H**	

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
SA 1.1.5) Water quality: Ensure that monitoring are conducted according to National Water Act						**H**	Check status of ecological reserve (SA 1.1.8), communicate with DWAF regarding water quality monitoring data
SA 1.1.6) Water quantity: Confirm legality of water abstraction trough communication with water use associations						**M**	
SA 1.1.7) Water quantity: Ensure that monitoring are conducted according to National Water Act						**M**	
SA 1.1.8) Water quantity: Confirm status of ecological reserve						**H**	
SA 1.1.9) Water quantity: Communicate with DWAF regarding water quality monitoring data						**M**	
SA 1.1.10) Alien impact: Communicate and motivate for upstream alien plant eradication program to ensure prioritization of the catchments and riparian areas						**H**	Forestry sector and WfW
SA 1.1.11) Evaluate potential for a alien fish eradication program in the Sabie catchment, off-channel storage dams and conservation area + strategy						**H**	
SA 1.1.12) Connectivity: Identify and map barriers up and downstream						**H**	
SA1.1.13) Connectivity: Monitor migration upstream and downstream Sabie river (Sabane, Marite, Mac Mac). Re-assessment in five years						**M**	See SA 1.2.2
SA 1.1.14) Utilization: Investigate the possible impact of utilization						**M**	

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective SA 1.2: To identify, prioritize and conduct research and monitoring on O. peringueyi							
SA1.2.1) Liaison and integration with existing monitoring programs						**M**	River health, DWAF Water Quality etc.
SA 1.2.2) Design and implement a monitoring program to monitor the up and downstream migration of *O. peringueyi.*						**h**	
SA 1.2.3) Develop monitoring program to include water quality, alien species, utilization, habitat modification, habitat integrity, population dynamics and fish health + implementation strategy						**H**	
SA 1.2.4) Lodge all data in existing national data-bases						**M**	
SA 1.2.5) Identify research priorities						**H**	
SA 1.2.6) Identify appropriate TPCs for long term monitoring of conservation effectiveness							
Objective SA 1.3: To establish aquatic reserve for the source area of O. peringueyi							
SA 1.3.1) Identify and map boundaries for the aquatic protected area (river length and buffer zone)						**H**	
SA 1.3.2) Link with existing conservation planning initiatives						**M**	
SA 1.3.3) Investigate a vehicle for protection in the Mac Mac River						**H**	Possibilities include PAA or stewardship program, Mpumalanga aquatic conservation plan, important water yield zone, Kruger park catchment, catchment to coast

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
SA 1.3.4) Set up management forum						**M**	Establishment should bear in mind the umbrella species concept
Objective SA 1.4: To secure upstream and downstream processes							
SA 1.4.1) Secure upstream and downstream processes						**H**	Monitoring program to guide if further action would be needed regarding improving connectivity. Source-sink and catchment integrity
SA 1.4.2) Initiate measures for improving connectivity for migration process						**H**	
SA 1.4.3) Communication with Kruger National Park						**M**	
SA 1.4.4) Active participation and liaison regarding general environmental management within the catchment and downstream						**H**	To secure upstream and downstream processes

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective SA 2.1: To identify and conserve species assemblages associated with O.peringueyi and river types that the species occurs in.							
SA 2.1.1) Identification and description of species assemblages						**M**	Confirmation of *O. peringueyi as suitable umbrella species*
SA 2.1.2) Identification of interactions within species assemblages						**M**	Confirmation of *O. peringueyi as suitable umbrella species*
SA 2.1.3) List all species being protected as a result of the conservation of *O. peringueyi*						**H**	
SA 2.1.4) Identification of future research programs						**H**	See SA 1.2

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective SA 2.2: To identify and conserve the river types, integrity and processes in O. peringueyi distribution range							
SA 2.2.1) List effects of degradation of river + mitigation						**M**	*O. peringueyi* is a key indicator of a ?-class category in the Sabie and tributaries (ecological management class) If the class changes, what would the effect be? Evaluation of goods and services lost going from a class B to a class C river
SA 2.2.2) Identification and description of habitat integrity, processes and river types						**M**	Confirmation of *O. peringueyi as suitable umbrella species*

KRA 3 – GOAL: To ensure that trough the conservation of the species associated goods and services are protected for the benefit of humankind.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective SA 3.1: To identify goods and services from the different river ecological categories and promote benefits to stakeholders							
SA 3.1.1) List the goods and services from a Class ? river and state the consequence of overutilization of the resource						**M**	Market value of ?-class river type to water users, land owners and conservation organizations (River health reports and other)

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective SA 4.1. To develop a stakeholder database							
SA 4.1.1) Identify local, regional, national and international stakeholders						**H**	
SA 4.1.2) Develop and coordinate a stakeholder database						**M**	
SA 4.1.3) Arrange information transfer to listed stakeholders						**M**	
Objective SA 4.2: To develop a stakeholder engagement strategy and implement it in the ESU							
SA 4.2.1) Appoint public participation manager						**H**	
SA 4.2.2) Background information survey done and presented to stakeholder group						**M**	

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
SA 4.2.3) Initial stakeholder meeting						**M**	
SA 4.2.4) Set up steering committee for ESU						**H**	
SA 4.2.5) Feedback to project management						**M**	

PONGOLO RIVER ESU (ESU CODE = PO)							
KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective PO 1.1: To identify and address threats to O. peringueyi long term survival							
PO 1.1.1) Water quality: Establish the extent of siltation, point source and diffuse sources						**H**	
PO 1.1.2) Water quality: Develop action plans to address them						**H**	
PO 1.1.3) Water quality: Communicate best practices to communities involved in association with Dept Agriculture						**M**	
PO 1.1.4) Water quality: Develop a strategy and implement measures to protect the riparian zone						**H**	
PO 1.1.5) Water quality: Develop a strategy and implement measures to establish and maintain a buffer-zone between agriculture and forestry areas and the riparian zone						**H**	
PO 1.1.6) Water quality: Ensure that monitoring are conducted according to National Water Act						**M**	

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
PO 1.1.7) Water quantity: Confirm legality of water abstraction trough communication with water use associations						**H**	
PO 1.1.8) Water quantity: Ensure that monitoring are conducted according to National Water Act						**H**	
PO 1.1.9) Water quantity: Confirm status of ecological reserve + monitoring						**H**	
PO 1.1.10) Alien impact: Communicate and motivate for upstream alien plant eradication program to ensure prioritization of the catchments and riparian areas						**H**	Forestry sector and WfW
PO 1.1.11) Alien impact: Evaluate the potential for an alien fish eradication program in the Bivane catchment, off-channel storage and conservation area						**M**	
PO 1.1.12) Connectivity: Identify and map barriers up and downstream + mitigation (feed into next point)						**H**	
PO 1.1.13) Connectivity: Monitor migration upstream and downstream Pongolo and Bivane rivers and downstream Pongolo River . Re-assessment in five years						**H**	See PO 1.2.2
PO 1.1.14) Utilization: Investigate the possible impact of utilization						**M**	
Objective PO 1.2: To identify, prioritize and conduct research and monitoring on O. peringueyi							
PO 1.2.1) Liaison and integration with existing monitoring programs						**M**	River health, DWAF Water Quality etc.
PO 1.2.2) Design and implement a monitoring program to monitor the up and downstream migration of *O. peringueyi.*						**H**	
PO 1.2.3) Develop monitoring program to include water quality, alien species, utilization, habitat modification, habitat integrity, population dynamics and fish health						**H**	
PO 1.2.4) Lodge all data in existing national data-bases						**M**	
PO 1.2.5) Identification of research priorities						**H**	
PO 1.2.6) Identify appropriate TPCs for long term monitoring of							

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
conservation effectiveness							
Objective PO 1.3: To establish aquatic protected area for the source area of O. peringueyi							
PO 1.3.1) Identify and map boundaries for the aquatic protected area (river length and buffer zone)						**H**	
PO 1.3.2) Link with existing conservation planning initiatives						**M**	Ithala Nature Reserve Management Plan, KZN Aquatic conservation plan, important water yield zone
PO 1.3.3) Investigate a vehicle for protection in the Bivane River						**H**	Possibilities include PAA or stewardship program
PO 1.3.4) Set up management forum						**M**	

KRA 1 – GOAL: To ensure the implementation of conservation measures for O. peringuyei in its distribution range in order to secure the long term survival of the species.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective PO 1.4: To secure upstream and downstream processes							
PO 1.4.1) Secure upstream and downstream processes						**M**	Monitoring program to guide if further action would be needed regarding improving connectivity
PO 1.4.2) Active participation and liaison regarding general environmental management within the catchment and downstream						**M**	To secure upstream and downstream processes
PO 1.4.3) Determine if it is an important catchment area + strategy						**H**	Declared?

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective PO 2.1: To identify and conserve species assemblages associated with O.peringueyi and river types that the species occurs in.							
PO 2.1.1) Identification and description of species assemblages						**M**	Confirmation of *O. peringueyi as suitable umbrella species*
PO 2.1.2) Identification of interactions within species assemblages						**M**	Confirmation of *O.*

KRA 2 – GOAL: To maintain or improve the diverse population patterns and ecological integrity of species assemblages and processes associated with the umbrella species, O. peringueyi.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
							peringueyi as suitable umbrella species
PO 2.1.3) List all species being protected as a result of the conservation of *O. peringueyi*						**M**	
PO 2.1.4) Identification of future research programs						**H**	See PO 1.2
Objective PO 2.2: To identify and conserve the river types, integrity and processes in O. peringueyi distribution range							
PO 2.2.1) List effects of degradation of river + mitigation						**H**	*O. peringueyi* is a key indicator of a ?-class category in the Bivane river (ecological management class) If the class changes, what would the effect be?
PO 2.2.2) Identification and description of habitat integrity, processes and river types						**M**	Confirmation of *O. peringueyi as suitable umbrella species*

KRA 3 – GOAL: To ensure that trough the conservation of the species associated goods and services are protected for the benefit of humankind.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective PO 3.1: To identify goods and services from the different river ecological categories and promote benefits to stakeholders							
PO 3.1.1) List the goods and services from a Class B/C river and state the consequence of overutilization of the resource						**M**	Market value of B/C-class river type to water users, land owners and conservation organizations. Establish if there are existing reports

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
Objective PO 4.1. To develop a stakeholder database							
PO 4.1.1) Identify local, regional, national and international stakeholders						**H**	
PO 4.1.2) Develop and coordinate a stakeholder database						**M**	
PO 4.1.3) Arrange information transfer to listed stakeholders						**M**	
Objective PO 4.2: To develop a stakeholder engagement strategy and implement it in the ESU							
PO 4.2.1) Appoint public participation manager						**H**	
PO 4.2.2) Background information survey done and presented to stakeholder group						**m**	

KRA 4 – GOAL: To ensure effective stakeholder engagement and communication throughout the implementation of the project.							
Actions	**Time-frame (years)**					**Priority (where H=high; M=moderate; and L=low)**	**Notes**
	1	**2**	**3**	**4**	**5**		
PO 4.2.3) Initial stakeholder meeting						**M**	
PO 4.2.4) Set up steering committee for ESU						**M**	
PO 4.2.5) Feedback to project management						**M**	

2.10 REFERENCES

CRASS, R.S. (1964). Notes on the freshwater fishes of Natal with descriptions of four new species. *Ann. Natal. Mus*., **14:** 405-458.

DEPARTMENT OF ENVIRONMENT AND TOURISM, Republic of South Africa. (2005). South Africa's National Biodiversity Strategy and Action Plan. Department: Environmental Affairs & Tourism, Republic of South Africa.

DEPARTMENT OF ENVIRONMENT AND TOURISM, Republic of South Africa. (2006). Draft Norms and standards for Biodiversity Management Plans for species (BMP-S) produced under the auspices of the National Environmental Management Biodiversity Act (NEMBA) No. 10 of 2004. Department: Environmental Affairs & Tourism, Republic of South Africa.

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

SKELTON, P.H. (1987). South African Red Data book – Fishes. *South African National Scientific Programmes Report,* 137: 1-199.

SKELTON, P.H., 1996. A review of *Opsaridium zambezense* (Pisces: Cyprinidae) form southern Africa with description of a new species from Malawi. *Icthyol. Explor. Freshwaters* **7(1)**: 59-84.

Section 3

Background report: a) The Southern Barred Minnow, *Opsaridium peringueyi* (Gilchrist & Thompson, 1913) and b) the rivers within the South African distribution range of the species.

A background report developed for a biodiversity management plan for species (BMP-S).

JA Venter, PSO Fouché, W Vlok, NAG Moyo, S Theron and P Grobler

Chapter 1: Introduction

JA Venter and PSO Fouché

3.1.1 INTRODUCTION AND PROJECT BACKGROUND

As stated in the section 1 of this report the conservation of biodiversity in southern Africa's highly diverse and important freshwater ecosystems is an important, but often neglected, conservation management priority. In addition it was *inter alia* pointed out by Darwall *et al.* (2009) who stated that the levels of threat to freshwater species are higher in South Africa than in other countries and that species information remains very limited for many parts of the region.

Water Research Commission funding was granted to develop a conservation framework applicable to southern African conditions for threatened fish species. This framework is intended to lead to effective conservation management and should serve as one of the tools for effective conservation management of threatened fish species. Because of its sensitivity rating (Kleynhans, 2007) and conservation status (Bills *et al.* 2007) the Southern Barred Minnow, *Opsaridium peringueyi,* was selected as an appropriate species.

As reported previously the project consisted of three phases and the first phase consisted of the collection of background information on the species.
Section three reports on the information collected and deals with:

a) the current distribution of *O. peringueyi* in its historical distribution range,
b) a characterization of the habitat and the habitat preference of *O. peringueyi,*
c) selected biological aspects of the species such as the feeding and breeding,
d) the genetic status of the species.

In addition the section contains a description of the river systems in the distribution range of the species. This description consists of a general and geomorphological description of the river, the potential threats in the river as well as a description of available habitat and the fish diversity.

3.1.2 SPECIES BACKGROUND

The Southern Barred Minnow, *Opsaridium peringueyi,* (Gilchrist & Thompson, 1913), forms part of the genus *Opsaridium* described by Peters in 1854 as "ray-finned fish in the family Cyprinidae family". The Cyprinidae is the largest family of freshwater fish, with over 2400 species in 220 genera. The genus is part of the African bariliins that are characterized by vertical bars on their body and are generally regarded shoaling, streamlined, active swimming and predatory. Three species of the genus namely *O. peringueyi*, *O. zambezense* and the recently described *O. tweddleorum* occur in southern Africa (Skelton,2001). Until recently the Northern Barred Minnow, *O. zambezense* was the only species recognized (Bell-Cross and Minshull, 1988) but a revision of the southern African forms has shown that the Southern Barred Minnow, *O. peringueyi* occurs in Zimbabwe and South Africa (Skelton, 1996). The three species each have a distinct distribution in southern Africa. Where the Dwarf Sanjika, *O. tweddleorum*, is restricted to the lower Zambezi the two other species are more widely distributed with *O. zambezense* occurring from in the Okavango, Zambezi, Revue and Buzi systems and *O. peringueyi* in an area from the Save River in the north to the Phongola River in the south. In Zimbabwe *O. peringueyi* is regarded as an endangered species where it may be extinct or close to extinction (Marshall and Gratwicke, 1999; Bills et al*.,* 2007). In earlier assessments in South Africa *O. peringueyi* the species was classified as "intermediate-rare" but was recently reclassified as "of least concern" in the 2007 IUCN classification (Bills *et al.* 2007).

According to Marshall and Gratwicke (1999) the *Opsaridium species* are shoaling fish "most frequently found in clear waters in strongly flowing rivers and are especially common in rocky stretches and in riffles" and they feed on macro-invertebrates such as chironomid and simuliid larvae, as well as adult insects taken from the surface. *O. peringueyi* prefers clean shallow perennial in stream pools of rivers or slow runs (15-50 cm) on a sandy or gravel substratum (Emery *et al.,* 2002). *O. zambezense* is typically found in running water and *O. tweddleorum* prefers clear fast flowing water. In the Nyagui River *O. zambezense* is abundant and it was found at altitudes between 1152-1328 m. The availability of riffles on the downstream section of the river appears to influence positively the abundance of this species. Other species associated with riffles in the Nyagui River included *B. trimaculatus*, *Chiloglanis neumanni*, *Labeobarbus cylindricus* and *Labeobarbus marequensis* (Kadye and Moyo, 2007).

As stated above the distribution range of *O. peringueyi* includes the Save, Limpopo, Incomati, Umfolozi and Phongola river systems (Skelton, 2001). However a relict population of this species was reported in the Lephalala River, a tributary of the Limpopo in the Waterberg Mountains in South Africa by Kleynhans and Hoffman (1992). In South Africa, this species is mostly found in areas below 1200 m altitude and it is found mainly along the lower escarpment and lowveld of Limpopo, Mpumalanga, Swaziland and in KwaZulu-Natal. The species is regionally extinct in Gauteng, South Africa (Bills *et al.* 2007). Beyond South Africa the barred minnow occurs in rivers as far north as the Zambezi River system in Zimbabwe and Mozambique (its downstream limits in Mozambique are not yet recorded), Malawi as well as in the Okavango, Upper Zambezi, the Kasai and Zambian/Zaire systems (Skelton, 1987).

Chapter 2: Conservation status and distribution

JA Venter, PSO Fouché and W Vlok.

3.2.1 CONSERVATION STATUS

During an earlier IUCN assessment in South Africa the species *O. peringueyi* was awarded the conservation status of "intermediate-rare" but it was recently reclassified into the Red Category of "Least concern" in southern Africa during the 2007 IUCN classification process (Bills *et al.* 2007). The outcome of this process and the deciding factors are shown in Table 3.2.1. During a BMP-S workshop for *O. peringueyi* held in October 2008 a process was followed where the IUCN red listing criteria was used to assess the species with the new data generated. The workshop formed part of this project and data used was obtained during the project. From the outcome of the 2008 it would appear as if IUCN rating of the species on a regional scale, in particular within South Africa, should be changed from "least concern" to "vulnerable". This re-assessment still needs to be submitted for inclusion in the IUCN listing.

3.2.2 PAST AND PRESENT DISTRIBUTION

3.2.2.1 Introduction

In addition to the fact that *O. peringueyi* has been collected from the Save, Limpopo, Incomati and Phongolo river systems Skelton (1996) reports that specimens were also collected from two relic populations in the upper catchments of the Limpopo River System in the Lephalala River in the Waterberg and the Pienaars and Hennops rivers in the Pretoria region respectively. Bills *et al.* (2007) confirms that the Gauteng population is regionally extinct.

In South Africa the species is confined to altitudes below 1200 m mainly along the lower escarpment and Lowveld of the Limpopo Province, Mpumalanga Province and KwaZulu-Natal Province Swaziland (Crass 1964; Gaigher 1973; Skelton 1987 and 1996).

Table 3.2.1: The IUCN Red List of Threatened Species listing information for *Opsaridium peringueyi* (Bills *et al. 2007)*

Taxonomy				
Kingdom	**Phylum**	**Class**	**Order**	**Family**
ANIMALIA	CHORDATA	ACTINOPTERYGII	CYPRINIFORMES	CYPRINIDAE
Scientific name:	*Opsaridium peringueyi*			
Species Authority:	(Gilchrist & Thompson, 1913)			
Common Name/s:	English – Southern Barred Minnow			
Taxonomic Notes:	Eschmeyer (1998) notes that this was recognised as a synonym of *O. zambezense* in 1984 (Leveque & Daget), but was noted as a valid species in 1996 (following Skelton).			
Assessment Information				
Red List Category & Criteria:	Least Concern ver 3.1			
Year Assessed:	2007			
Assessor/s	Bills, R., Engelbrecht J. & Marshall, B.E.			
Evaluator/s:	Snoeks, J. (Freshwater Fish Red List Authority) & Darwall, W. (Freshwater Biodiversity Assessment Programme)			
Justification: Although reductions have occurred within the Limpopo River system in Zimbabwe it is widespread and fairly common throughout the remainder of its range.				
Geographic Range				
Range Description:	Save River in Zimbabwe south to the Phongolo River in KwaZulu-Natal, South Africa. No recent records in Zimbabwe and possibly extinct there.			
Countries:	Native: Mozambique; South Africa (Gauteng – Regionally Extinct, KwaZulu-Natal, Limpopo Province, Mpumalanga); Swaziland Possibly extinct: Zimbabwe			
Population:	During the 1980/90s numbers assessed in Mpumalanga were very low. A significant increase in population numbers has occurred during the last decade. In recent Swaziland surveys it was abundant in suitable swift flowing habitats.			
Population Trend:	? Unknown			
Habitat and Ecology				
Habitat and ecology:	Typically found in pools below fast flowing waters, juveniles often in very shallow water running over sand or gravel bars.			
Systems:	Freshwater			
Threats				
Major threats:	Flow regulation and water pollution in parts of the Limpopo River have significantly reduced the species range. In Zimbabwe regulation of flows by dams, sedimentation of habitats, pollution from agriculture and alien fishes (*Micropterus* spp.) have probably effected its decline.			
Conservation Actions				
Conservation actions:	River health programmes and in-stream flow requirement assessments are in operation in most areas in South Africa.			

3.2.2.2 Historic distribution data.

3.2.2.2.1 Methodology

Historical distribution data was collected from academic institutions, museums and conservation organizations as well as from other literature sources. The initial goal was to determine the coordinates for the collection sites from the historical data. Where these coordinates were not available the location of the site in relation to farms, dams, roads or other recognizable features were used to allocate coordinates to a collection site. In some cases expert mapping was used to

create a point feature based on the knowledge of a relevant expert. Point-data features were created in ArcGIS 9.2 (ESRI, 2006) where each point represented a historical collection locality. Polygons were created to highlight these rivers as historic distribution areas. The secondary goal was to collect other relevant information that is attached to each historical collection site. This included habitat, water quality, associated fish species, invertebrates present, pollution, predators, number of fish found, number of fish sampled, etc. This data was to be attached to the point data feature in ArcGIS in the form of an attribute table (ESRI, 2006). A distribution map was created in ArcGIS and then exported in for use in the report (Figure 3.2.1).

3.2.2.2.2 Results

The individual data sources are discussed below and summarised in table 3.2.2. The map of the historic distribution drawn from the collected data is shown in figure 3.2.1. No coordinates or farm names could be found for the samples that were taken from relic populations in the Lephalala, Pienaars and Hennops rivers.

a) Data from the South African Institute for Aquatic Biodiversity (SAIAB)

This was the main data source (SAIAB) whose dataset also included data of specimens held in the Albany Museum, Grahamstown. The dataset included one shape file of all *O. peringueyi* as well as a shape file with the *O. zambezense* sample sites. One data point near St Lucia was removed after discussions with SAIAB and was considered a misplacement of species or coordinate data.

b) Additional data

Four data points were included from Weeks *et al.* (1996) and sixteen from the data of Russell (1997). The South African National Parks Board data added a total of twenty data points to the data set and consisted mainly of data sampled by Deacon and Pienaar as well as River Health Programme (RHP) site monitoring data (Deacon, *pers com*.[1]) Other inputs consisted of: a) Fifteen data points which originated from the data base of Limpopo Province Nature Conservation (Angliss, *pers com*.[2]), b) Seventeen data points from the data base of Mpumalanga Parks Board (Engelbrecht, *pers com*.[3], c) Ten data points from Ezemvelo KZN Wildlife (Rivers-Moore, *pers com*.[4]) and d) Thirteen data points that were mainly from the upper Luvuvhu River system (Fouché, *pers com*.[5]).

[1] Dr. Andrew Deacon – South African National Parks, Skukuza.
[2] Mr. Mick Angliss - Limpopo Dept of Economic Development Environment and Tourism, Polokwane
[3] Dr. Johan Engelbrecht – Mpumalanga Parks and Tourism Agency, Lydenburg
[4] Dr. Nick Rivers-Moore – Ezemvelo KwaZulu-Natal Wildlife, Pietermaritzburg
[5] Dr. Paul Fouché – University of Venda, Thohoyandou

c) Expert mapping data.

Where necessary expert mapping, rather than actual data, was used to construct the data points with the help of experienced regional experts. In this way twenty two data points were constructed for sites in Mpumalanga (Engelbrecht, pers com.[3]).

d) Historic Transvaal Provincial Administration (TPA) data.

The twenty-three data points obtained from the TPA data set were originally in quarter degree squares and had to be converted. This was done by overlaying a 1:250 000 topographical map over the quarter degree squares layer. The data point was then created by linking the quarter degree squares to the locality name. Although not precise, the method does give a reasonable estimate as to where the collection site was positioned.

Although an attempt was made to collect additional information attached to each historical collection site most of the data sources however did not have additional information. This was disappointing as this was data that could have assisted in determining temporal changes in habitat and water quality.

Table 3.2.2: Sources or *Opsaridium peringueyi* historic distribution data, format of data received and name of data feature in map.

SOURCE NR.	SOURCE	FORMAT	DATA FEATURE IN MAP
1	South African Institute for Aquatic Biodiversity	GIS Shapefile	SAIAB Data *O peringueyi*
2	Russell, 1997	Literature	*Opsaridium* additional data
3	Weeks *et al.,* 1996	Literature	*Opsaridium* additional data
4	SA National Parks	Excel data	*Opsaridium* additional data
5	Limpopo Department of Nature Conservation	Excel data	*Opsaridium* additional data
6	Mpumalanga Parks Board	a) Excel data b) Expert mapping	a) *Opsaridium* additional data b) Expert mapping data
7	University of Venda	Excel data	*Opsaridium* additional data
8	Transvaal Provincial Administration	Excel data	*Opsaridium* TPA historic data
9	Ezemvelo KZN Wildlife	Excel data	*Opsaridium* additional data

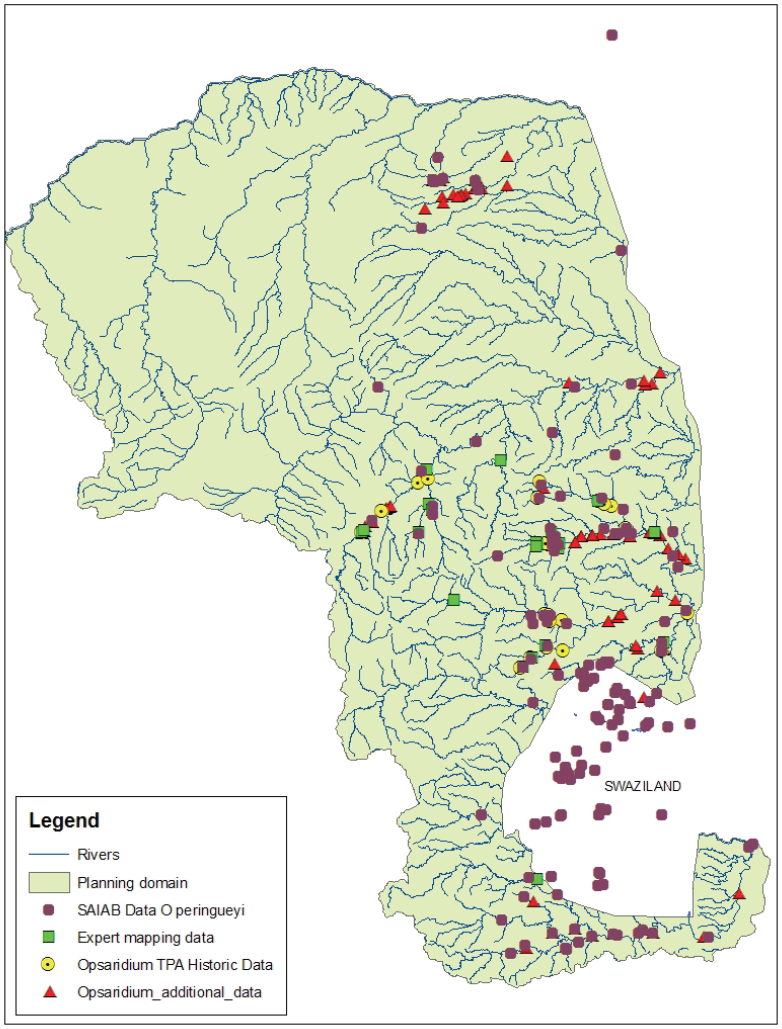


Figure 3.2.1: The historic distribution of *Opsaridium peringueyi* in South Africa and Swaziland.

3.2.2.3 Sampling of historic distribution sites

In an attempt to determine the current distribution of *O. peringueyi* it was decided to sample the historic sites. Where several sample sites were in close proximity in the same river reach, a representative site was selected and only this site was sampled. Where original sites could not be located a representative site in the general area, based on the original coordinates, was selected and sampled. The method of collection depended on the biotope type and the biotope specific sampling protocol suggested by Kleynhans (2008) was used. In fast-deep and fast-shallow biotopes the fish were electro-narcotized, using a 220V AC generator, and collected with scoop nets. In the slow-deep and slow–shallow biotopes, that were clear of snags, a small seine net (15 m long X 1,5 m deep with 10 mm stretched mesh) was used. A pole-seine (2,5 m long X 1,5 m deep with 10 mm stretched

mesh) was used in the small pools, backwaters and in particular where sampling had to be done under and amongst vegetation. No time constraint was exercised and the biotopes were thoroughly sampled. The number of specimens of each species collected per biotope was recorded on a field form.

As a result a total of 64 sites (Table 3.2.3) were sampled and *O. peringueyi* was present at fourteen of these sites and the resultant map is shown in figure 3.2.2.

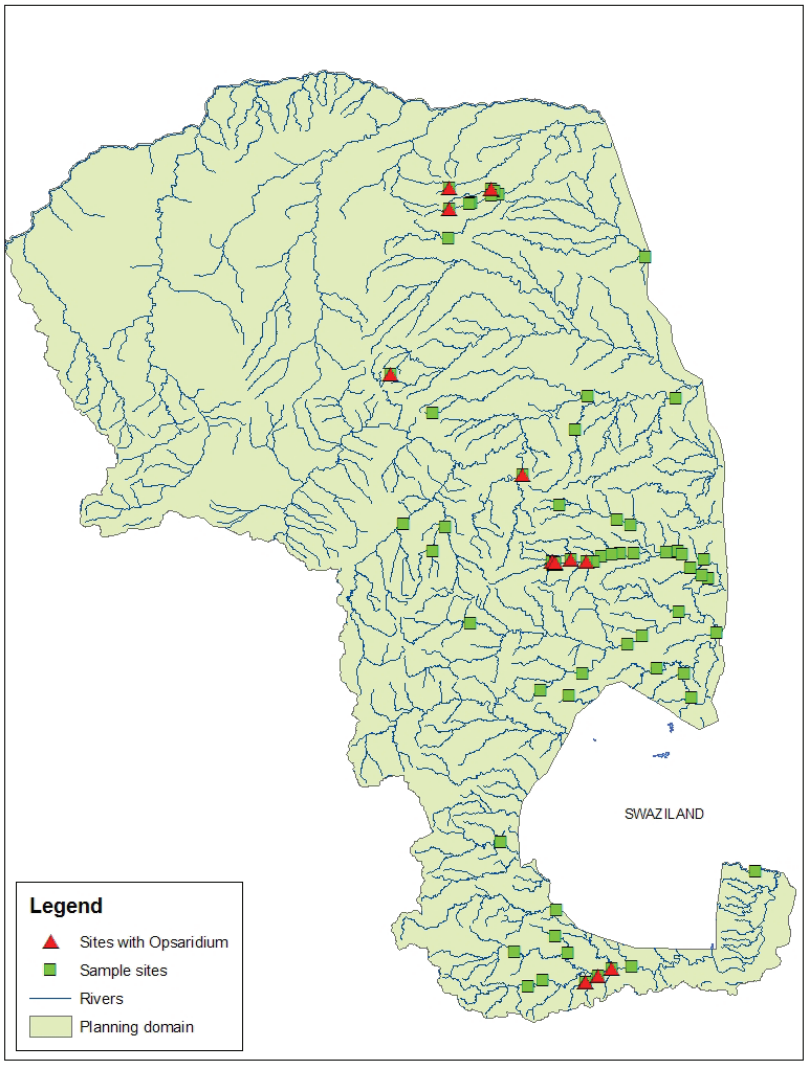


Figure 3.2.2: Location of the *Opsaridium peringueyi* sampling sites surveyed.

Table 3.2.3: The sample sites that were sampled during the project

SITE NR	SITE NAME	RIVER	TRIBUTRY	FIELD WORKERS	LAT	LON	DATE
OPS1	Bivane/Nsingana confluence (PON2)	Pongola	Bivaan	J.Venter/W. Vlok	-27.4948	30.95156	25/08/2005
OPS2	Pongola Ithala Wilderness area (PON8)	Pongola		J.Venter/W. Vlok	-27.4268	31.35878	23/08/2005
OPS3	Bivaan at Natal spa (PON11)	Pongola	Bivaan	J.Venter/W. Vlok	-27.5293	30.86142	24/08/2005
OPS4	Ithala River (PON12)	Pongola	Ithala	J.Venter/W. Vlok	-27.5034	31.20072	22/08/2005
OPS5	Pongola Ithala picnic site (PON13)	Pongola		J.Venter/W. Vlok	-27.4663	31.27756	23/08/2005
OPS6	Pongola/Wit confluence (PON15)	Pongola		J.Venter/W. Vlok	-27.3305	31.09947	25/08/2005
OPS7	Lower Sabie Causeway (KNP1)	Sabie		PFouche/ JVenter	-25.1221	31.92506	19/6/2006
OPS8	Lubyelubye (KNP2)	Sabie		PFouche/ JVenter	-25.1	31.88575	19/6/2006
OPS9	Sabie Ifr (KNP3)	Sabie		PFouche/ JVenter	-25.0581	31.81828	20/6/2006
OPS10	Leapard Site A(KNP4a)	Sabie		PFouche/ JVenter	-24.9621	31.73711	20/6/2006
OPS11	Leapard Site B (KNP4b)	Sabie		PFouche/ JVenter	-24.9611	31.74308	21/6/2006
OPS12	Nwantindlophu(KNP5)	Sabie		PFouche/ JVenter	-24.9782	31.76967	21/6/2006
OPS13	Sekurakwane(KNP6)	Sabie		PFouche/ JVenter	-24.9888	31.29175	22/6/2006
OPS14	Kruger gate gauging weir(KNP7)	Sabie		PFouche/ JVenter	-24.9722	31.48861	22/6/2006
OPS15	Sabie fish site (KNP 8)	Sabie		PFouche/ WVlok/ Jventer	-24.9651	31.67822	21/8/2006
OPS16	Lisbon (KNP9)	Sabie		PFouche/ WVlok/ Jventer	-24.97	31.40639	21/8/2006
OPS17	Mtshawa (KNP10)	Sabie		PFouche/ WVlok/ Jventer	-24.9771	31.35781	22/8/2006
OPS18	Sabiehoek Pumpstation (KNP11)	Sabie		PFouche/ WVlok/ Jventer	-25.0186	31.24922	22/8/2006
OPS19	Malelane bridge (KNP12)	Crocodile		PFouche/ W Vlok/JVenter	-25.4611	31.5345	23/8/2006
OPS20	Mvoveni (KNP13)	Crocodile		PFouche/ W Vlok/JVenter	-25.5061	31.44639	23/8/2006
OPS21	Mbyanti (KNP15)	Crocodile		PFouche/ W Vlok/JVenter	-25.3156	31.74928	24/8/2006
OPS22	Weir down-stream of Confluence LUV1	Luvuvhu	Mutshindudi	P. Fouche/ W.Vlok	-22.8541	30.68637	1/8/2006
OPS23	Mphaphuli Cycad Reserve LUV 2	Luvuvhu	Mukase	P. Fouche/ W.Vlok	-22.8175	30.6485	1/8/2006
OPS24	Bridge pump LUV3	Luvuvhu	Mbwedi	P. Fouche/ W.Vlok	-22.8357	30.66143	1/8/2006
OPS25	Malavuwe Bridge LUV 4	Luvuvhu	Mutshindudi	P. Fouche/ W.Vlok	-22.8586	30.64408	2/8/2006
OPS26	Tar Bridge LUV 5	Luvuvhu	Tshinane	P. Fouche/ W.Vlok	-22.9006	30.52702	2/8/2006
OPS27	Mutale/ Tshirovha Confluence LUV 6	Luvuvhu	Mutale	P. Fouche/ W.Vlok	-22.8152	30.39602	3/8/2006
OPS28	Hydro scheme LUV 8	Luvuvhu	Mutshindudi	P. Fouche/ W.Vlok	-22.9391	30.40098	3/8/2006
OPS29	Balule KNP16	Olifants		P. Fouche/ W.Vlok	-24.0538	31.73048	13/09/2006
OPS30	Old Mamba KNP17	Olifants		P. Fouche/ W.Vlok	-24.0419	31.21197	14/09/2006
OPS31	Matumi MAC1	Sabie	Mac Mac	PFouche/ JVenter	-25.0234	31.00194	20/03/2007
OPS32	Sabane SAB1	Sabie	Sabane	PFouche/ JVenter	-25.0329	31.02144	20/03/2007
OPS33	Confluence CON1	Sabie		PFouche/ JVenter	-25.0291	31.02572	21/03/2007
OPS34	Hoxane	Sabie		PFouche/ JVenter	-25.0193	31.20503	21/03/2007
OPS 35	Sabie birding site	Sabie	Mac Mac	PFouche/ JVenter	-25.0061	30.89926	22/05/2007
OPS36	Marite	Sabie	Marite	PFouche/ JVenter	-25.008	31.11589	22/05/2007
OPS37	Taylors crossing'	Sabie	Sand	PFouche/ W.Vlok/ JVenter	-24.8046	31.46853	23/05/2007

SITE NR	SITE NAME	RIVER	TRIBUTRY	FIELD WORKERS	LAT	LON	DATE
OPS38	Lodge	Sabie	Sand	PFouche/ W.Vlok/ JVenter	-24.7708	31.38547	23/05/2007
OPS39	Klaserie	Klaserie	Klaserie	PFouche/ W.Vlok/ JVenter	-24.2423	31.13878	24/05/2007
OPS40	Groot Sand	Sabie	Groot Sand	P. Fouche/ W.Vlok	-24.6881	31.04956	25/05/2007
OPS41	Tshino	Luvuvhu	Luvuvhu	P. Fouche/ W.Vlok	-23.1133	30.38975	19/06/2007
OPS42	Causeway	Shingwedzi	Dzombo	P. Fouche/ W.Vlok	-23.2218	31.5518	20/06/2007
OPS43	Sterkstroom	Spekboom		P. Fouche/ W.Vlok	-24.8153	30.37628	27/09/2007
OPS44	Spitskop	Steelpoort		P. Fouche/ W.Vlok	-24.7979	30.12399	25/09/2007
OPS45	Waterval	Watervals		P. Fouche/ W.Vlok	-24.9568	30.30186	26/09/2007
OPS46	Boshalte	Crocodile	Sterkspruit	P. Fouche/ W.Vlok	-25.3834	30.52492	25/09/2007
OPS47	Komati/Croc Confluence(Kom1)	Komati		PFouche/ W.Vlok/ JVenter	-25.4386	31.97333	25/10/2007
OPS48	Tonga(Kom2)	Komati		PFouche/ W.Vlok/ JVenter	-25.6805	31.78303	25/10/2007
OPS49	Mabondweni(Kom3)	Komati	Mlumati	PFouche/ W.Vlok/ JVenter	-25.6497	31.62214	26/10/2007
OPS50	FigTree weir(Kom4)	Komati		PFouche/ W.Vlok/ JVenter	-25.822	31.82608	26/10/2007
OPS51	Tshivhulani	Luvuvhu		P.Fouche	-22.9055	30.51867	09/09/2007
OPS52	Amsterdam	Usuthu	Ngwempisi	P.Fouche/ J.Venter	-26.6776	30.70214	05/05/2008
OPS53	Alma	Pongola	Assegaai	P.Fouche/ J.Venter	-27.0778	31.02956	05/05/2008
OPS54	CTC	Pongola	Pongola	P.Fouche/ J.Venter	-27.324	30.78336	06/05/2008
OPS55	Witrivier	Pongola	Witrivier	P.Fouche/ J.Venter	-27.2331	31.02492	06/05/2008
OPS56	Impala Weir	Pongola		P.Fouche/ J.Venter	-27.4133	31.47444	07/05/2008
OPS57	Ndumu Redcliffs	Usuthu	Usuthu	P.Fouche/ J.Venter	-26.8486	32.20528	08/05/2008
OPS58	Ithala River (Recent)	Pongola	Ithala	P.Fouche/ J.Venter	-27.5034	31.20072	09/05/2008
OPS59	Highlands farm	Komati	Nlomati	P. Fouche/ W.Vlok	-25.8145	31.10564	09/06/2008
OPS60	Aerodrome	Crocodile	Queens	P. Fouche/ W.Vlok	-25.7819	30.93673	09/06/2008
OPS61	Kaap	Crocodile	Kaap	P. Fouche/ W.Vlok	-25.6811	31.18178	09/06/2008
OPS62	Swadini	Olifants	Blyde	P. Fouche/ W.Vlok	-24.5051	30.83258	10/06/2008
OPS63	Appel	Letaba	Groot Letaba	P. Fouche/ W.Vlok	-23.9151	30.05195	24/06/2008
OPS64	Lekgalametsi	Olifants	Selati	P. Fouche/ W.Vlok	-24.1438	30.30131	24/06/2008

3.2.3 CHANGE OF *O. PERINGUEYI* DISTRIBUTION OVER TIME

The historic distribution data and new data obtained from sampling for this project was categorized into four groups a) A pre 1990 group which includes all sites where *O. peringueyi* was found before 1990, b) A between 1991 and 2000 group which includes all sites where *O. peringueyi* was found after 1990 but before 2000, c) A post 2000 group which includes all the sites where *O. peringueyi* was found post 2000 excluding the sites sampled during the project and d) the current distribution group which includes sites where *O. peringueyi* was found during course of the project.

The results (Figure 3.2.3) show a general decline in the number of sites where *O. peringueyi* were found. This should be interpreted with caution as it could be a reflection of sampling effort or other factors that influence sampling success. In general the results show that *O. peringueyi* current distribution range is limited to sites which are located at the transition zone between mountain stream and middle reach.

There is however evidence that *O. peringueyi* have disappeared from the lower reaches of the Olifants River system as no specimens have been collected after the pre-1990 period. This is in particular applicable to the sites in Kruger National Park, where sampling effort was still high during the period between 1991 and 2000.

3.2.3.1 A historic and new distribution map for O. peringueyi distribution in South Africa

The historic and current distribution was mapped using tertiary catchments as spatial units (Figure 3.2.4). Two point layers were created using the *O. peringueyi* distribution point data sets namely a pre-2000 data and post-2000 data set. This resulted one layer showing the historic distribution range and one layer displaying the current distribution of *O. peringueyi.* Subsequently the difference in area size was calculated to determine change over time and the results show that the distribution range of *O. peringueyi* has shrunk with 59%.

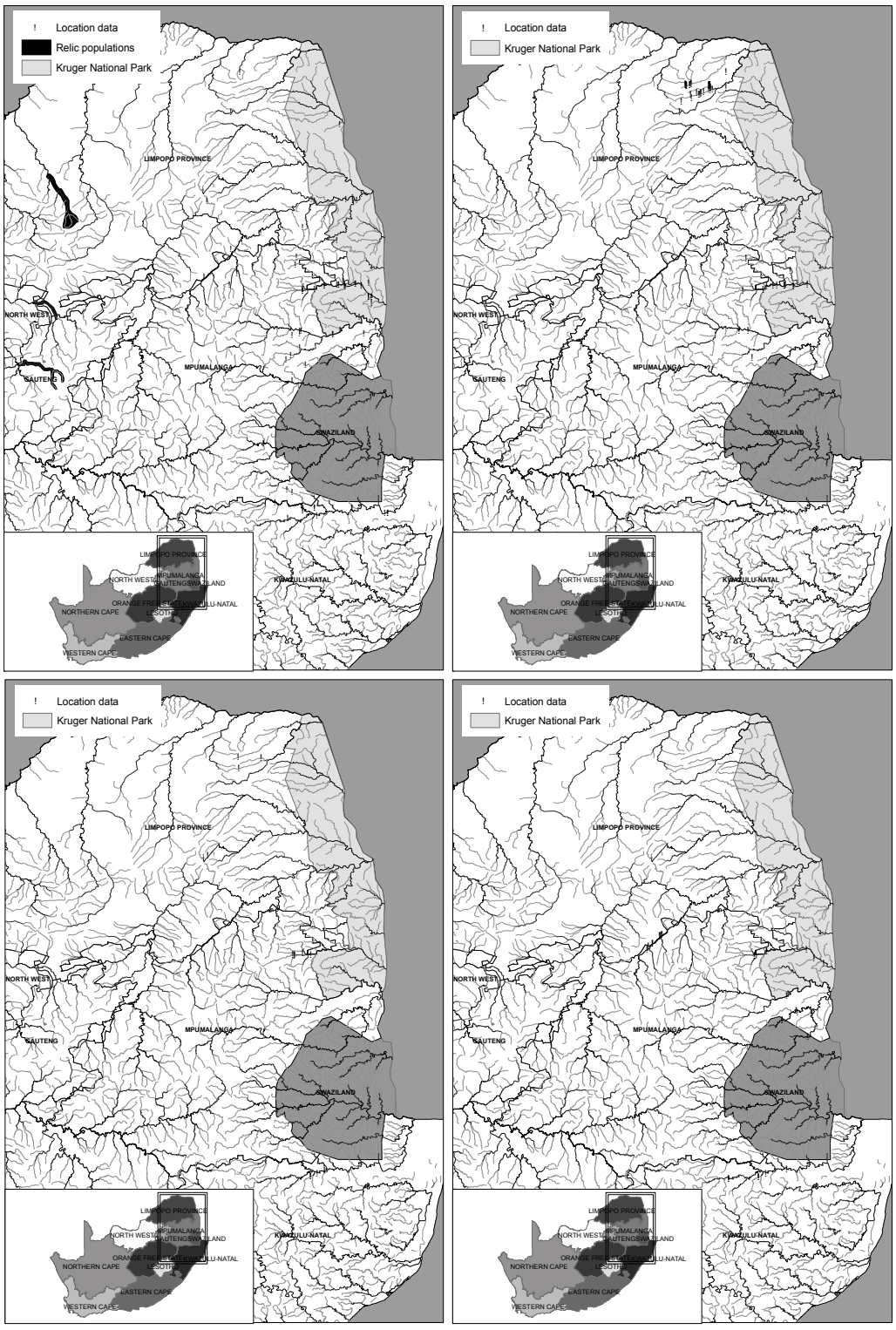


Figure 3.2.3: The sample sites where *Opsaridium peringueyi* has been found over time categorized into time periods: a) The "pre 1990" period which includes all sites where *O. peringueyi* was found before 1990; b) The "between 1990 and 2000" period where *O. peringueyi* was found during 1990 but before 2000; c) The "post 2000" period which includes all the sites where *O. peringueyi* was found after 2000 excluding the sites sampled during the project; d) The "current" distribution which includes sites where *O. peringueyi* was found during the project.

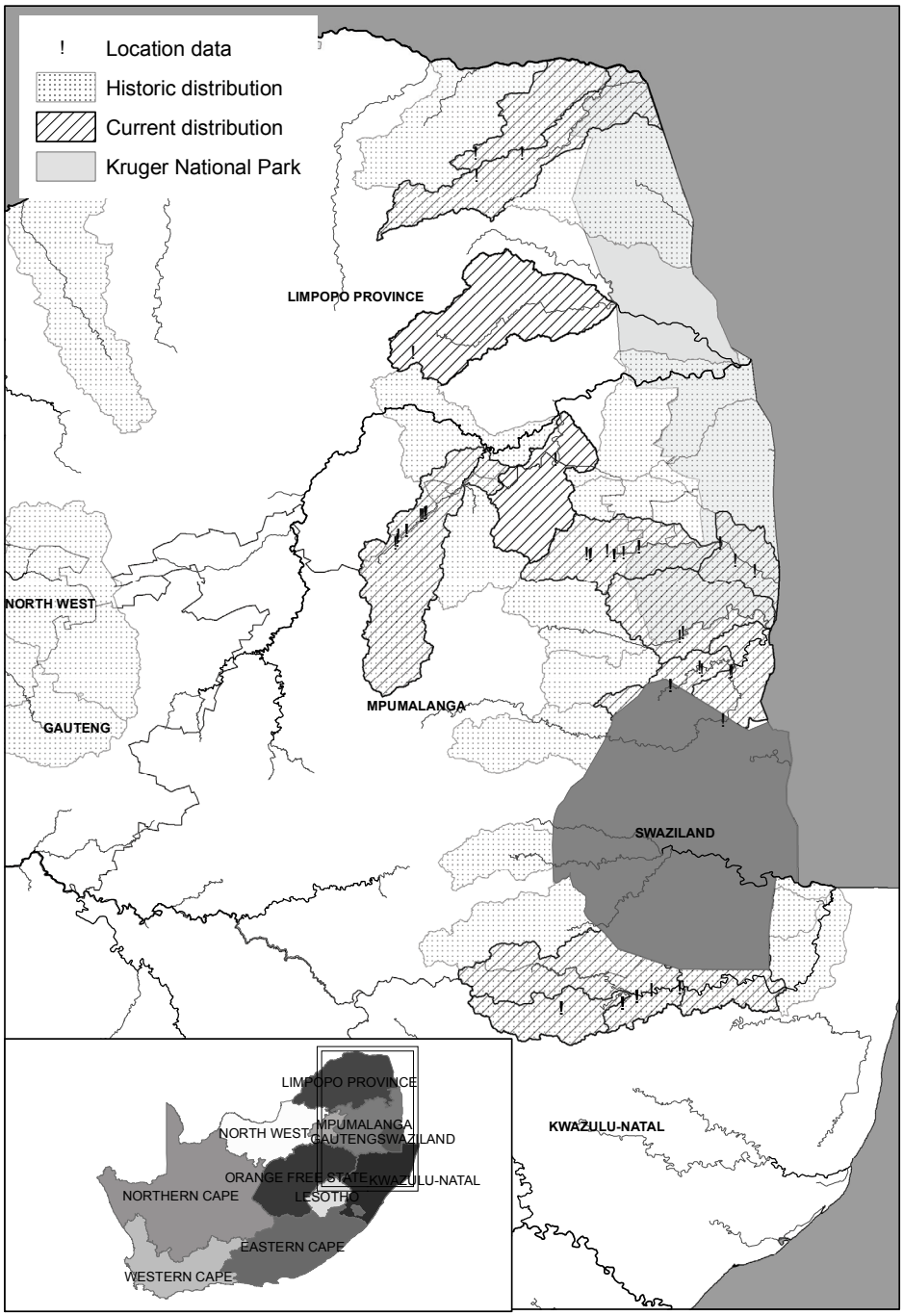


Figure 3.2.4: The historic distribution and current distribution of *Opsaridium peringueyi* in South Africa was mapped using tertiary catchments as spatial units.

3.2.4 CONSERVATION PLANNING.

In order to determine the best practice for the conservation planning a workshop was conducted. This workshop took place during the Biodiversity Forum meeting of March 2008 arranged by the South African National Biodiversity Institute (SANBI) as most of the major role players were already part of the meeting. The workshop was attended by members of the team, regional and provincial freshwater experts and an aquatic conservation planning expert of the CSIR. The main conclusion of the workshop was that a formal spatial conservation planning process should not be used but rather that a focused planning exercise around the few areas where significant populations of *O. peringueyi* are still present should be applied

It was also concluded that:

- The threat drivers in the system should be identified, that the drivers of decline be established and that suggestions for mitigation be made.
- Priority areas be identified.
- Fish sanctuaries be identified and established.
- Special features (e.g. wetlands) be identified and aligned to the priority/sanctuary areas.
- The “species management plan” should align with existing conservation plans and initiatives.

During the Biodiversity Management Plan for Species (BMP-S) workshop held in October 2008 at Skukuza these conclusions were discussed. As a result priority areas, fish sanctuaries and special features were identified and an attempt was made to align these with priority conservation areas within the provincial biodiversity conservation plans.

3.2.5 CONCLUSION

In general it appears that the distribution of *O. peringueyi* had changed over time. A new distribution map was created that show that *O. peringueyi* distribution range has shrunk with more than 50% (based on tertiary catchment area size). Inadequate data on water quality and river conditions associated with the historic data makes it difficult to quantify actual change of habitat quality over time. During the BMP-S workshop a focused planning exercise were completed where priority conservation areas for *O. peringueyi* were identified.

Chapter 3: Population genetics

P Grobler

3.3.1 RATIONALE FOR A GENETIC SURVEY OF THE DISTRIBUTION OF GENETIC DIVERSITY IN *OPSARIDIUM PERINGUEYI* IN SOUTHERN AFRICA

The objectives for a detailed genetic analysis of *Opsaridium peringueyi* were:

- To screen for possible genetic structure within *O. peringueyi*, i.e. to investigate whether different genetic variants exist in geographically isolated populations. This work is in line with the Evolutionary Significant Unit (ESU) concept, a concept now widely recognized in international and South African conservation programmes for all taxa (Waples, 1991; 1995; Moritz, 1994; 2002; Nielsen, 1995; Roe and Lydeard, 1998; Frankham *et al*., 2002).
- To determine levels of genetic diversity in isolated populations. This data will serve to: (i) identify the best possible source populations for any future breeding and augmentation programmes, since populations with the best levels of genetic diversity can then be selected for breeding; and (ii) reduced levels of genetic diversity can assist to identify populations that has experiences genetic bottlenecks as a result of human influences.
- To elucidate overall systematic relationships within the genus *Opsaridium*.
- To use trends and patterns observed in *O. peringueyi* populations to formulate wider strategies for conservation of other southern African freshwater fish species.

3.3.2 GENETIC ANALYSIS

3.3.2.1 Samples used

The following samples were available for optimization and analysis:

- Muscle and fin clippings of *O. peringueyi* from a range of localities sampled within the project.
- Specimens from Swaziland, Okavango and the KZN additional localities were obtained from SAIAB.

3.3.2.2 DNA isolation

DNA was isolated from *O. peringueyi* samples using the Roche Applied Science *High Pure Template Preparation Kit*. Skeletal muscle (approximately 40 mg per individual) or fin clips were used as a

source for DNA extraction. The extraction technique was based on the standard protocol for DNA isolation from mammalian tissue, but with extended incubation time (overnight rather than 1 hour). The quality and quantity of DNA isolated was verified using a NanoDrop ND-1000 spectrophotometer (Figure 3.3.1). Yields of 50-140 ng/µl were achieved (average = 82.51ng/µl), which is highly suitable for PCR (sequencing) procedures. Concentrations of all DNA samples were standardized to 25ng/µl prior to PCR reactions.

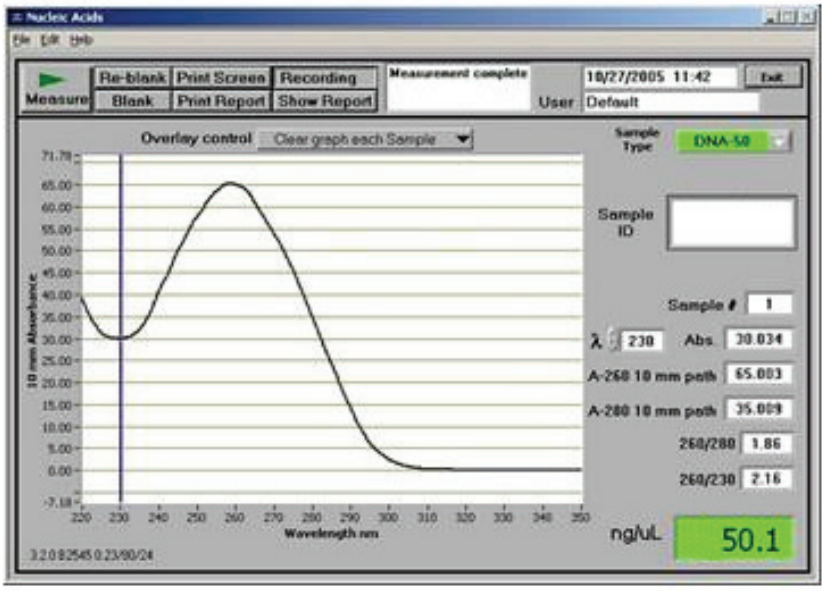


Figure 3.3.1: Typical output after quantification of DNA yield using the NanoDrop ND-1000 spectrophotometer (yield = 50.1 ng/µl in this instance).

3.3.2.3 Selection of a phylogenetically useful gene

Genetic analysis of *O. peringueyi* initially concentrated on the identification of a suitable marker for phylogenetic analysis, and optimization of suitable techniques, in preparation for routine screening of samples.

The ND complex

Miya *et al*. (2005) compared 15 mtDNA genes to determine which is the most suitable for phylogenetic studies. The criteria used were ease of alignment, informative variation, sufficient length, and easy of amplification and sequencing. From a panel of candidate gene areas, the ND4 and ND5 genes were unequivocally chosen as the most suitable for phylogenetic studies on species of the Cypriniformes. (*ND-4* and *ND-5* codes for the 4th and 5th subunits of NADH dehydrogenase). These authors then developed 15 primers for amplification of fractions of the *ND-4/ ND-5* area. The finalized primers contain several degenerate sites to allow for cross species amplification within the

Cypriniformes. *Opsaridium* is not among the seven genera in the panel of genera used by Miya *et al.* (2005), but general experience with cross species application of PCR primers suggested that some of the *ND4/ND5* flanking regions may be conserved in *Opsaridium*. In this regard, Miya *et al*. (2005) suggested that these primers could be applied to the *Cypriniformes Tree of Life Project*, a project which aims to generate markers usable over as many as 1000 targeted species.

Amplification of ND-4/ND-5 in Opsaridium:

Six primers were selected to test for successful amplification of *ND-4 / ND-5* in *O. peringueyi*, i.e. to investigate the degree of conservation of flanking regions in *O.peringueyi* compared to other members of the Cypriniformes. The primers are L10681, L12328, L13559, H11618, H13393 and H14473. Suitable combinations of these six primers as forward and reverse primers can potentially amplify all of the length of the *ND-4/ND-5* genes (Figure 3.3.2).

Figure 3.3.2: Areas of the *ND-4/ND-5* genes potentially amplified by the six primers selected for cross application in *Opsaridium*. Figure adapted from Miya *et al*. (2005).

Consistent amplification was achieved using primers L12328 and H13393 (boxed in Figure 3.3.2). This primer pair flanks a region of the *ND-5* region of the *ND* gene complex. The sequences used for these primers are: L12328 5'-AAC TCT TGG TGC AAM TCC AAG-3'; and H13393 5'- CCT ATT TTK CGG ATG TCT TGY TC-3'. The symbols M, K and Y denote degenerate positions using the IUPAC code.

Following optimization the following reaction mixture were used: 2 µl DNA, using isolated DNA standardized at 25 mg / µl; 1x buffer; 2.5 mM $MgCl_2$; 0.4 mM dNTP mix; 1.5U Taq Polymerase; 1 µl of each primer (with primers diluted to 10 µM); and with ddH_2O added to a total reaction volume of 25 µl. Reaction conditions consisted of an initial denaturation phase of 8 minutes at 94°C, followed by 35 cycles each consisting of: 94°c for 40 seconds, 55°C for 1 minute (after experimenting with temperatures of 50-60°C), and 72°C for 40 seconds. This was followed by a final 72°C for 10 minutes.

Using the primer pair L12328/H13393, reaction mixture and reactions conditions described above, consistent amplification of a 1000bp region of the *ND* gene complex, representing *ND-5*, was achieved in *O. peringueyi* (Figure 3.3.3).

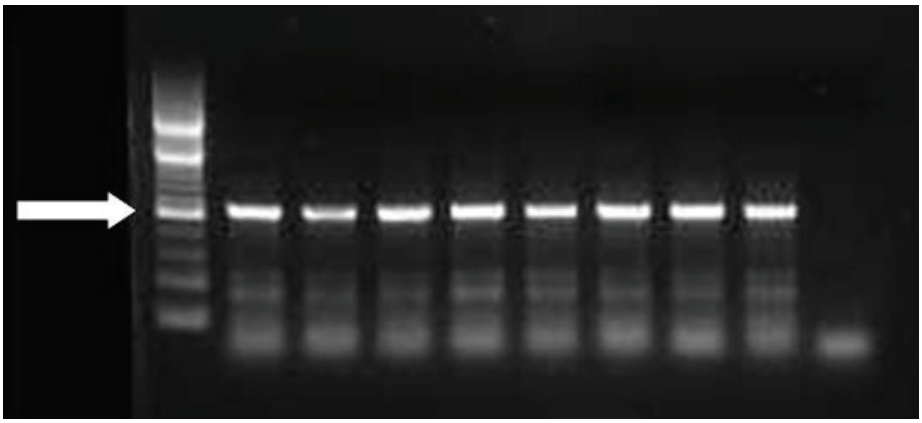


Figure 3.3.3: Amplification of the *ND-4* mtDNA gene region in *Opsaridium peringueyi*. Lane 1 = size standard; lanes 2-9 = amplified *ND-5* in eight samples of *O. peringueyi*; lane 10 = control PCR reaction with no DNA, indicating no contamination. Arrow indicates the 1000bp band in the size standard.

Actual nucleotide sequences of these PCR products were determined using a standard Big Dye © based protocol, followed by analyses on an automated sequencer. PCR products first were first purified using a Qiagen DNA purification kit to remove any remaining primers. The cleaned PCR products then were sequenced with a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems), following the standard reaction mixtures and conditions supplied by the manufacturers.

Results were unfortunately inconclusive. Despite numerous attempts at full optimization, it was not possible to obtain clear sequences for *O. peringueyi* (Figure 3.3.4). Troubleshooting involved the following: (i) continued experimentation with annealing temperatures (50-60^{o}C); (ii) numerous repetitions of PCR and sequencing reactions (average 2-3 runs per week over several months); and (iii) having part or all of the procedure done in another laboratory at the University of Johannesburg. The results were consistent: similar, non-scorable sequencing products.

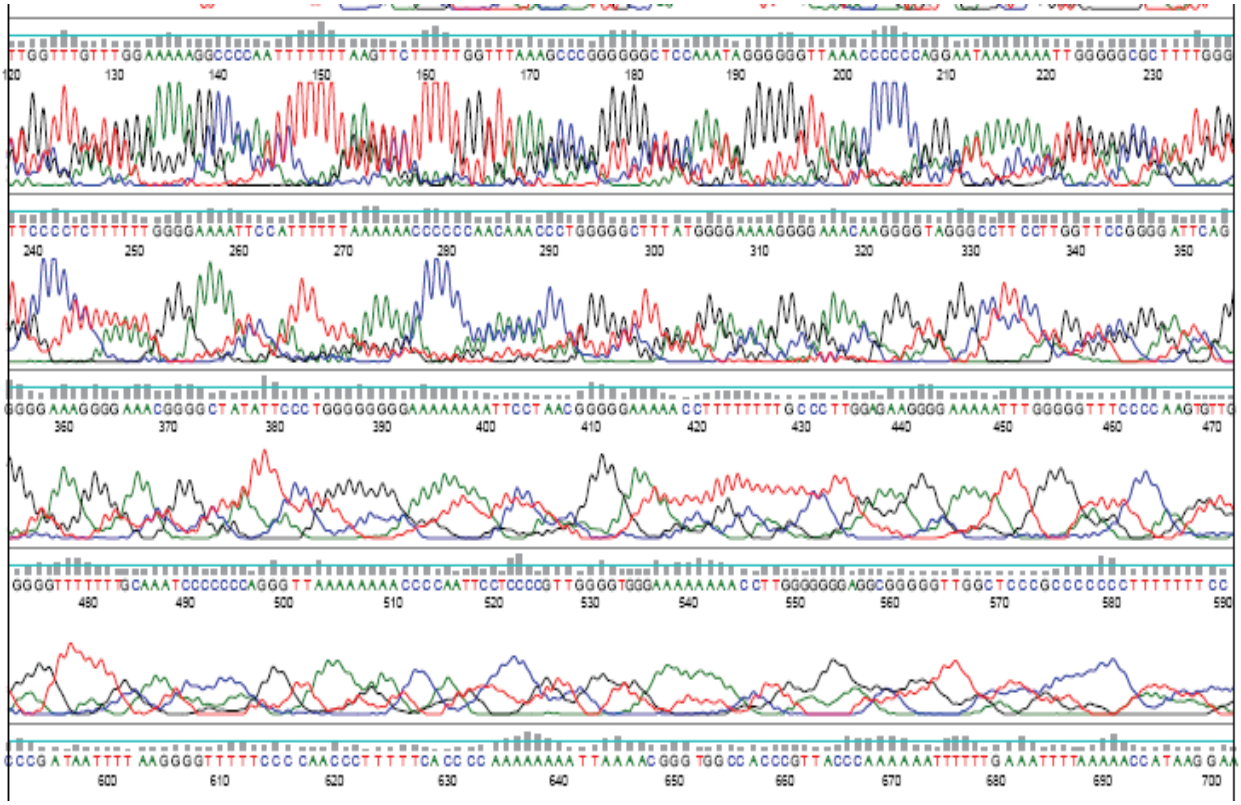


Figure 3.3.4. Persistent problems with sequencing products of *ND* in *Opsaridium peringueyi*. The sequences could not be cleared of artefacts, despite numerous attempts.

The mitochondrial DNA control region

Following the lack of success with the ND complex in *O. peringueyi*, it was decided to switch to another gene. After an analysis of suitable candidate genes, the mtDNA control region was selected. This gene region was previously used by Bloomer and Impson (2000) on an indigenous fish species (*Pseudobarbus*). As indicated by these authors, this gene region is often the most variable part of the mtDNA genome (Brown, 1985), and it has been used on a wide range of freshwater fish species, including Cichlids (Bowers *et al*., 1994), pupfish (Duvernall & Turner, 1998), brown trout (Giuffra *et al*., 1994) and many others.

Amplification of the mtDNA control region in Opsaridium

Following Bloomer and Impson (2000) the vertebrate primers L15925 (5'-TACACTGGTCTTGTAAACC-3') and H16499 (5'-CTTGAAGTAGGAACCAGAT-3') were used in an attempt to amplify the mtDNA control region in *O. peringueyi*. Reaction mixtures and conditions followed the aforementioned authors.

As with the ND gene, results were initially positive and amplification of the genes were definitely successful. However, despite numerous attempts to optimize conditions, clear sequences suitable for interpretation and statistical analysis could not be achieved after sequencing reactions.

Possible reasons for the failure to obtain genetic results for Opsaridium

There are a number of possible reasons why genetic results were not obtained during this study. The most plausible explanation is however as follows: primers and markers designed for other species were used throughout this investigation. While it is unlikely that *O. peringueyi* is so different from these species that the primers cannot be made to work, considerable optimization (and thus time) may be required before successful sequencing is ultimately achieved. This is the result of differences in the flanking regions (where primers are to anneal) in *O. peringueyi* compared to the species for which the primers were developed. The turnover of students who worked on the project was also perhaps too high but this was unavoidable, with some students being in the department for one year only, and others finding alternative employment and thus deserting the project leading to a lack of continuity.

3.3.3 MANAGEMENT STRATEGIES FOR *O. PERINGYUEI*

Recovery plans for many imperiled aquatic species often include relocation of individuals from demographically robust populations to demographically imperiled ones (Grobler *et al*., 2006). The impact of evolutionary and ecological factors on such relocation programs must be carefully scrutinized (Villella *et al*., 1998). Genetic structuring of geographically separated populations may be linked to significant adaptive differentiation. If so, relocation without regard to locally adapted genetic factors may result in reduced fitness in progeny. In addition, re-stocking programmes should incorporate the requirement for sufficient genetic diversity in founder populations. The need for genetic diversity is well documented, and relocated populations with low diversity may have a reduced ability to thrive, or survive stochastic perturbations (Villella *et al.,* 1998).

It is generally expected that there will be a correlation between spatial distribution and degree of differentiation. In other words, populations that are geographically close together should be genetically very similar, whereas populations situated further apart should be genetically more different (Figure 3.3.5. This model, however, assumes an absence of any barriers to gene flow other

than absolute geographic distance. However, the model can be disrupted by processes such as selection and isolation and in reality, it can be expected that the actual pattern of differentiation among population of *O. peringueyi* will show deviations from the idealized model. Such deviations from a simple pattern of isolation-by-distance may be the result of historical fragmentation (geological events involving drainages) or more recent anthropogenic influences (dams and habitat degradation). This makes the model useful in the sense that when spatial distribution and genetic relatedness are compared, and there is an obvious deviation from expectations (Close e.g. proximity coupled with high genetic differentiation – Figure 3.3.5(ii)), it serves as a clear indication that non-random influences are at work which may result in uniqueness in populations.

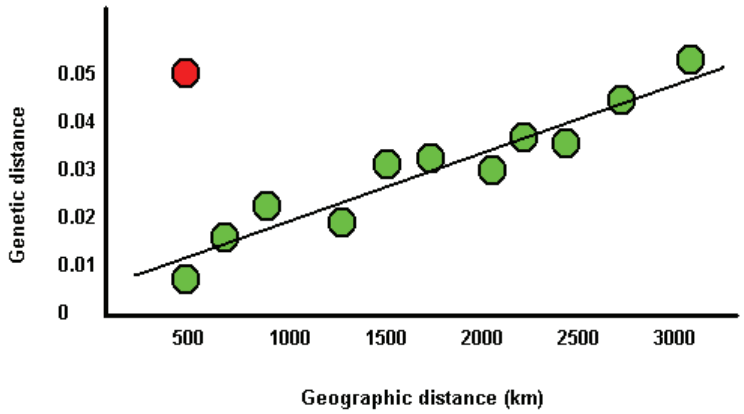


Figure 3.3.5. Isolation by distance. (i) Correlation between geographic and genetics distance (green dots); (ii) a population (red dot) that deviates from the expected model of isolation by distance, resulting from a unique selective influence or a historic event.

It was anticipated that the genetic data generated during the current study would facilitate the identification of populations with unique genetic characteristics. That would have enabled the writers of this report to assign specific a status (level of uniqueness, if any) to each of the populations sampled, in line with the criteria proposed by Moritz (2002) and other authors, or declare that all populations are monomorphic. However, Moritz (2002) also cautioned that molecular criteria impose arbitrary thresholds and categories on an evolutionary process that is in reality based on a continuum of divergence. Ecological data can therefore provide valuable pointers to management guidelines in the absence of molecular data, and even when molecular data is available; these should be backed-up using ecological data. Of particular importance in this regard is the work of Waples (1991) who proposed that populations are ESUs when they are reproductively

isolated from other conspecific units and represent an important component of the evolutionary legacy of the species. To meet the latter criterion, the population must:

- be genetically distinct;
- occupy a unique habitat;
- exhibit unique adaptation to its environment; or
- pose a significant loss to the ecological or genetic diversity of the species if it should become extinct.

It is thus recommend that the available biological, environmental and historical data be scrutinized to assess possible levels of population genetic fragmentation and identify appropriate units for conservation. Most notably, such scrutiny should include investigation of potentially unique life history traits, morphological characteristics, habitat use and historic events, since such multi-faceted data sets can provide much needed insight into evolutionary processes (Mulvey *et al*., 1997; Roe and Lydeard, 1998). If unique conditions are observed for specific populations, it would be wise to restrict artificial gene flow between such populations and random donor populations.

It s that should be noted there can be negative consequences from erroneously designating a population unit as unique when it is not. Such an error could promote gene pool fragmentation and loss through attrition of larger real significant units. Erroneous assignment of populations as ESUs also ignores metapopulation structure and does not adequately consider population viability. Roe & Lydeard (1998) suggested that incorrect application of the ESU concept could hinder rather than aid in the recognition of biodiversity. Nevertheless, it is recommended that the precautionary principle be applied as far as possible in the absence of hard molecular data. It is thus suggested that in the short to medium term, translocations be limited to involve only individuals from the same drainage, and even be limited to individuals from similar areas within drainages (in terms of habitat conditions).

Chapter 4: Habitat selection

PSO Fouché, JA Venter, W Vlok and S Theron

3.4.1 INTRODUCTION

Werner *et al.* (1976) pointed out that the patterns of habitat utilization and habitat partitioning are not known in all groups of organisms and where known the majority of knowledge is about terrestrial organisms. However a number of investigators have investigated habitat preference in freshwater fish and have *inter alia* shown that a) intraspecific differences exist in the spatial distribution of size classes (Werner *et al.*, 1976), b) that species could be segregated on the basis of habitat preference and c) that habitat utilization was so similar in each stream that it was possible to predict fish assemblages (Wikramanayake and Moyle, 1989). Facey and Grossman (1992) was of the opinion that habitat use is velocity related. As far as the cyprinids are concerned, Felley and Hill (1983) showed that seasonal and velocity related differences in habitat preference were evident. According to the literature *Opsaridium peringueyi* mostly occurs below an altitude of 1200 m.a.s.l. which therefore includes the lower escarpment and lowveld of Limpopo, Mpumalanga, Swaziland and KwaZulu-Natal where according to Crass (1964), Gaigher (1973) and Pienaar (1978) it is found in "clean, shallow pools of rivers on sandy or gravel substrates". Schulz (1992) stated that the species occur in water with a depth of between 15 and 50 cm and a temperature of 15-20°C (cited in Skelton 1996). A survey of the literature shows that little detailed knowledge is available of the ecology, which includes the habitat preference and the niche differentiation, of most of the indigenous fish of southern Africa and *O. peringueyi* is no exception. In this study it was hypothesized that *O. peringueyi* has specific habitat preferences which are related to its morphological and flow characteristics of the particular zones of rivers in which it occurs. Therefore the aim of this component of the project was to investigate the large scale habitat preference of *O. peringueyi.*

3.4.2 METHODS

The sixty four sites identified in chapter 2 of this section of the report (Figure 3.2.2) formed the sites that were surveyed in this component of the study. Throughout this chapter two aspects should be borne in mind namely: a) That each site was only surveyed once during the project and b) the level of biotope characterization.

3.4.2.1 Habitat preference

3.4.2.1.1 General habitat characteristics

At each site the pH, dissolved oxygen, electrical conductivity, total dissolved solids and temperature was determined with handheld Eutech meters. In addition a water sample was collected for analyses.

3.4.2.1.2 Habitat description and mapping of the biotopes

Each site was photographed and the possible habitats, based on the criteria listed in table 3.4.1, were identified and demarcated by judging the velocities and depths. The actual depths and velocities in each habitat were then measured and the habitat was then subdivided into units or microhabitats that are referred to as *"biotopes"* in this study. This decision to use this term instead of micro-habitat was based on the description of Rowntree and Wadeson (1998) that used the term biotope for "spatially distinct instream flow environments determined by temporally variable hydraulic and substrate characteristics" and that of Davies and Day (1998) who refer to a biotope as "an area of uniform environmental conditions" and who regard a habitat as a "combination of biotopes that make up the living space of an organism". A sketch map to illustrate the habitat heterogeneity at the site was then drawn on which the biotopes were delineated and numbered.

Table 3.4.1: The Velocity-depth classes proposed by Kleynhans (2007).

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

3.4.2.1.3 The micro-habitat characteristics within the biotopes

The maximum length and width of each biotope were measured from which the approximate surface area was later calculated. The water depth was then measured at five randomly selected points throughout the biotope. At the same points the velocity was determined at just below the upper surface of the water and directly above the substrate using a Pasco Explorer 2000 velocity meter in meters per second.

To determine the substrate composition, the substrate type was determined at points in each biotope where water depth and velocity was determined. The substrate was classified as bedrock,

boulder, cobble, pebble, gravel, sand or sediment according practical description listed in table 3.4.2. From the recorded data the percentage of each component was calculated and the dominant substrate class identified. In each one of the recorded biotopes the overhanging vegetation, undercut banks, root wads and aquatic macrophytes were identified, using the criteria shown in table 3.4.3, and the extent estimated and scored (Table 3.4.4).

Table3.4.2: Substrate classification (Adapted from Rowntree and Wadeson, 2000).

Substrate class	Size (mm)
Bedrock	N/a
Boulder	> 256
Cobble	64-256
Gravel	2-64
Sand	0,06-2
Silt and clay (sediment)	< 0,06

Table 3.4.3: Classification of the estimated cover types (Kleynhans *et al.*, 2007).

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

Table 3.4.4: Abundance scoring of cover types (adapted from Kleynhans *et al*., 2007).

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

3.4.2.1.4. Collection of fish

As stated in chapter 2 the method of collection depended on the biotope type and the sampling protocol suggested by Kleynhans (2008) was used. Methods of collection included electro-narcotization in fast-deep and fast-shallow biotopes, seine netting in the slow-deep and slow–

shallow biotopes and pole-seine netting in the small pools, backwaters and in particular where sampling had to be done under and amongst vegetation. All fish collected were identified using the key from Skelton (2001). The species and numbers collected were recorded, per biotope number, on site. The fork length of the *O. peringueyi* specimens collected was also measured and a fin clipping removed for genetic analyses. Except for voucher specimens of the species and *O. peringueyi* specimens for stomach content and gonad analyses all other specimens were returned to the environment.

3.4.2.1.5 Statistical analyses and habitat preference of the species

In order to enable an understanding of the habitat preference of the species a number of research questions were formulated. These questions were: a) what local scale environmental variables are the best predictors of *O. peringueyi* presence and abundance? b) What regional scale environmental variables are the best predictors of *O. peringueyi* presence and abundance? c) Which other fish species associate with *O. peringueyi* and is there an assemblage of particular fish that associated with *O. peringueyi* that can act as possible indicators of their presence?

Based on the above, the data analyses focused on two spatial scales namely regional (sites) and local (biotopes). Total species abundance was determined for each site and biotope. Non-metric multi-dimensional scaling (MDS) was used to display the unconstrained relationships between fish assemblages at sites and in biotopes respectively.

An attempt was also made to classify fish assemblages using hierarchical agglomerative cluster analysis with group average linking in Primer v.6. The robustness of the groups identified was tested by randomly permuting the similarity matrix that is used to construct the cluster classification.

Relationship of fish assemblages and environmental variable were examined with redundancy analysis, RDA, a constrained method of ordination using CANOCO V4.5 (Ter Braak & Šmilauer, 2002). Species turnover between sites were low enough <3 (2.1) to allow the use of ordination methods based on linear models of species responses, *viz*. RDA. A manual forward selection procedure was used to identify environmental variables that significantly explained fish assemblage structure. This was done at two spatial scales local (with reference to biotopes) and regional (incorporating variables that does not vary locally). Species sample relationships were also displayed as bi-plots to determine which species contributed to differences between assemblages. Only species with more than 70% of their variability explained by the bi-plot were included. Species are depicted as arrows pointing in the direction of the steepest increase in abundance (Botes *et al*., 2006; Lepš and Šmilauer, 2003).

Characteristic species of the groups proposed by the cluster analysis were identified with the indicator value method (Dufrêne and Legendre, 1997) based on the species abundance matrix. This method assesses the fidelity and specificity (calculated as a frequency) of a species for a particular group compared to all other groups expressed as a percentage indicator value (IndVal). Species with a high IndVal scores are not only very specific to a given group but also has a high probability of being surveyed in that locality (McGeoch and Chown 1998; Van Rensburg *et al*., 2000). Indicator species were assessed based on all the species collected. Dufrêne and Legendre's (1997) random reallocation procedure of sites among site groups was used to test the significance of the IndVal measures for each species. Species with significant IndVals > 70% (Van Rensburg *et al*. 2000), were then regarded as characteristic (i.e. indicator) species for the group, sites and environmental variables in question, and species with significant IndVals > 50% were regarded as detectors.

3.4.3 RESULTS

3.4.3.1 Mapping of the biotopes

A detailed map was drawn at each of the surveyed sites which the demarcation and numbering of the "biotopes" and an example of these maps are shown in figure 3.4.1.

3.4.3.2. Statistical analyses

O. peringueyi was present at only eighteen percent of the sites sampled and at regional level the sites with *O. peringueyi* seem to be more similar to each other than they are to other sites without *O. peringueyi* (Figure 3.4.2). The same pattern was observed at the local, or biotope, level (Figure 3.4.3). It was tested whether *O. peringueyi* was responsible for these similarities by removing it from the matrix and constructing another similarity matrix and correlating this with the original similarity matrix that contained *O. peringueyi* as part of its analysis.

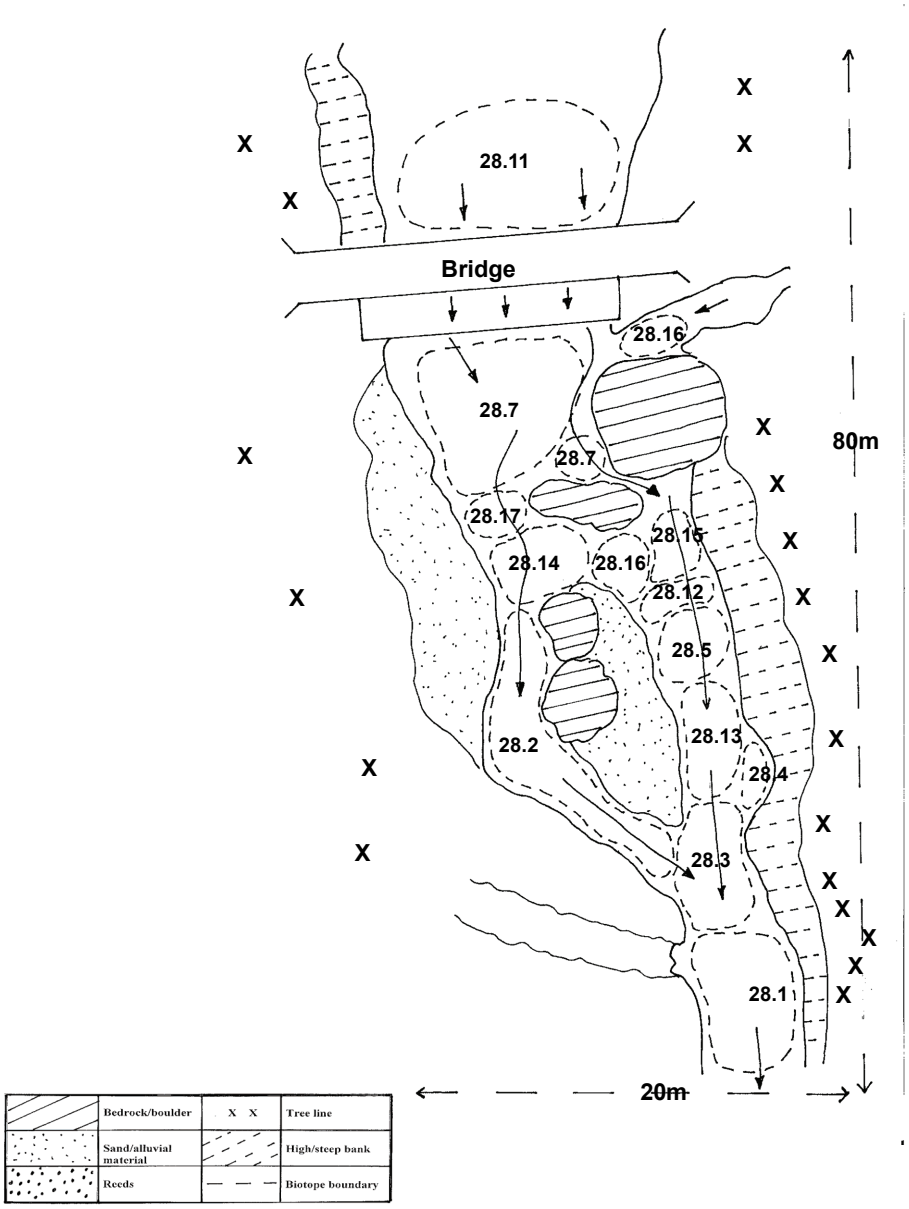


Figure 3.4.1: Sketch map drawn at Hydro bridge (OPS 28) in the Mutshindudi River which is an example of the map drawn at each of the surveyed sites and shows the demarcation and numbering of the "biotopes. The arrows indicate the direction of flow and the X-s the tree line.

The correlation between the two matrices were highly significant R = 0.992 ($p < 0.001$), suggesting that *O. peringueyi* had no significant influence on the results of our ordinations and are therefore embedded within a larger and robust assemblage. It therefore implies that it should be able to identify typical fish communities that could act as indicators of potential *O. peringueyi* sites.

Cluster analysis identified seven distinct groups and, all records of *O. peringueyi* were restricted to group "**g"** shown in figures 3.4.4 A and B. Table 6.4 lists *Labeobarbus marequensis* as the indicator

species of this group, with *Chiloglanis pretoriae* a detector. An evaluation of taxa that are good indicators of *O. peringueyi* sites, *sensu stricto*, revealed no other species, except for *Amphilius uranoscopus* and *Barbus euteania* that could act as detector species.

Regional environmental variables explain 21.9% of variation in fish assemblage structure. Four environmental variables explain significant amounts of systematic variation and are, in order of importance: altitude, TDS, width of stream and pH (Table 3.4.7). The first two axes of the bi-plot explain 10.4% in fish assemblage variation (Figure 3.4.5). Altitude as a predictive variable is the only environmental factor that produces a significant response model based on the generalized additive model, *O. peringueyi* have a uni-modal response to altitude with an optimum at 600 m above sea level (Figure 3.4.7).

Local scale environmental variables explain 24.8% of variation in fish assemblage structure. Four environmental variables explain significant amounts of systematic variation and are, in order of importance: sand, maximum velocity, root wads and gravel (Table 3.4.8). The first two axes of the bi-plot explain 8.5% in fish assemblage variation (Figure 3.4.9). Sand as a predictive variable is the only environmental factor that produces a significant response model based on the generalized additive model for *O. peringueyi* that have a uni-modal response to percentage sand cover with an optimum 80% (Figure 3.4.8).

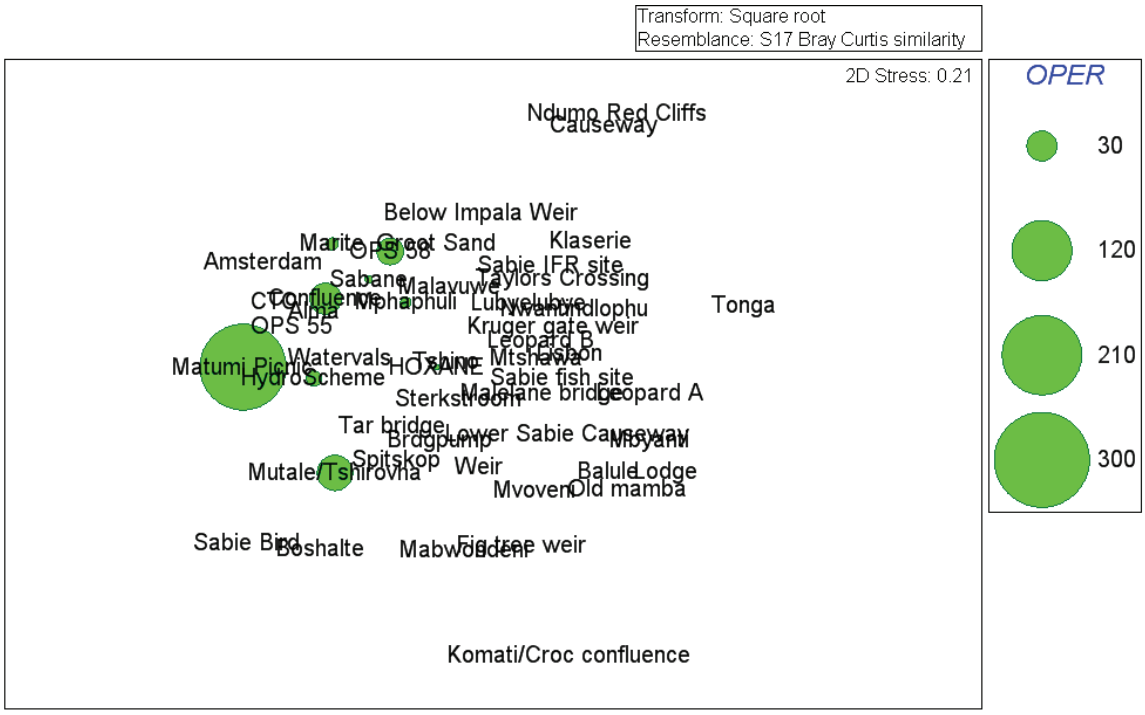


Figure 3.4.2: MDS ordination of fish assemblage composition at each of the sites, where the bubble size represents *Opsaridium peringueyi* abundances at each site. The names refer to the site names.

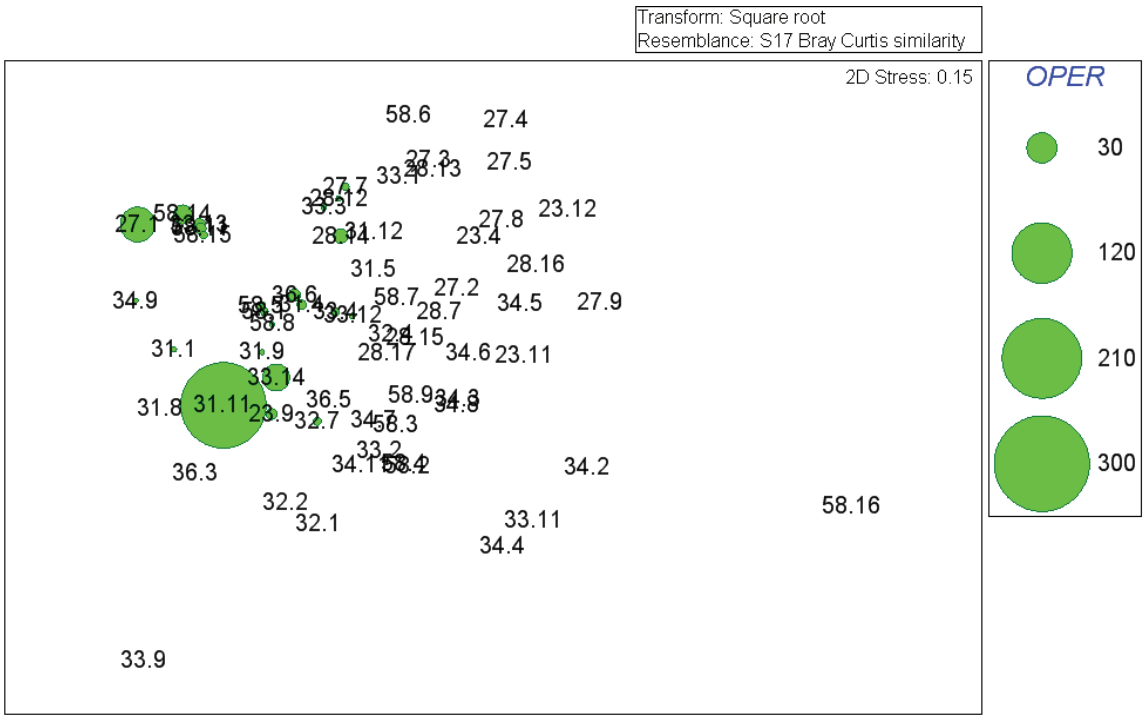


Figure 3.4.3. MDS ordination of fish assemblage composition in each of the biotopes in sites where *Opsaridium peringueyi* were recorded, bubble sizes represent *O. peringueyi* abundances in each biotope. The numbers are the numbers of the biotopes as shown in figure 3.4.1.

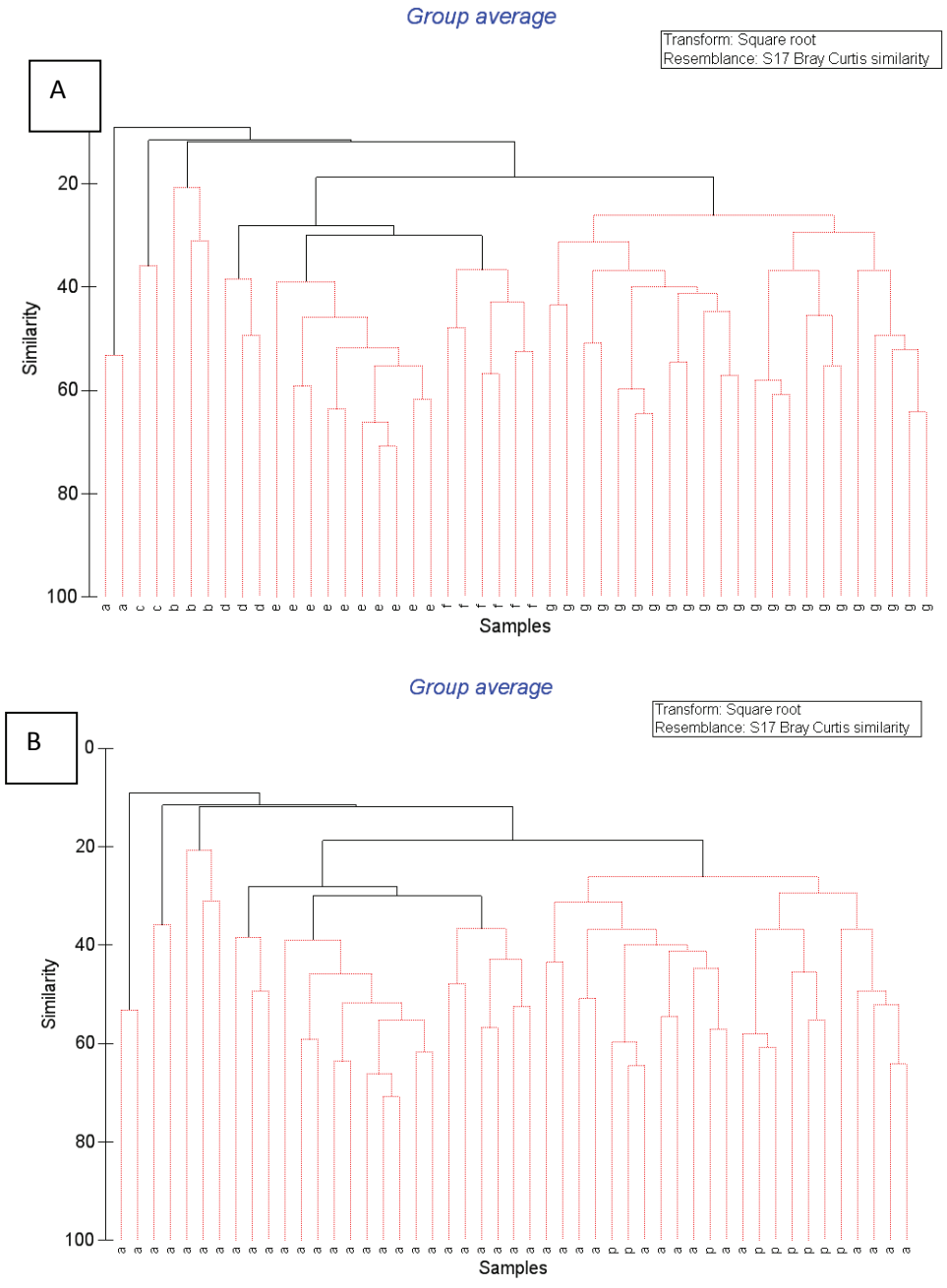


Figure 3.4.4: Hierarchical agglomerative cluster analysis based on group average linking for all 49 sites, identifying 7 groups (a-g), (B) with all the sites where *O. peringueyi* was collected indicated by p. Black lines represent classifications and groups for which there are significant support

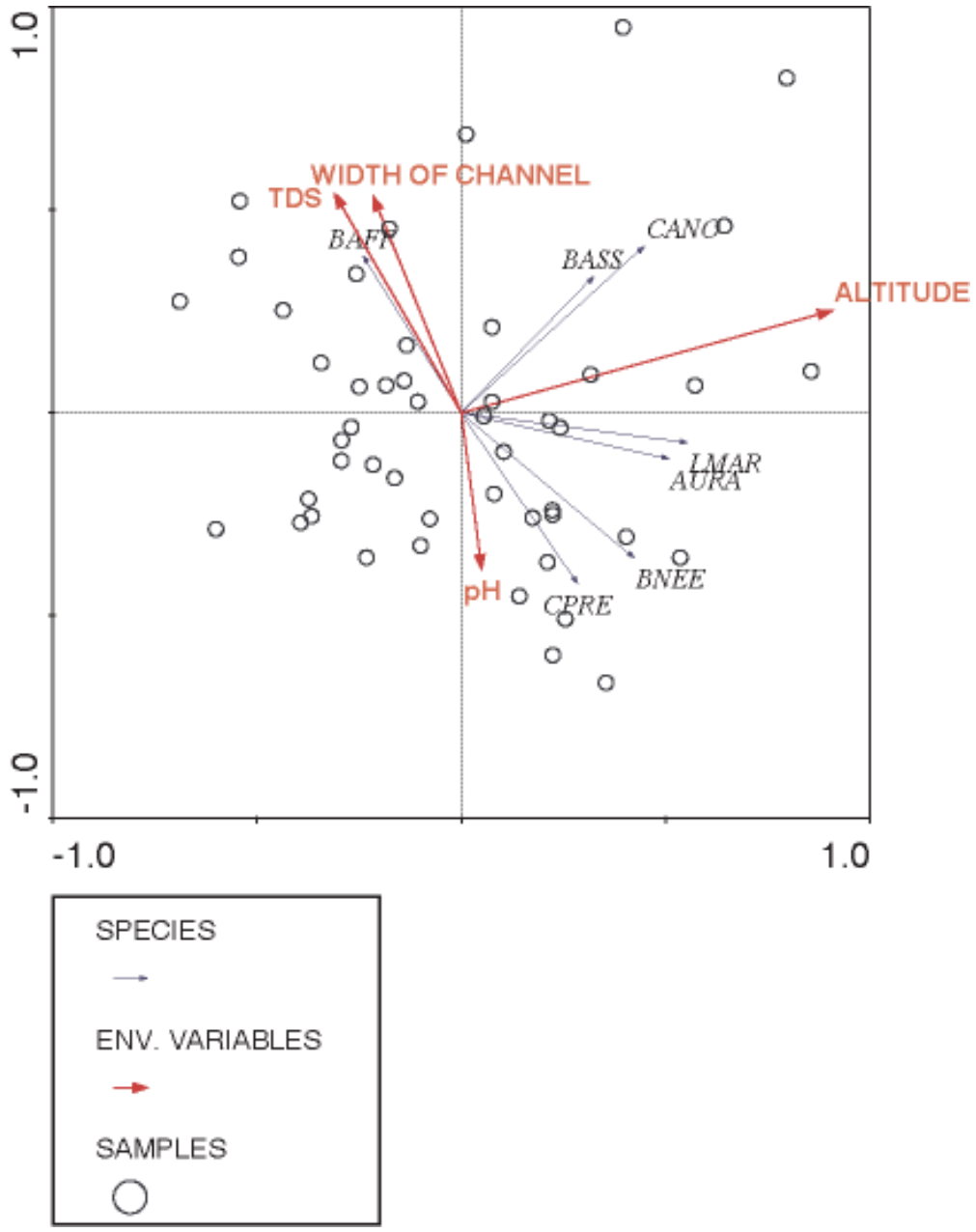


Figure 3.4.5: Tri-plot of sites, species and environmental variables, based on RDA analysis. Length of arrows indicates direction of increase in values.

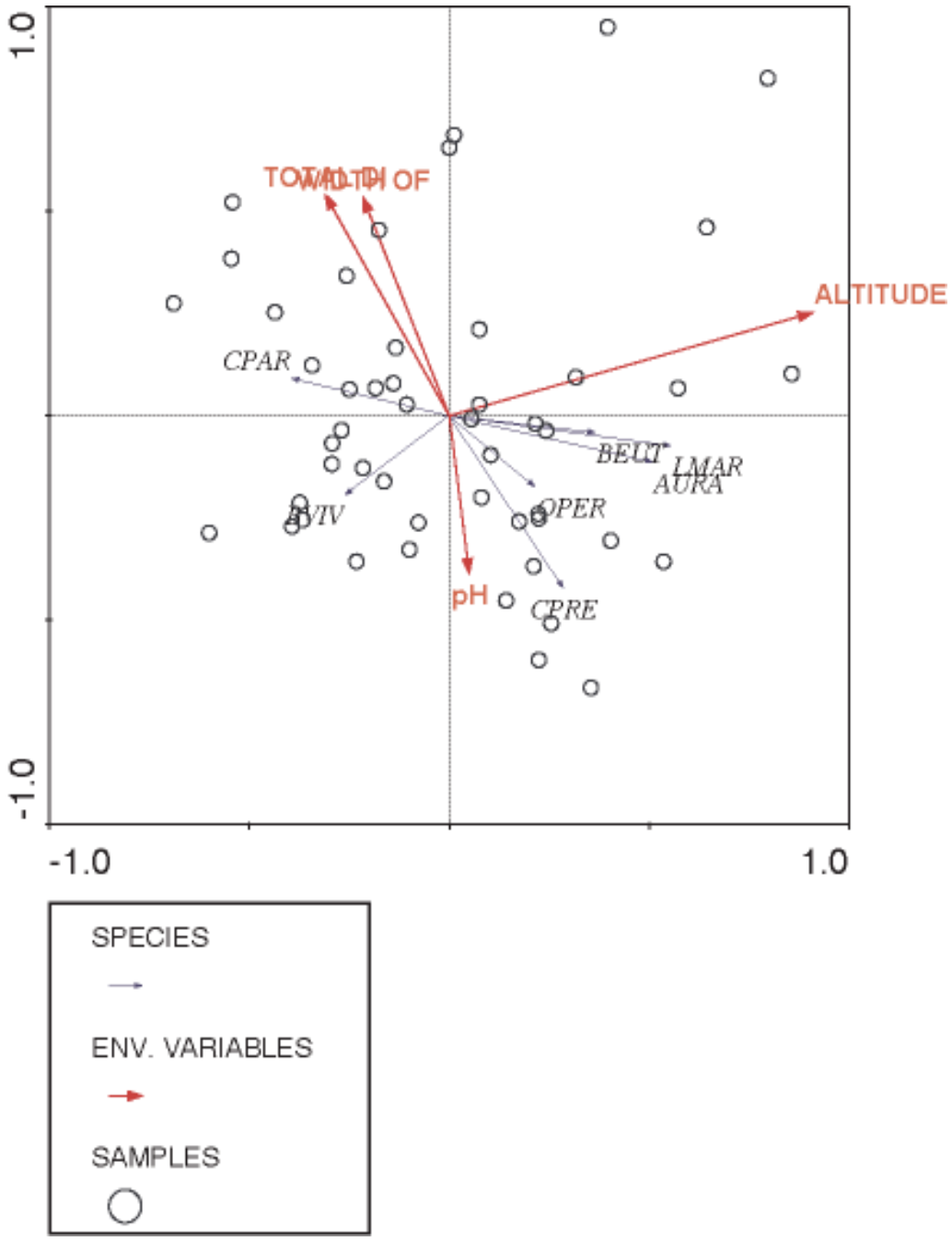


Figure 3.4.6: Tri-plot of with species that had significant indicator values (IndVal).

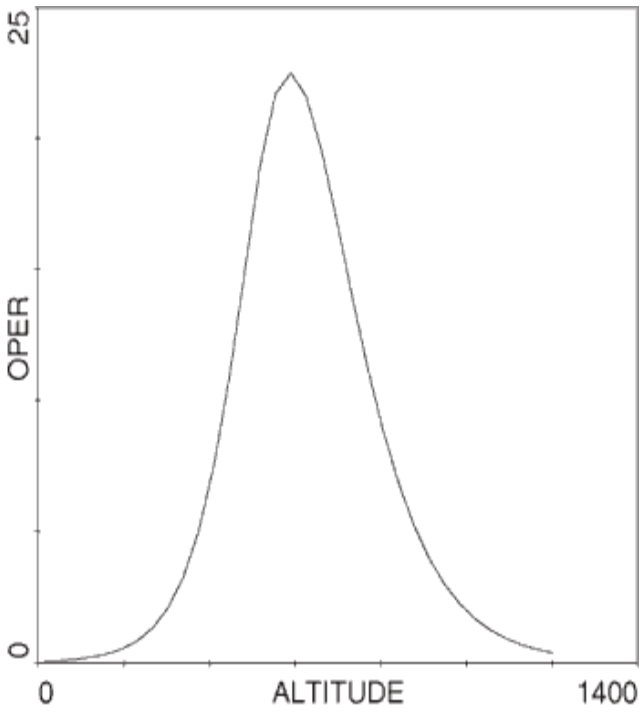


Figure 3.4.7: The response of *Opsaridium peringueyi* to altitude.

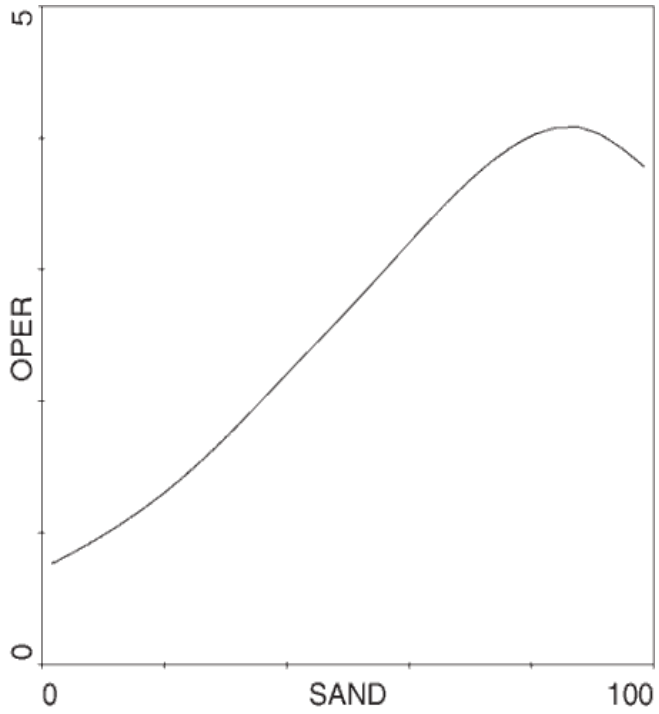


Figure 3.4.8: The response of *Opsaridium peringueyi* to sand.

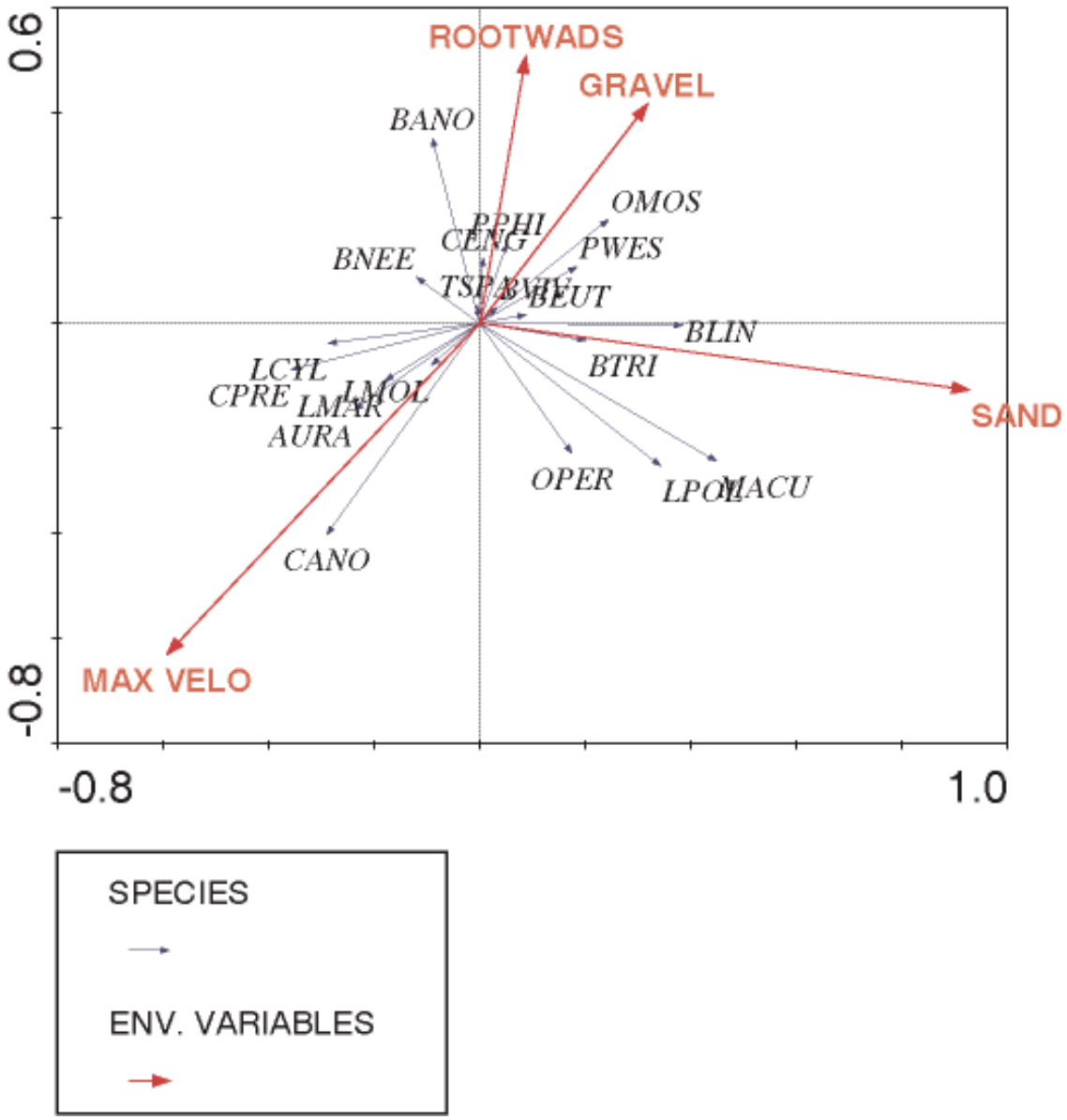


Figure 3.4.9: Bi-plot of species and environmental variables based on the association with biotopes in sites where Opsaridium peringueyi were collected based on RDA analysis. The length of the arrows indicates the direction of increase in values. The names of the species are indicated by the national abbreviations and Max Velo indicates the maximum velocity of the water.

**Table 3.4.5: Results of the SIMPER analyses. (AURA: *Amphilius uranoscopus*, BEUT: *Barbus euteania,* BVIV: *Barbus viviparus*, CANO: *Chiloglanis anoterus,* CPRE: *Chiloglanis pretoriae,* CPAR: *Chiloglanis paratus*, LMAR: *Labeobarbus marequensis*, LCYL: *Labeo cylindricus*, MACU: *Micralestes acutidens*, OMOS: *Oreochromis mossambicus,* PPHI: *Pseudocrenilabrus philander*, OPER: *Opsaridium peringueyi.*

Species	Average Abundance	Average similarity	Sim/SD	Contribution (%)	Cumulative (%)	similarity
Sites without O. peringueyi, Average similarity: 23.03						
LMAR	2.39	5.35	0.83	23.24	23.24	
LCYL	1.95	3.73	0.69	16.19	39.43	
CPAR	2.21	2.93	0.47	12.73	52.16	
BVIV	2.22	1.75	0.44	7.61	59.77	
PPHI	1.13	1.69	0.53	7.33	67.09	
Sites with O. peringueyi, Average similarity: 38.63						
LMAR	3.19	10.85	2.42	28.1	28.1	
OPER	4.7	9.61	1.64	24.88	52.98	
AURA	1.78	3.73	0.94	9.65	62.63	
BEUT	1.54	2.66	0.73	6.89	69.52	
MACU	3.27	2.62	0.34	6.77	76.3	
CPRE	1.47	2.32	0.68	6.01	82.31	
CANO	1.34	2.2	0.58	5.7	88.01	

Average dissimilarity = 80.00 between sites with and without OPER

Species	Group without Av.Abund	Group with Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
OPER	0	4.7	10.41	1.3	13.01	13.01
MACU	0.68	3.27	6.98	0.79	8.72	21.74
LMAR	2.39	3.19	5.28	1.24	6.59	28.33
CPAR	2.21	0	4.74	0.66	5.92	34.25
LCYL	1.95	0.81	4.36	1.08	5.45	39.71
CPRE	0.93	1.47	4.25	0.95	5.31	45.02
CANO	0.95	1.34	4.2	0.92	5.25	50.26
BVIV	2.22	0.27	3.93	0.7	4.91	55.18
AURA	0.39	1.78	3.81	1.21	4.76	59.94
PPHI	1.13	1.4	3.64	1.04	4.55	64.48
OMOS	1.39	0.75	3.62	0.88	4.53	69.01
BEUT	0.27	1.54	3.48	1.05	4.34	73.36

Table 3.4.6: Percentage indicator values (taxa with IndVal > 70% are indicator species and taxa with IndVal>50% are detector species) of fish species relative to their habitat fidelity and frequency. AURA: *Amphilius uranoscopus*, BEUT: *Barbus euteania,* BVIV: *Barbus viviparus*, CPRE: *Chiloglanis pretoriae,* CPAR: *Chiloglanis paratus*, LMAR: *Labeobarbus marequensis*, LCYL: *Labeo cylindricus*, MACU: *Micralestes acutidens*, OMOS: *Oreochromis mossambicus,* PPHI: *Pseudocrenilabrus philander*, OPER: *Opsaridium peringueyi.*

Group G	% IndVal	Complement of group G	% IndVal	Opsaridium sites	% IndVal	Complement of G	% IndVal	G w/o OPS sites	% IndVal
AURA	49.72	BVIV	52.46	AURA	48.85	CPAR	59.53	LMAR	59.47
CPRE	55.84	CPAR	61.69	BEUT	53.93				
LMAR	82.04			OPER	100.00				
OPER	39.13								

Table 3.4.7: Significant species-environmental correlation coefficients at a regional (Sites) scale(R-values, Ter Braak & Šmilauer 2002) from redundancy analysis for sites. The significance of the R- values was determined using Monte Carlo permutation tests (P = significance and F = test statistic).

Variable	Variation explained	P	F
Altitude	0.0604	0.002	3.019
TDS	0.092	0.068	1.583
Width of channel	0.12	0.086	1.471
pH	0.03	0.028	1.471
Total variance explained by environmental variables	0.219		

Table 3.4.8: Significant species-environmental correlation coefficients (R-values, Ter Braak & Šmilauer 2002) from redundancy analysis for biotopes. The significance of the R- values was determined using Monte Carlo permutation tests (P = significance and F = test statistic).

Variable	Variation explained	P	F
Sand	0.049	0.002	2.93
Maximum Velocity	0.079	0.046	1.81
Rootwads	0.107	0.024	1.75
Gravel	1.35	0.028	1.71
Total variation explained by environmental variables	0.248		

Chapter 5: Fine scale habitat selection

PSO Fouché, JA Venter and S Theron

3.5.1 INTRODUCTION

It is generally accepted that aquatic species can survive within certain ranges of physical environmental conditions that have been determined by its evolutionary past (Davies and Day, 1998). Within an aquatic environment a mosaic pattern similar to that observed in a terrestrial environment is evident. Where in the terrestrial environment the patches making up the mosaic consists of patches of grass and clumps of trees, in the aquatic environment these patches result from an array of environmental components, which *inter alia* includes substrate, water depth, water velocity and cover. The preferential occupation or use of habitat is referred to by a number of terms in the literature. It would appear that the terms habitat utilization (Werner *et al.*, 1976), habitat partitioning (Werner, op cit.), habitat preference (Gaigher, 1973; Felley and Hill, 1983) and habitat use (Wikramanayake and Moyle, 1989; Facey and Grossman, 1992; Wood and Bayne, 1995) are all used to describe a similar aspect. In freshwater fish it is accepted that, in addition to general habitat preference, seasonal variation in habitat selection occurs (Fouché *et al*., 2005). These variations may be driven by various factors which can include biological events such as breeding or evasive actions to avoid adverse conditions (Engelbrecht, pers com.[6]).

In earlier results in chapter 4 of this section of the project report it was shown that at a regional scale the Southern Barred Minnow, *Opsaridium peringueyi* does display definite habitat preferences such as for example the presence of sand in the habitat where they occur. It should however be pointed out that in that component of the project each site was surveyed only once and that various sites were surveyed at different times of day and during different seasons. In addition the habitat types at each site were not studied in detail due to time constraints. It was hypothesized that *O. peringueyi* is specific in its selection of habitat and only occur in specific biotopes within an area. It was furthermore hypothesized that this species also adapted or changed their habitat selection on a seasonal basis.

This component of the project therefore intended to determine the fine scale habitat preference and fine scale habitat selection of *O. peringueyi* at both temporal and spatial scale. At the spatial

[6] Dr. Johan Engelbrecht – Mpumalanga Parks and Tourism Agency, Lydenburg

scale it was decided to work at a fine or “micro-habitat” scale and at the temporal scale it was decided to establish whether variation in seasonal habitat preference could be established.

3.5.2 METHODS

3.5.2.1 Introduction

Two potential sites for this study were initially identified. These sites were part of the historic sites identified and are the following: a) a site at the confluence of the Mutale and Tshirovha rivers in the upper Luvuvhu River catchment and b) the second site, at the Matumi picnic site in the Mac Mac River. Both sites were regarded as suitable but after consideration it was decided to use the Matumi site as the main site where all the “micro-habitat” or fine scale habitat selection study would be done. The Mutale/Tshirovha site could then be used for comparison at a later stage if needed.

3.5.2.2 The selected study site

The site is located at Matumi (S 25.02339, E 31.001944) in the Mac Mac River *ca* 2 km upstream of the confluence with the Sabie River (Figure 3.5.1). This site is also referred to as OPS 31 in other components of this report. The topography at the site is typical of the foot hill slopes of the Mpumalanga Drakensberg escarpment with deep valleys and undulating terrain. Previous sampling done during the “habitat selection” (Chapter 4) of the project showed that *O. peringueyi* was abundant at the site. Although land use in the area mainly consists of forestry, the vegetation on both river banks is predominantly indigenous. Local impacts consist of a picnic site *ca* 300 m upstream of the site and a wooden hut present at the site.

3.5.2.3 Identification and delineation of fish habitat

During an initial site visit the site was photographed and the possible habitats, based on the criteria listed in table 3.4.1, were identified and demarcated by judging the velocities and depths. The actual depths and velocities in each habitat were then measured. The subdivision was then re-assessed and where needed the initial habitat type was either subdivided or combined into new units. Because these units were then “spatially and hydraulically distinct instream flow environments” (Rowntree and Wadeson, 1998) they were referred to as biotopes as was the case in chapter 4.

Figure 3.5.1: The location of the Matumi study site.

These biotopes were demarcated and their position and boundaries indicated on a sketch map where they were delineated and numbered. Within each biotope the substrate was identified and classified, using the criteria listed in Table 3.4.2, at five randomly selected points within the biotope. From this data the substrate composition was then determined and each component expressed as a percentage of the total.

3.5.2.4 Survey frequency and survey protocol

It was decided to survey the site monthly for a period of at least twelve months. A protocol that was to be followed during the monthly surveys was developed and consisted of the following:

Day 1 – Orientation and identification of biotopes.

a) When arriving at the site the biotopes and their boundaries, as indicated on the final sketch map were "ground truthed" and the survey team familiarized themselves with the biotopes.

b) The water quality parameters which included pH, temperature, electrical conductivity, total dissolved solids and dissolved oxygen levels were determined using handheld Eutech meters.

c) In each biotope the velocity was determined with a Pasco velocity meter in meters per second at three randomly selected points. The depth was measured in centimeters with a meter rule at the same points.

Days 2, 3 and 4 – Camera monitoring within the biotopes.

The monitoring protocol was structured in such a way that each biotope was monitored three times during the three day period at three different daylight periods. "Daylight" was arbitrarily divided into three three-hour periods: a) *Early morning* (7h00-10h00) b) *Midday* 12h00-15h00 and c) A*fternoon* 15h00-18h00. The site was also divided into three sections and each section was to be monitored once in each three-hour period. The first section, section A, was the furthest downstream (Figure 3.5.2). The protocol is illustrated in table 3.5.1.

Table 3.5.1: Summary of the camera monitoring protocol in the biotopes. A, B and C refers to sections at the site

	Time of "daylight".		
Day	**Early morning (7-10h00)**	**Mid day (12-15h00)**	**Afternoon (15-18h000**
2	First section (A)	Second section (B)	Last section (C)
3	Second section (B)	Last section (C)	First section (A)
4	Last section (C)	First section (A)	Second section (B)

Day 5: Biomonitoring.

On the last day of the monitoring the following sampling also took place:

a) Specimens, for vouchers purposes and species confirmation, were collected using the following methods. In shallow biotopes that were less than 0,5 meters in depth, specimens were electro-fished. In deep biotopes specimens were collected with both seine and cast nets. One drag of the seine net and ten efforts with a cast net was regarded as sufficient. All the specimens were identified on site using a key (Skelton, 2001) and voucher samples, which consisted of one specimen of each species, were preserved in 10% formalin.

b) The *in situ* water quality parameters which included pH, temperature, electrical conductivity, total dissolved solids and dissolved oxygen levels were determined using handheld Eutech meters.

3.5.2.5. Camera work

Each of the biotopes was surveyed extensively with an underwater camera. The camera unit was attached to a 3 meter rod and this was handled by a team member to survey each biotope. Where necessary the lights on the camera were switched on the facilitate observation. The camera was attached by cable to a screen handled by a second team member on the river bank. The second team member identified and recorded the specimens observed and in addition guided the camera handler to stay within the boundaries of the biotope that was being surveyed.

3.5.2.6. Data analyses

The data collected during the 2008 survey was statistically analysed to establish whether habitat preferences did occur and if seasonal trends existed. Prior to analyses biotope characteristics were used to classify the biotope into one of the four velocity-depth classes (Table 3.4.1). In addition the dominant substrate type was then added to the velocity-depth characteristics and each biotope was classified on the basis of the three aspects namely depth, velocity and dominant substrate. A frequency distribution of abundances was done to establish habitat preferences and to ascertain whether differences, when observed were significant the data was subjected to a Kruskal-Wallis analyses. To investigate seasonal trends three dimensional histograms were plotted of the occurrence and abundance of the species during the months surveyed. This was done for both biotope classification systems.

3.5.3 RESULTS

3.5.3.1 Survey effort

Mapping of the biotopes.

During the initial site survey in March 2007 a map of the identified biotopes was drawn (Figure 3.5.2) and this was used for the surveys from April to October 2007. Based on the experience gained during the 2007 survey the map was adapted and figure 3.5.3 shows the final map produced. A comparison of the two maps shows that biotope identification and delineation in the second format (Figure 3.5.2) was done on a finer scale which resulted in a further and final subdivision of the biotopes. The second version of the map was used in the 2008 survey.

Survey frequency

The site was surveyed on a monthly basis from March to October 2007 and again from April 2008 to December 2008. Because of the change in the biotope delineation at the end of 2007 it resulted in two sets of data which will be dealt separately. Where the 2007 results are used for general discussions the results obtained in the 2008 survey will in addition be used in the statistical analyses.

3.5.3.2 The results of the 2007 survey

3.5.3.2.1 The mapped biotopes and the substrate composition within the biotopes

Figure 3.5.2 shows where the thirteen identified biotopes are situated within the site and also shows where the three monitoring sections begin and end. Table 3.5.2 shows the substrate composition, and dominant substrates, within the biotopes.

3.5.3.2.2 The velocity-depth classes awarded to the biotopes

Table 3.5.3 shows the velocity-depth classes awarded to the biotopes. These classes are based on the dominant values of velocity and depth measured during the course of the whole survey. It is important to note that with the exception of biotopes 5, 11 and 12 (Table 3.5.5.3) the classification of the rest of the biotopes remained unchanged throughout the survey period.

Table 3.5.2: The substrate composition of the biotopes delineated in the 2007 survey at the Matumi site in the Mac Mac River where BR:bedrock, B:boulders, C:cobbles, G:gravel and S: sand. The dominant substrate component is printed in bold.

Biotope number	Substrate	Biotope number	Substrate	Biotope number	Substrate	Biotope number	Substrate
1	**S**	5	**C/**S	9	**B/C/**S	13	C/G/**S**
2	**S**	6	**B/**S	10	B/**C/**S		
3	B/C/**S**	7	BR/**B/**CS	11	B/**C/**G/S		
4	**BR/**B/C	8	BR/**B/**C/S	12	**B**/C		

3.5.3.2.3 The fish observed in the biotopes during the monthly surveys

Tables 3.5.4 to 3.5.10 show the fish species recorded in the biotopes during the period April to October 2007. The abbreviations for the species names are in accordance to that currently in use in South Africa (Kleynhans, 2007) and are listed in Table 3.5.11.

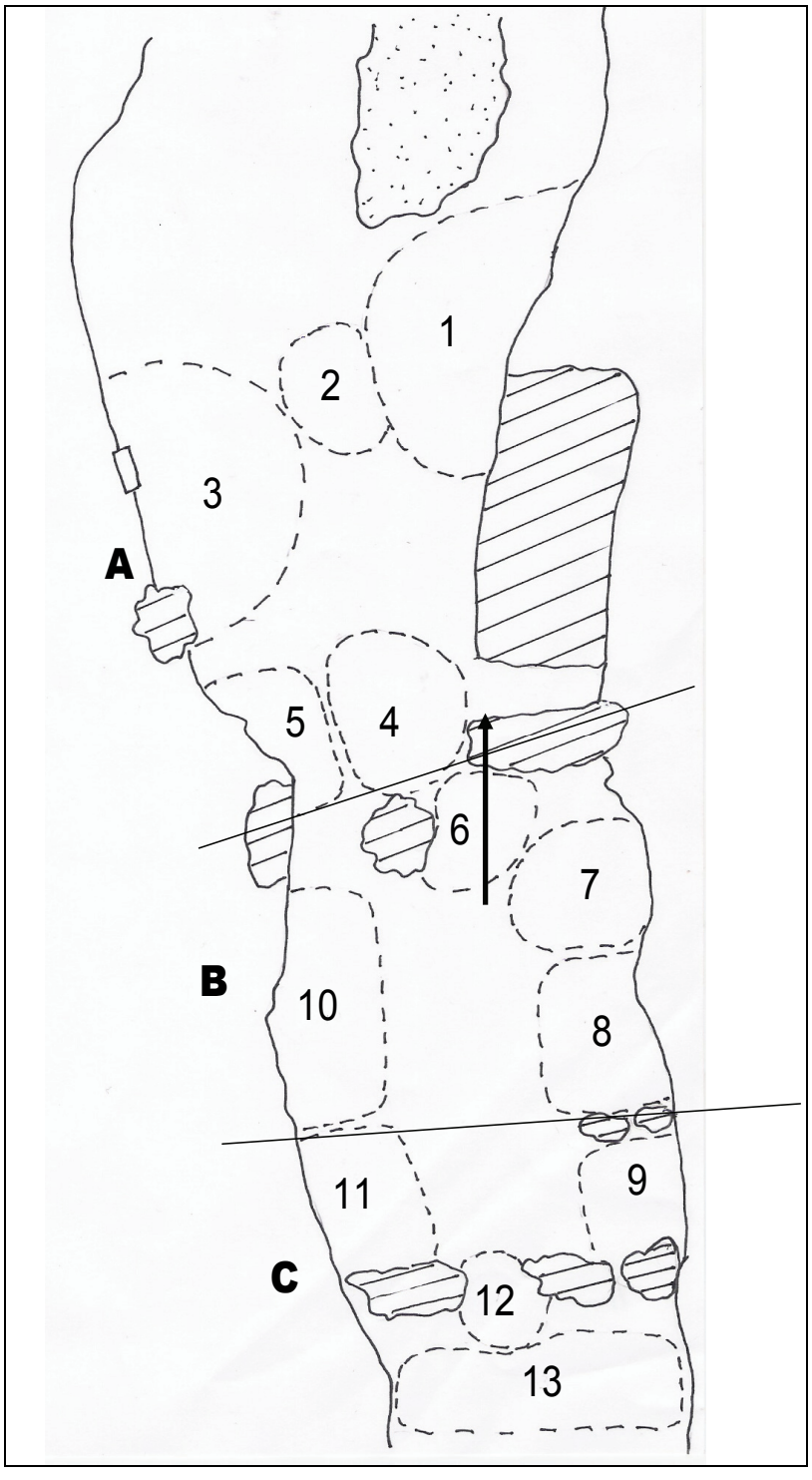


Figure 3.5.2: Sketch map indicating the biotopes identified at the Matumi site in the Mac Mac River during first survey period from April to October 2007. The arrow indicates the direction of the flow. A, B and C are the three survey sections. The dotted area consists of sand while the areas with diagonal lines are areas of bedrock or boulders.

Table 3.5.3: The velocity-depth classes awarded to the biotopes at the Matumi site in the Mac Mac River for the period April to October 2007. (SD=slow-deep, SS = slow-shallow, FD = fast deep and FS = fast-shallow)

	April	May	June	July	August	September	October
Biotope no.	**Velocity-Depth class**	**Velocity-Depth class**	**Velocity-Depth class**	**Velocity-Depth class**	**Velocity-Depth class**	**Velocity-Depth class**	**Velocity-Depth class**
1	SD	SD	SD	SD	SD	SD	SD
2	SD	SD	SD	SD	SD	SD	SD
3	SD	SD	SD	SD	SD	SD	SD
4	FD	FD	FD	FD	FD	FD	FD
5	FS	FS	FS	FS	FS	FS	FD
6	FD	FD	FD	FD	FD	FD	FD
7	FD	FD	FD	FD	FD	FD	FD
8	FD	FD	FD	FD	FD	FD	FD
9	FD	FD	FD	FD	FD	FD	FD
10	FS	FS	FS	FS	FS	FS	FS
11	SS	SS	FS	FS	FS	FS	FS
12	FS	FS	SD	SD	FD	FD	FD
13	FS	FS	FS	FS	FS	FS	FS

Table 3.5.4: The fish species observed during April 2007 in each biotope. Where [A] – single specimens, [B] – small school (<5) and [C] – large school (>5). (BEUT: *Barbus eutaenia,* MACU; *Micralestes acutidens,* OPER: *Opsaridium peringueyi,* VNEL: *Varichorinus nelspruitensis).*

Biotope	Morning			Mid-day			Afternoon		
no.	**Day1**	**Day2**	**Day3**	**Day1**	**Day2**	**Day3**	**Day1**	**Day2**	**Day3**
1	BEUT[A] OPER[B]								
2	VNEL[C]								VNEL[C]
3	BEUT[B] VNEL[C]							BEUT[A] OPER[A] MACU[A] VNEL[C]	
4									
5									
6									
7				VNEL[A] OPER[A]					
8									
9					BEUT[A]				
10									
11					MACU[B]				
12									
13									

Table 3.5.5: The fish species observed during May 2007 in each biotope. Where [A] – single specimens, [B] – small school (<5) and [C] – large school (>5). (BEUT: *Barbus eutaenia,* MACU; *Micralestes acutidens,* OPER: *Opsaridium peringueyi,* VNEL: *Varichorinus nelspruitensis).*

Biotope no.	Morning			Mid-day			Afternoon		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
1						BEUT[B] OPER[B]		BEUT[A] OPER[B]	
2	VNEL[C]					VNEL[C]			VNEL[C]
3	BEUT[B]					BEUT[A] OPER[B] VNEL[C]		BEUT[B] OPER[B] VNEL[C]	
4				OPER[B]					
5									
6									OPER[B]
7									
8				VNEL[B]					BEUT[B] VNEL[A]
9		VNEL[B]							
10									
11									
12									
13		VNEL[B]		VNEL[B]	VNEL[B]				VNEL[A]

Table 3.5.6: The fish species observed during June2007 in each biotope. Where [A] – single specimens, [B] – small school (<5) and [C] – large school (>5). (BEUT: *Barbus eutaenia,* MACU; *Micralestes acutidens,* OPER: *Opsaridium peringueyi,* VNEL: *Varichorinus nelspruitensis).*

Biotope no.	Morning			Mid-day			Afternoon		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
1						BEUT[B] OPER[B]		BEUT[B] OPER[B] VNEL[B]	
2	VNEL[C]					VNEL[C]			
3	BEUT[B] VNEL[C]					BEUT[B] OPER[B] VNEL[C]		BEUT[B] VNEL[C]	VNEL[A]
4				VNEL[A]					
5									
6									OPER[B]
7									
8				VNEL[A] BEUT[A]					BEUT[B]
9					BEUT[B]		VNEL[A]		
10									MACU[A]
11							OPER[B]		
12									
13		VNEL[B]		OPER[A] MACU[A]	BEUT[B] VNEL[A]		VNEL[B]		VNEL[B]

Table 3.5.7: The fish species observed during July 2007 in each biotope. Where [A] – single specimens, [B] – small school (<5) and [C] – large school (>5). (BEUT: *Barbus eutaenia,* MACU; *Micralestes acutidens,* OPER: *Opsaridium peringueyi,* VNEL: *Varichorinus nelspruitensis).*

Biotope no.	Morning			Mid-day			Afternoon		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
1					BEUT[B] OPER[B]	BEUT[B] OPER[C]			
2	VNEL[C]				VNEL[C]	VNEL[B]			
3	BEUT[B] OPER[B] VNEL[C]				BEUT[B] OPER[B] VNEL[C]	BEUT[B] VNEL[C]			
4									
5									
6									
7									
8									
9									
10									OPER[B] MACU[A]
11									
12									
13			VNEL[A]	VNEL[A]			BEUT[B] VNEL[A]		VNEL[A]

Table 3.5.8: The fish species observed during August 2007 in each biotope. Where [A] – single specimens, [B] – small school (<5) and [C] – large school (>5). (BEUT: *Barbus eutaenia,* MACU; *Micralestes acutidens,* OPER: *Opsaridium peringueyi,* VNEL: *Varichorinus nelspruitensis).*

Biotope no.	Morning			Mid-day			Afternoon		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
1	BEUT[C] OPER[C]				VNEL[A]	BEUT[B] OPER[B]		BEUT[B] OPER[B]	
2	VNEL[C]					VNEL[C]		VNEL[C]	
3	BEUT[B] VNEL[C]		VNEL[B]			BEUT[B] VNEL[C]		BEUT[B] OPER[B] MACU[A] VNEL[C]	
4		BEUT[B]		OPER[A]					OPER[A]
5									
6									
7									
8				VNEL[A]					BEUT[B] MACU[B]
9							BEUT[B]		
10				OPER[5]					
11					OPER[A]				
12							MACU[B]		
13		BEUT[B] VNEL[A]		OPER[A]	BEUT[B] OPER[B] VNEL[B]		OPER[B]		

Table 3.5.9: The fish species observed during April 2007 in each biotope. Where [A] – single specimens, [B] – small school (<5) and [C] – large school (>5). (BEUT: *Barbus eutaenia,* MACU; *Micralestes acutidens,* OPER: *Opsaridium peringueyi,* VNEL: *Varichorinus nelspruitensis).*

Biotope no.	Morning			Mid-day			Afternoon		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
1				OPER[B]					
2						VNEL[C]			
3	BEUT[B] OPER[A] VNEL[C]					BEUT[B] OPER[B] VNEL[C]		BEUT[B] OPER[B] VNEL[C]	
4									
5									
6									
7									
8									
9					BEUT[A]				
10									OPER[B]
11								VNEL[C]	
12									
13				BEUT[B] MACU[B] VNEL[A]	VNEL[A]		BEUT[B] OPER[B]		BEUT[B] VNEL[A]

Table 3.5.10: The fish species observed during April 2007 in each biotope. Where [A] – single specimens, [B] – small school (<5) and [C] – large school (>5). (BEUT: *Barbus eutaenia,* MACU; *Micralestes acutidens,* OPER: *Opsaridium peringueyi,* VNEL: *Varichorinus nelspruitensis).*

Biotope no.	Morning			Mid-day			Afternoon		
	Day1	Day2	Day3	Day1	Day2	Day3	Day1	Day2	Day3
1	BEUT[B] OPER[B] VNEL[C]					BEUT[B] OPER[B] VNEL[C]		BEUT[B] OPER[B]	
2	VNEL[C]					VNEL[C]		VNEL[C]	
3	BEUT[B] VNEL[C]							BEUT[B] OPER[B] VNEL[C]	
4				OPER[A]					VNEL[A]
5									OPER[A]
6		VNEL[B]							
7									
8									
9							VNEL[B]		
10		MACU[A]		OPER[B]					BEUT[B] MACU[B]
11									
12									
13		VNEL[A]	VNEL[A]	BEUT[B]	OPER[B] VNEL[A] BEUT[B]		VNEL[A]		VNEL[A] OPER[B]

Table 3.5.11: The abbreviations used for the species observed in the surveys

Abbreviation	Species
BEUT	*Barbus eutenia*
MACU	*Micralestes acutidens*
OPER	*Opsaridium peringueyi*
VNEL	*Varichorhinus nelspruitiensis*

An analysis of the results shown in tables 3.5.4 to 3.5.10 shows that during the survey period specimens of *O. peringueyi* were observed a total of fourty five times. Single specimens of the species were observed in 25% of the total observations and small schools, which consisted of up to five specimens formed the bulk, or 76%, of the observation. "Large" schools, which consisted of more than five specimens, only formed 4% of the observations. Table 3.5.12 shows that the highest frequency of occurrence of *O. peringueyi* was recorded in biotopes 1 and 3 followed by biotope 13. Both biotopes 1 and 3 were slow-deep biotopes dominated by sand. Table 3.5.12 also shows that no specimens were collected in biotopes 8, 9 and 12. These three biotopes were classified as fast-deep and where 12 had no sand present biotopes 8 and 9 had very little sand. Biotope 4 where the frequency of occurrence was 19% was one of the biotopes without sand where *O. peringueyi* was observed (Table3.5.13).

Table 3.5.12: The percentage frequency of occurrence of *Opsaridium peringueyi* in the biotopes during the period April 2007 to October 2007 at the Matumi site in the Mac Mac River.

Biotope no.	Number of times present.	Percentage frequency of occurrence
1	14	66.7
2	0	0.0
3	11	52.4
4	4	19.0
5	1	4.8
6	1	4.8
7	1	4.8
8	0	0.0
9	0	0.0
10	4	19.0
11	2	9.5
12	0	0.0
13	7	33.3

Table 3.5.13: The percentage frequency of occurrence of *Opsaridium peringueyi* in the biotopes during the monthly surveys done in the period April 2007 to October 2007 at the Matumi site in the Mac Mac River.

Biotope no.	April	May	June	July	August	September	October
1	4.8	9.5	9.5	9.5	14.3	4.8	14.3
2							
3	4.8	9.5	4.8	9.5	4.8	14.3	4.8
4		4.8			9.5		4.8
5							4.8
6			4.8				
7	4.8						
8							
9							
10				4.8	4.8	4.8	4.8
11			4.8				
12							
13			4.8		14.3	4.8	9.5

3.5.3.3 The results of the 2008 survey

3.5.3.3.1 The mapped biotopes and the substrate within the biotopes.

Figure 3.5.3 shows the twenty-eight biotopes identified and demarcated in April 2008 while Table 3.5.14 shows the substrate composition within these biotopes. Figures 3.5.4, 3.5.5 and 3.5.6 show the location of the finally selected biotopes in the three arbitrarily chosen sections of the site. Table 3.5.14 shows that the substrate composition of the site is typically that of a mountain foothill in that is dominated by coarse alluvial material. Although sand is present at the site in the majority of the biotopes it dominated in the slow flowing biotopes. The lack of silt in the biotopes should be noted.

Table 3.5.14: A summary of the substrate composition in the biotopes at the Matumi site in the Mac Mac River. Each substrate category is expressed as a percentage of the total substrate composition in the biotope.

Biotope number	Bedrock	Boulder	Cobble	Gravel	Sand	Silt
1					100	
2					100	
3					100	
4					100	
5		17	50		33	
6			100			
7			34		66	
8			60		40	
9	40	20	40			
10					100	
11	80				20	
12	50	25	25			
13	50		50			
14	90			10		
15		90			10	
16		70	20		10	
17		80			20	
18		25	75			
19		50	50			
20	20		80			
21				50	50	
22		50	50			
23		40	20		40	
24	10	40	40		10	
25	90				10	
26		80	20			
27		80	20			
28			20	20	60	

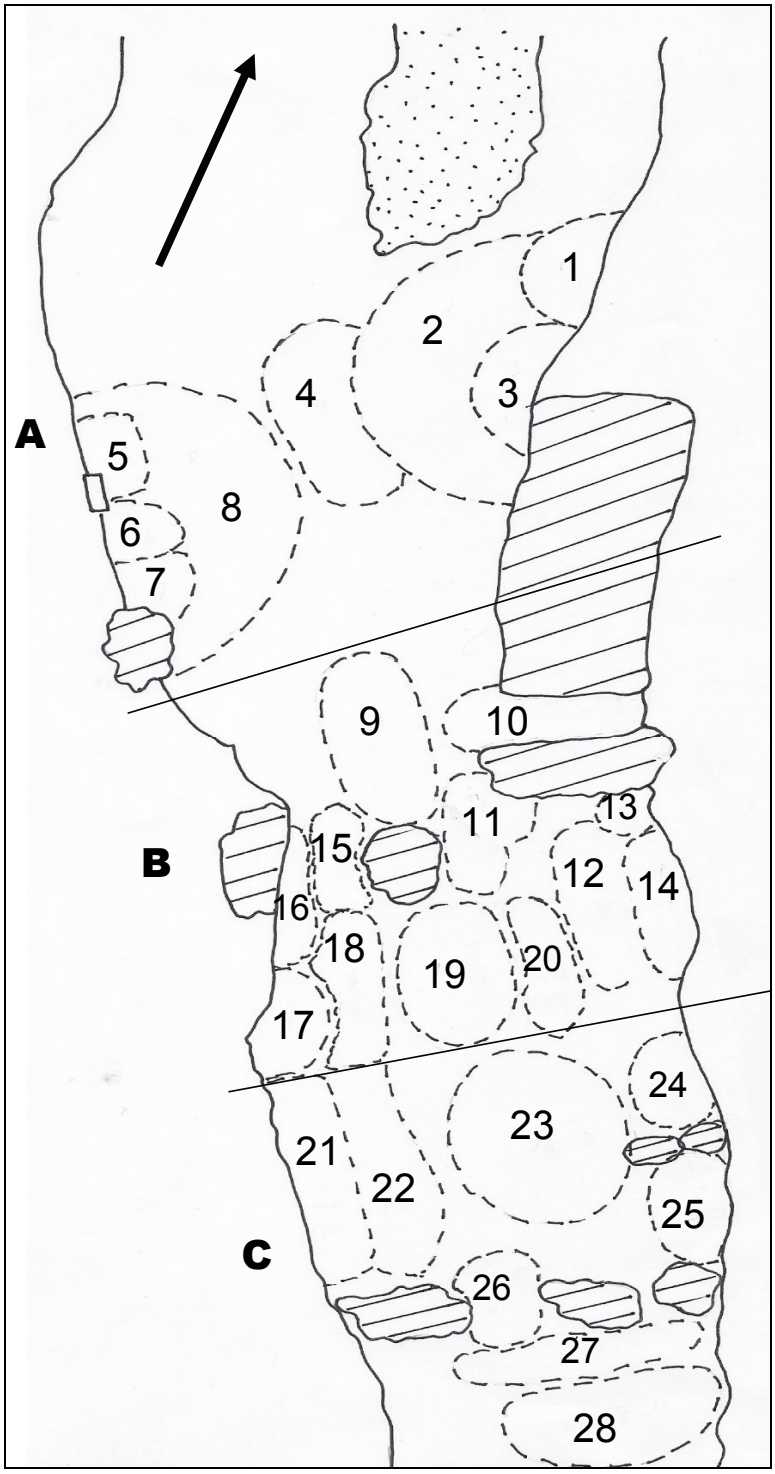


Figure 3.5.3: Sketch map indicating the biotopes identified at the Matumi site in the Mac Mac River during first survey period from April to October 2008. The arrow indicates the direction of the flow. A, B and C are the three survey sections. The dotted area consists of sand while the areas with diagonal lines are areas of bedrock or boulders

Figure 3.5.4: An illustration of the location of the biotopes in the lowest downstream section of the Matumi site in the Mac Mac River. The numbers indicate the biotopes shown in figure 3.5.3

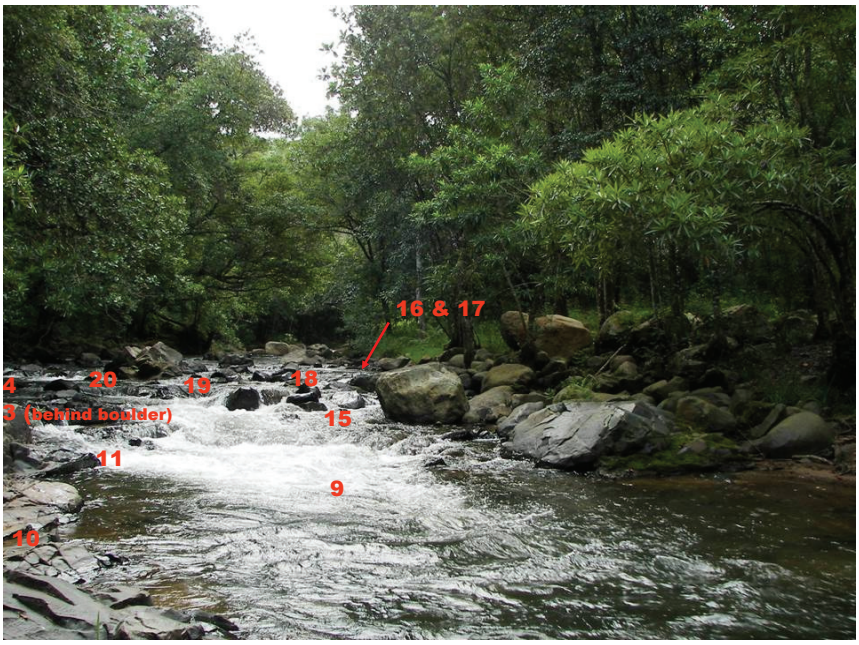


Figure 3.5.5: An illustration of the location of the biotopes in the middle section of the Matumi site in the Mac Mac River. The numbers indicate the biotopes shown in figure 3.5.3

Figure 3.5.6: An illustration of the location of the biotopes in the upstream section of the Matumi site in the Mac Mac River. The numbers indicate the biotopes shown in figure 3.5.3

3.5.3.3.2 Depth-velocity classification of the biotopes based on the final subdivision

Although the depth and the velocity of the biotopes showed temporal changes over the survey periods Table 3.5.15 shows the heterogeneity as observed in April 2008. This classification was extended with the incorporation of the absence or presence of sand within the biotope. This decision was based on the findings of chapter 4 of this report which showed that *"sand as a predictive variable is the only environmental factor that produced a significant response model for the presence of the species within a habitat"*. This latter classification resulted in eight classes (Table 3.5.16) and both classification systems were used in the data analyses.

3.5.3.3.3 Changes in flow and depth over the survey periods

While Figure 3.5.7 shows the seasonal changes in the average depth and velocity recorded at the site for the period April to December 2008 figure 3.5.8 shows how the average depths and velocities varied within each of the twenty-eight biotopes for the same period. Figure 3.5.8 shows the typical decline in both parameters from April to May followed by a steady incline which commenced from August when the rainy season begins. Figure 3.5.8 shows that between the biotopes conditions were extremely variable.

Table 3.5.16: The velocity-depth-sand classification of the biotopes at the Matumi site in the Mac Mac River as observed in the April 2008 survey where S indicates the presence of sand and N the absence of sand in the biotope

Biotope number	Velocity–depth–substrate classification	Biotope number	Velocity–depth – substrate classification
1	SSS	15	FDS
2	FDS	16	FDS
3	SSS	17	FSS
4	FDS	18	FSN
5	SDS	19	SSN
6	SDN	20	FDN
7	FDS	21	SSS
8	FDS	22	FDN
9	FDN	23	FDS
10	SSS	24	FDS
11	FSS	25	FDS
12	SSN	26	FDN
13	SSN	27	FDN
14	FDN	28	FDS

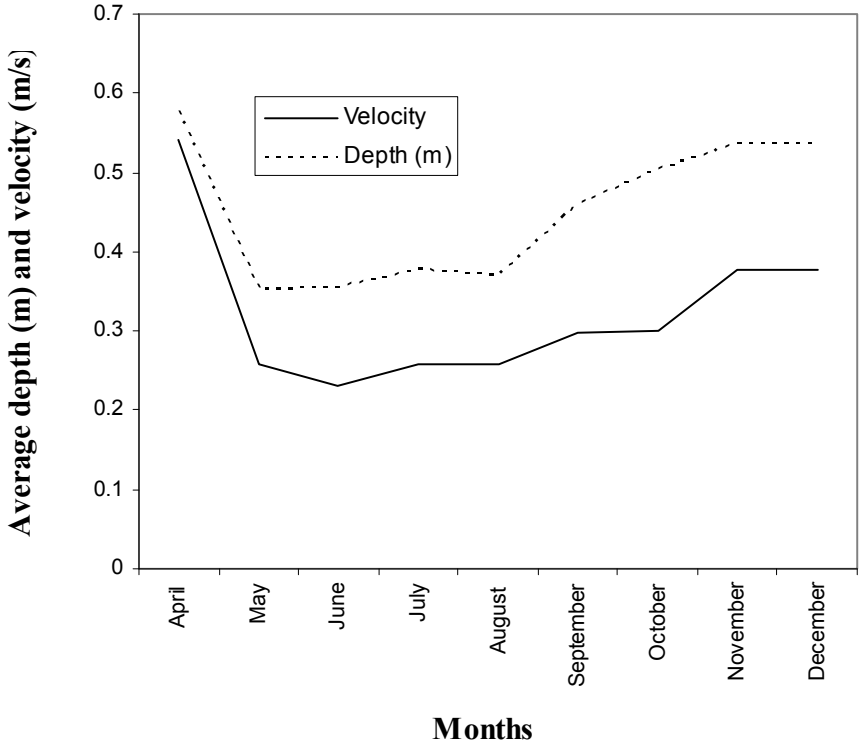


Figure 3.5.7: Average velocities and depths recorded *in situ* at the Matumi site in the Mac Mac River for the period April 2008 to December 2008.

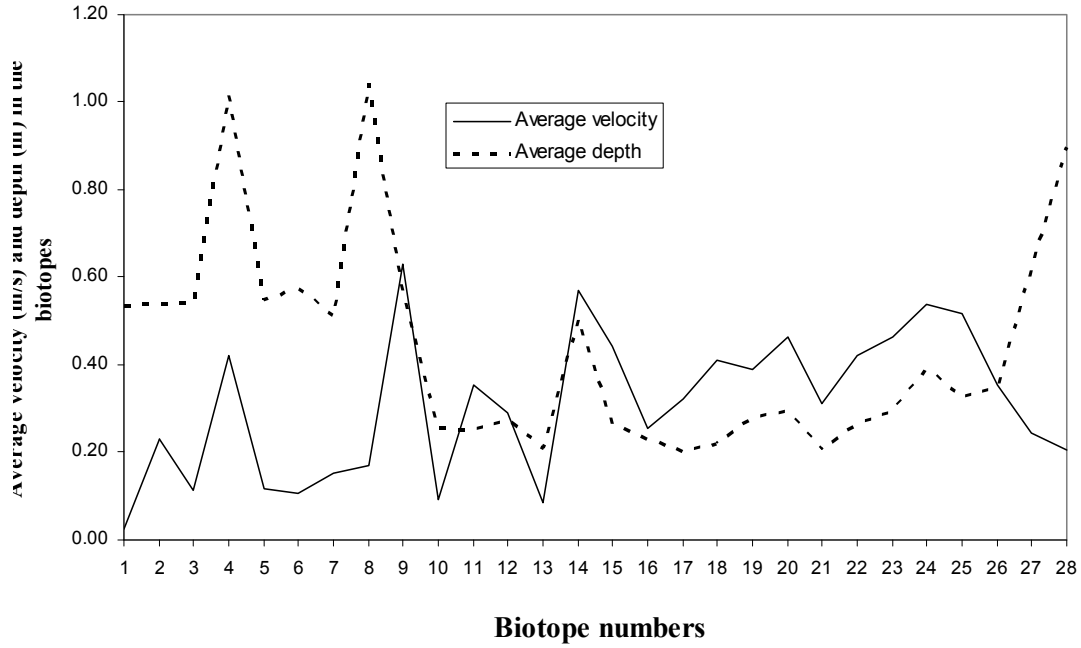


Figure 3.5.8: Average velocities and depths recorded in the biotopes at the Matumi site in the Mac Mac River for the period April 2008 to December 2008.

3.5.3.3.4 The fish collected in the biotopes

During the 2008 survey three species that were not observed in the 2007 survey were recorded in the biotopes. These were two cyprinid species, namely *Labeobarbus marequensis* and *L. polylepis* and one of the chiloglanids namely *Chiloglanis anoterus*. Figure 3.5.9 shows the percentage frequency of occurrence of *O. peringueyi* as observed in the individual biotopes throughout the whole survey period of 2008. It should be noted that in fact biotopes 5,6,7 and 8 form part of the largest pool in the site and if their frequencies of occurrence are combined it would be rated as the highest of all.

3.5.3.3.5 Seasonal fish frequency of occurrence in the biotopes

Table 3.5.17 shows the actual number of *O. peringueyi* specimens that were collected in each of the biotopes and as indicated in figure 3.5.9 large numbers of specimens were collected in the deep sandy biotopes 2, 6 and 8. It should be noted from these results that seasonal differences were observed in these and the other biotopes. Of note are also those biotopes where no specimens were collected at all namely biotopes 4, 10, 13, 16, 17, 18, 24 and 28. Biotope 4 is of interest as it is also one of the biotopes in which no specimens were collected during the 2007 survey.

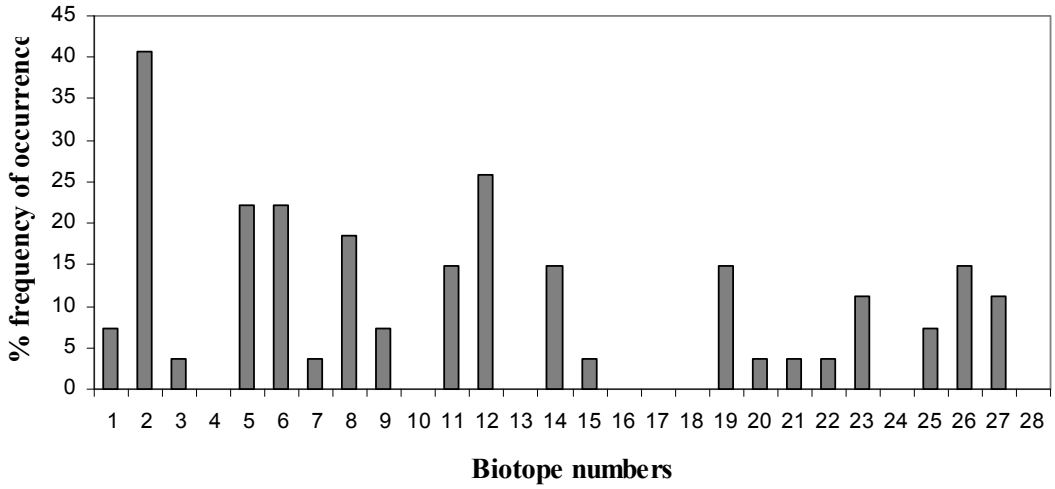


Figure 3.5.9: Percentage frequency of *Opsaridium peringueyi* collected in the biotopes at the Matumi site from April to December 2008.

Table 3.5.17: Abundance of *Opsaridium peringueyi* specimens collected in the demarcated biotopes during the period April to December 2008.

Biotope number	April	May	June	July	August	September	October	November	December
1	3		10						
2	30	8	3	5	10	20	8	35	35
3								10	
4									
5	15	6	5			8		2	5
6				20	20	31		5	8
7						8			
8		40	20			5	29	41	
9			1					20	
10									
11			3		10	3			
12	2			5	10	5		27	6
13									
14		2			5		9		11
15			2						
16									
17									
18									
19			6				9	10	8
20									2
21								5	
22		3							
23			3			3		7	
24									
25	1	5							
26	20					2	5	1	
27		3		9		5			
28									

The frequency distribution of abundances, which include transformed abundances, as shown in Figure 3.5.10 clearly shows that the data is dominated by absences. "Absences" refer to velocity-depth classes where no specimens of *O. peringueyi were* observed. This resulted in a right-skewed distribution pattern as observed figure 3.5.10.

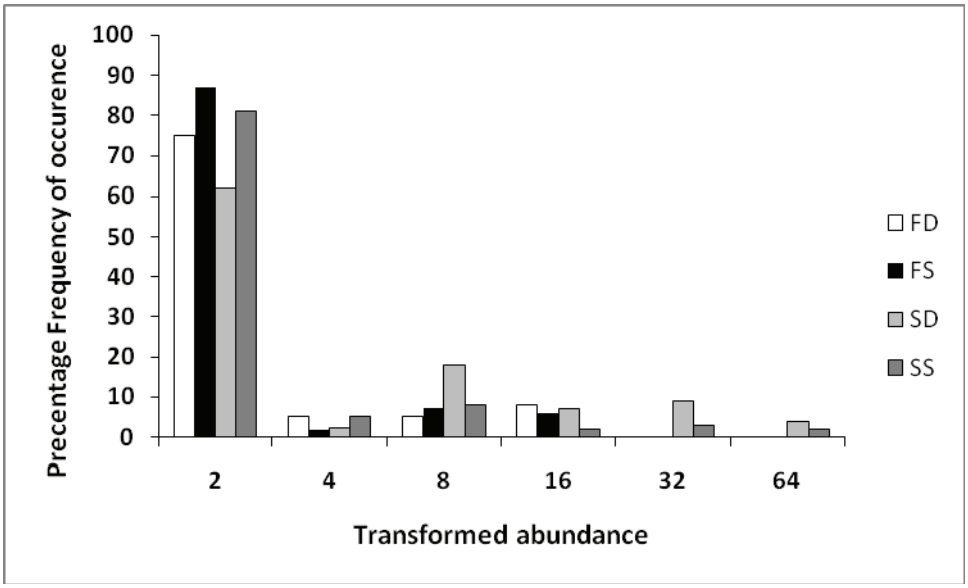


Figure 3.5.10: Frequency distribution of *Opsaridium peringueyi* in the velocity-depth grouped biotopes surveyed from April to December 2008.

Because the abundance distribution does not approach normal distributions, a non-parametric Kruskal-Wallis test was done to determine whether differences in the abundances of *O. peringueyi* in the different velocity-classes existed. The result of the Kruskal-Wallis test of 13.300 at $p < 0.004$ was highly significant for the four-level velocity-depth class classification. Although weaker, it was also significant for the eight-level biotope classification (17.5 at $p<0.015$) where substrate was added on to depth and velocity.

Figures 3.5.11 and 3.5.12 are three-dimensional histograms in which abundance and the number of replicates of each recorded incidence of abundance in the biotopes is plotted as a function of the date of sampling. In the figures the replicates are indicated as “counts”. While figure 3.5.11 includes the “absences”, which are the biotopes with no specimens, they are excluded from figure 3.5.12. In the fast-deep (FD) component of figures 3.5.11 and 3.5.12 the highest abundances were observed in April, October and November 2008.

However the replicates of all the abundances never surpassed a count of 1.2. In the slow-deep (SD) component of figures 3.5.11 and 3.5.12 the highest abundances were recorded between July and October 2008 and it is important to note that in a number of instances of replicates higher than 1.2 exceeded that of the fast-deep biotopes.

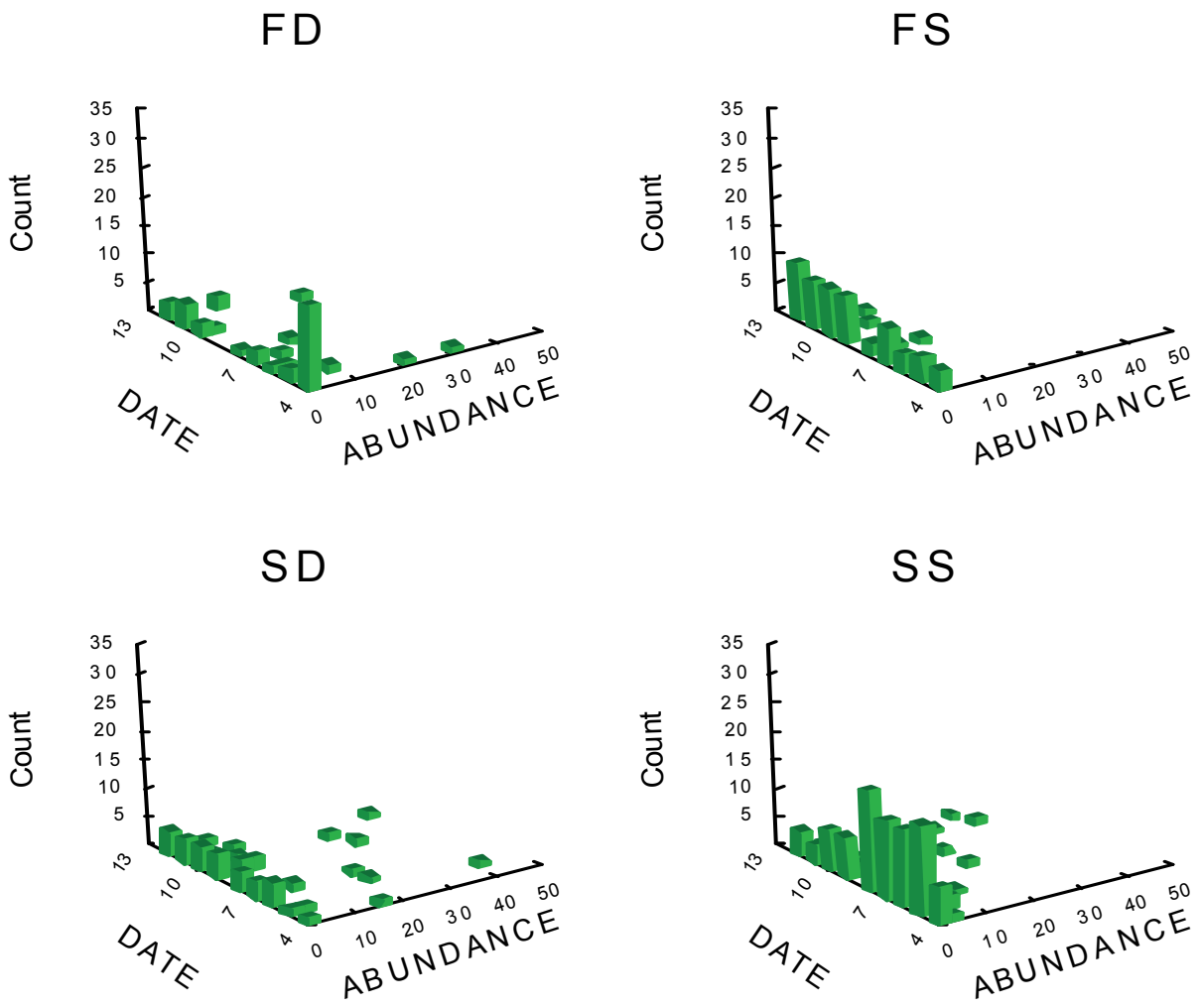


Figure 3.5.11: Histogram of occurrence and abundance of *Opsaridium peringueyi* in the Biotopes when grouped into velocity-depth classes for the period April to December 2008 with the counts on the y-axis reflecting the number of replicates of the abundance scores.

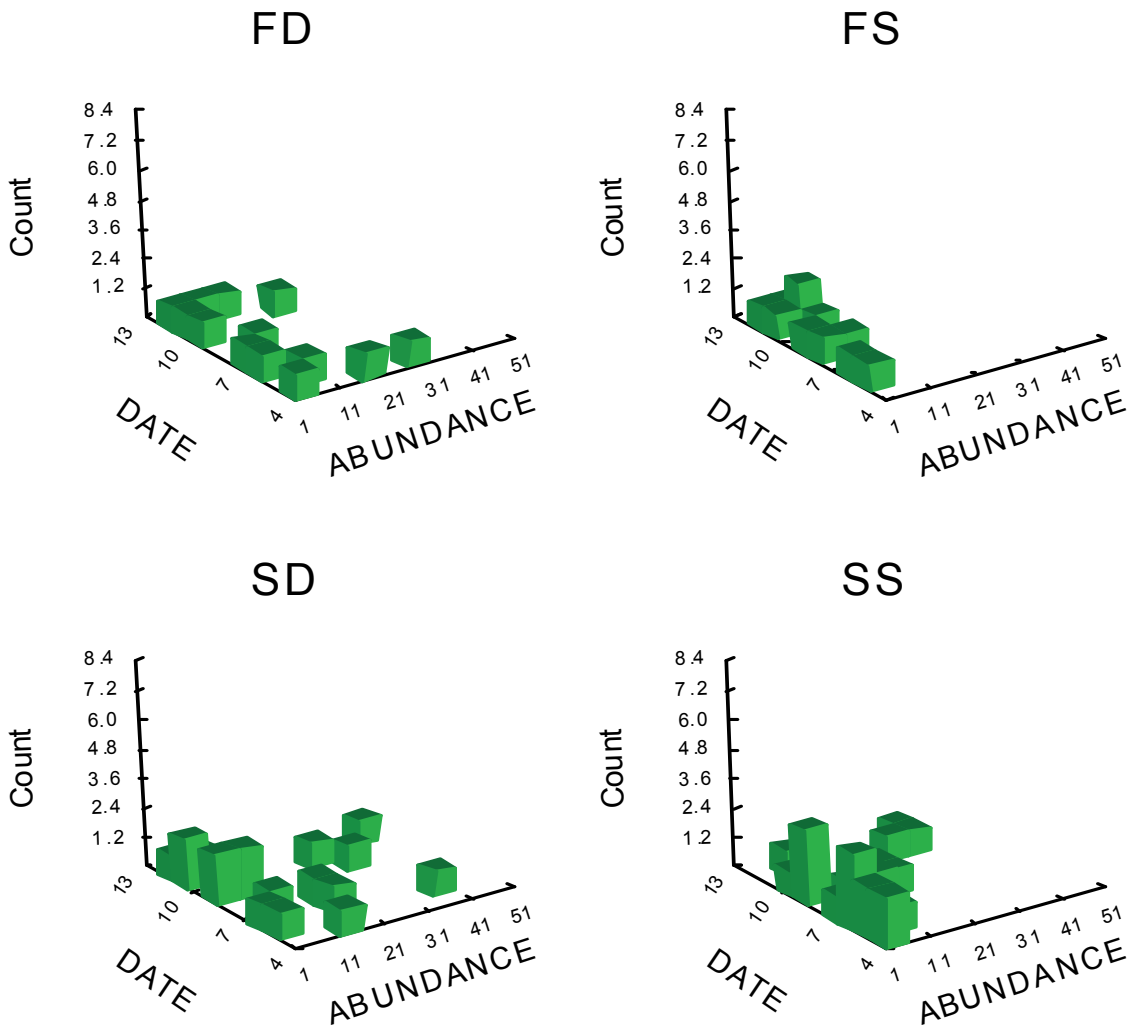


Figure 3.5.12: Histogram of occurrence and abundance (excluding "absences") of *Opsaridium peringueyi* in the biotopes grouped into velocity-depth classes for the period April to December 2008 with the counts on the y- axis reflecting the number of replicates of the abundance scores.

The results obtained in the slow-deep (SD) biotopes are of special interest with figure 3.5.11 showing that slow-deep biotopes has the lowest count of replicates of "absence". In addition figure 3.5.12 shows that the replicate count of the lower abundances increases from May 2008 onwards, indicating that the species was present throughout. The fast- shallow biotopes (FS) with the low abundance (Figures 3.5.11 and 3.5.12) and the high replicate counts, which increase from April onwards, indicate that this is the "least preferred" biotope of the species.

The slow-shallow (SS) biotopes seems to be more preferable with higher abundances recorded between July and November (Figure 3.5.12). Figure 3.5.13, where the absence or presence of sand is included in the biotope classification, shows that in the slow-deep and fast-deep biotopes with sand had the highest abundances. To a lesser extent this also applied for the slow-shallow biotopes. In the least-preferred biotopes, namely fast-shallow, the presence or absence of sand had no or little influence. Although this is the case the trends do not differ.

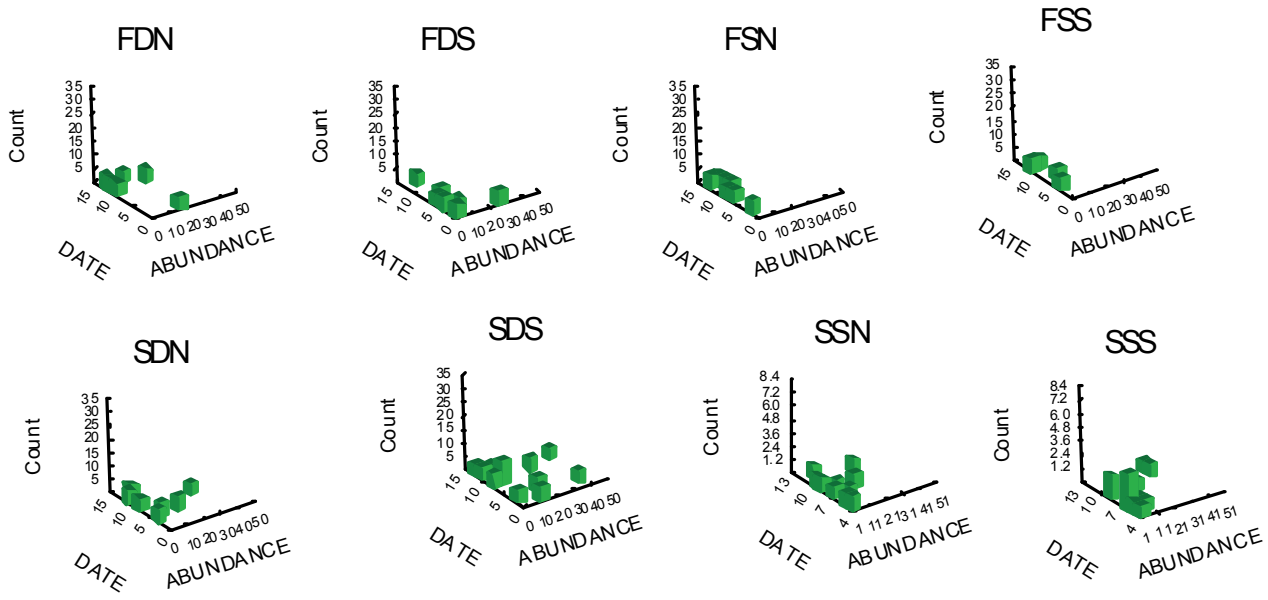


Figure 3.5.13: Histogram of occurrence and abundance of *Opsaridium peringueyi* in the biotopes grouped into velocity-depth-sand classes for the period April to December 2008 with the counts on the y- axis reflecting the number of replicates of the abundance scores. (S =sand, N=No sand).

3.5.4 CONCLUSION

The results obtained in the 2007 surveys show that in deep habitat the species form schools and that in the majority of instances schools or large groups of *Barbus eutaenia* intermingle with *O. peringueyi*. *Varichorhinus nelspruitensis* co-occur in the same habitat but it was observed that this larger sized species does not mix with the two species mentioned above. The 2007 results, and in particular the presence of schools, show that *O. peringueyi* shows a preference for deep biotopes and in the rest of the biotopes they occur as single specimens or as small groups. To an extent a

seasonal pattern was observed with the highest frequency of occurrence occurring in the slow deep biotopes at first between May to July followed by an increase from August to September.

The results obtained in 2008 confirm the 2007 results and in particular shows that the species prefer deep biotopes. In essence this agrees with the findings obtained in the “habitat” component of this project. The 2008 results, as was the case with the 2007 survey results, show that fast-shallow biotopes are the least preferred by the species with slow-shallow habitat falling in the middle category. The 2008 results also show that seasonal differences in biotope preference were apparent. Within the preferred deep biotopes the abundance in the slow-deep biotopes was high during colder months when the velocity and depth is at its lowest. During high flow periods when the water depth increased and temperatures rose, the highest abundances were recorded in the fast deep biotopes. It is important to note that it was also observed that within the preferred deep biotopes the highest abundances and replicates were observed in biotopes where sand was present. This was also applicable to the slow-shallow biotopes and agrees with the findings in the “habitat selection”(Chapter 4) component of the project.

Chapter 6: Reproductive biology

PSO Fouché and JA Venter

3.6.1. INTRODUCTION

In any conservation exercise extended knowledge of the proposed indicator species is imperative. This knowledge should include aspects such as the current status of populations, threats to the species and the biology of the species. To establish the current status and in particular to determine possible responses to environmental threats, implies that monitoring should occur. Many biomonitoring indices, such as the South African Fish Response Assessment Index (FRAI) "are assessment indices based on the environmental intolerances and preferences of the reference fish assemblage and the response of the constituent species of the assemblage to particular groups of environmental determinants or drivers" (Kleynhans, 2007). The same author in addition states that "to relate drivers and the resulting fish habitat template to the stress response of fish, the life-history requirements and environmental preferences of species must be considered". As is the case with any animal, the life history or cycle of a fish consists of a "number of periods starting at the embryo and ending in a period of senility, during which the organism slows down and eventually dies" (Nikolsky, 1963). One of the periods, namely the adult period occurs when the organism is able to reproduce, forms the focus of this component of the study. Reproduction is regarded as the link in the life cycle of a fish which, in connection with other links, ensures the continuation of the species (Nikolsky, 1963). The number of eggs produced, or fecundity, in fish is regarded as an adaptation that ensures the survival of the species (Cambray, 1992) and although fish in general have a high fecundity variation between species is considerable (Nikolsky, 1963). In addition fecundity can be increased by fractional spawning, which is when only groups of the eggs in an ovary are "ripe" at different times. A further important link in the life cycle of fish is its growth (Helfman *et al*., 2000) and the closely related dynamics of fat deposition and the condition status of the fish. Where condition refers to the mass to length ratio of the fish, fat deposition not only plays a quantitative role in the condition of the fish, but forms an important stored reserve for periods of high activity such as migration and reproduction (Shul'man, 1974).

A study of the available literature on *Opsaridium peringueyi* (Jubb, 1967; Pienaar, 1978 ; Bell-Cross and Minshull, 1988; Schulz, 1992; Marshall and Gratwicke, 1999; Schulz and Schoonbee 1999;

Skelton 2001; Gratwicke *et al*., 2003) has shown that very little detailed knowledge of the reproductive biology of the species, and in particular on the aspects listed, exist.

The aim of this component of the project was therefore to obtain detailed knowledge on aspects of the reproductive biology of the species. These included aspects such as the seasonal fat deposition and changes in condition, seasonal reproductive trends, ova counts and size distribution, body length at sexual maturity and the spawning chronology. This was to be done by collecting specimens at selected sites and analyzing these aspects.

3.6.2. MATERIALS AND METHODS

3.6.2.1 Selected sites

Originally four sites where *O. peringueyi* historical occur and where the species was collected in the distribution surveys of this project (Chapter 3) were identified as possible sites for this sub-project (Table 3.6.1). These sites were also selected because of the observed abundance of the species. After careful consideration, which included the logistical problems experienced at OPS 23, it was decided to only use OPS 27, OPS 28 and OPS 31.

Table 3.6.1: The sites suggested for the reproductive studies of *Opsaridium peringueyi.*

				Coordinates			
Site number	**Name**	**River**	**Tributary**	**DS**	**MS**	**DE**	**ME**
OPS23	Mphaphuli Cycad Reserve	Luvuvhu	Mukase	22	49.051	30	38.91
OPS27	Mutale/ Tshirovha Confluence	Luvuvhu	Mutale	22	48.911	30	23.761
OPS28	Hydro scheme	Luvuvhu	Mutshindudi	22	56.343	30	24.059
OPS31	Matumi	Sabie	Mac-Mac	25	1.403	31	0.117

3.6.2.2 Survey frequency

Each site was surveyed regularly at monthly intervals. Where OPS 31 was surveyed as part of the "fine scale habitat selection" component of the project (Chapter 3), the two other sites were surveyed specifically for the purpose of this component of the project.

3.6.2.3 Collection and preservation of fish specimens

Collection procedures were selected to correspond with habitat characteristics as proposed by Kleynhans (2007). Procedures included the use of a bagged seine net in pools and back-waters, electro-fishing in riffles and rapids and pole-seining under and amongst vegetation. All the specimens collected were identified using the key proposed by Skelton (2001) and the fork length of each *O. peringueyi* specimen was determined to the nearest millimeter on a measuring board. One or two representative specimens from each 10 mm interval fork length of *O. peringueyi* were retained, preserved in 10% formalin and transported to the laboratory. All other specimens were returned to the river.

3.6.2.4 Laboratory analyses

In the laboratory the mass of each specimen was determined to the nearest milligram and recorded and the fork length again determined to the nearest millimeter. Each specimen was dissected open with a section on the mid-ventral line from just anterior of the anal opening through the pelvic and pectoral girdles to posterior of the branchiostomal membrane (Willers, 1991).

3.6.2.4.1 Visual observation of the condition and gonadal development

In order to express the condition of the fish two aspects namely i) the scale of intestinal fat content and ii) the condition factor were considered. While the scale of fat deposition was based on the visual observation, as described below, the condition factor was calculated and the method is described later.

Observation and classification of the fat deposition

After the viscera have been exposed the fat deposits surrounding the intestines were assessed and classified according to the scale proposed by Nikolsky (1963). The scale and its criteria are shown in Table 3.6.2.

Table 3.6.2: The scale of fat deposition (Adapted from Nikolsky, 1963)

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

Observation and classification of gonadal development

In the process of preparing for spawning, the gonads and in particular the gametes within them grow and ripen or mature up to a point where the gametes are ready to be released in order to facilitate fertilization. The maturing process is cyclic and a continuum but with repeating steps in which the gametes start to develop, then grow in size after which they are discarded. This process is repeated between spawning events and the length of time between spawning events varies between species. Although the maturing process is continuous, various stages in the process have been identified. A number of scales, such as the one proposed by Nikolsky (1963) have been developed to describe the stages which represent the state of the gonads in general terms.

Table 3.6.3. The gonadal maturity classes of fish. Adapted from De Villiers (1991)

Maturity Classes		Description
1.	Virgin	Sexual organs small, both ovaries and testes are white, no eggs visible
2.	Developing	Size increase in both male and female, colour changes to cream; eggs visible. Eggs are of various sizes
3.	Ripe	Size increase in both male and female. Testes appear swollen and are cream in colour. Ovaries increase dramatically and occupy large amount or volume of abdominal cavity. Large eggs are visible.
4.	Spent	A marginal size decrease in testis, but still cream in colour, Ovaries decrease in size and in size of eggs.

Once exposed, the gross assessment of gonadal development was based on the classification proposed by de Villiers (1991) (Table 3.6.3) with the exception that is was decided to replace the terms "virgin", "developing" and "ripe" with the terms "*Latent*" (LA), "*Maturing*" (MA) and "*Mature*" (M) respectively. For the purpose of this study the latent, maturing and mature classes are also referred to as classes 1, 2 and 3. Fish that were in classes 2 and 3 was regarded as sexually mature. Class 4, which consisted of the gonads of fish that had participated in spawning events are referred to as "spent". The number of specimens in each of the visually classified gonad stages observed during monthly surveys was recorded.

3.6.2.4.2 Calculation of the condition factor and gonadal development of the species

3.6.2.4.2.1 Condition of the fish

The condition of the fish was established by calculating the body mass to body length ratio as a percentage using the formula proposed by Nikolsky (1963):

$$CF = \frac{\text{Fish mass}}{\text{Fork length}} \quad X \quad 100$$

In addition, a second condition factor that took the length relation into consideration was also applied, which in this study is referred to as the length related condition factor (LCF). This factor was calculated with the use of the formula:

$$LCF = \frac{\text{Fish mass}}{L^b} \quad X \quad 100$$

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

Although the length to mass relationship in cyprinids is often expressed as $L^b = L^3$ (Hamman, 1974; Fouché, 1995) it was decided to establish this relationship for *O. peringueyi* prior to calculating the LCF.

3.6.2.4.2.2 Seasonal reproductive trends

During dissection one of the gonads was removed, blotted dry and the mass determined to the nearest milligram. The samples were then preserved in 4% formalin for future analyses.
To establish the seasonal trends, or reproductive seasonality, a number of methods were employed:

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

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

From this the mean GSI was calculated from each collection date (Glazier and Taber, 1980).

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

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

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

3.6.2.4.2.3 Ova counts and size distribution

A sub-sample, *ca* 3 mm^3, of the preserved gonad sample was cut out and the mass was determined to the nearest milligram. This sub-sample was then placed in a vial containing 4% formalin and shaken vigorously to separate the ova and the connective tissue (Gaigher, 1976). The excess connective tissue was then physically removed with tweezers and by repeated filling and decanting with water. After separation, all the liquid was decanted and 1 *ml* distilled water added to the sample. The sample was thoroughly stirred and a 0,1 *ml* sub-sample was then extracted and placed on a microscope slide. The diameter of the ova was measured with a calibrated ocular micrometer at 400X magnification on a light microscope. A minimum of 50 ova were measured to the nearest 0,01 mm.

3.6.2.4.2.4 Length at sexual maturity

Fish with gonads in classes 2 and 3 (Table 3.6.2) were regarded as sexually mature as opposed to fish with gonads in class 1 that were regarded as immature. Length at sexual maturity was determined as the length at which 50% of the fish had maturing or mature gonads. In addition length at sexual maturity was calculated by plotting the calculated maturity coefficient as a function of fork length (Gaigher, 1969).

3.6.2.4.2.5 Spawning chronology

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

3.6.3. RESULTS

3.6.3.1 Frequency of the surveys and the number of specimens collected

Where site OPS 31 was surveyed in March, April, May, June, July, September, October, November and December 2008, sites OPS 27 and OPS 28 was surveyed from April to October of the same year. Because of the low numbers of fish collected at site OPS 27 it was decided to exclude this site from the calculations. Figures 3.6.1 and 3.6.2 show the length distribution of the specimens collected at the two remaining sites. Figure 3.6.1 shows that at site OPS 31 specimens up to a fork length of 110 mm were collected with the majority of specimens in the three fork length classes between 41 and 70 mm. Although no specimens longer than 80 mm were collected at site OPS 28 a similar distribution as at OPS 31 of specimens in the fork length classes between 41 and 70 mm were present.

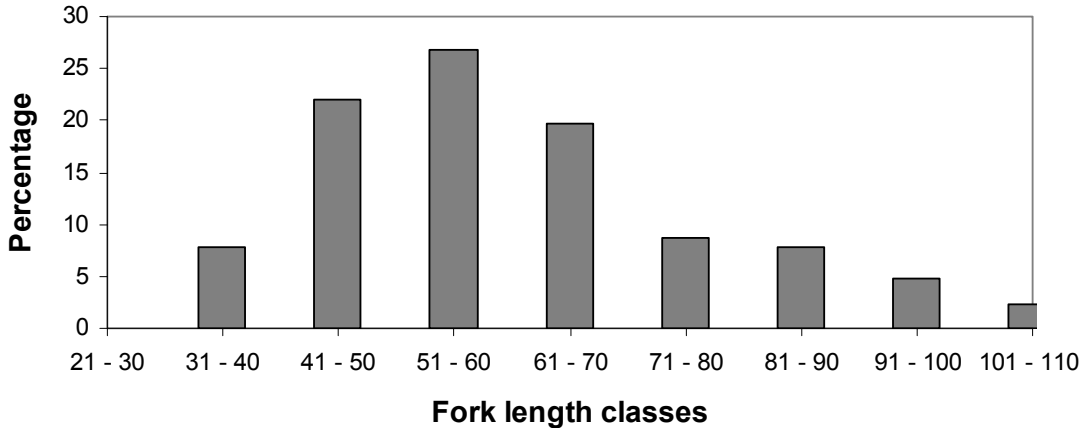


Figure 3.6.1: The number of *Opsaridium peringueyi* in the fork length classes (expressed as a percentage of the total number of specimens collected) at site OPS 31 in the Mac Mac River in the period from March to December 2008.

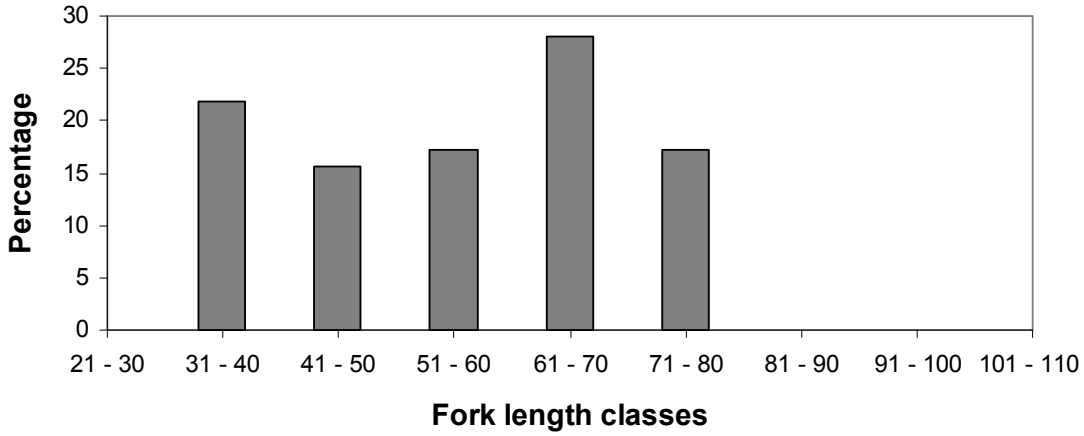


Figure 3.6.2: The number of *Opsaridium peringueyi* in the fork length classes (expressed as a percentage of the total number of specimens collected) at site OPS 28 in the Mutshindudi River in the period from April to October 2008.

3.6.3.2 The length mass relationship of the two populations

The scatter plots in Figures 3.6.3 and 3.6.4 8.3 show that the length-mass relationship of both samples was similar at the sites. The fitted trend lines show that in both cases the mass is directly related to a cube of the fork length. This relationship was then used in later calculations of the length condition factor (LCF).

When the changes in the calculated slope of the fitted trend line are considered seemingly distinct growth phases, or stanzas, could be identified in the specimens collected at OPS31 (Table 3.6.3). The first stanza consists of fish with an average fork length of less than 60 mm. The second stanza, which includes specimens between 60 and 70 mm, is distinguished by a marked increase at the average fork length of *ca* 62 mm. A similar increase in the calculated slope, at an average fork length of 73 mm, marks the onset of the third stanza. The onset of a fourth stanza is at 95 mm. A marked increase at 64 mm is also observed at site OPS 28 (Table3.6. 4).

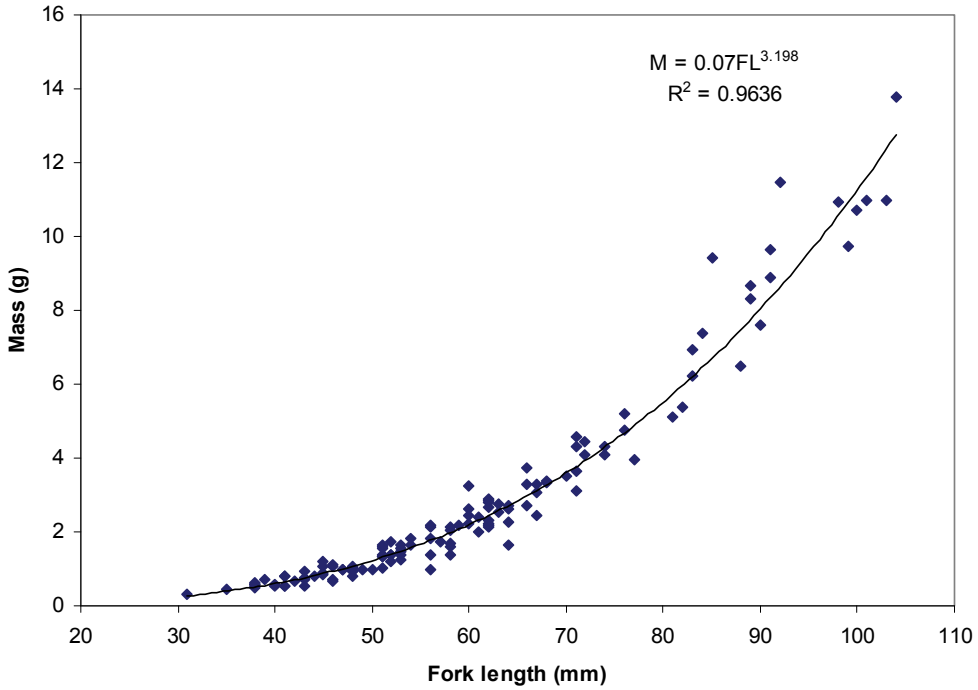


Figure 3.6.3: The length-mass relationship of *Opsaridium peringueyi* collected at site OPS 31 in the Mac Mac River.

Table 3.6.4: Calculation of the observed inclines at the inflection points observed in the fitted exponential trend line in the length:mass relationship of *Opsaridium peringueyi* collected from the OPS 31 site in the Mac Mac River

Average fork length at inflection point. (mm)	Average mass at inflection point (g)	Increase in X-axis value	Increase in Y-axis value.	Calculated slope of fitted trend line
37.5	0.54	-	-	-
45.25	0.866	7.75	0.326	0.042
54.824	1.709	9.574	0.843	0.088
62.2	2.708	9.376	0.999	0.106
73.181	4.233	8.981	1.525	0.170
85.40	7.145	12.219	2.912	0.238
95.167	10.23	9.767	3.085	0.316
102.667	11.913	7.5	1.683	0.224

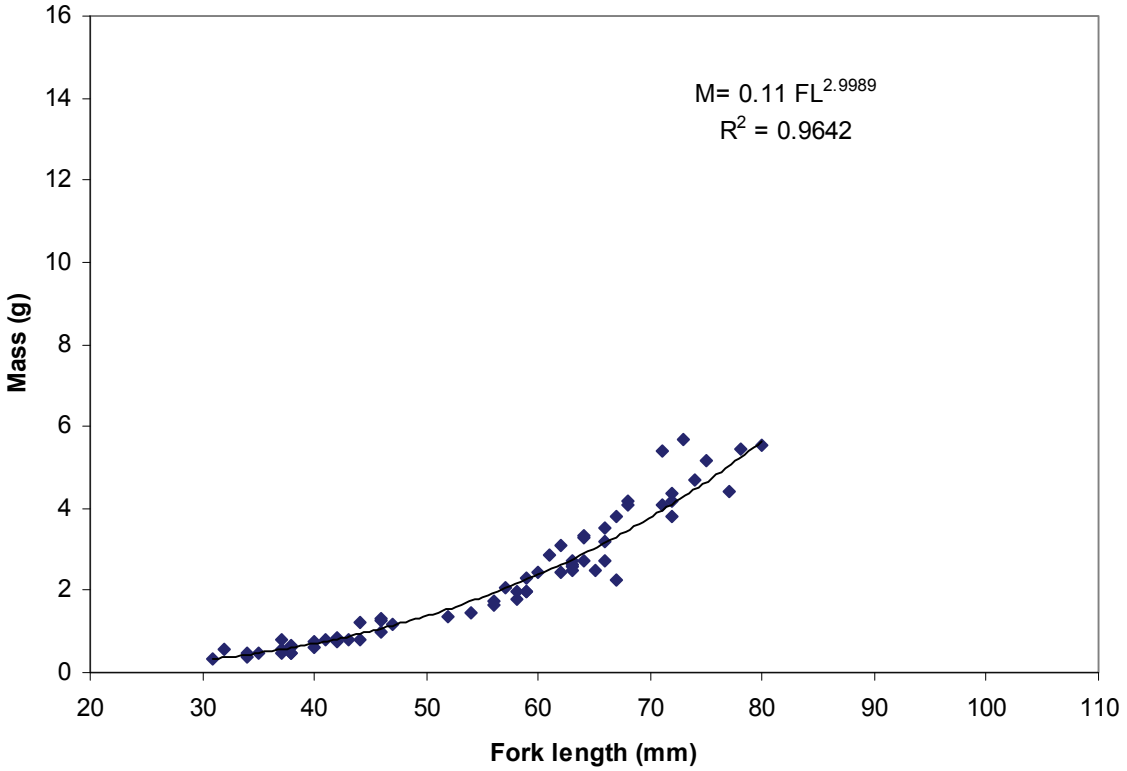


Figure 3.6.4: The length-mass relationship of *Opsaridium peringueyi* collected at site OPS 28 in the Mutshindudi River.

Table 3.6.5 : Calculation of the observed inclines at the inflection points observed in the fitted exponential trend line in the length:mass relationship of *Opsaridium peringueyi*

Average fork length at inflection point. (mm)	Average mass at inflection point (g)	Increase in X-axis value	Increase in Y-axis value.	Calculated slope of fitted trend line
36.36	0.542	-	-	-
44.1	0.991	7.74	0.449	0.058
57.09	1.878	12.99	0.887	0.068
64.556	3.0188	7.466	1.1408	0.153
74.091	4.8	9.535	1.7812	0.187

3.6.3.3 The condition of the species

Tables 3.6.5 and 3.6.6 show the results obtained with the analyses of the condition factors, the gonadosomatic index and the maturity coefficient at both sites. In the case of the LCF no distinct seasonal pattern could be observed at the two sites. The condition factor (CF) did however show a distinct increase in between July and August at site OPS 28. The relationship between the CF and the

GSI of the female specimens at this site is illustrated in figure 3.6.6 and this shows that the increase in the CF at site OPS 28 does not persist as long as the increased GSI. This is an indication that the increase in the condition factor could be attributed to aspects other than an increase in gonadal mass. This pattern is not so distinct at site OPS 31. At site OPS 31 the increase in the CF was also not as pronounced as at site OPS 28 (Figure 3.6.5).

Table 3.6.6: Calculated monthly range and averages of the condition factors, gonadosomatic index and maturity coefficients of *Opsaridium peringueyi* collected at site OPS 31 in the Mac Mac River during 2008

Condition factor (CF)									
	March	April	May	June	July	September	October	November	December
Mean	3.877	6.415	2.879	6.522	4.722	4.859	5.025	5.207	4.076
S.D.	3	3.696	0.905	3.988	2.214	2.715	2.981	2.881	2.421
Min	1.721	2.413	1.41	1.587	1.675	1.959	2.584	2.229	1.902
Max	8.771	13.24	4.629	11.153	10.71	10.651	12.467	9.758	9.753
N	9	16	15	8	19	13	15	14	19
(LCF)									
	March	April	May	June	July	September	October	November	December
Mean	1.114	1.187	0.955	0.971	0.971	0.969	1.01	1.057	1.078
S. D.	0.13	0.187	0.189	0.149	0.098	0.112	0.239	0.079	0.159
Min	0.931	1.01	0.552	0.749	0.831	0.816	0.633	0.967	0.707
Max	1.246	1.532	1.244	1.162	1.185	1.144	1.473	1.178	1.279
N	9	16	15	8	19	13	15	14	19
Gonadosomatic Index (GSI) males and females									
	March	April	May	June	July	September	October	November	December
Mean	1.418	0.929	1.169	1.428	1.491	1.404	8.187	4.477	0.866
S.D.	0.783	0.811	0.836	0.661	0.922	0.873	5.587	3.779	0.485
Min	0.901	0.082	0.387	0.668	0.179	0.339	0.715	0.722	0.254
Max	3.33	2.502	3.01	2.216	3.776	2.464	13.483	10.737	1.466
N	9	16	15	8	19	13	15	14	19
Gonadosomatic Index (GSI) females									
	March	April	May	June	July	September	October	November	December
Mean	1.076	0.991	2	1.42	2.04	2.199	9.793	6.139	1.171
S.D.	0.179	0.767	0.994	0.773	0.764	0.164	4.595	3.554	0.465
Min	0.9	0.198	0.387	0.661	1.268	2.052	1.073	1.693	0.349
Max	1.37	2.501	3.01	2.216	3.776	2.414	13.483	10.37	1.457
N	7	12	6	6	10	7	6	4	5
Maturity Coefficient (MC)									
	March	April	May	June	July	September	October	November	December
Mean	0.114	0.12	0.147	0.14	0.192	0.186	1.325	0.628	0.129
S. D.	0.019	0.096	0.01	0.067	0.007	0.072	0.233	0.356	0.051
Min	0.092	0.024	0.041	0.077	0.118	0.083	1.043	0.164	0.041
Max	0.144	0.309	0.264	0.238	0.389	0.252	1.629	1.029	0.166
N	7	12	6	6	10	7	6	4	5

Table 3.6.7: Calculated monthly range and averages of the condition factors, gonadosomatic index and maturity coefficients of *Opsaridium peringueyi* collected at site OPS 28 in the Mutshindudi River during 2008

Condition factor (CF)						
	April	June	July	August	September	October
Mean	3.409	3.239	2.639	4.228	5.739	2.662
Std Dev	1,417	1.864	2.373	1.196	1.047	1.67
Min	1.818	1.118	1.286	1.758	4.286	1.129
Max	6.338	7.949	7.605	6.147	7.794	5.253
N	7	16	7	13	10	7
(LCF)						
	April	June	July	August	September	October
Mean	1.008	1.07	1.117	1.017	1.245	1.276
Std Dev	0.096	0.12	0.192	0.149	0.107	0.246
Min	0.921	0.875	0.893	0.741	1.08	1.052
Max	1.158	1.284	1.509	1.329	1.462	1.709
N	7	16	7	13	10	7
Gonadosomatic Index (GSI) males and females						
	April	June	July	August	September	October
Mean	0.874	1.196	1.293	2.682	7.723	11.233
Std Dev	0.489	0.559	0.861	1.634	4.213	3.841
Min	0.296	0.357	0.757	1.101	0.996	8.517
Max	1.639	2.106	2.286	7.397	13.316	13.949
N	7	16	3	13	10	2
Gonadosomatic Index (GSI) females						
	April	**June**	**July**	**August**	**September**	**October**
Mean	1.178	1.352	2,286	2.526	9.753	11.233
Std Dev	0.484	0.514		0.577	2.626	3.841
Min	0.675	0.357		1.864	5.969	8.517
Max	1.639	2.106		3.756	13.316	13.949
N	3	9	1	9	7	2
Maturity Coefficient (MC) females						
	April	**June**	**July**	**August**	**September**	**October**
Mean	0.121	0.146	0.235	0.254	1.201	1.245
Std Dev	0.061	0.058		0.074	0.322	0.494
Min	0.063	0.036		0.152	0.757	0.896
Max	0.184	0.225		0.364	1.559	1.594
N	3	9	1	9	7	2

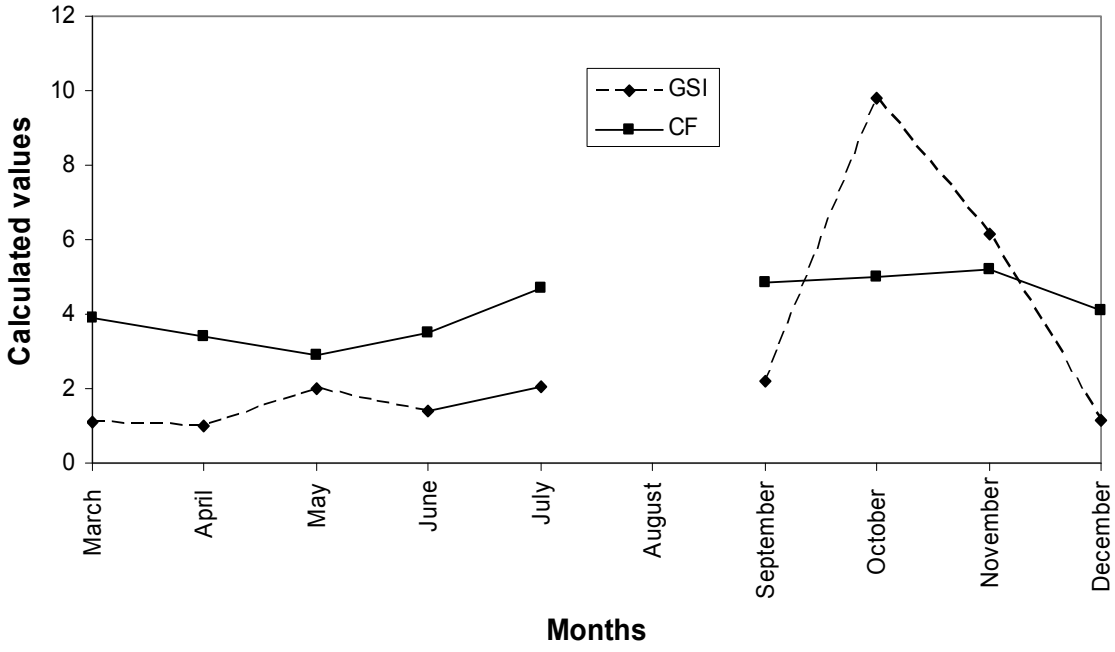


Figure 3.6.5: A comparison between the calculated GSI values of female *Opsaridium peringueyi* and he calculated condition factor (CF) of all specimens collected at site OPS 31 in the Mac Mac River.

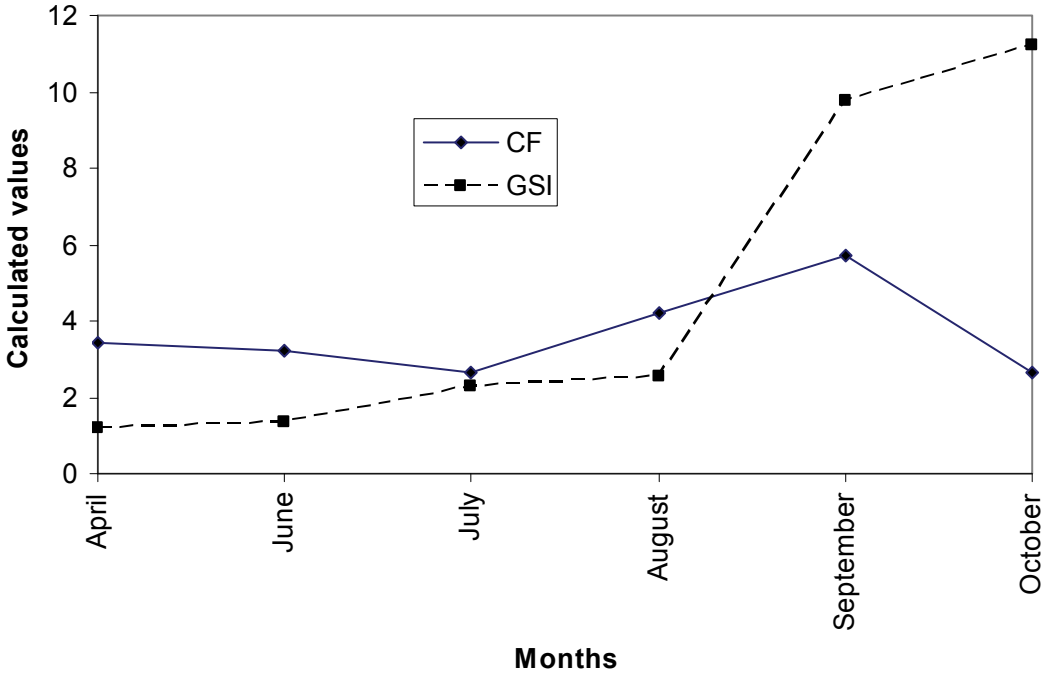


Figure 3.6.6: A comparison between the calculated GSI values of female *Opsaridium peringueyi* and the calculated condition factor (CF) of all specimens collected at site OPS 28 in the Mutshindudi River.

3.6.3.4 The sexual development of the species.

Frequency of spawning events

Tables 3.6.5 and 3.6.6 show that a pronounced increase in the GSI of the combined sexes occurred at both sites. At site OPS 31 this increase is slightly later, during October, than at site OPS 28 where it occurred during September. It should also be noted, and in particular when the GSI of OPS 31 is considered, that this increase only occurred once during the survey period. This is an indication that spawning in this species only occurs once per annum. When the GSI of the female specimens are considered (Figure 3.6.7) the difference in timing and the uni-modality of the spawning event is clearly illustrated. The slight increase observed in May 2008 at site OPS 31 is not considered as sufficient evidence of a second spawning event.

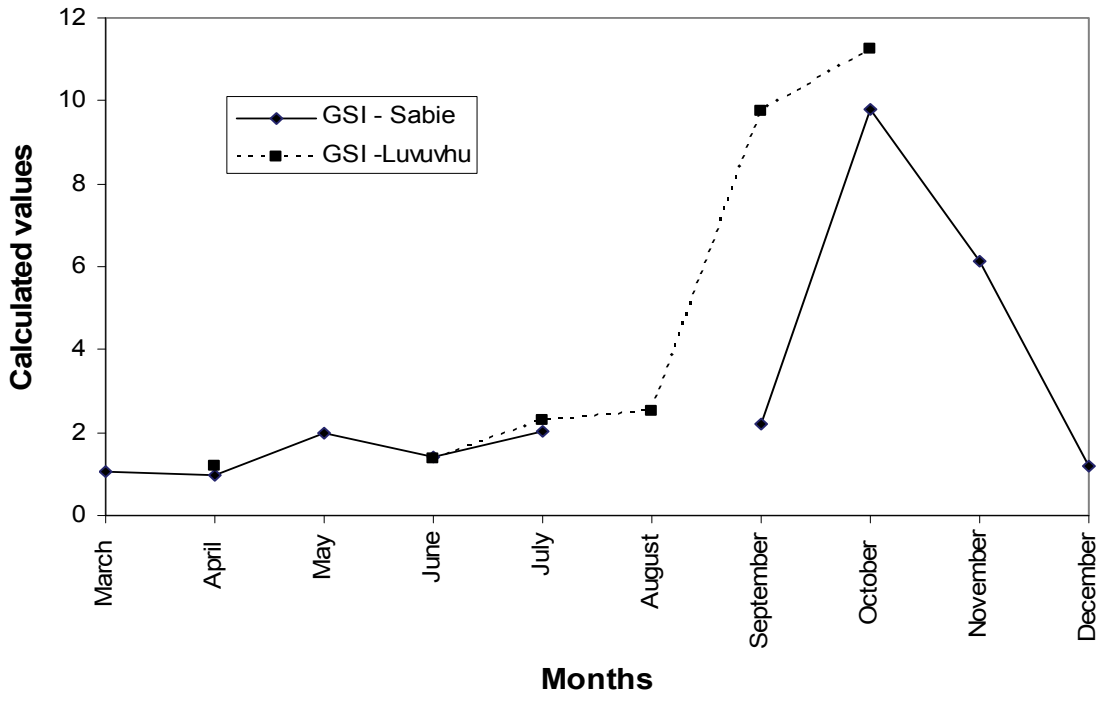


Figure 3.6.7: A comparison of the calculated GSI values of female *Opsaridium peringueyi* collected in the Mac Mac and Luvuvhu River systems during 2008. (Sabie: site OPS 31, and Luvuvhu: site OPS 28)

Length at maturity

At both sites it is observed that the point at which 50% of the females can be regarded as sexually mature is reached by specimens in the fork length class 61-70 mm (Figures 3.6.8 and 3.6.10). In the case of the females collected at site OPS 31 this length is confirmed by the marked increase in the maturity coefficient that occurs at fork lengths longer than 70 mm (Figure 3.6.9). At both sites the point where 50% of the male specimens were classified as mature occurred at a fork length between 50-60 mm (Figures 3.6.11 and 3.6.12).

Based on the above it can be accepted that females reached maturity at an approximate body length of 70 mm. Analyses of the diameter distribution and the consequent maturity classification of the ova (Figures 3.6.13 and 3.6.14) underpins the fact that females are sexually mature at 70 mm. On the other hand males are sexually mature at an approximate length of 50 mm.

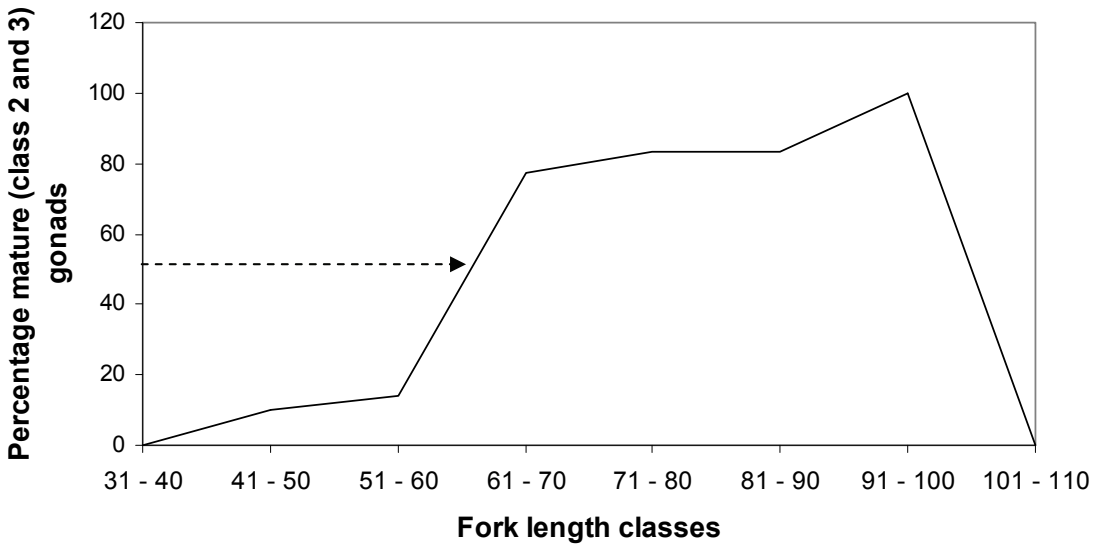


Figure 3.6.8: Percentage of mature females, based on visual gonadal classification, in the fork length classes of *O. peringueyi* collected at site OPS 31 in the Mac Mac River during 2008.

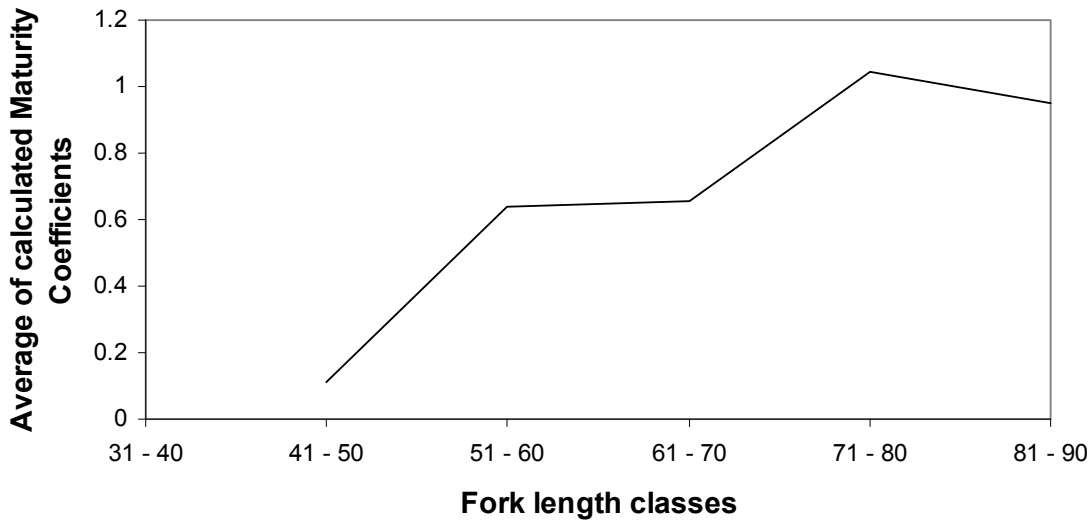


Figure 3.6.9: Maturity coefficient of the fork length classes of female specimens of *Opsaridium peringueyi* collected at site OPS 31 in the Mac Mac River during 2008.

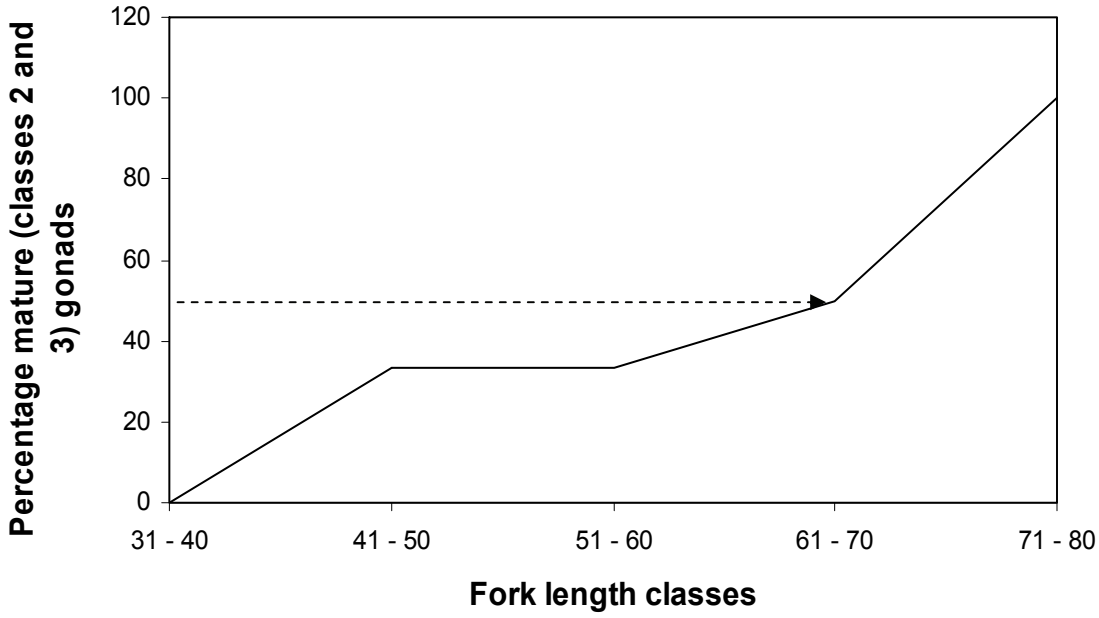


Figure 3.6.10: Percentage of mature females, based on visual gonadal classification, in the fork length classes of *Opsaridium peringueyi* collected at site OPS 28 in the Mutshindudi River during 2008.

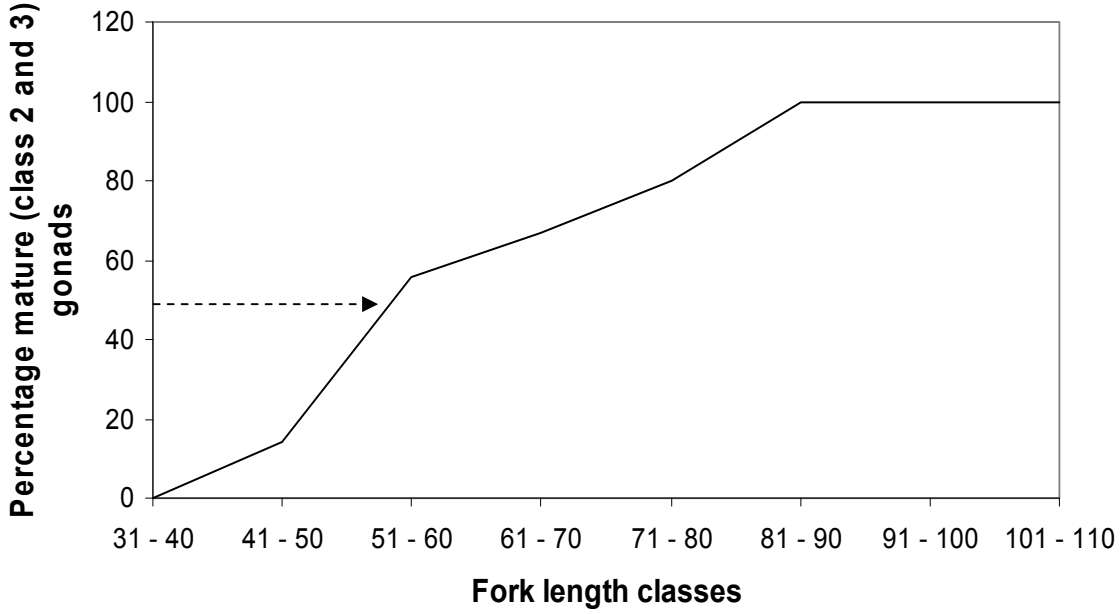


Figure 3.6.11: Percentage of mature males, based on visual gonadal classification, in the fork length classes of *Opsaridium peringueyi* collected at site OPS 31 in the Mac Mac River during 2008.

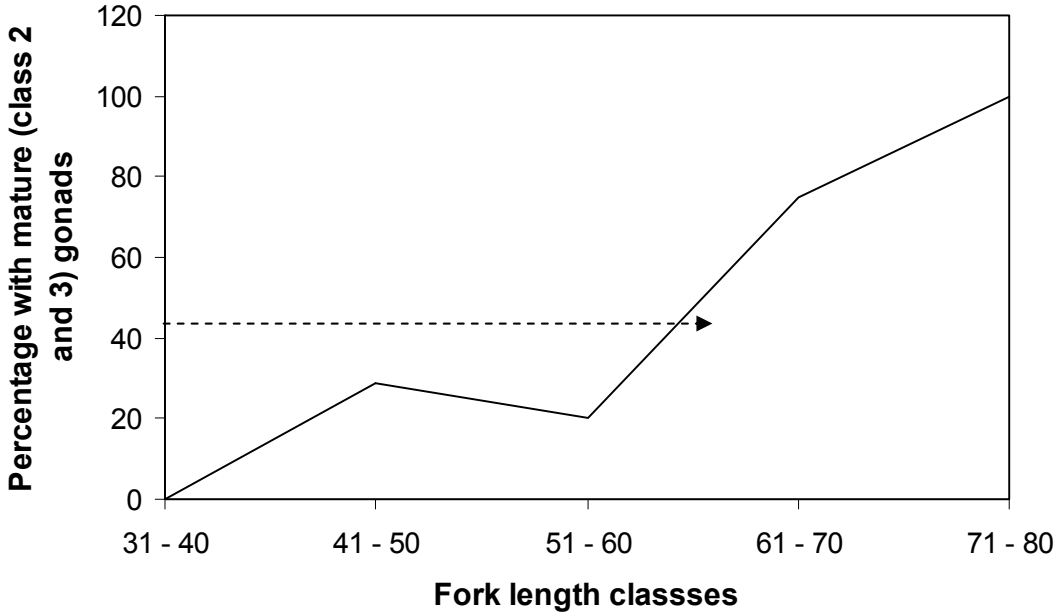


Figure 3.6.12: Percentage of mature males, based on visual gonadal classification, in the fork length classes of *Opsaridium peringueyi* collected at site OPS 28 in the Mutshindudi River during 2008.

The measured "egg" diameters ranged from 0,125 to 1,8 mm and the microscopical investigation showed distinct morphological differences between the various sizes. Based on these differences

three different egg size classes were identified. The smallest size class, from 0,125 up to 0,5 mm in diameter, consisted of cells where the nucleus could still be identified and the cytoplasm appeared homogenous. In the second size class, with diameters up to 0,875 mm, the cytoplasmic component took on a grainy appearance with distinct patches of yolk forming. In the largest size class, where eggs had a diameter in excess of 0,875 mm, the whole structure was dark and opaque and no specific structures such as the nucleus were visible. Based on the above, the eggs less than 0,5 mm in diameter were regarded as recruitment eggs with no signs of yolk development and are referred to oocytes in the text. The second group of eggs was regarded as developing or maturing ova and was in various stages of vitellogegenesis (De Villiers, 1991) with proteinaceous yolk granules forming in the cytoplasm (Helfman *et al.,* 1997). Eggs over 1 mm in diameter, which exhibited dense yolks, were regarded as the mature ova.

Figures 3.6.13 and 3.6.14 show the average distribution of the three classes of eggs within the various fork length classes. Figure 3.6.13 shows that the case of the female specimens collected in the Mac Mac River the first observation of maturing and mature eggs were in the 51-60 mm size class. It is however in the next size class, 61-70 mm, where substantial numbers of maturing and mature ova are observed. This is the same fork length class at which 50% of the females are sexually mature and this confirms that females mature at a fork length of ca 70 mm. The results obtained at site OPS 28 in the Luvuvhu River were similar.

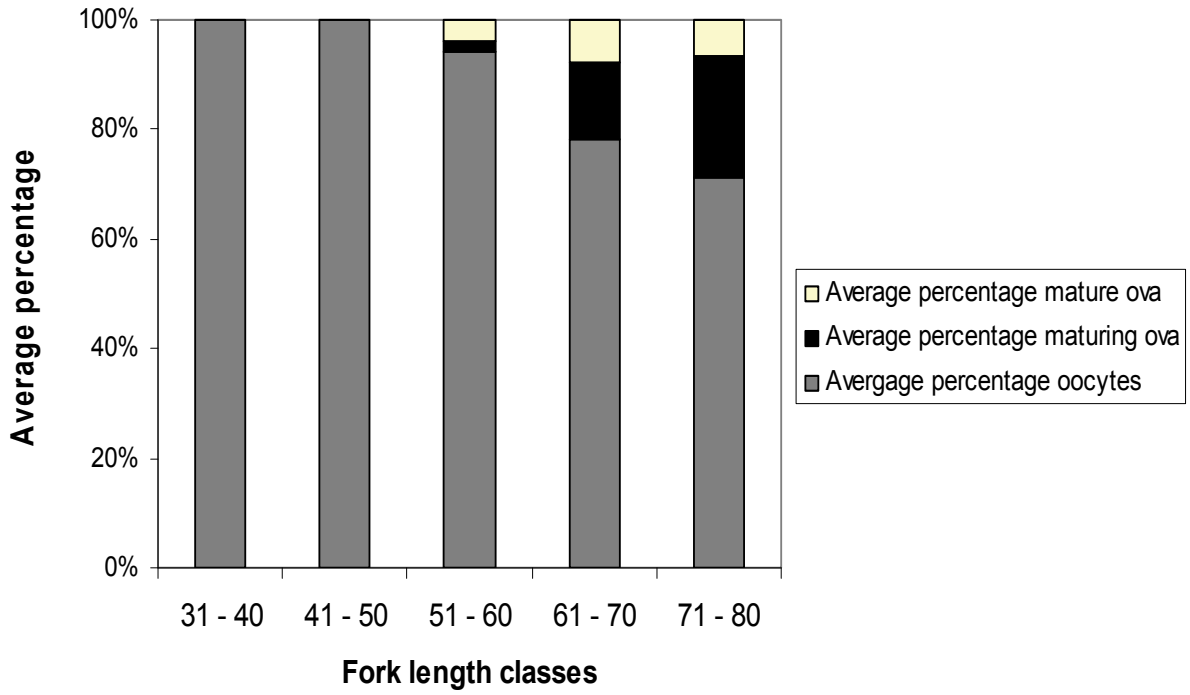


Figure 3.6.13: Average of egg sizes in the fork length classes of *O. peringueyi* collected at site OPS 31 in the Mac Mac River during 2008.

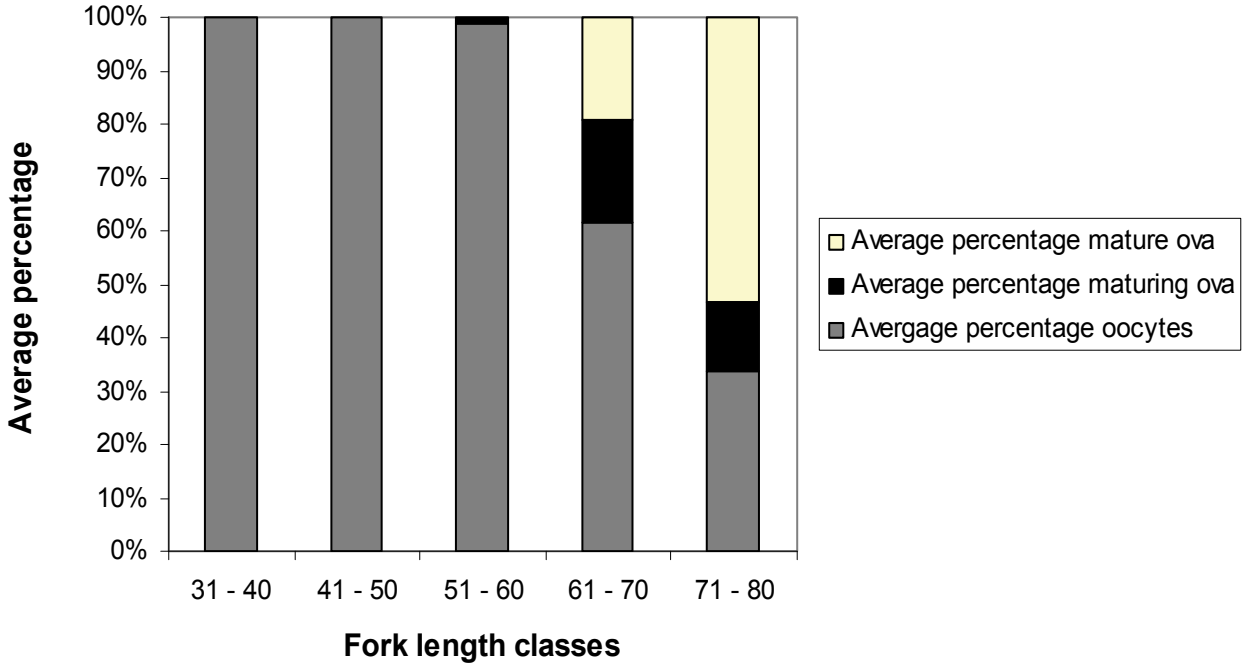


Figure 3.6.14: Average of egg sizes in the fork length classes of *Opsaridium peringueyi* collected at site OPS 28 in the Mutshindudi River during 2008.

3.6.4. CONCLUSION

From the results obtained it can be concluded that *O. peringueyi* in both the Mac Mac and Mutshindudi rivers breed only once per year during spring or early summer. This is not only underpinned by analyses of gonadal development, which includes the GSI and the maturity coefficient, but also by the distribution of the egg sizes within the maturing and mature gonads. The latter aspect to an extent could suggest that the species is a fractional spawner and that the spawning event could extend over a prolonged period. Although the measured maximum egg diameter of 1,8 mm is larger maximum diameter of 0,68 mm reported by Schulz (1992) the measurements in the current study was done on 43 females from OPS 31 and 33 from OPS 28 as opposed to a total of 15 females in the study of Schulz (1992). The size of eggs varies from fish species to species and can range from fractions of millimeters to 7 mm in diameter as observed in salmon (Nikolsky, 1963). Schulz and Schoonbee (1999) reported that in *Barbus brevipinis* egg size varied between 0,25 and 1,25 mm whereas in *B. treurensis* it varied between 0,44 and 1,15 mm. The size of ova is often regarded as a reproductive tactic of fish where larger egg and their larvae are more likely to survive than smaller ones (Duarte and Alcaraz, 1989). In larger eggs the size is related to the amount of yolk present in the mature egg which is available for the embryo. In essence it implies that the more yolk is present the longer the embryo can rely on this internal feed source, and thus grow larger in size, before switching to external feeding.

The breeding of *O. peringueyi* in September in the Mutshindudi River occurs slightly earlier than the event in the Mac Mac which occurs late in October. In both instances the increase in the GSI values are accompanied by an increase in the condition factor (CF).

Data from both river systems indicate that males, as is the case with a number of fish species are sexually mature at a smaller body size than females. In the case of the males the indicative 50% of individuals that are sexually mature are observed before the specimen is 60 mm in length. In the case of the females it occurs when the specimens are approximately 70 mm long.

Chapter 7: Artificial breeding.

NAG Moyo and S Theron

3.7.1. BREEDING PROTOCOL

3.7.1.1 Introduction

The captive breeding of *Opsaridium peringueyi* poses some serious challenges because of the paucity of the information on its basic biology. *O. peringueyi* is a cyprinid, and a number of studies have been carried out on the breeding biology of some cyprinids in Southern Africa (Hamman, 1974). Roux (2008) focused on the reproductive strategy of *Labeobarbus polylepis,* which belongs to the collective group of species known as "yellowfish" in South Africa. The main objective of this part of the project was to develop a protocol for the artificial breeding of *O. peringueyi.*

3.7.1.2 The experimental set-up

The main approach was to create a breeding environment which simulates the natural habitat, as was described in chapter 4 of this project, in which *O. peringueyi* occur. The system must be able to simulate natural seasonal abiotic conditions but must also make provision to regulate flow, depth and temperature. The manipulation of these factors stimulated the onset of breeding condition. A great deal of experimentation was done on different systems and only the most successful applications are discussed in this report.

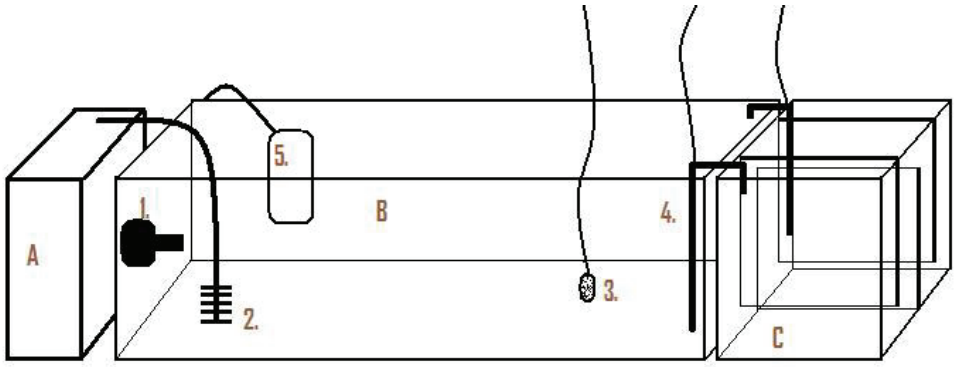
The final breeding set up consisted of the following components:

- Brood stock housing tanks (Figure 3.7.1)
- Conditioning tanks for the selected brooders (Figure 3.7.2).
- Spawning tanks (Figure 3.7.3).
- Nursery tanks.

In total the system consisted of two brood stock housing tanks, three conditioning tanks, six spawning tanks and a ten nursery tanks.

a) Brood stock housing tanks

As is shown in figure 3.7.1 this component consisted of a 250 litre glass aquarium with an attached gravel filter (Figure 3.7.2) through which water quality could be maintained. While the air stone provided additional oxygen the temperature could be lowered with the adjoining chiller. Within the total breeding system two brood stock housing tanks were employed.



A - ELECTRICAL CHILLER
B - 250L GLASS AQUARIUM
C - GLASS PARTITIONED GRAVEL FILTER

1. POWERHEAD
2. CHILLER PROBE
3. AIRSTONE
4. AIR DRIVEN IN- AND OUT FLOW PIPES
5. UV LIGHT FILTER

Figure 3.7.1: Technical layout of the brood stock housing tanks.

b) Conditioning tanks

Selected brooders were conditioned in rectangular 350 litre fibre-glass tanks (Figure 3.7.3). The tanks were housed in a climate controlled fish breeding green house at a mean room temperature of 23^{o} C. Water temperatures were maintained at approximately 22 ^{o} C. Each tank was equipped with an air venturi power head to ensure adequate aeration and flow. Water was circulated through the under gravel biological filter, filled with coarse gravel filter medium, by a submersible pump or power head.

c) Spawning tanks

The tanks in which spawning occurred were small, 100 litre, glass aquaria (Figure 3.7.4) in which a spawning bed was constructed in a tray that contained breeding substrate. In addition flow was directed onto the spawning bed by a power head. A heater was fitted to control the water temperature and an air stone provided additional oxygen. Water quality was maintained by circulating the water through an exterior canister filter.

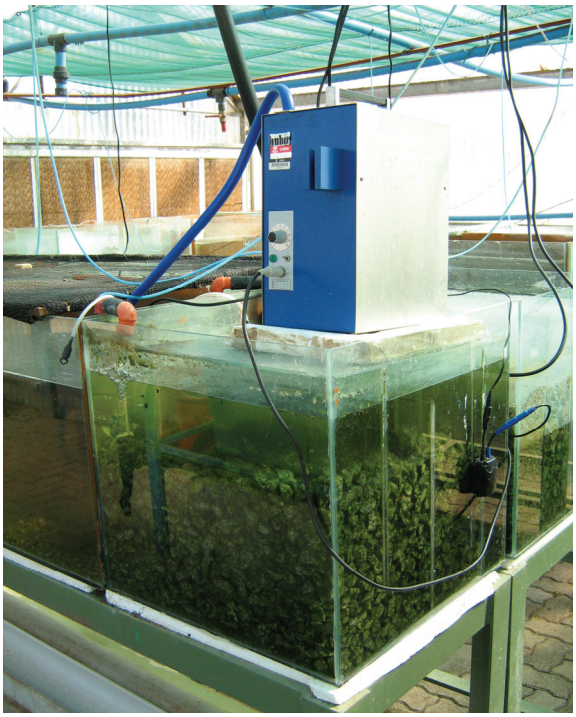
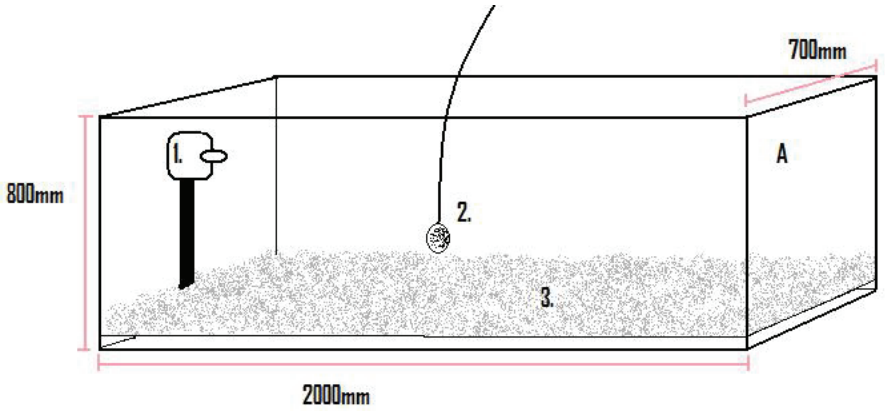


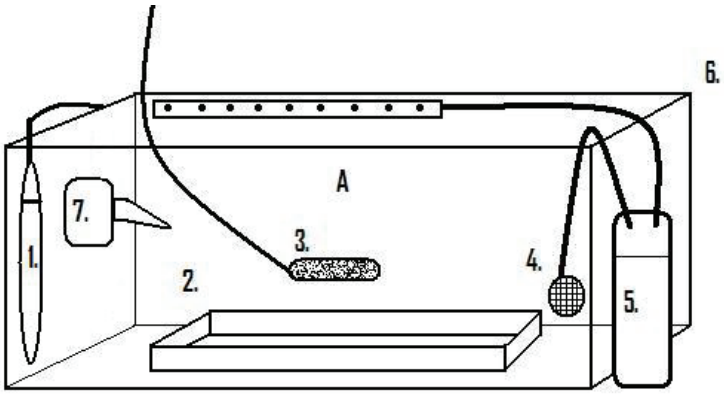
Figure 3.7.2: The gravel filter and chiller utilized in the brood stock housing.



A - 350L Fibreglass tank

1. Air venturi Powerhead.
2. Large airstone
3. Under gravel filter - recurculated by Powerhead.

Figure 3.7.3: Technical layout of the conditioning tanks.



A - 100L glass aquarium

1. Heater
2. Spawning tray
3. Airstone
4. Banjo (Prevents fry/eggs from entering filter).
5. Recurculating canister filter.
6. Cover glass (Insulater)
7. Powerhead (Adjusted to spread flow over spawning tray).

Figure 3.7.4: Technical layout for spawning the tank.

The nursery tanks

These tanks were small 20 litre sterilized glass aquarium fitted with an aged sponge filter, thermostat heater, air pump and a small enclosed UV light.

3.7.1.3 The breeding process

Up to a maximum fifty specimens were kept in each brood stock housing tank to ensure a ready supply of healthy brood fish in non-breeding condition. The fish were allowed to acclimatize for a period of two weeks. They were fed a starter feed of a mix of mosquito larvae, water beetles and daphnia at 5% of the fish body weight per day. A precautionary medicinal dose of Malachite Green at 0,05 ml per 100 litres water was given on day 2, 4 & 11 after initial stocking to prohibit any transport-stress related diseases. Fish showing early symptoms of disease were removed, quarantined and subsequently treated. White spot and fungus were the main fish diseases that were noticed.

Specimens were maintained in non-breeding condition by lowering water temperatures to 18 ˚C. It also appeared as if the lower water temperatures also ensured that the large number of males in the aquarium were more tolerant towards each other.

Water quality was maintained by airlifting water through the large partitioned gravel filter boxes (Figure 3.7.2) which provided sufficient biological filtration. An 8 watt UV-light filter was employed to limit bacteria build up. The power head was utilized to ensure flow and an air stone, driven by a commercial blower, maintained oxygen levels. According Skelton (1986) both flow and high oxygen levels are characteristic of *O. peringueyi* habitat.

Potential brooders were selected based on specimen size and transferred to the conditioning tanks. The brood fish were stocked at a ratio of 2 males per 4 females per tank. The reasoning behind this was based on personal observation which indicated that when two males were present they would change to breeding coloration (Figure 3.7.5) and start displaying aggressive behaviour towards each other for territorial space in the tank. In addition they would start attracting females into their selected area. It is postulated that this behaviour also serves as a breeding cue for the females.

Figure 3.7.5: Male breeding coloration.

Correct feeding is absolutely crucial to induce breeding and the main feeding strategy was to feed mainly live food to the fish, as *O. peringueyi* exclusively feeds on invertebrates as shown by Fouché and Gaigher (1998). The food composition consisted mainly of the following: brine shrimp, water fleas, blood worm, mosquito larvae, juvenile earth worms and small water beetles.

Most of the live food were harvested from six 5 000 litre circular plastic culture dams using plankton collecting nets with a 123 micron mesh size. The culture dams were enriched by adding chicken manure to facilitate the growth of phytoplankton which in turn served as a food source for zooplankton and aquatic invertebrates. Enrichment of the ponds was staggered, with two ponds being enriched simultaneously, to ensure a continuous supply of food. The invertebrate component showed peak production three weeks after adding the manure and then afterwards indicated a steady decline as the cycle loses nutrients (Schroeder, 1980). The culture dams were enriched at a ratio of 10 kg chicken manure to 5000 litres of water. The manure was placed in bags made out of 40% shade cloth netting to ensure a slow release of nutrients.

The fish were fed twice a day, at approximately 5% to the fish's body weight per feeding, with the bulk of the food supplied in the morning. This has been shown by Fouché and Gaigher (1998) to be the preferred foraging time for *O. peringueyi*, through studies done on sampling stomach contents during different times of the day. According to Ingram et al. (2007) feed can also be given until satiation as the breeding form improves, enlivening the environmental cue of plentiful foraging.

Spawning behaviour was manifested when males started chasing the females. After approximately two weeks of maintaining the water temperatures at 22^{o}C and controlled feeding, the abdomens of females noticeably appeared swollen. This was an indication of increased gonadal development. In

addition the females were weighed at five day intervals and an average daily increase of 0.050 mg after three weeks corresponded with the swollen abdomens.

At this point the breeding specimens were transferred to the spawning tanks and stocked at a ratio of 1 male per 2 females per tank. This decision was based on prior experience where larger tanks had very erratic results. In the smaller tanks it was possible to monitor and manipulate the breeding cue process more accurately and to observe the breeding behaviour of the fish more closely. Although the tanks were housed in a climate controlled room, the main features of each tank are important and consisted of: a) A heater to maintain water temperatures at 22 ˚C, b) a spawning bed tray with suitable substrate (1/3 aquarium gravel & 2/3 washed river sand), c) a Banjo which is an apparatus used in the commercial aquarium trade and which prevents eggs/fry from entering recirculation filtration systems and d) an air venturi power head which is a submersible pump with a fully adjustable nozzle, to direct flow directly on to the spawning substratum.

In order to simulate natural events that stimulate breeding the water of each tank consisted out of 1/3 original conditioning tank water and 2/3 rain or aged tap water. This resulted in the tank water hardness and conductivity measures; falling much lower than the original conditioning tanks water. This simulates the natural cue of rain water influx into the river system.

The following conditions were found to be the most suitable for artificial breeding of *O. peringueyi* and produced the best results: a pH of between 6.5 and 7.5, water with a hardness of 60 -150 dpm, a temperature of 22^{o}C, a flow velocity of 0,2 to 0,3 ms^{-1} directed onto the breeding substrate and a breeding substrate that consisted of a mixture of sand and gravel.

Spawning was therefore induced through simulating the natural conditions found in the wild during the breeding season. Once spawning started to occur two approaches were available:

(i) The first approach was that brood fish were left to spawn naturally in the tank and then removed after the spawning ritual has been completed. The eggs were then left to hatch within the tank after which they were removed and placed in nursery aquarium.

(ii) The second approach was to strip the eggs from females and artificially fertilize them (The process is discussed in 7.1.4).

After spawning had occurred, the brood fish were returned to conditioning tanks and the tank with the eggs covered to ensure complete darkness.

Following the general principles of egg incubation the water was renewed in order to provide oxygen and after hatching the new born larvae was separated from the remaining egg-shells and dead eggs. The latter is of utmost importance in order to avoid fungal infections of hatchlings and consequent larval mortalities. Hatching occurred within approximately 48 hours.

Figure 3.7.6: *Opsaridium peringueyi* larva approximately 24 hours old and 1,9 mm in length.

When the larvae hatched (Figure 3.7.6) they displayed a constant burrowing action which seems to be aggravated by light and *vice versa.* The first swimming action was observed after 3 days and at day 5 the post-natal yolk sacs had been absorbed. From this point they are then fed as a starter feed on cultured infusaria. The infusaria was cultured by placing pond water and a few lettuce leaves in a jar. After four days the water in jar turned cloudy indicating that a population boom had occurred and that a productive culture was present. One culture maintained in a one litre jar, will be more than sufficient for one spawning. The hatchlings were fed by extracting the cloudy culture water with a syringe from the jar and squirting it into the nursery tank in close proximity of the fry. As the larvae increased in size they fed on *Artemia* larvae and micro worms (*Anguillilula silusae*) cultured and separated using the method described by Parameshwaran *et al*. (2001). According to Kaiser et al. (2003) live foods should produce a higher survival and growth rates and another option that may be looked at is to feed these juveniles on a mixture of processed and live foods.

As far as general aquarium maintenance is concerned the following was regarded as important: a) The aquarium water was monitored daily for pH, dissolved oxygen levels, total dissolved solids and ammonia, b) The banjos were cleaned daily. c) Any waste build-up, such as unfertilized eggs, egg shells, unutilized food and dead fry were siphoned off on a regular basis as when required.

3.7.1.4. Artificial breeding

As soon as the first spawning behaviour was observed in breeding pairs the specimens were closely observed to determine whether females displayed inflamed genital papillae. The fish were then removed and the female stripped for eggs. The female was anesthetized by placing in 5 litres of water in which 1 ml of 2-Phenoxyetanol had been dissolved. The stripping of the female spawners was then carried out by gently pressing the abdomen with the thumb from the pectoral fin towards the genital papillae.

Males could not be stripped and consequently the sperm could only be obtained by sacrificing a male. The male was killed and the body surface thoroughly dried. The testis was then dissected out, placed in a Petri dish and cut into small pieces using a scalpel. The milt was then is pressed out and diluted with 10 ml saline solution. This solution was and an equal volume of aquarium water then added to the stripped eggs in a Petri dish. Mixing was achieved by gently shaking the Petri dish with a "quivering" action. The eggs were found to be slightly adhesive but the adhesiveness was lost after 60 seconds of stirring and rinsing with aquarium water. After about 4 minutes, which is the adjudged time for fertilization to occur and the sperm to lose activity, the fertilized eggs were then deemed ready for incubation and placed in the nursery tank. The eggs and consequently the hatched larvae were kept in low light conditions as exposure to direct sunlight or fluorescent light can kill the developing larvae.

Hormone induced propagation was done in order to ascertain the effectiveness on the species and to compare the difference in production between natural and induced breeding. For hormone induced reproduction *Choluron* and *Aquaspawn* were used. Each fish were injected under the dorsal fin using with 0, 02 ml of the desired hormonal inductive agent. Males were injected at least five hours prior to the female fish. After approximately 10 hours female readiness were checked by lightly pressing on the abdomen to release the eggs. The process of final maturation and ovulation cannot be stopped or reversed after administration of the correct hormone dosage. Once these processes start the eggs were stripped and the milt manually removed. The eggs were then fertilized as described above.

3.7.2 FACTORS AFFECTING REPRODUCTION

3.7.2.1 Introduction

Reproduction of cyprinids is of interest because of the worldwide importance of this fish. Cyprinids are prized as food in Japan, China, India and many European countries. In North America, the acceptance of cyprinids as food is slow. There is an interest in the ecology and physiology of cyprinids such as shiners, minnows and chubs. This is because piscivorous game fish, such as salmonids, pikes and bass, feed extensively on these species (Munro *et al.*, 1990).

Reproduction in cyprinids is cyclical and timed to ensure maximum survival of the young. It is difficult to make generalizations about the reproductive cycles and the timing of spawning of the entire family Cyprinidae because its members have a cosmopolitan distribution (Munro *et al.*, 1990). The onset of spawning in various cyprinids species in the temperate zones ranges from early spring to late summer. Many species start their gonadal recrudescence in the fall, mature their gonads throughout winter and complete the final maturation stages in early spring. Goldfish, *Carrasius auratus*, follows this pattern and spawn in late spring to early summer. The European common carp exhibit yet another pattern. Spawning usually occurs in early summer, recrudescence is initiated immediately and gonadal growth is complete in 6 to 8 weeks. The gonads are then in a resting state throughout winter, and the final maturation stages are completed in early spring before spawning (Munro *et al.*, 1990).

In more southerly latitudes, many cyprinids have protracted spawning seasons that are associated with the rainy season of the year, especially in arid zones. Extended breeding seasons have been reported for several South African cyprinids such as *Barbus anoplus, Labeo capensis* and *L. umbratus* as well as *Cyprinus carpio*. Species that have extended spawning seasons are usually multiple spawners, individual females producing several clutches of eggs. It is difficult to predict the pattern of the reproductive cycle of a fish from latitude alone since even within the same water system fish do not necessarily follow the same reproductive pattern (Munro *et al.*, 1990).

Generally cyprinids require flowing water in the rivers to reproduce. This is supported by Roux (2008) who studied the reproduction strategy of the smallscale yellowfish (*Labeobarbus polylepis*). He observed that man-made effects restricted preferred breeding conditions for the *L. polylepis*. The anthropogenic effects disturbed flow regimes which impacted on the environmental cues that are a

pre-requisite for successful reproduction. A definite spawning behaviour pattern observed during his study only occurred when environmental cues (daylight length, water temperature and constant low flow) coincided with optimal habitat requirements for spawning. He also observed that stream velocity, depth, substrate composition and layout of spawning beds suggest that this species is highly selective in terms of its habitat requirements for breeding. Roux, 2008 concluded that *L. polylepis* is a highly specialized breeder that is sensitive to river regulation.

On the other hand, habitat alteration and deterioration appears to be the major threat affecting the status of populations of *O. peringueyi* species. Many formerly perennial rivers of the Transvaal Lowveld now stop flowing during the dry season due to impoundment and extraction of water for agricultural purposes. Turbidity and siltation through soil erosion from overgrazing and crop cultivation occurs when the rivers start flowing again at the beginning of the rainy season. Turbidity and siltation probably affect the sight, feeding and breeding success of the species (Skelton, 1987).

In Malawi, the breeding success of a related species is adversely affected by siltation. The construction of many weirs and dams in the rivers of the eastern Transvaal, Swaziland and Natal is a major obstruction to fish movement. Other threats to this *Opsaridium* species are industrial and agricultural pollution as well as the choking of such rivers by the alien plant *Eichhornia crassipes* (Skelton, 1987).

Few studies have been undertaken on *O. peringueyi* yet but it has already been on the IUCN red data list. There is evidence that the geographical range of this species has shrunk (Skelton, 1993) therefore detailed studies on O. peringueyi are needed to conserve this species. This study investigated flow rate, temperature, conductivity and substrate to determine if they have any influence on the reproduction of *O. peringueyi*. The environmental stimuli triggering reproduction in *O peringueyi* is not known. This study is an attempt to identify and quantify the environmental cues important in the reproduction of *O. peringueyi*.

3.7.2.2 Objectives

- To determine the effect of flow rate on reproduction of *O. peringueyi.*
- To determine the effect of substrate on reproduction of *O. peringueyi.*
- To determine the effect of conductivity on reproduction of *O. peringueyi.*
- To determine the effect of temperature on reproduction of *O. peringueyi.*

3.7.2.3 Null hypothesis

- Flow rate has no effect on reproduction of *O. peringueyi*.
- Substrate has no effect on reproduction of *O. peringueyi.*
- Conductivity has no effect on reproduction of *O. peringueyi.*
- Temperature has no effect on reproduction of *O. peringueyi.*

3.7.2.4 Materials and methods

The *O. peringueyi* used in this project were obtained from site OPS 31 in the Mac Mac River. The temperature variations in the river ranged from 15 to 21°C and water was taken directly from the river into the plastic tank used for transportation. Ice was added into the tank every one and half hours to maintain the temperature at 17°C and also to supply sufficient oxygen because oxygen dissolves easily in cold water. The fish were then transferred into 250 litre brood stock housing tanks at the Aquaculture Research Unit of University of Limpopo Turfloop campus to acclimatize them for the conditions of captivity. The actual experiments were performed in the 100 litre spawning tanks. Four experiments were performed with the first one investigating flow rate, the second one investigating temperature, the third one investigating conductivity and the fourth one investigated substrate to determine if the mentioned factors have an influence in the natural reproduction of *O. peringueyi*.

Three tanks were set aside to investigate the effect of flow rate on natural reproduction of *O. peringueyi*. These tanks were labelled 1A, 1B and 1C. There was no flow in tank 1A, tank 1B had a low flow rate of 0.8 m/s while tank 1C had a strong flow rate of 0.28 m/s. These different flow rates were achieved by putting one VA 250B submersible pump in tank 1B producing a flow rate of 0.8 m/s while two of the submersible pumps were put in tank 1C to produce a flow rate of 0.28 m/s. The flow rates were measured with a PS 2000 Pasco flow meter. All the three tanks had a temperature of 21°C, standard conductivity of 0.783 µS/cm and a substrate of 2/3 sand and 1/3 gravel.

Three tanks were also set aside to investigate the effect of temperature on natural reproduction of *O. peringueyi*. The three tanks were labeled 2A, 2B and 2C. Temperatures of 18, 22 and 25°C were maintained in 2A, 2B and 2C respectively. A chiller was used to drop the temperature in tank 2A to 18°C while thermostat heaters were used to maintain the temperature of 2B and 2C at 22°C and

25°C respectively. All the tanks had flow rate of 0.23 m/s, standard conductivity of 0.783 µS/cm and a substrate of 2/3 sand and 1/3 gravel.

Conductivity was the third factor investigated to determine its influence on natural reproduction of *O. peringueyi*. Two tanks which were labeled 3A and 3B were used for this experiment. Tank 3A had a standard of conductivity 0.783 µS/cm made up of only aged tap water while tank 3B had altered conductivity of 0.291 µS/cm, i.e. 1/3 aged tap water and 2/3 rain water. A flow rate of 0.23 m/s, a temperature of 21°C and a substrate of 2/3 sand and 1/3 gravel were similar in all the 3 tanks

Substrate was the last factor investigated to determine if it had an influence in the natural reproduction of *O.* peringueyi. Three tanks labeled 4A, 4B and 4C were set aside for this experiment. Tank 4A had a substrate of ⅔ sand and ⅓ gravel, tank 4B had a gravel substrate while tank 4C had no substrate. All the 3 tanks had a flow rate of 0.23 m/s, a temperature of 21°C and a standard conductivity of 0.783 µS/cm.
In each of the tanks used to investigate flow rate, temperature, conductivity and substrate if they influence natural reproduction of *O. peringueyi*, an external canister filter 2211 and glass lids were used. The filter was used to extract excess debris in the water as well as re-circulating the water to provide additional oxygen while the glass lids were used to stop the fishes from jumping to the outside.

In each experimental tank, the *O. peringueyi* were stocked at a ratio of one male is to one female. The males had a mean length of 96 mm and mean weight of 11 g while the females had a mean length of 74 mm and mean weight of 5.25 g. The breeding pair was fed with a mixture of natural food to condition them. The mixture of the natural food included Chironomidae, Simuliidae, Culicidae and Dytiscidae.

The experiments were undertaken towards the end of the *O. peringueyi* breeding season to observe the effect of photoperiod on natural reproduction of this fish. The first two experiments for investigating flow rate and temperature were undertaken for 9 days while the experiments for conductivity and substrate were undertaken for 16 days. The fishes were observed on a daily basis to determine if they show a breeding colour (BC) characterized by red flushes on the operculum, ventral part (stomach) as well as on the anal and caudal fins. The *O. peringueyi* was also observed to check if a male chases the female (C), if the female swims together with the male, i.e. spawning behaviour (SB), and also to observe whether they are spawning, i.e. spawning ritual (SR), these

results were scored as either breeding category no (N) intermediate (I) or yes (Y). BC, C, SB and SR were allocated breeding stage scores of 1, 2, 3 and 4 respectively. N, I and Y were allocated scores of 0, 5 and 10 respectively. Breeding score was obtained by multiplying the breeding stage score with the breeding category score. Total breeding score was obtained by adding all the breeding scores.

3.7.2.5 Results

It was observed that a male attains his breeding, first by reddening of the operculum (gill cover). The ventral part, anal and caudal fins also start to turn bright red as breeding conditions in the aquarium improves. Males at peak of breeding condition show a distinct white tip on the dorsal fin. General appearance of males and females of the body colour and the intensity is most prominent during the breeding period. Less dominant males were observed to have reduced or no breeding colour.

Male which has attained his breeding colour chased the female actively around the aquarium. At first the female avoided the male while feeding actively. The female then showed a slight increase in body weight indicating development of eggs. As the female got in a peak breeding condition too, she avoided the male less and started swimming together with the male over the substrate at the bottom of the aquarium, both fish fed actively during that stage.

When the female has chosen an adequate spawning area for breeding, both fish moved in parallel and started pressing right against each other. They then went into a vibrating motion, releasing a cloud of sand caused by digging of their anal fins. Only a couple of eggs were released at a time (3-6). If breeding conditions are a favourable, breeding motion can continue up to 30 times per day. It was also observed that a female *O. peringueyi* had different sizes of eggs in its ovaries after it was dissected, this indicated that *O. peringueyi* is a multiple spawner.

O. peringueyi did not show any breeding activity at a flow rate of 0 m/s. Breeding activity increased at a flow rate of 0.08 m/s and it was at its highest at a flow rate of 0.26 m/s (Table 3.7.1 and figure 3.7.7). *O. peringueyi* did not show any breeding activity at 18°C. Breeding activity was highest at 22°C and declined at a temperature of 26°C as compared to the temperature of 22°C (Table 3.7.2 and figure 3.7.8).

Breeding activity was low at a standard conductivity of 0.783 µS/cm. Breeding activity was highest at an altered conductivity of 0.291 µS/cm (Table 3.7.3 and figure 3.7.9). *O. peringueyi* showed breeding

activity at an uncovered and gravel substrate but the breeding activity was highest at a substrate of 2/3 sand and 1/3 gravel (Figure 3.7.10).

Table 3.7.1: Effects of flow rate on natural reproduction of *Opsaridium peringueyi*

	Tank	Breeding stage			
	1A (no flow)	1	2	3	4
24/02	Breeding score	0	0	0	0
25/02		0	0	0	0
26/02		0	0	0	0
27/02		0	0	0	0
28/02		0	0	0	0
01/03		0	0	0	0
02/03		0	0	0	0
03/03		0	0	0	0
04/04		0	0	0	0
Total score		0			
	1B(0.08 m/s)	1	2	3	4
24/02	Breeding score	5	10	0	0
25/02		5	10	0	0
26/02		5	20	0	0
27/02		5	20	0	0
28/02		5	20	30	0
01/03		5	10	15	0
02/03		5	10	15	0
03/03		5	10	15	0
04/04		5	10	0	0
Total score		240			
	1C(0.28 m/s)	1	2	3	4
24/02	Breeding score	5	20	0	0
25/02		5	20	30	0
26/02		5	20	0	0
27/02		5	20	15	0
28/02		5	20	30	0
01/03		5	20	15	0
02/03		5	20	15	0
03/03		5	20	15	0
04/04		5	20	15	0
Total score		360			

Table 3.7.2: Effects of temperature on natural reproduction of *Opsaridium peringueyi*

	2A (18°C)	1	2	3	4
24/02	Breeding score	0	0	0	0
25/02		0	0	0	0
26/02		0	0	0	0
27/02		0	0	0	0
28/02		0	0	0	0
01/03		0	0	0	0
02/03		0	0	0	0
03/03		0	0	0	0
04/04		0	0	0	0
Total score		0			
	2B (22°C)	1	2	3	4
24/02	Breeding score	10	20	30	40
25/02		10	20	30	40
26/02		10	20	30	0
27/02		10	20	30	20
28/02		10	20	30	20
01/03		10	20	30	20
02/03		10	20	30	20
03/03		10	20	30	20
04/04		10	20	30	20
Total score		865			
	2C (26°C)	1	2	3	4
24/02	Breeding score	0	10	0	0
25/02		0	10	0	0
26/02		5	10	0	0
27/02		5	10	0	0
28/02		5	10	0	0
01/03		5	10	0	0
02/03		5	10	0	0
03/03		5	10	0	0
04/04		5	10	0	0
Total score		125			

Table 3.7.3: Effects of conductivity on natural reproduction of *Opsaridium peringueyi*

	3A (Standard)	1	2	3	4
05/03		0	0	0	0
06/03		0	0	0	0
07/03		0	0	0	0
08/03		5	10	0	0
09/03		5	10	0	0
10/03		5	10	0	0
11/03		5	10	0	0
12/03		5	10	0	0
13/03		5	10	0	0
14/03		5	10	0	0
15/03	Breeding score	5	10	0	0
16/03		5	10	15	0
17/03		5	10	0	0
18/03		5	10	0	0
19/03		5	10	0	0
20/03		5	10	15	0
Total score		225			
	3B (Altered)	1	2	3	4
05/03		10	20	30	20
06/03		10	20	30	20
07/03		10	20	30	20
08/03		10	20	30	20
09/03		10	20	30	20
10/03		10	20	30	0
11/03		10	20	30	40
12/03		10	20	30	0
13/03		10	20	30	0
14/03		10	20	30	0
15/03	Breeding score	10	20	30	0
16/03		10	20	30	0
17/03		10	20	30	0
18/03		10	20	30	0
19/03		10	20	15	0
20/03		10	20	30	0
Total score		1085			

Table 3.7.4: Effects of substrate on natural reproduction of *Opsaridium peringueyi*

	4A	1	2	3	4
05/03		0	20	0	0
06/03		0	20	0	0
07/03		0	20	0	0
08/03		0	20	0	0
09/03		0	20	30	0
10/03		0	20	0	0
11/03		0	20	15	0
12/03		0	20	0	0
13/03		0	20	0	0
14/03		0	20	15	0
15/03	Breeding score	0	20	0	0
16/03		0	20	0	0
17/03		0	20	0	0
18/03		0	20	0	0
19/03		0	20	15	0
20/03		0	20	0	0
Total score		385			

	4B	1	2	3	4
05/03		0	20	0	0
06/03		0	0	0	0
07/03		0	0	0	0
08/03		0	0	0	0
09/03		0	20	0	0
10/03		0	0	0	0
11/03		0	20	0	0
12/03		0	20	0	0
13/03		0	10	0	0
14/03		0	20	0	0
15/03	Breeding score	5	20	0	0
16/03		5	20	0	0
17/03		5	20	0	0
18/03		5	20	0	0
19/03		5	20	0	0
20/03		5	20	0	0
Total score		260			

	4C	1	2	3	4
05/03		0	0	0	0
06/03		0	20	0	0
07/03		0	20	0	0
08/03		0	20	0	0
09/03		0	20	0	0
10/03		0	20	0	0
11/03		0	20	0	0
12/03		0	20	0	0
13/03		0	20	0	0
14/03		0	20	0	0
15/03	Breeding score	0	20	0	0
16/03		0	20	0	0
17/03		0	20	0	0
18/03		0	20	0	0
19/03		0	20	0	0
20/03		0	20	0	0
Total score		300			

Table 3.7.5: Total breeding scores of tank 1A, 1B and 1C.

	Total breeding score
1A	0
1B	240
1C	360

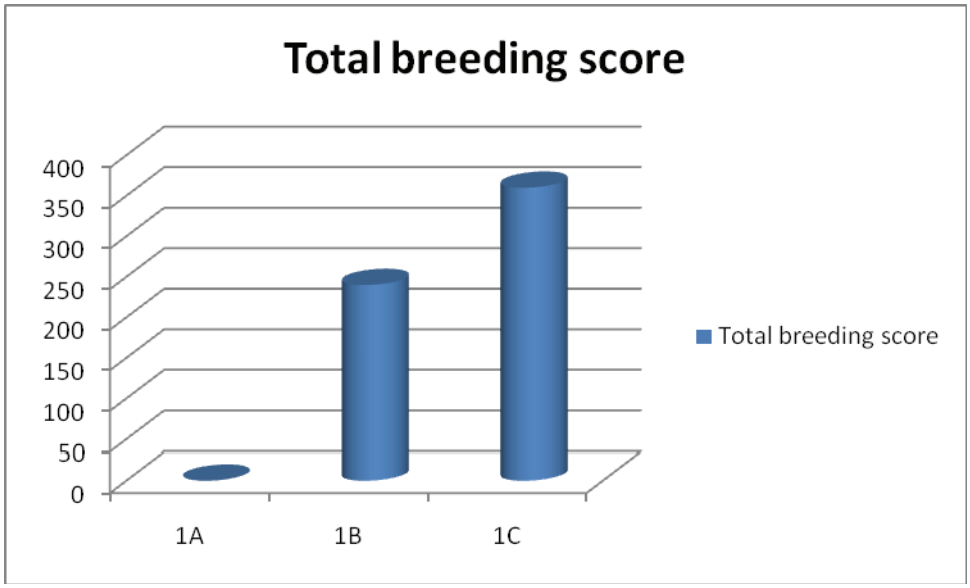


Figure 3.7.7: Comparison of different flow rates on natural reproduction of *Opsaridium peringueyi*.

Table 3.7.6: Total breeding scores of tank 2A, 2B and 2C.

	Total breeding score
2A	0
2B	865
2C	125

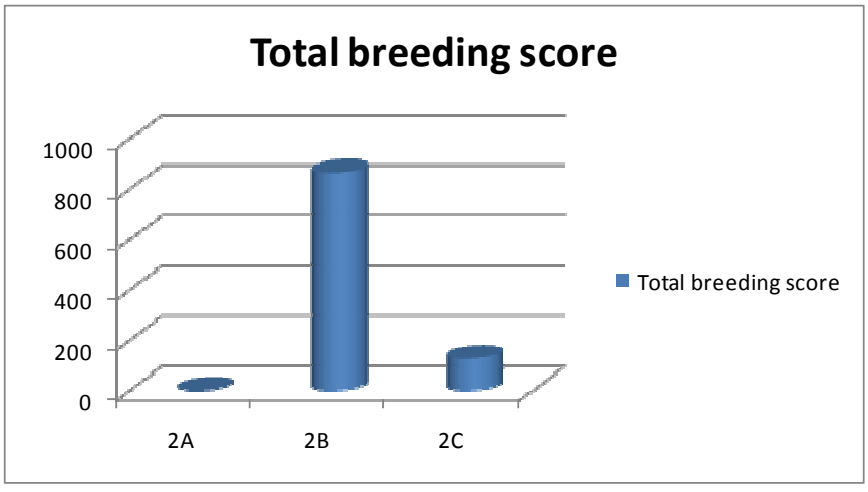


Figure 3.7.8: Comparison of different temperatures on natural reproduction of *Opsaridium peringueyi*

Table 3.7.7: Total breeding scores of tank 3A and 3B.

	Total breeding score
3A	225
3B	1085

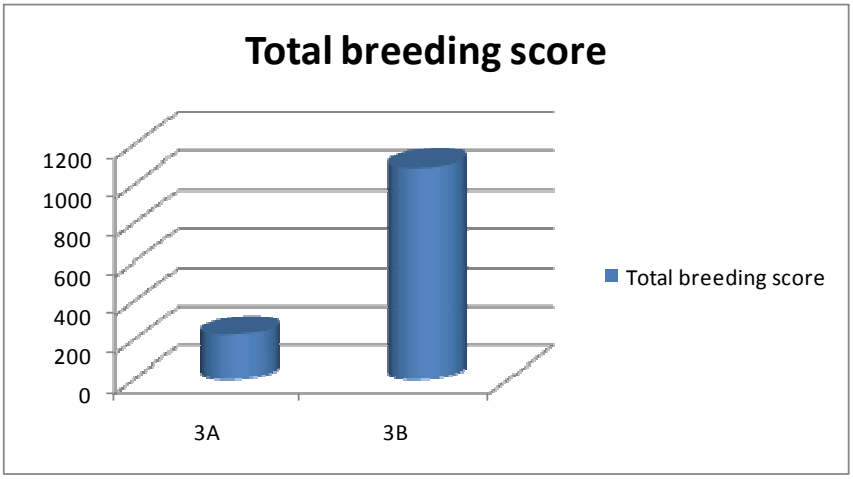


Figure 3.7.9: Comparison of different states of conductivity on natural reproduction of *Opsaridium peringueyi.*

Table 3.7.8: Total breeding scores of tank 4A, 4B and 4C.

	Total breeding score
4A	385
4B	260
4C	300

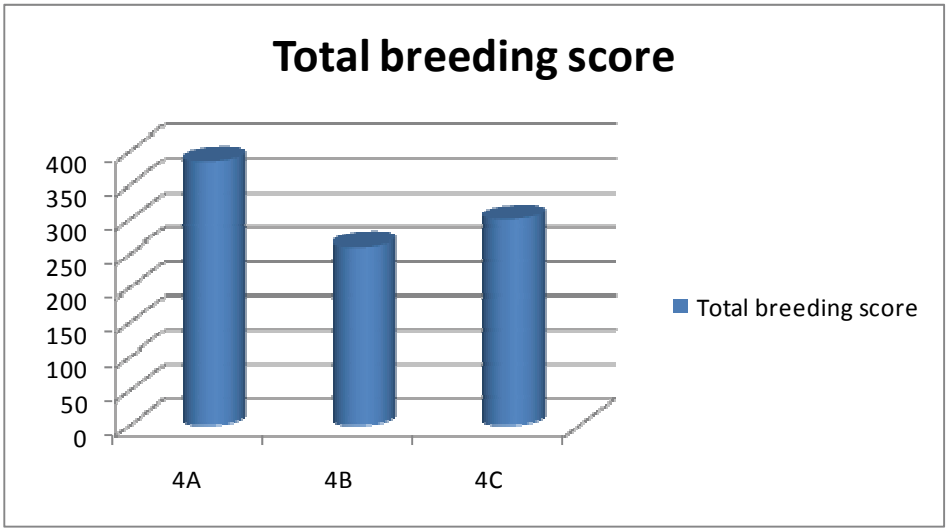


Figure 3.7.10: Comparison of different types of substrates on natural reproduction of *Opsaridium peringueyi*

3.7.2.6 Discussion

Flow rate had an influence in the reproduction of *O. peringueyi*. This is proven in the results section which indicates that breeding activity of *O. peringueyi* increased with increasing flow rate. That was because tank 1C which had a the strongest flow rate of 0.28 m/s showed the highest breeding score as compared to tank 1A and 1B which had the low and medium breeding scores respectively. This work is supported by Roux (2008) who observed that stream velocity is important in breeding success of a stream cyprinid *L. polylepis*.

Temperature also had an influence on reproduction of *O. peringueyi*. Breeding activity was highest at a temperature of 22°C where the breeding pair spawned and produced 12 eggs per spawn. In tank 2A, where the temperature was 18 °C there was no breeding activity observed while the breeding activity in tank 2C which had a temperature of 26 °C only shown the chasing behaviour. Kapoor *et al.*

(1975) indicated that temperature influences metabolic rate of fishes, i.e. the metabolic rate had to be at optimum range for a specific fish species (*O. peringueyi*) in order for it to breed. *O. peringueyi* prefers a temperature range between 15 and 25 °C (Skelton, 1996). This explains why the breeding pair did not breed at a temperature of 18 and 26 °C. At 18 °C the water was too cold resulting in low metabolic rates. At 26 °C, the temperature was not favourable for the *O. peringueyi* as it was 1 °C above its preferred temperature range as described by Skelton (1996).

This study also showed that conductivity influences reproduction of *O. peringueyi*. Breeding activity in the experiment that investigated conductivity was highest in tank 3B which had altered conductivity of 1/3 aged tap water and 2/3 rain water. Breeding pair spawned and produced 19 eggs per spawn. Munro *et al*. (1990) indicated that in southerly latitudes many cyprinids have protracted spawning seasons that are associated with the rainy season of the year and this was the case in this study as the ⅔ rain water simulated fresh rains that the *O. peringueyi* in their novel environment. Tank 3A which had a standard conductivity also showed some signs of breeding activity although the signs were not as high as in tank 3B.

The fourth factor which was proven to have an influence on reproduction of *O. peringueyi* was substrate. Breeding activity in tank 4A was slightly higher as compared to tank 4B and 4C. This proved that the breeding pair favoured a substrate of 2/3 sand and 1/3 gravel. This scenario was proven by Crass (1964), Pienaar (1968, 1978), Gaigher (1973) who explained that *O. peringueyi* prefers habitats with a sandy or gravel substrate.

Besides the objectives that which are discussed above, it was also observed that photoperiod had an influence on the natural reproduction of *O. peringueyi*. This is because breeding activity decreased as the seasons changed from autumn approaching winter. That was observed in experiments which investigated conductivity and substrate as they were undertaken towards the end of March. It was noted that males which did not attain a breeding colour also showed chasing behaviour that can lead to spawning if it happens for a prolonged period of time. This chasing behaviour among the males with and without breeding colour was linked to their genetic makeup but further studies are required verify the statement. Breeding colour faded in the males after 20% percent water change was done in the tanks. In tank 2B and 3B where the breeding pair spawned, different sizes of eggs were observed which indicated that *O. peringueyi* is a multiple spawner.

3.7.2.7 Conclusion

This study showed that *O. peringueyi* prefers to breed in water with strong flow, a temperature of 22°C, altered conductivity which simulated rain water their natural environment as well as a mixture of gravel and sandy substrate. All this factors were proven under experimental conditions in glass tanks therefore these observations can be in the conservation planning framework of *O. peringueyi* that can be undertaken on a large scale in the wild.

Chapter 8: Feeding biology

NAG Moyo and S Theron

3.8.1 INTRODUCTION

Skelton (1996) found that *O. peringueyi* primarily feeds on benthic invertebrates, mainly chironomid and simuliid larvae, and adult insects taken from the surface. *O. peringueyi* is a flow-dependent species and suffers as a result of weirs, channels and water abstraction (Anon, 2001). Habitat alteration and deterioration appears to be the major threat affecting the status of populations of this species. Many formerly perennial rivers of the Transvaal Lowveld now cease flowing during the dry season due to impoundment and extraction of water for agricultural purposes. Turbidity and siltation through erosion from overgrazing and crop cultivation occurs when the rivers start flowing again at the beginning of the rainy season. Turbidity and siltation probably affect the sight feeding and breeding success of the species (Skelton, 1987). Other threats to this species are industrial and agricultural pollution, and the choking of such rivers by the alien plant *Eichhornia crassipes* (Skelton, 1987).

Very little work has been done on this species yet it has already been on the red data list and there is evidence that the geographical range of this species has shrunk (Skelton, 1993). There is also paucity in the literature of studies which investigate the feeding and growth of *O. peringueyi*. The main objective of this study is to investigate some aspects of the feeding and growth of *O. peringueyi*.

3.8.2 LITERATURE REVIEW

3.8.2.1 Temperature

Temperature variation in water bodies depends on their geographical location, latitude and altitude. Fouché *et al.* (2005) explained that altitude as a predictive variable is the only environmental factor that produces a significant response model based on the generalized additive model; *O. peringueyi* has a unimodal response to altitude with an optimum at 600 m above the sea level.

In the temperate regions the daily maximum temperatures rarely reach over 25°C, but in the high altitude tropics (e.g. in Kenya, Tanzania, Malawi, Zimbabwe in Africa) the thermal conditions are similar to those of temperate region. Water temperature affects fish activity, behavior, growth and reproduction. The optimal temperature tolerances of *Opsaridium peringueyi* are between 15 and 25°C (Skelton, 1996).

Temperature affects all chemical and biological processes. The metabolic rate of fish doubles for every rise of 10°C. Therefore, temperature has a direct effect on important factors such as growth, oxygen demand, food requirements and food conversion efficiency. The higher the temperature, the greater the requirement for oxygen and food which result in a faster growth rate. If the temperature is to exceed the critical level for a particular species, fish may become stressed, more vulnerable to disease, stop growing and can die.

Cui and Wootton (1982) explained that the standard metabolic rate is related to food, temperature and body size. In winter the metabolic rate of most fish slows down and speeds up again as the water becomes warmer in spring. This dependence on water temperature also affects the fish immune system, wound healing and digestion. O'Hara (1988) found that metabolic rates of smaller fish are less affected by temperature change than those of larger fish.

It is generally known that feeding activity of cyprinids decreases with decreasing water temperature. During a study of the feeding activity of silver crucian carp in Lake Egirdir feeding activity were the least in winter because of low temperatures and the greatest in spring (Balik *et al.,* 2003).

3.8.2.2 Feeding ecology and diet

Gerald (1976) explained that the efficiency with which food is converted has been shown to be influenced by both external and internal factors. Many fish are known to pass through periods of starvation and to utilize energy stored in their bodies. Previous investigators have concluded that restoration of feeding after varying periods of starvation affected rate of feeding, digestion, absorption and conversion.

Cyprinids lack a stomach and pyloric caecae, so the digestive tract is greatly simplified. However this species-rich family developed a set of movably interconnected pharyngeal jaws, powered by body muscles. Each pharyngeal jaw bears a maximum of three rows of large, often left-right interdigitating teeth impacting on the skull base. They also developed oral roof, the palatal organ with a highly

sensomotoric capacity enabling the sorting of food and non-food (Sibbing, 1991). Prey motion is essential to induce attack in cyprinids.

Schlosser and Ebel (1989) did experiments in an artificial stream on Gould Creek and indicated cyprinids influenced invertebrates' abundance, but the effect of cyprinid predation was variable among habitats. Invertebrate abundance decreased most in structurally complex pools but exhibited little response to cyprinid predation in shallow riffle and raceway habitats. Because predation intensity varied among habitats, pool-dwelling invertebrates such as chironomidae and crustacean decreased more in the presence of cyprinid predation than riffle dwelling hydropsychidae and simuliidae. However if simuliidae occurred in pool-habitats, they were strongly selected by cyprinids, resulting in a significant depression in prey size in pools.

Anon (2007) explained that Southern Africa has an extremely high diversity of insects, with more than 80 000 species already recorded. Insects are the most abundant and successful terrestrial species occupying almost every type of habitat except the ocean. They form part of a wider ecosystem and if lost the complexities and abundance of other life will be affected. Tamatamah (2007) explained that species in the *pool guild* in Wami river, Tanzania, which were represented by *Opsaridium, Brycinus, Micralestes, Synodontis, Bagrus* and *Barbus* in the study, are slightly more limnophilic in habit and generally seek to inhabit the slack regions of back eddies where emergent and floating vegetation may occur. They tend to be insectivorous, feeding on the drift dislodged from the riffles or on insects falling into the river from riparian vegetation. They may be either limnophilic, breeding in the riffles, or phytophilic, attaching their eggs to vegetation. They usually have well defined home ranges, and habitats delimited by depth, current strength and the distribution of vegetation. These species are also disturbed by changes to the flow regime that desiccate the pools or leave them for long periods without flow so they become anoxic. They also generally rely on the delicate balance between pool and riffle of the main channel and respond negatively to any influence that changes this balance. Again this guild can be affected by loss of longitudinal connectivity resulting from changes in flow regime of the river.

According to Fouché *et al.* (2005) the distribution of fish in a river is regarded to be determined by the flow regime, which would include factors such as inter alia, velocity and depth. On a micro-scale, factors such as the availability of cover and food complicate matters and needs to be investigated. This study will look at the different feeds (live and artificial) and find the preferred food for *O. peringueyi* at different temperatures.

Information on the growth of this fish is an important pre-requisite for understanding the dynamics of their populations. For fish that are exploited by man growth is one of the parameters used to estimate biological production and yield.

There are three basic approaches to assess the age and growth of fish:

- An empirical approach which is the direct measurement of the growth of fish of known or partly known age,
- The analysis of length frequency distribution or statistical approach and
- The interpretation of regular periodic growth checks or anatomical approach.

Most studies on the aging of fish in South Africa have been done using scales (e.g. Le Roux, 1961; Batchelor, 1978 and Hecht, 1986). Schulz (1992) successfully aged *O .peringueyi* using scales. However, his sample size was very small. One of the objectives of the present study is to provide growth data on *O. peringueyi.*

3.8.3 OBJECTIVES

- To determine the diet of *O. peringueyi* from different localities.
- To determine the effect of temperature on the feeding activity of *O. peringueyi*
- To determine the effect of temperature on the swimming activity of *O. peringueyi*
- To determine the preferred feed by *O. peringueyi*
- To determine the effect of hunger on food selection by *O. peringueyi*.
- To determine the length at age classes of *O. peringueyi.*

3.8.4 NULL HYPOTHESIS

- Temperature has no effect on the feeding activity of *O. peringueyi*
- Temperature has no effect on the swimming activity of *O. peringueyi*
- *O. peringueyi* does not show preference for any particular feed
- Hunger has no effect on food consumption by *O. peringueyi*

3.8.5 MOTIVATION OF STUDY

Little information is recorded on *O. peringueyi*, which is becoming extinct in South Africa river systems. There is very little known about the feeding habits of this species. As there is an increase in the threat posed to *O. peringueyi*, it is vital to assess its conservation status. The purpose of this study is to investigate some aspects of the feeding ecology and growth of *O. peringueyi* in order to assist in the development of a conservation plan.

3.8.6 MATERIAL AND METHODS

3.8.6.1 Diet of *O. peringueyi*

Seine nets and electro-fishing gear were used to collect fish from the Mac Mac River, the confluence of the Mac Mac, Sabie and Sabane Rivers (OPS), the Marite River (OPS), the Hoxane weir on the Sabie River(OPS) and the Luvuvuhu River system (OPS). The fish were dissected and the stomachs removed, then immersed in 5% formalin. After fixation for 20 hours the stomachs were routinely examined under a low power dissection microscope. The frequency of occurrence of the different prey items was determined according to Hyslop (1980) omitting empty stomachs. The number of stomachs containing one or more individuals of a food item was recorded. This number was the expressed as a percentage of all the guts, which gave the percentage composition by frequency of occurrence. The frequency of occurrence method gives little indication of the relative amount or bulk. However, it gives a picture of the food spectrum. The intestinal index was obtained by dividing gut length with standard length, this was then multiplied by 100. The width of the mouth aperture was expressed as percentage of standard length.

3.8.6.2 Study Site and tank preparation

The fish used in the experiment were collected from the Mac Mac River near Hazy view. The fish were caught twice in a day at 10 am and 3 pm. The temperature variations in the river were between 15 and 21°C and water was taken directly from the river into the plastic tank used for transportation. Ice was thrown in the water to maintain the temperature at 17°C. The fish were transported in 1000 L plastic tank filled with 400 litres of water on the back of a van. An air pump was used to supply oxygen for the fish.

The experiment was conducted at the University of Limpopo (Turfloop campus), Animal Unit. The feeding habits of *O. peringueyi* were investigated. Rectangular tanks with a length of 145 cm, width of 38 cm and average depth of 45 cm were used. The tanks were filled with water to full capacity. Thermostatic heaters were placed in the tanks to regulate temperature. Temperatures of 10, 15, 20, 24, 28 and 30°C were used and the experiment was replicated twice. Filters were used in each tank. Dissolved oxygen concentrations were generated by the air stones placed in the tanks. Fish were kept in separation tanks and acclimatized to temperature of 20°C prior to stocking in experimental tanks.

3.8.6.3 Stocking Density in Tank

Mixed-sex *O. peringueyi* of size 14 g were randomly selected. Fish were starved prior to stocking and five *O. peringueyi* were put in each tank. The fish were fed three different diets namely mosquito larvae (*Culex tarsalis*), frozen *Artemia* (*Artemia salina*) and water beetles (*Cybister tripunctatus)*. The feed ration was 5% body weight daily. One diet was given to the fish for three consecutive days. Feeding activity was measured at different temperatures by counting the number of food items consumed per minute. At the end the fish were given three diets at the same time to determine preference for any particular diet.

Swimming activity was measured at different temperatures (10, 15, 20, 24, 28 and 30°C) by observing tail fin beats per minute. Swimming activity was measured to determine the condition of the fish.
To test the food intake of hungry fish, the fish were starved for 24 hrs, 12 hrs, 6 hrs and 30 min.

3.8.6.4 Aging of *O. peringueyi*

Six scales were removed from the first row of scales above the lateral line. The scales were cleared by soaking them in water and were then mounted on microscope slides. A serial number was given to each slide before being examined twice under the microscope. At the first examination the number of growth rings were counted and recorded. The same was done on the second examination. If the results of the two examinations were inconsistent the scale was rejected. The standard length, weight and sex of each specimen was also determined. The von Bertalanffy model was used to describe growth:

$$\mathbf{L_t = L_\infty \{ 1 - exp [- k (t - t_0)] \}}$$

Where Lt is length at time t, k is the growth coefficient, $L\infty$ is the asymptotic length and t_o is the hypothetical age at zero length. These parameters were estimated using the Length Based Fish Stock Assessment (LFSA) package of BASIC computer programs.

3.8.6.5 Statistical analysis

One-way ANOVA was used to determine the effect of temperature on feeding and swimming activity of *O. peringueyi* through the SPSS 14.0 program.

3.8.7 RESULTS

O. peringueyi has a terminal mouth with a wide mouth gap of 10%. The relatively big eyes indicate that it's a visual predator. It has an extendible lower jaw and there are no teeth on the jaws. The stomach is simple with an average intestinal length of 125%. The diet of *O. peringueyi* was dominated by Beatidae (Table 3.8.1). Heptageniidae and Simulidae also occurred frequently. Feeding activity increased with temperature up to 24°C and decline thereafter (Table 3.8.2). Temperature had a significant effect on feeding activity (ANOVA, $p < 0.05$).

Swimming activity increased with temperature up to 24°C and declined thereafter (Table 3.8.3). Temperature has a statistically significant effect on swimming activity (ANOVA, $p< 0.05$). *O. peringueyi* showed significant X^2, $p< 0.05$ preference for mosquito larvae, at all temperatures (Table 3.8.4). *O. peringueyi* showed no preference X^2, $p< 0.05$ for any particular diet after being starved for 24 hours (Table 3.8.5).

Table 3.8.1: Percentage frequency of prey items in the diet of *Opsaridium peringueyi.*

Species	% occurrence
Beatidae	28
Caenidae	4
Coenagrionidae	4
Gomphidae	5
Belostimatidae	5
Gerridae	6
Notonectidae	4
Culicidae	11
Dryspidae	3
Hydrophilidae	4
Chironomidae	5
Atyidae	1
Heptageniidae	15

Table 3.8.2: Different feeds given to *Opsaridium peringueyi* at different temperatures

Feeds	Tank 1(10°C)	Tank 2 (15°C)	Tank 3 (20°C)	Tank 4 (24°C)	Tank 5 (28°C)	Tank 6 (30°C)
Mosquito Larvae	24	32	34	38	30	25
Beetles	14	27	22	31	21	18
Frozen Artemia	12	16	15	20	15	16

Table 3.8.3: Swimming activity of *O. peringueyi* at different temperatures

	Tank 1(10°C)	Tank 2 (15°C)	Tank 3 (20°C)	Tank 4 (24°C)	Tank 5 (28°C)	Tank 6 (30°C)
Tail fin beats	104	123	130	148	137	134

Table 3.8.4: Food preferences by *O. peringueyi*

Temperature	Mosquito Larvae	Water Beetles	*Artemia*
10°C	6	1	1
15°C	10	2	3
20°C	9	2	2
24°C	11	3	3
28°C	8	2	2
30°C	7	1	2

Table 3.8.5: Effect of hunger on food selection by *Opsaridium peringueyi.*

	24 hrs Starvation	12hrs Starvation	6 hrs Starvation	30 min Starvation
Mosquito Larvae	6	6	8	10
Water Beetles	6	5	7	3
Frozen *Artemia*	6	4	6	2

Table 3.8.6 (a): Length and age data of *Opsaridium peringueyi* .

Mean length (mm)	Age (years)
28	1
43	2
53	3
62	4
67	5
70	6

Table 3.8.6(b): Length and age data of male *Opsaridium peringueyi.*

Mean length (mm)	Age (years)
30	1
45	2
58	3
66	4
71	5
75	6
77	7

The von Bertalanffy method was fitted to the length and age data (Table 3.8.6a and b). The growth model for the female fish was:

$$Lt = 90.5\ [1 - exp\ 0.21\ (t + 0.53)]$$

For the male fish the growth model was:

$$Lt = 115.3\ [1 - exp\ 0.25\ (t + 0.61)]$$

Males grow at a faster rate and have a higher $L\infty$ than females.

3.8.8 DISCUSSION

O. peringueyi feeds predominantly on insects both on the water surface and in mid water, it will occasionally feed on food items on the bottom. The major food items identified in the stomachs of *O. peringueyi* are Beatidae, Heptagenidae and Simulidae. These invertebrates are closely associated with riffles. *O. peringueyi* is a shoaling fish species with adult and juvenile fish congregating together. There was no obvious ontogenetic shift in fish that were more than one year old. The mouth of *O. peringueyi* is quite wide and well adapted to "grabbing" prey. The intestinal index obtained is typical of insectivorous fish (Keast and Webb, 1966).

Temperature had an effect on the feeding activity of *O. peringueyi*. Feeding activity increased with increasing water temperature and decreased with decreasing water temperature, and these results are supported by Sandfoss (2003) who explained that feeding activity in cyprinids decreases with decreasing water temperature. Kapoor *et al.* (1975) explained that temperature influences the feeding activity via its effect on standard metabolic rate.

The best feeding activity of *O. peringueyi* was obtained at 24°C which is within the temperature range of *O. peringueyi* which is 15°C and 25°C (Skelton, 1996). At l0°C the feeding activity was low

due to the low metabolic rate caused by cold water temperature. Feeding activity generally decreased beyond 24°C as the temperature was not within its optimum preferred temperature.

Kapoor *et al.* (1975) also explained that the level of swimming activity is influenced by temperature. Temperature and feeding have a direct relationship as swimming requires energy and food consumption increases the level of activity. In this experiment similar results were found as swimming activity increased with increasing water temperature and were at its peak at 24°C which was also the same optimum temperature for feeding activity.

At 30°C swimming and feeding activity of *O. peringueyi* declined. *O. peringueyi* is found at high altitudes, high temperatures can cause stress which may lead to diseases. Rottmann *et al.* (1992) reported that physiological stress is a primary contributing factor of fish disease and mortality in aquaculture. Stress conditions such as undesirable temperature can result in decreased resistance by the fish, resulting in the spread of disease and parasite infestation. Indeed the fish that were kept at 30°C eventually died from fungal infections.

At 12 and 24 hours starvation, the fish did not show any selection, they went for all the three diets that were offered. Prolonged starvation makes a fish to be euryphagous until the level of satiation. At 30 minutes and six hours the fish selected preferred diet (mosquito larvae) over the other food items. The reason might be that fish which are full would select only their preferred diet over others due to the fish having a choice because of its stomach fullness. Priyadarshana *et al.* (2006) observed inconsistent feeding behaviour after satiation in cyprinids. This may have been partly due to different sizes of prey and, therefore, fish exercising a certain degree of prey selection. Prolonged starvation of a fish causes a maximum hunger state which remains at a constant level and hunger motivates the fish to feed to satiation.

Kalinin and Rantin (no date) explained that metabolic rate decreased markedly after only 2 days of food deprivation in the black bass, *Micropterus salmoides*. These authors attributed this reduction, at least partially, to the decrease in spontaneous activity. They also explained that many fishes subjected to longer periods of starvation have also shown significant decreases in their metabolic rate.

Therefore it can be concluded that *O. peringueyi* is an opportunistic feeder within a defined niche and it feeds at all levels in the water column. Opportunistic feeders are animals that eat whatever

food is convenient at the time. In the experiment the food were found at different levels in the tank, mosquito larvae at the surface, water beetles in the mid water and A*rtemia salina* at the bottom. Food abundance in the river is greatly affected by the changes in the river systems which then results in the disappearance of some species.

In both the male and female *O. peringueyi* were the growth rate faster in the first year and decreased progressively thereafter. *O. peringueyi* displays sexually dimorphic characteristics. The females grow more slowly and mature earlier than the males. This divergence in the growth rate of the sexes begins early and becomes more pronounced after sexual maturity. These inter-sex differences in *O. peringueyi* may probably be attributed in part to the fact that females may be maturing earlier and channel their energy to reproduction rather than growth. Observations from this study suggest that feeding habits and growth studies have important implications for the management and conservation of this species.

Chapter 9: River systems

PSO Fouché, JA Venter, W Vlok and S Theron

3.9.1 INTRODUCTION

As part of the process to develop a conservation framework for a species the assimilation of detailed knowledge concerning both the historical and current distribution of *Opsaridium peringueyi* in its historical distribution range is of outmost importance. At the same time such the assimilation should include the characterization of the habitat and threats to the existence of the species in the rivers within the distribution range. It is also imperative that the historical sites where the species have been recorded, the geomorphological aspects of the sites and the potential threats within the river reaches be recorded. In addition the site heterogeneity with regard to available fish habitat and the substrate diversity should be investigated and recorded.

According to the literature the historic distribution range of *O. peringueyi* within South Africa included the Luvuvhu, Shingwedzi, Letaba, Olifants, Sabie, Crocodile, Komati, Usutu and Mphongolo rivers and it was decided to establish the aspects listed in these rivers. At the same time the fish assemblages at each site were to be identified.

3.9.2 METHODS

Geomorphology

For the study, the methodology developed for the geomorphological index survey for the River Health Programme (RHP) was followed. This entailed the completion of the geomorphological index form for each site. Photographs of the different features, the hydraulic biotopes and river banks were taken and the completed forms were used to determine the bank stability index, the habitat diversity index, the habitat cover index and the total habitat index. The data were further captured in a site description and various parameters from the completed forms were used to compile the final report.

Table 3.9.1.1: The geomorphological zonation of river channels (Adapted from Rowntree and Wadeson, 1999).

Longitudinal zone	Macro-reach characteristics			Characteristic channel features
	Valley form	Gradient class	Zone class	
Zonation associated with a "normal" profile				
Source zone	V10	not specified	S	Low gradient, upland plateau or upland basin able to store water. Spongy or peaty hydromorphic soils.
Mountain headwater stream	V1, V3	> 0.1	A	Very steep gradient streams dominated by vertical flow over bedrock with waterfalls and plunge pools. Normally first or second order. Reach types include bedrock fall and cascades.
Mountain stream	V1, V3	0.04-0.99	B	Steep gradient stream dominated by bedrock and boulders, locally cobble or coarse gravels in pools. Reach types include cascades, bedrock fall, step-pool, Approximate equal distribution of 'vertical' and 'horizontal' flow components.
Transitional	V2, V3, V4, V6	0.02-0.039	C	Moderately steep stream dominated by bedrock or boulder. Reach types include plain-bed, pool-rapid or pool riffle. Confined or semi-confined valley floor with limited flood plain development.
Upper Foothills	V4, V6	0.005-0.019	D	Moderately steep, cobble-bed or mixed bedrock-cobble bed channel, with plain-bed, pool-riffle or pool-rapid reach types. Length of pools and riffles/rapids similar. Narrow flood plain of sand, gravel or cobble often present.
Lower Foothills	V8, V10	0.001-0.005	E	Lower gradient mixed bed alluvial channel with sand and gravel dominating the bed, locally may be bedrock controlled. Reach types typically include pool- riffle or pool-rapid, sand bars common in pools. Pools of significantly greater extent than rapids or riffles. Flood plain often present.
Lowland river	V4, V8, V10	0.0001- 0.001	F	Low gradient alluvial fine bed channel, typically regime reach type. May be confined, but fully developed meandering pattern within a distinct flood plain develops in unconfined reaches where there is an increased silt content in bed or banks.
Additional zones associated with a rejuvenated profile				
Rejuvenated bedrock fall/ cascades	V1, V4	>0.02	A/B/Cr	Moderate to steep gradient, confined channel (gorge) resulting from uplift in the middle to lower reaches of the long profile, limited lateral development of alluvial features, reach types include bedrock fall, cascades and pool-rapid.
Rejuvenated foothills	V2, V3, V4, V6	0.001-0.02	D/Er	Steepened section within middle reaches of the river caused by uplift, often within or downstream of gorge; characteristics similar to foothills (gravel/cobble bed rivers with pool-riffle/ pool-rapid morphology) but of a higher order. A compound channel is often present with an active channel contained within a macro channel activated only during infrequent flood events. A limited flood plain may be present between the active and macro-channel.
Upland flood plain	V8, V10	< 0.005	Fr	An upland low gradient channel often associated with uplifted plateau areas as occur beneath the eastern escarpment.

Potential threats

Available spatial data was used to investigate the impacts of land transformation and land use on the different river catchments. The GIS layers that were used were the following: a settlement layer (displaying all towns, suburbs, villages and community dwellings); a transformed land layer (with forestry plantations, small holdings, commercial irrigated, commercial dry-land, built-up industrial/transport/residential); a large dams layer (all large dams); a gauging weir layer (displaying all DWAF gauging weirs); and lastly a protected areas layer (displaying national parks; provincial nature reserves and private nature reserves and game parks). The combination of these GIS layers was then displayed as a map for each river system. This map should be interpreted with caution as

most of the layers have been created with remote sensing methods with limited ground-truthing. The layers also originated in the period between 1995 and 1998 and development since then could create a different picture. In addition the impacts at the site were identified and rated according to the protocol prescribed for the Index of Habitat Integrity (Kleynhans *et al*., 2008). These results are shown in tabular format for each river system.

Biotopes and site heterogeneity

The methods involved here consisted of an initial visual identification of the biotopes followed by a process of demarcation and mapping of these biotope types within the selected site. The biotopes were also classified according to the prescriptions of Kleynhans (2008) into depth-velocity classes. In all the biotopes the cut-off point between shallow and deep was 0,5 m where all biotopes with depths below 0,5 meters was regarded as shallow. In the case of velocity the cut-off point between fast and slow was 0,3 m/sec. For each site in the river system the number of each biotope or velocity depth class was determined and expressed as a percentage of the total number of biotopes. These results are presented in tabular format as the "diversity of available fish habitat". The biotopes were then surveyed and the substrate type (Rowntree and Wadeson, 1999) determined at five randomly selected points in each biotope. Each biotope was then classified according to the dominant substrate present. Within each site the substrate diversity shown by a count of the number of times a specific substrate type was encountered in each biotope. Both these latter aspects (*viz.* substrate dominance and substrate diversity) are reported in tabular format.

Fish

In each biotope fish were collected using the most appropriate method or methods for the particular biotope type. These methods included electro-fishing as well as seine and cast net sampling.

3.9.3 THE LUVUVHU RIVER

3.9.3.1 River description

The Luvuvhu catchment forms part of the larger Limpopo River system where the Luvuvhu River is a major tributary of the Limpopo River (Figure 3.9.1.1). The catchment area is ca 6000 km^2 and stretches for more than 250 km from Louis Trichardt to Pafuri in the Kruger National Park (KNP). The Soutpansberg mountain range is one of the most prominent features of the catchment and the

majority of the tributaries originate in its southern slopes. The main tributaries are Latonyanda, Dzindi and Mutshindudi rivers. The only tributary on the northern slopes is the Mutale River.

A small portion of the catchment along the watershed between Louis Trichardt and Thohoyandou, receives a mean annual precipitation (MAP) in excess of 1200 mm. Most of the remainder of the catchment receives between 500 and 1000 mm per year. The major tributaries contribute 73% of the natural run-off that enters the river. In the very arid areas that exist in the extreme east and the west, of the catchment the rainfall is less than 500 mm per annum and therefore the resulting tributaries in these areas are non-perennial.

Historically the water of the river was regarded to be of good quality with for example conductivity (EC) that ranged from 13-16 mSm. This EC was found to be dominated by total alkalinity as well as by the sodium, chloride and to some extent calcium concentrations. Concentrations of all these ions were fairly uniform along the length of the river. The nutrient concentrations were found to be similarly low with the same longitudinal tendency as for the ions.

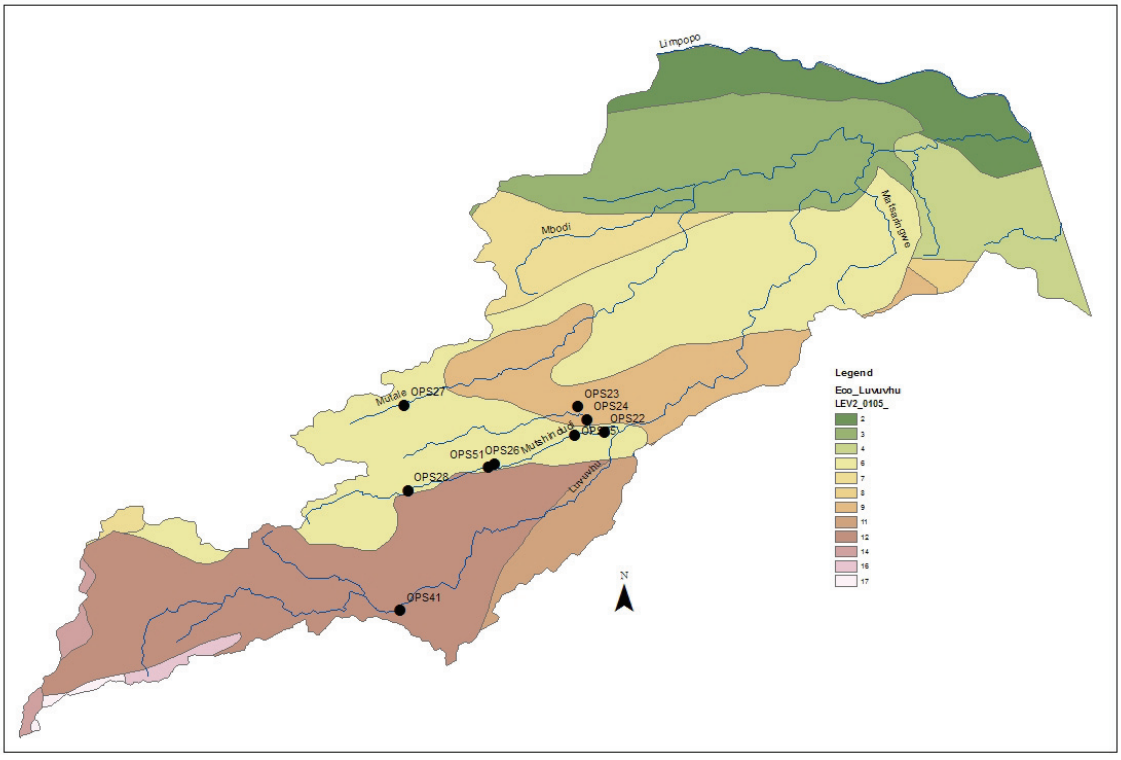


Figure 3.9.1.1: The project sample sites in the Luvuvhu River. Tributaries and Level 2 Ecoregions are indicated.

3.9.3.2 Geomorphology description

The geomorphological aspects observed at the sites are listed in table 3.9.1.2.

3.9.3.3 Potential threats

The impacts in the Luvuvhu River are listed for the reaches between two sites and indicate the impacts on the lower site. The comments are of the specific aspects observed during surveys (Table 3.9.1.3). The use of the available spatial data and the relevant GIS layers resulted in the map shown in figure 3.9.1.2. The caution mentioned previously and the period in which the layers originated is again brought to the attention. In the Luvuvhu River, erosion in general is not a problem, although forestry is present in the mountain areas. The main contributing activities to the problem originate from subsistence farming and run-off from settlements. The main problem experienced in this river is nutrient enrichment mainly from sewerage and run-off from settlements. In the river channel, sand mining is a problem. The local communities use the sand for building material and to manufacture bricks.

3.9.3.4 Biotopes en site heterogeneity

In table 3.9.1.4 the heterogeneity, based on velocity and depth, of the sites is shown. In table 3.9.1.5 the dominant substrate in each of biotopes sampled is shown while the actual substrate of these biotopes diversity is shown in table 3.9.1.6.

3.9.3.5 Fish

The fish collected in each of the habitat classes is shown in table 3.9.1.7 while the actual fish diversity observed at the site is summarized in table 3.9.1.8.

3.9.3.6 Discussion

The high degree of habitat diversity, particularly with regard to substrate and flow aspects, of the Luvuvhu River provides what can be regarded as suitable habitat for *O. peringueyi*. The presence of sand in the fast flowing habitats, where boulders and cobbles dominate represents what is regarded

as the necessary habitat for the species. The upper reaches are more diverse than the sites in the lower reaches and therefore more suitable.

Two of the sites where *O. peringueyi* were recorded, OPS 23 and 27 have the potential to serve as conservation areas for the species. Both sites are in the headwaters of the Luvuvhu River system in the Mukhase and Mutale rivers respectively. Site 23 is in the Mphaphuli Nature Reserve which at this point is hardly impacted at all and has the highest potential to be utilized as a sanctuary for the species. Site 27 on the other hand is in the Mutale River and has a similar potential for conservation of the species but is currently under threat of anthropogenic activities. Site 28 is also as diverse as the two other sites but is highly impacted as is evident in the large amounts of silt found in the slow deep habitats.

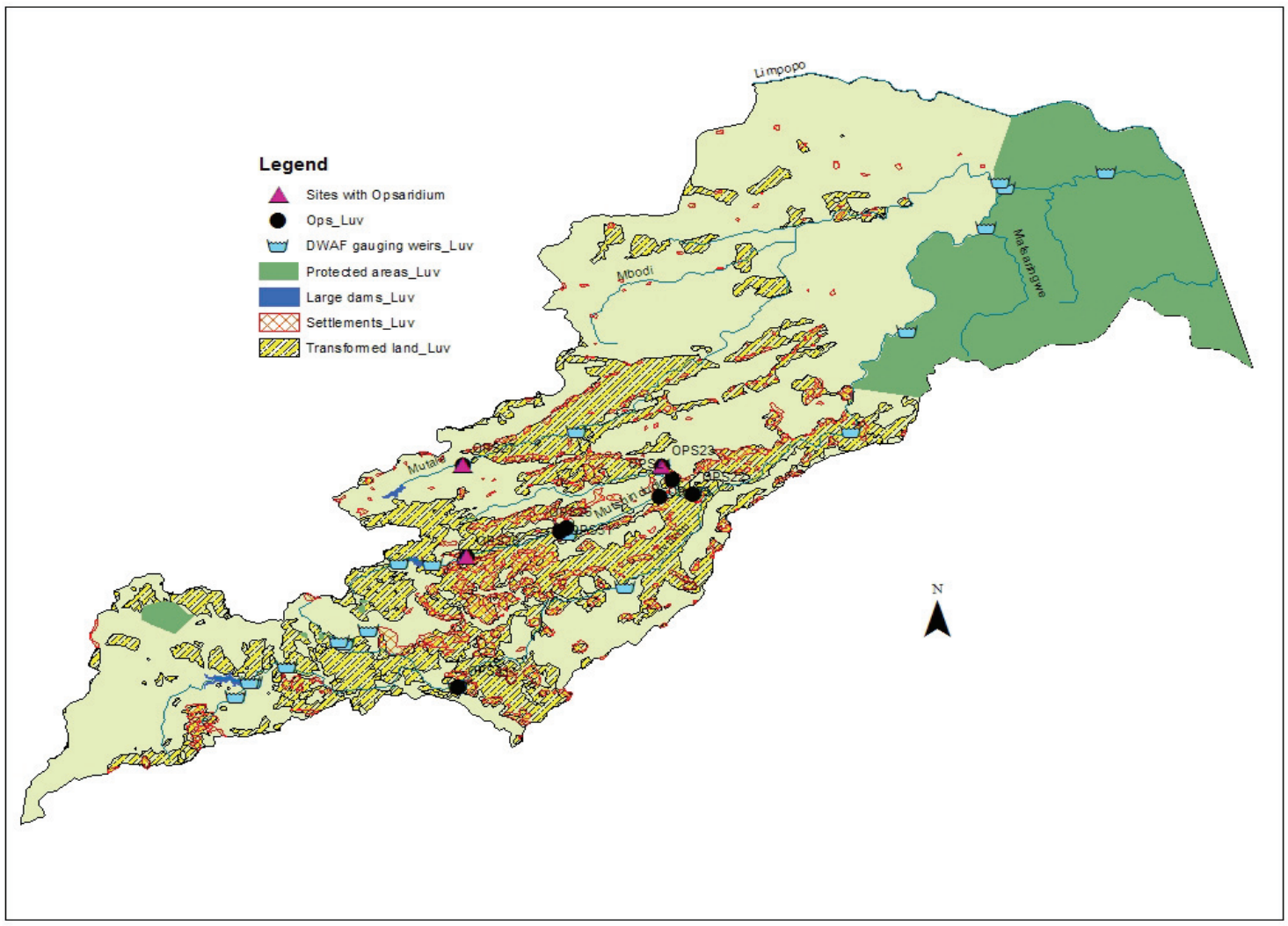


Figure 3.9.1.2: The Luvuvhu River catchment with associated land-use impacts.

Table 3.9.1.2: Geomorphological aspects for the sites in the Luvuvhu River.

Site number	Flow regime during survey	Valley form	Channel dimension	Channel type	Morphological units	Reach type
27	Moderate	Incised plain	50-75 m	Mixed, alluvial	Rock step, rapid boulder, riffle, run, shallow pool	Cascade, pool-rapid, plain bed
28	Moderate	V-shaped valley	50-75 m	Mixed	Rock step, rapid bedrock, rapid boulder, step (cobble/boulder), riffle, shallow pool, deep alluvial and boulder pool	Pool-rapid, pool riffle
26	Moderate	Incised plain	30-50 m	Mixed	Rapid bedrock, rapid boulder, riffle, run, shallow pool, deep alluvial pool	Pool-rapid
25	Moderate	Incised plain	50-75 m	Mixed	Rapid boulder, step, riffle, shallow pool, deep pool, backwater	Pool-riffle
24	Moderate	Incised plain	30-50 m	Mixed	Rapid, riffle, shallow pool, deep pool, backwater.	Pool-riffle
23	Moderate	V-shaped valley	15-30 m	Mixed	Rock step, bedrock rapid, bedrock pool, bedrock run, rapid, riffle, run, shallow pool, deep pool.	Cascade, pool-riffle
22	Moderate	Incised plain	50-75 m	Mixed	Bedrock, rapid, bedrock pool, bedrock pavement, boulder rapid, riffle, shallow pool.	Flat bed rock, pool-riffle
41	Low	Incised plain	50-75 m	Mixed alluvial	Riffle, run, shallow pool, backwater	Pool-riffle

Table 3.9.1.3: The impacts observed between the different sites in the Luvuvhu River.

Site number	Erosion and siltation	Flow reduction and impoundments	Habitat modification	Water Quality	Abstraction	Vegetation clearance	Land use
27	Limited erosion	Weir		Moderate sewerage	Moderate	Moderate	Subsistence farming
28	Severe erosion		Moderate	Limited nutrient enrichment	Severe		Subsistence farming Forestry Sand mining Poor land use
26				Limited nutrient enrichment			Poor land use Subsistence farming Sand mining
51				Limited nutrient enrichment			Poor land use Settlements Subsistence farming Sand mining
25				Limited nutrient enrichment			Poor land use Subsistence farming Sand mining
24					Limited		Settlements Subsistence farming Grazing Forestry
23	Limited erosion					Clearance	
22		Weir		Limited nutrient enrichment			Poor land use Subsistence farming Sand mining
41	Limited erosion	Weir	Moderate	Moderate nutrient enrichment Sewerage	Severe		Commercial farming Settlements Sand mining

Table 3.9.1.4: Summary of the diversity of available fish habitat at the sites in the Luvuvhu River system. The numbers represent the specific biotope type as a percentage of the total number of biotopes at the site.

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 22	Mutshindudi	44	15	23	15
OPS 23	Mukhase	18	28	9	45
OPS 24	Mbwedi	28	58	0	14
OPS 25	Mutshindudi	38	38	12	12
OPS 26	Tshinane	25	12	25	38
OPS 27	Mutale	20	20	50	10
OPS 28	Mutshindudi	50	29	0	21
OPS 41	Tshino	50	20	0	30

Table 3.9.1.5: Summary of the dominant substrate in the surveyed biotopes at sites in the Luvuvhu River system. (Where A =bedrock, B = boulder, C = cobble, D = gravel, E = sand and F = silt dominated).

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 22	Mutshindudi	AAAABB	AE	AAA	AE
OPS 23	Mukhase	AA	ACC	A	AACEF
OPS 24	Mbwedi	CC	CCCC		C
OPS 25	Mutshindudi	CCC	BFF	C	F
OPS 26	Tshinane	CC	C	BBB	BEE
OPS 27	Mutale	C	BC	BBCCC	E
OPS 28	Mutshindudi	BBBBCEEE	CCCC		BCF
OPS 41	Tshino	CCE	C		CC

Table 3.9.1.6: Summary of the substrate diversity in the biotopes at the sites in the Luvuvhu River system. The numbers are counts at random points in the biotopes where the substrate occurs.

Site no	River/ Tributary	Shallow												Deep											
		Fast						Slow						Fast						Slow					
		BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si
OPS 22	Mutshindudi	4	2	3	1	1		1				2	1	3	2			1		1				2	
OPS 23	Mukhase	2		1		1		2	1	3		3	1					1		2	1	3	1	5	2
OPS 24	Mbwedi			2		1				4		2										1			
OPS 25	Mutshindudi			3					1			1	2			1								1	1
OPS 26	Tshinane		1	2		1			1	1		1			3	3					3	1		2	2
OPS 27	Mutale								1	2		1			5	4		2						1	1
OPS 28	Mutshindudi		7	6					4	4		1									2	3		3	1
OPS 41	Tshino		2	3	1				1	1												2		2	

Table 3.9.1.7: The fish species collected in the biotopes at the sites in the Luvuvhu River system. The terms fast, slow, shallow and deep are adapted from Kleynhans (2007). The abbreviations for the species are listed in appendix I.

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 22	Mutshindudi	AMAR, BMAR, CPAR CPRE, CSWI, LCYL, LMOL	BMAR, BVIV, LCYL,	BMAR, LCYL, , LMOL	
OPS 23	Mukhase	CPRE, LCYL	AURA, BNEE,		AURA, BEUT, BLIN, BMAR, BVIV, OPER, MACU, MMAC, PPHI
OPS 24	Mbwedi	AURA, BMAR, BVIV, CPRE, LCYL, LMOL, MACU, MBRE	CGAR, PPHI, TREN		CGAR, LCYL
OPS 25	Mutshindudi	BMA, BVIV, CPRE	BNEE, BVIV, OMOS	BMAR, BVIV, BTRI, CPRE, MMAC	
OPS 26	Tshinane	AURA, BMAR, BNEE, CPRE, LCYL, LMOL, MMAC	AURA, BMAR, BNEE, CPRE, LMOL	BMAR, CPRE, LCYL,LMOL,	BMAR, CGAR, LCYL
OPS 27	Mutale		AURA, BMAR, LCYL, BNEE, CPRE,	AURA, BNEE, CPRE, LCYL, OPER	OPER
OPS 28	Mutshindudi	AURA, BEUT, BMAR, BNEE, CPRE, OPER	AURA, BEUT, OMOS		AURA, OMOS
OPS 41	Tshino	BMAR, CPRE, LCYL, OMOS, PPHI	BMAR, CPRE, OMOS		BMAR, CGAR, OMOS, PPHI, TREN

Table 3.9.1.8: Summary of fish biodiversity at the sites in the Luvuvhu River system. The abbreviations for the species are listed in appendix I.

	Site numbers							
Species	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**41**
AURA		X	X		X	X	X	
BAFR								
BEUT		X					X	
BLIN		X						
BMAR	X	X	X	X	X	X	X	X
BNEE		X		X	X	X	X	
BPAU								
BPOL								
BRAD								
BTRI				X				
BVIV		X	X	X				
CANO								
CGAR			X		X			X
CPAR	X							
CPRE	X	X	X	X	X	X	X	X
CSWI	X							
GGUI								
LCYL	X	X	X		X	X	X	X
LMOL	X		X		X			
LRUD								
MACU		X	X					
MBRE			X					
MMAC		X		X	X			
OMOS				X			X	X
OPER			X			X	X	
PCAT								
PPHI		X						X
SINT								
TREN			X					X
TSPA								

3.9.4 THE SHINGWEDZI RIVER

3.9.4.1 River description

The Shingwedzi River lies in one of the drier sub-catchments of where the rainfall varies between 400 and 600 mm per annum with a mean annual run-off of only 90 million m^3 per annum (Figure 3.9.2.1). It is a non-perennial river and the major components and its tributaries originate outside the park with impacts reflecting inside the park. It should be borne in mind that this was based on an estimated population of 135,000 people in the sub-catchment. If trends in population are similar in this region as elsewhere in South Africa it would be correct to surmise that the Shingwedzi River is

under severe pressure and that this will increase in the near future. Visual proof of this pressure was already visible when travelling through the area during the survey.

3.9.4.2 Geomorphology description

Table 3.9.2.1 shows the specific results for the Shingwedzi River.

3.9.4.3 Potential threats

The impacts in the Shingwedzi River impacts were assessed in the same way as was done in the Luvuvhu River. In the Shingwedzi River, very little impacts were observed. The site sampled is in the Dzombo River, a tributary that originates within the boundaries of the KNP. The impacts are related to restricted local impacts, the most important being the impoundment upstream of the sampling site. Limited siltation and nutrient enrichment was also evident.

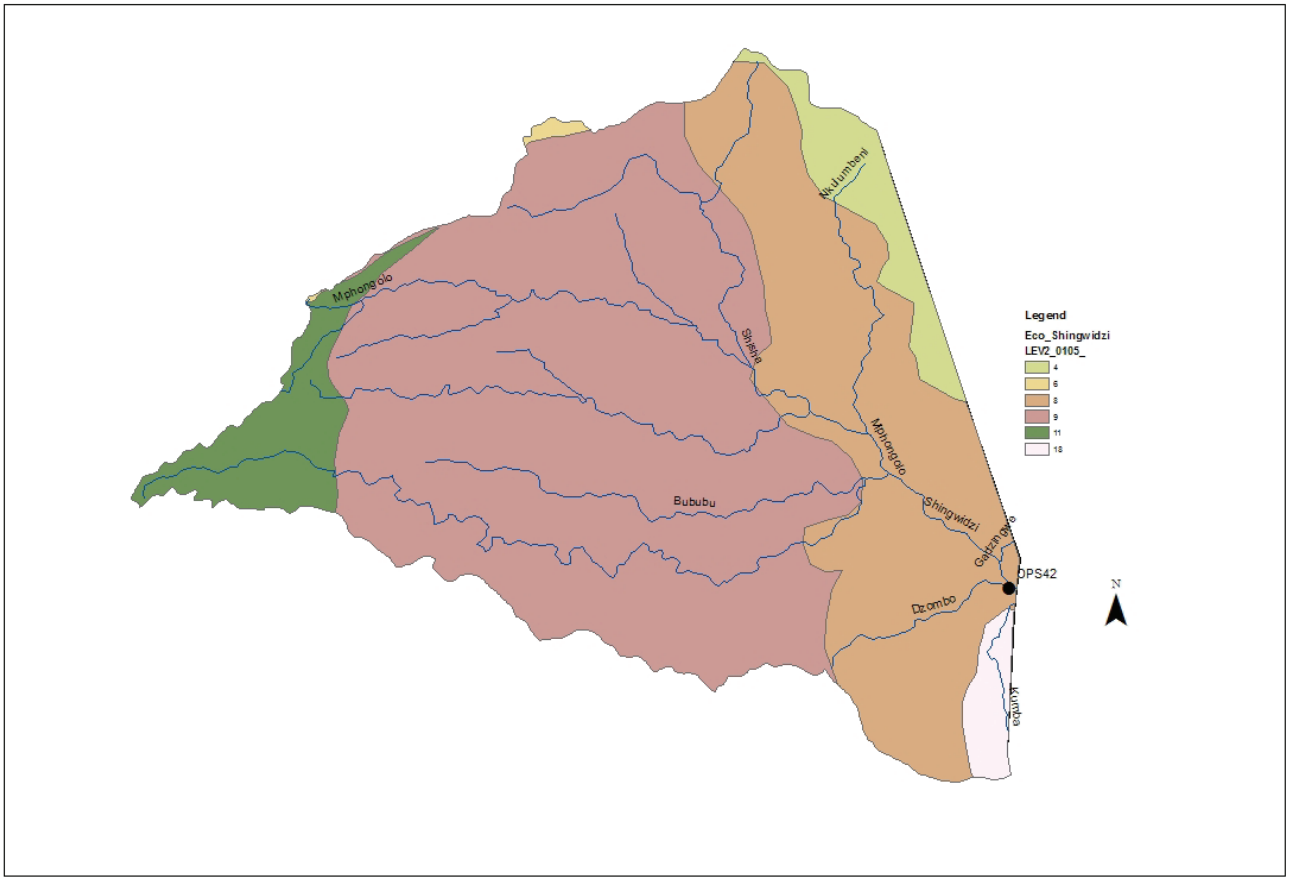


Figure 3.9.2.1: The project sample sites in the Shingwedzi River. Tributaries and Level 2 Ecoregions are indicated.

3.9.4.4 Biotopes and site heterogeneity

The results, shown in tables 3.9.2.3 to 3.9.2.5 shown that the site is homogenous with regard to the biotope diversity and that within the biotope the substrate is dominated by sand.

3.9.4.5 Fish

The results fish survey are shown in 3.9.2.6 and it shows that seven species were collected at the site.

Table 3.9.2.1: Some geomorphological aspects of the site in the Shingwedzi River.

Site number	Flow regime	Valley form	Channel dimension	Channels type	Morphological units	Reach type	Comments
OPS 42	Low	Incised plain	30-50 m	Mixed	Bedrock pool, bedrock run, shallow pool, deep pool	Flat bedrock, flatbed	

Table 3.9.2.2: The impacts observed between the different sites in the Shingwedzi River.

Site number	Erosion and siltation	Flow reduction and impoundments	Habitat modification	Water quality	Abstraction	Vegetation clearance	Land use	Comments
OPS 42	Low – roads	High due to dam upstream	Low	Moderate – some enrichment	Low	None	Conservation	

Table 3.9.2.3: Summary of the diversity of available fish habitat at the sites in the Shingwedzi River system. The numbers represent the specific biotope type as a percentage of the total number of biotopes at the site.

	Shallow			Deep	
Site number	River/Tributary	Fast	Slow	Fast	Slow
OPS 42	Dzombo				100

Table 3.9.2.4: Summary of the dominant substrate in the surveyed biotopes at sites in the Shingwedzi River system. (Where A =bedrock, B = boulder, C = cobble, D = gravel, E = sand and F = silt dominated).

	Shallow			Deep	
Site number	River/Tributary	Fast	Slow	Fast	Slow
OPS 42	Dzombo				F

Table 3.9.2.5: Summary of the substrate diversity in the biotopes at the sites in the Shingwedzi River system.

Site number	River/Tributary	Shallow												Deep											
		Fast						Slow						Fast						Slow					
		BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si
OPS 42	Dzombo																								1

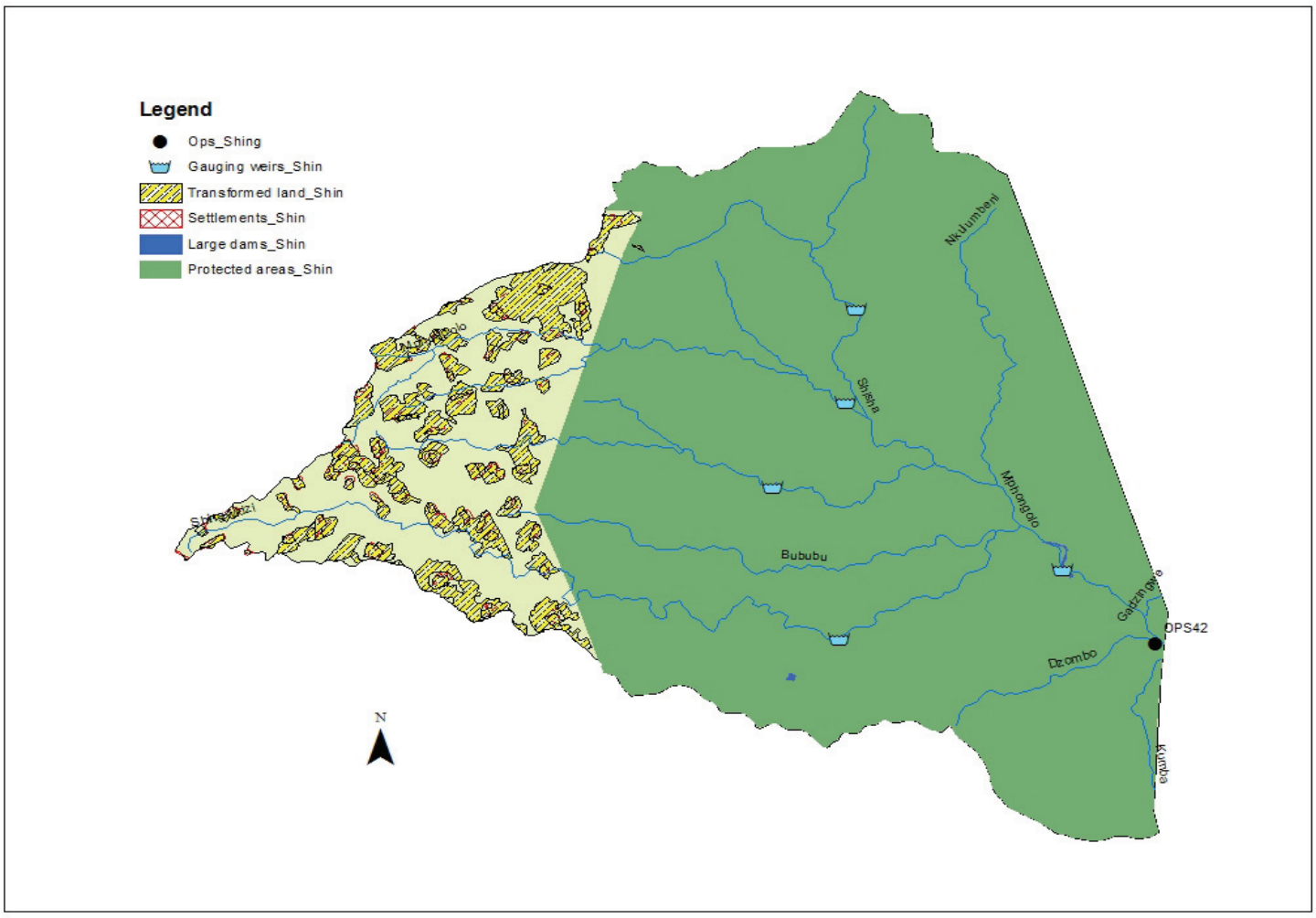


Figure 3.9.2.2: The Shingwedzi catchment with associated land-use impacts

Table 3.9.2.6: The fish species collected in the biotopes at the sites in the Shingwedzi River system. The abbreviations for the species are listed in appendix I.

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 42	Dzombo				BPAU, BTRI, CGAR, LROS, LRUD, OMOS, TREN

3.9.4.6 Discussion

The Dzombo River is a small tributary which originates in the Kruger National Park except for a dam upstream from the confluence there is little other impacts. Stream flow or the lack thereof is however a concern. It is also questionable whether this site is representative of the river. On the other hand the Shingwedzi River, into which the Dzombo River flows a few hundred meters from the site, is sand and bedrock dominated.

3.9.5 THE LETABA AND OLIFANTS RIVERS

3.9.5.1 River description

The Olifants River originates in the Mpumalanga Highveld where it flows in an easterly direction through the KNP. It subsequently joins the Limpopo River (Figure 3.9.3.1). The Groot Letaba originates in the Wolkberg and is joined by its main tributary, the Klein Letaba River at the western boundary of KNP. Downstream the Groot Letaba River is joined by the Olifants River. The Letaba River Catchment covers an area of 13400 km2 and the topography of the catchment is characterized by in the southern part by rolling sloped hills, where it makes its way through the Drakensberg to the plains of the Lowveld. The geology in this catchment consists primarily of hard rock formations, with the occurrence of the Bushveld Igneous Complex as the main characteristic. The soils of the area are variable and highly erodible. The climate varies from temperate in the southern Highveld, to sub-tropical in the east of the escarpment. Precipitation is characterized as summer rainfall, with approximately 500 mm in the Lowveld and up to 1000 mm in the mountains.

3.9.5.2 Geomorphology description

The results (Table 3.9.3.1) show the diverse character of the component of the river that was surveyed with bedrock dominated narrow channels in the upper reaches to wider alluvial beds lower down.

3.9.5.3 Potential threats

Among other threats (Table 3.9.3.2) In the Letaba/Olifants River a host of pollution issues were noted. In the higher reaches, erosion is a problem and this is related to forestry and poor agricultural activities in the mountainous areas. The system is further impacted by habitat modification and abstraction and sewerage inputs are very high throughout the system. Return flows from agriculture further contribute to the degradation of water quality and mines in the Highveld regions of the Olifants River and on the western border of the Kruger National Park are also contributors to the poor water quality. In the lower reaches of the system, water abstraction, lodges and camps in the conservation areas add pollutants to the river.

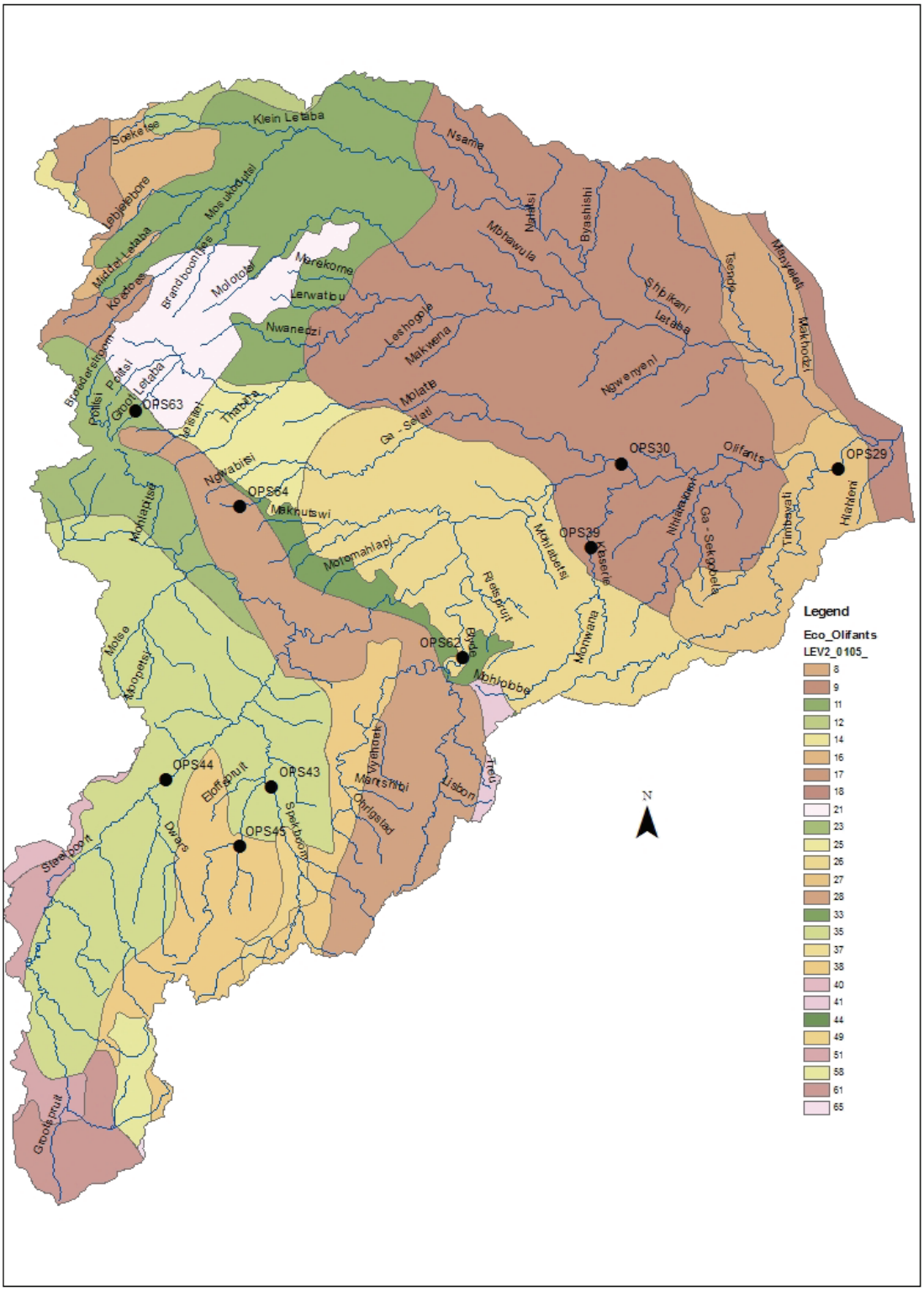


Figure 3.9.3.1: The project sample sites in the Letaba and Olifants Rivers. The tributaries and Level 2 Ecoregions are indicated

3.9.5.4 Biotopes en site heterogeneity

The results, summarized in tables 3.9.3.3 to 3.9.3.5, show a vast array of diversity throughout the two rivers. It should however be noted that in the biotopes, both in the Blyde and Groot Letaba river, where O*. peringueyi* were collected the substrate consisted of a mixture of cobbles and sand.

3.9.5.5 Fish.

The results obtained (Tables 3.9.3.6 and 3.9.3.7) show that twenty-one species were collected with varying degrees of diversity at the sites.

3.9.5.6 Discussion

Although the two sites where *O. peringueyi* was collected in this river system do have potential as conservation areas for the species, the challenges are far greater than in the Luvuvhu system. The site in the Blyde River (OPS 62) is directly downstream of the Blyde dam and as such subject to the typical problems associated with flow regulation. The lack of sand in the shallow and slow deep habitats, which is regarded as a key habitat component for the species, is evident of flow control. The site in the Groot-Letaba (OPS 63) also has potential but is prone to sewage disposal (as was evident on site) and is subject to a very high degree of anthropogenic activity directly upstream.

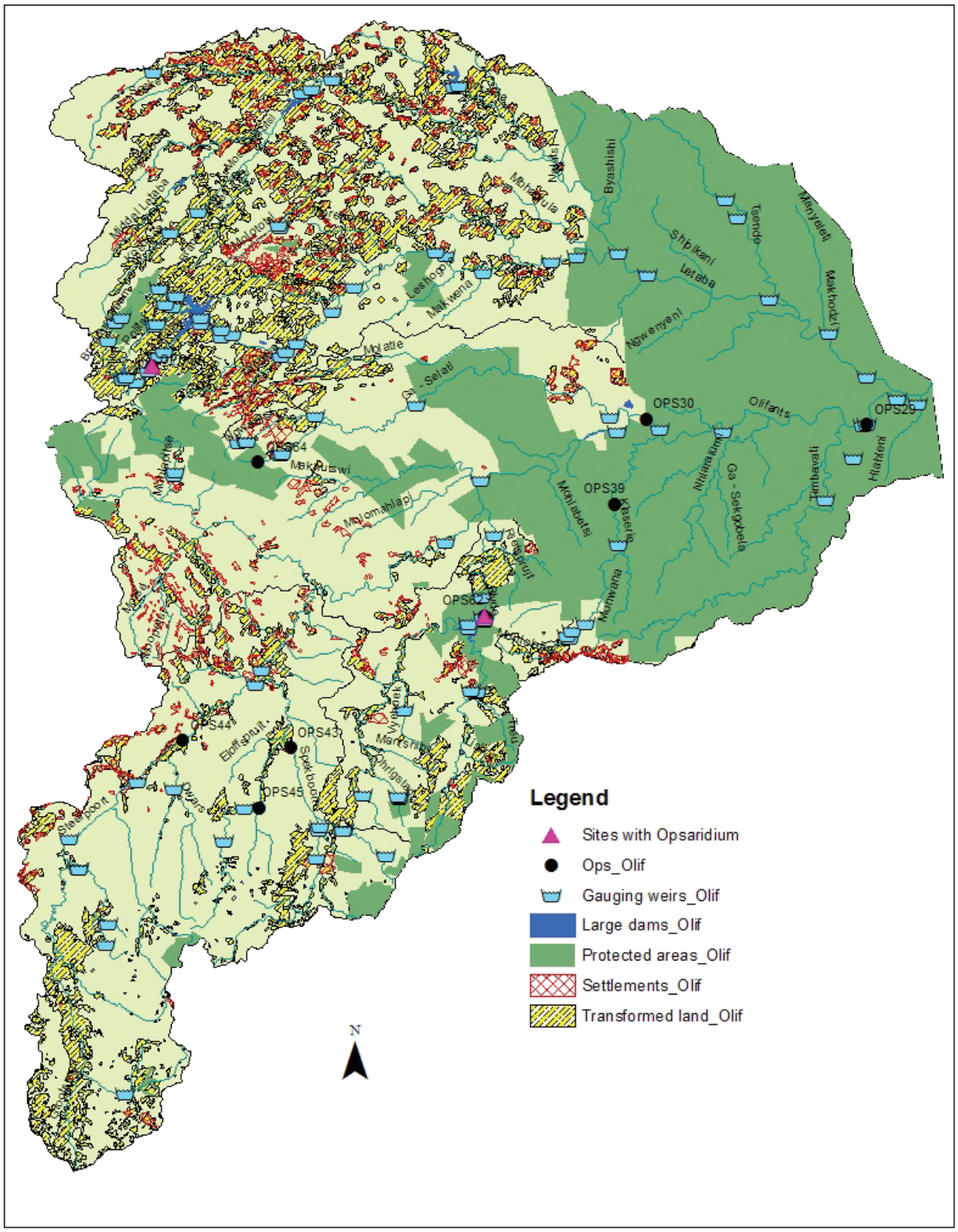


Figure 3.9.3.2: The lower Olifants/Letaba catchments with associated land-use impacts

Table 3.9.3.1: Some geomorphological aspects for the sites in the Letaba/Olifants River.

Site number	Flow regime	Valley form	Channel dimension	Channels type	Morphological units	Reach type
63	Low	V-shaped valley	50-75 m	Bedrock	Bedrock pool, bedrock pavement, riffle, run, shallow pool	Pool-riffle
64	Low	Incised plain	15-30 m	Alluvial	Riffle, run, shallow pool, deep pool, flat sand bed	Pool-riffle
30	Medium	Incised plain	150-300 m	Mixed	Rapid (bedrock, boulder), bedrock run, bedrock backwater, plane bed, riffle, rapid, run, shallow pool, deep pool, flat sand bed, backwater	Flat bedrock, pool-riffle
44	Low	Incised plain	75-100 m	Alluvial	Step, plane bed, riffle, run, deep pool, flat sand bed	Pool-riffle
45	Low	Foothill floodplain	100-150 m	Alluvial	Riffle, run, shallow pool, backwater	Pool-riffle
43	Low	Incised plain	100-150 m	Alluvial	Riffle, run, deep pool	Pool-riffle
62	Medium	Incised plain	75-100 m	Bedrock	Bedrock pool, bedrock pavement, bedrock run, bedrock backwater, riffle, run, shallow pool, deep pool, backwater	Flat bedrock, pool-riffle
39	Low	Incised plain	150-300 m	Bedrock	Bedrock pool, bedrock pavement, bedrock run, backwater, rapid, run, shallow pool, backwater	Flat bedrock, pool-riffle
29	Medium	Incised plain	150-300 m	Mixed	Bedrock pavement, bedrock run, riffle, rapid, run, shallow pool, deep pool, backwater	Flat bedrock, pool-riffle

Table 3.9.3.2: The impacts observed between the different sites in the Letaba/Olifants River.

Site number	Erosion and siltation	Flow reduction and impoundments	Habitat modification	WQ	Abstraction	Vegetation clearance	Land use	
63	Severe erosion and siltation from roads and forestry	Severe, dams and weirs	Extensive	Sewerage from informal housing, saw mills return flows	High	Severe – forestry	Forestry, agricultural, settlements, roads, saw mills	Alien fish
64	Medium – roads and clearance of vegetation	Limited, weir	Limited	Slightly impacted – agriculture and housing	Severe – all water diverted into canal	Moderate, forestry, agriculture and wood collection	Agriculture (subsistence and commercial), informal settlement, reserve housing	
30	Limited from outside KNP	Severe, large dams and weirs	Severe	Very poor – mines, sewerage, agriculture	High	High outside KNP	Mines, commercial and subsistence farming, sand mining	
44	Limited	Moderate	Moderate – high	Poor – mines, agriculture, sewerage, settlements, nutrient enrichment	Moderate	Moderate – high	Agriculture, mines, settlements	
45	Limited	Limited	Moderate – abstraction and crossings	Fairly good	Low	Low	Agriculture	
43	Limited	Limited	Moderate	Nutrient enrichment from agriculture and sewerage	Low	Low	Agriculture and housing	
62	Moderate	High – dam	Severe	Poor – agriculture, holiday resort, old mines	High	Moderate	Agriculture, holiday resort, forestry	Alien fish
39	Moderate – forestry, rural areas	Moderate	Limited	Moderate – rural settlements, lodges	High	Severe	Lodges, agriculture, forestry	Alien fish
29	Limited from roads	Weirs in KNP	Limited – low bridge crossings	Limited in KNP from camps	Limited	Very low	Camps	

Table 3.9.3.3: Summary of the diversity of the available fish habitat at the sites in the Letaba-Olifants River system. (The numbers represent the specific biotope as a percentage of the total biotopes at the site).

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 29	Olifants	50		50	
OPS 30	Olifants	50		50	
OPS 39	Klaserie	50	30		20
OPS 43	Spekboom	38	50		12
OPS 44	Steelpoort		60		40
OPS 45	Watervals		100		
OPS 62	Blyde	60	10	10	20
OPS 63	Groot Letaba	69	8	15	8
OPS 64	Ga-Selati	66			34

Table 3.9.3.4: Summary of the dominant substrate in the surveyed biotopes at sites in the Letaba-Olifants River system. (Where A =bedrock, B = boulder, C = cobble, D = gravel, E = sand and F = silt dominated).

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 29	Olifants	AAA		AACD	
OPS 30	Olifants	AC		AC	
OPS 39	Klaserie	DEEEE	CEE		CE
OPS 43	Spekboom	CCC	EEEE		B
OPS 44	Steelpoort		CCC		CC
OPS 45	Watervals		CCCCF		
OPS 62	Blyde	ABCCCC	C	A	CE
OPS 63	Groot Letaba	CCCCCCCCC	C	DD	F
OPS 64	Ga-Selati	CC			D

Table 3.9.3.5: Summary of the substrate diversity in the biotopes at the sites in the Letaba-Olifants River system.

Site number	River/ Tributary	Shallow												Deep											
		Fast						Slow						Fast						Slow					
		BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si
OPS 29	Olifants	2				3								2		1	1	2							
OPS 30	Olifants	1		2	1									1	1	2		1							
OPS 39	Klaserie	2	1	1		3	2	2		1		2								2		1	1	2	1
OPS 43	Spekboom		1	3	1	1				3	1	4									1	1	1		
OPS 44	Steelpoort		2	3																1	2	2			
OPS 45	Watervals								1	5	4	4													
OPS 62	Blyde	1	5	5						1	1			1	1							1		2	2
OPS 63	Groot Letaba		5	7	7	5			1	1	1	1			2	2	2	2						1	1
OPS 64	Ga-Selati		2	2	2	1																1	1	1	

Table 3.9.3.6: The fish species collected in the biotopes at the sites in the Letaba-Olifants river system.

Site number	River/ Tributary	Shallow		Deep	
		Fast	Slow	Fast	Slow
OPS 29	Olifants	BMAR, CPAR, LCYL		CPAR, CSWI, LCYL	
OPS 30	Olifants	CGAR, CPAR, LCYL		CPAR, LCYL	
OPS 39	Klaserie		BPAU, BTRI, BVIV, LCYL, MACU, OMOS, PPHI,		BLIN,BPAU,BTRI, BVIV,OMOS, PCAT,LCYL, MACU
OPS 43	Spekboom	BMAR, CPAR, CPRE, LCYL	BMAR, CPAR, PPHI		BEUT, BBRE, BMAR, BLIN, BPAU, PPHI,
OPS 44	Steelpoort		BMAR	BMAR, CPAR, CPRE, LMOL,	
OPS 45	Watervals		AURA, BBRE, BEUT, BLIN, BMAR, BNEE, BTRI, CPAR, CPRE,		
OPS 62	Blyde	BMAR, BRAD, CPRE, LCYL			OPER, PPHI
OPS 63	Groot Letaba	BMAR, CPRE, OPER,	PPHI	AURA, BMAR, CPRE,	
OPS 64	Ga-Selati	BMAR, LCYL			BEUT,BLIN, BMAR, BVIV, PPHI

Table 3.9.3.7: Summary of fish biodiversity at the sites in the Letaba-Olifants River system

Species	29	30	39	43	44	45	62	63	64
AURA						X		X	
BBRE				X		X			
BEUT				X		X			X
BLIN			X	X		X			X
BMAR	X			X	X	X	X	X	X
BNEE						X			
BPAU			X	X					
BRAD							X		
BTRI			X			X			
BVIV			X						X
CGAR		X							
CPAR	X	X		X	X				
CPRE				X	X	X	X	X	
CSWI	X								
GGUI									
LCYL	X	X	X	X			X		X
LMOL					X				
MACU			X						
OMOS			X						
OPER							X	X	
PCAT			X						
PPHI			X	X		X	X	X	X

3.9.6 THE SABIE RIVER

3.9.6.1 River description

The Sabie River catchment covers 7096 km^2, of which the Sand River Sub-Catchment contributes 1910 km^2 (Figure 2.5.1). The topography of Sabie Catchment is dominated consists of the Drakensberg escarpment in the west and north-west, from where the Sabie River and some of its major tributaries originate. Other important geographic features are the Lowveld to the east and the Mankeli Hills in the south. In the upper reaches the catchment falls from 1000 m.a.s.l at Graskop, to around 600 m.a.s.l at Hazyview, only a few kilometres downstream and then to 250 m at Skukuza. The variation in mean annual precipitation is as dramatic with rainfall in the Sabie River is situated in a humid mountainous region with high precipitation (2000 mm/annum). The rainfall decreases towards in the semi arid Low-veld (550 mm/annum) (Woodhouse, 1995). The main tributaries are the Sand, Marite and Mac Mac rivers. Whereas the Marite originates in similar rainfall conditions as the main stem, the flow in Sand River is extremely variable and nearly doubles the variability of the Sabie River downstream of the Sabie-Sand confluence.

3.9.6.2 Geomorphology description

The results are shown in table 3.9.4.1.

3.9.6.3 Potential threats

The impacts in the Sabie-Sand River system were assessed in the same way as was done in the Luvuvhu River and the results are shown in table 3.9.4.2. Spatial data, as described in paragraph 3.9.2.4., was used to investigate the impacts of land transformation and land use. In the Sabie/Sand River system, erosion is a problem in the upper reaches and this is linked to the land use, which is mainly forestry in the mountainous areas. Further downstream there is a slight improvement and the erosion here can be linked to the roads in the area. Habitat modification is rated as moderate to high throughout the system and is linked to the roads, bridges and impoundments. The water quality is fairly poor as a result of the sewerage and run-off from the settlements and other activities. Further downstream lodges contribute to the problem. A slight improvement in water quality is observed in the conservation areas, but the build- up of pollutants in the sediments are of concern.

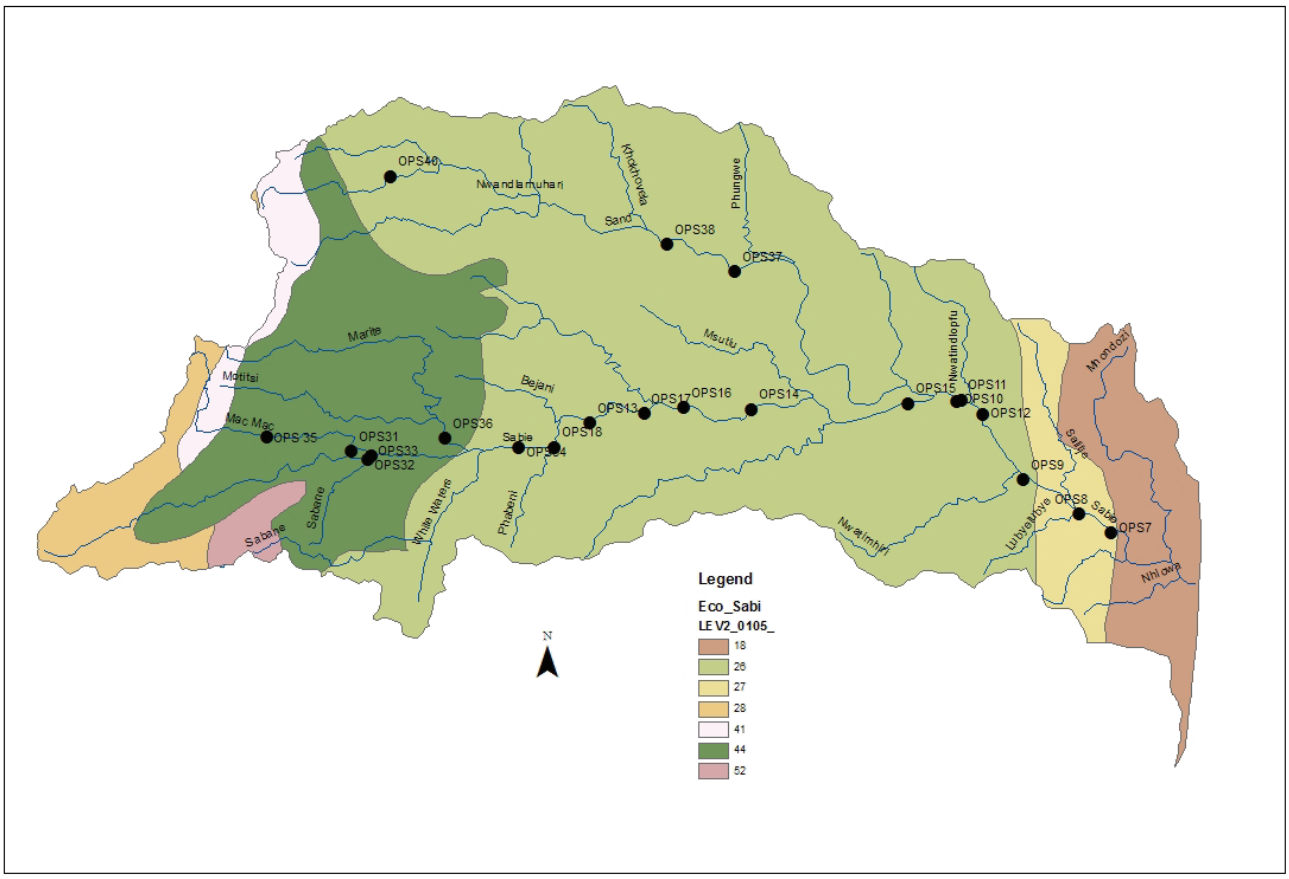


Figure 3.9.4.1: The project sample sites in the Sabie river. Tributaries and Level 2 Ecoregions are also indicated

3.9.6.4 Biotopes and site heterogeneity.

The results obtained are shown in table 3.9.4.3, 3.9.4.4 and 3.9.4.5.

3.9.6.5 Fish

The results are shown in tables 3.9.4.6 and 3.9.4.7.

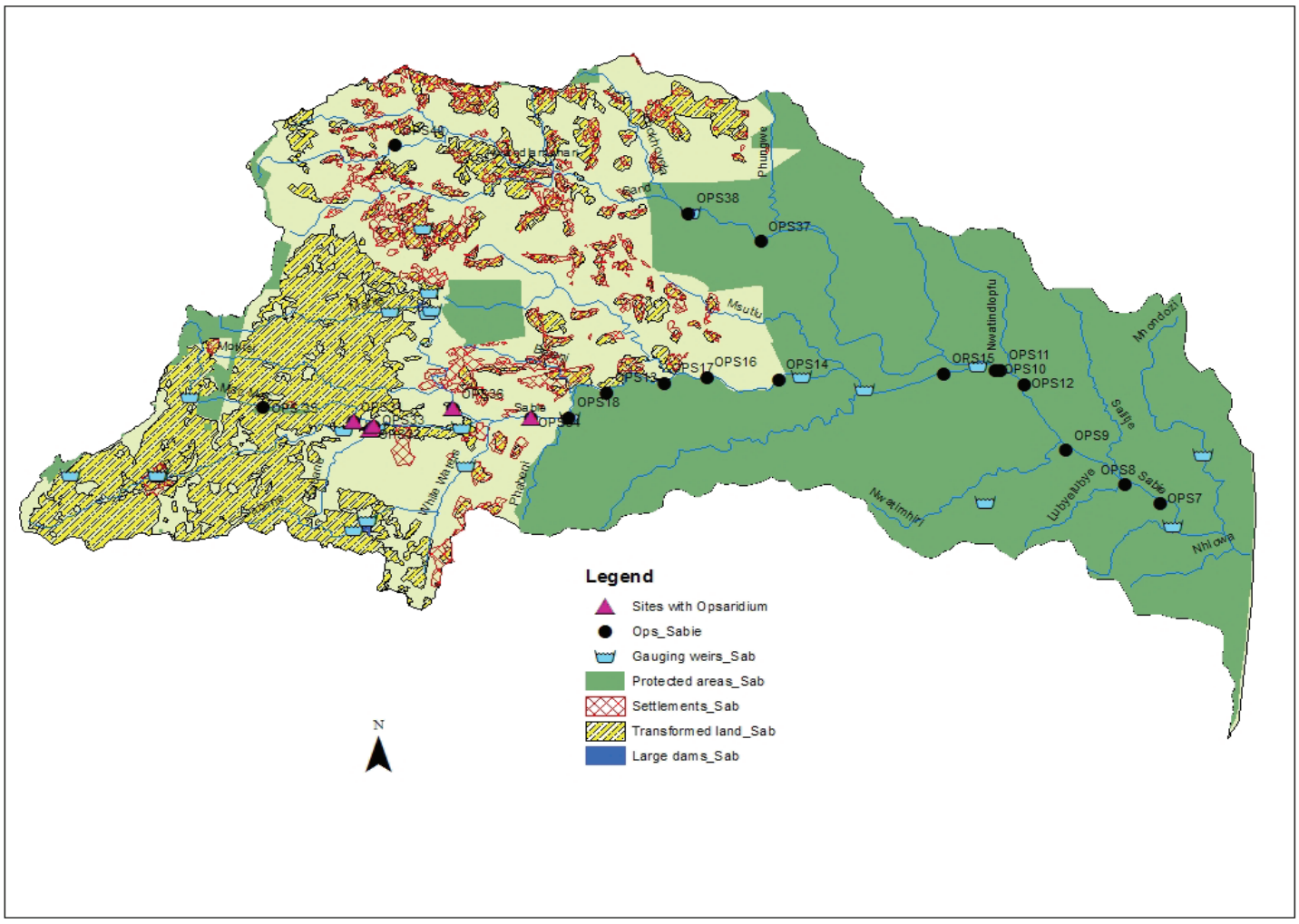


Figure 3.9.4.2: The Sabie River catchment with associated land-use impacts

Table 3.9.4.1: Some geomorphological aspects for the sites in the Sabie/Sand River.

Site number	Flow regime	Valley form	Channel dimension	Channel type	Morphological units	Reach type
40	Low	V-shaped valley	50-75 m	Bedrock	Bedrock rapid, bedrock pool, bedrock pavement, bedrock run	Flat bedrock
38	Low	Incised plain	150-300 m	Bedrock, alluvial	Rock step, bedrock pool, bedrock run, backwater, riffle, run, shallow pool, deep pool	Flat bedrock, pool-riffle
37	Low	Incised plain	150-300 m	Bedrock, alluvial	Rock step, bedrock pool, bedrock run, bedrock backwater, rapid, run, shallow pool, backwater	Flat bedrock, pool-riffle
35	Medium	Alternating slopes	30-50 m	Fixed boulder bed	Riffle, rapid, shallow pool, deep pool, flat sand bed, backwater	Pool-riffle
31	Medium	V-shaped valley	30-50 m	Fixed boulder bed	Step, plane bed, riffle, rapid, run, shallow pool, deep pool, flat sand bed, backwater	Pool-riffle
32	Low	Incised plain	15-30 m	Bedrock, mixed	Plane bed, shallow pool, flat sand bed, backwater	Step-pool, flat bed
33	Medium	Incised plain	30-50 m	Fixed boulder bed	Riffle, rapid, run, shallow pool, flat sand bed	Plane bed, pool riffle
36	Medium	V-shaped valley	50-75 m	Bedrock	Rock steps, bedrock rapid, bedrock pool, bedrock backwater	Cascade
34	Medium	Incised plain	50-75 m	Alluvial	Step, plane bed, riffle, rapid, run, shallow pool, flat sand bed	Pool-riffle
18	Medium	Incised plain	100-150 m	Bedrock	Rock steps, bedrock pool, bedrock pavement, bedrock run, bedrock backwater, step, plane bed, riffle, rapid, run, shallow pool, deep pool, backwater	Cascade, pool-riffle
16	Medium	Incised valley	150-300 m	Bedrock	Rock steps, bedrock rapid, bedrock pool, bedrock run, bedrock backwater, run, deep pool, flat sand bed, backwater	Cascade, pool-riffle
15	Medium	Incised plain	100-150 m	Bedrock	Rock steps, bedrock rapid, bedrock pool, bedrock run, bedrock backwater, riffle, shallow pool, deep pool, backwater	Cascade, pool-riffle
10	Medium	Foothill floodplain	50-75 m	Alluvial	Flat sand bed	Flat bed
11	High	Foothill floodplain	150-300 m	Mixed	Rock steps, bedrock rapid, bedrock pool, bedrock pavement, bedrock run, bedrock backwater, rapid, shallow pool, flat sand bed	Flat bedrock, pool-riffle
12	High	Foothill floodplain	100-150 m	Bedrock, mixed	Bedrock rapid, bedrock pool, bedrock pavement, bedrock run, bedrock backwater, plane bed, run, shallow pool, deep pool, flat sand bed, backwater	Flat bedrock, pool-riffle
9	Medium	Incised plain	100-150 m	Mixed	Step, riffle, shallow pool, deep pool, flat sand bed, backwater	Pool-riffle
8	High	Incised plain	100-150 m	Bedrock, mixed	Rock step, bedrock rapid, bedrock run, bedrock backwater, shallow pool, flat sand bed, backwater	Cascade, flat bed
7	High	Incised plain	100-150 m	Mixed	Bedrock rapid, bedrock backwater, step, riffle, rapid, shallow pool, deep pool, flat sand bed, backwater	Cascade, pool-riffle/pool-rapid

Table 2.4.2: The impacts observed between the different sites in the Sabie/Sand River

Site number	Erosion and siltation	Flow reduction and impoundments	Habitat modification	Water Quality	Abstraction	Vegetation clearance	Land use
40	Moderate – high	Low	Moderate	High impact – sewerage and enrichment	Low	Moderate – forestry	Settlements, forestry, subsistence farming
38	Moderate	Moderate – high	High	High impacts – sewerage, agriculture, lodges	Moderate	Moderate	Lodges, settlements, agriculture, sand mining
37	Low	Very low	Low	Moderate – sewerage, siltation	Low	Very limited	Lodges
35	Moderate – high	High	High	Moderate	High	High	Forestry
31	High	Low	Moderate	Moderate impacts	Low	High	Forestry, village
32	High	Moderate	High	High – nutrient enrichment	High	High	Forestry, extensive agriculture, recreation
33	High	Moderate – high	High	High	High	High	Forestry, lodges, recreation, commercial farming
36	High	High	High	Moderate – high	Low	High	Settlements, forestry, commercial agriculture
34	Moderate - high	High	High	High – sewerage, siltation	High	High	Commercial farming, sand mining, lodges, golf courses
18	Moderate	Low	Moderate	Moderate – high, return flows and run-off	Moderate – high	Low	Agriculture, settlements, lodges
13	High	Low	Moderate	High – enrichment, sediments	Low	Low	Conservation
17	Moderate - roads, run-off	Low	Low - moderate	Moderate – high – agriculture return flows	Moderate	Moderate	Agriculture, settlements, lodges
16	Moderate - roads, run-off	Low	Low - moderate	Moderate – high – agriculture return flows	Moderate	Moderate	Agriculture, settlements, lodges
14	Moderate - high	Moderate - weirs	High – weirs, bridges, roads	High – sewerage, return flows, agriculture, siltation	Moderate – high	Moderate - high	Lodges, settlements, agriculture
15	Low - roads	Moderate – weirs and bridges	Low – bridges and weirs	Low – siltation and some outside influences, camp	Low	Low	Camp, conservation
10	Low - roads	Moderate – bridges and causeways	Low – bridges and causeways	Low – moderate, enrichments from camps	Low – moderate	Low	Camps, conservation
11	Low - roads	Moderate – bridges and causeways	Low – bridges and causeways	Low – moderate, enrichments from camps	Low – moderate	Low	Camps, conservation
12	Low - roads	Moderate – bridges and causeways	Low – bridges and causeways	Low – moderate, enrichments from camps	Low – moderate	Low	Camps, conservation
9	Low - roads	Moderate – bridges and causeways	Low – bridges and causeways	Low – moderate, enrichments from camps	Low – moderate	Low	Camps, conservation
8	Low - roads	Moderate – bridges and causeways	Low – bridges and causeways	Low – moderate, enrichments from camps	Low – moderate	Low	Camps, conservation
7	Low - roads	Moderate – bridges and causeways	Low – bridges and causeways	Low – moderate, enrichments from camps	Low – moderate	Low	Camps, conservation

Table 3.9.4.3: Summary of the diversity of the available fish habitat at the sites in the Sabie-Sand River system. (The numbers represent the specific biotope as a percentage of the total biotopes at the site).

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 7	Sabie	17	42	25	16
OPS 8	Sabie	16	34	16	34
OPS 9	Sabie	44	44	12	0
OPS 10	Sabie	67	0	34	0
OPS 11	Sabie	19	27	27	27
OPS 12	Sabie	28	16	28	28
OPS 13	Sabie	X	X	X	X
OPS 14	Sabie	67	17	13	0
OPS 15	Sabie	30	0	60	10
OPS 16	Sabie	20	30	50	0
OPS 17	Sabie	40	20	30	10
OPS 18	Sabie	X	X	X	X
OPS 31	Mac Mac	33	33	22	12
OPS 32	Sabane	25	63	0	12
OPS 33	Sabie	61	17	0	13
OPS 34	Sabie	50	33	0	17
OPS 35	Mac Mac	30	50	0	20
OPS 36	Marite	17	0	16	67
OPS 37	Sand	33	34	8	25
OPS 38	Sand	28	57	0	15
OPS 40	Groot Sand	28	28	16	28

Table 3.9.4.4: Summary of the dominant substrate in the biotopes at the sites in the Sabie- Sand River system. (Where A=bedrock, B = boulder, C = cobble, D = gravel, E = sand and F = silt).

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	Slow
OPS 7	Sabie	BC	BCDDE	BBC	BB
OPS 8	Sabie	B	EE	B	AE
OPS 9	Sabie	CCDE	DEEE	A	
OPS 10	Sabie	DEEE		ED	
OPS 11	Sabie	A	EE	AAAA	AAE
OPS 12	Sabie	ABE	EE	AB	EE
OPS 13	Sabie				
OPS 14	Sabie	BBCC	E	E	
OPS 15	Sabie	AAC		AAABDE	E
OPS 16	Sabie	AB	EEE	BBBCC	
OPS 17	Sabie	AACE	AE	BCE	E
OPS 18	Sabie				
OPS 31	Mac Mac	ACE	AEE	AC	E
OPS 32	Sabane	B	BCE		E
OPS 33	Sabie	ACCCCCC	EE		EC
OPS 34	Sabie	AACCC	CCCD		E
OPS 35	Mac Mac	CCC	DDDDE		EE
OPS 36	Marite	E	B	B	BEE
OPS 37	Sand	AAAA	AAEE	E	AEE
OPS 38	Sand	AA	CCEE		E
OPS 40	Groot Sand	CE	EE	A	AE

3.9.6.6 Discussion

This is probably the most diverse of the river systems surveyed. Within the river the three typical reaches/zones namely mountain stream, middle reach and lower reach were easy to identify. *O*

peringueyi were only collected in the sites that were situated in the mountain reach and the upstream parts of the middle reaches. In both these areas the substrate not only consisted of large alluvial particles (boulders and cobbles) but the presence of sand (and in particular coarse sand) was evident in most of the biotopes at the sites (OPS 31, 32, 33, 34 and 36.) Further downstream of these sites the river dramatically changes character and although sand is present throughout all downstream sites, the presence of bedrock and the absence of large alluvial particles should be noted.

It is evident that in this river the highest conservation potential exists within the reach where site 33 is located. Although also impacted by human activity and forestation, these impacts can be regarded as “limited” and has the potential to be managed or mitigated. The physico-chemical characteristics of this river reach is regarded as ideal for the survival of the species.

Table 3.9.4.5: Summary of the substrate diversity in the biotopes at the sites in the Sabie – Sand River system.

		Shallow												Deep											
Site number	**River/Tributary**	**Fast**						**Slow**						**Fast**						**Slow**					
		BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si
OPS 7	Sabie		1	2	1	1			3	4	3	2	1		2	2	1	1			2	2			
OPS 8	Sabie	2	2	1								2	2							2					
OPS 9	Sabie	1	3	3	3	1	1				2	3	3	1				1							
OPS 10	Sabie				4	4											2	2							
OPS 11	Sabie	1				1		1				2		4	1	1	2	1	1	2			2	2	
OPS 12	Sabie	2	1			2		1		1	1	2	2	1	3	1	2	1			1	1	1	1	
OPS 13	Sabie																								
OPS 14	Sabie		3	3	3	1						1	1			1	1	1							
OPS 15	Sabie	2	1	1										4	2	2	3	2						1	
OPS 16	Sabie	4	2	1	2	2		3				5		1	4	4	2	1							
OPS 17	Sabie													2	1	1		1		1			1		
OPS 18	Sabie																								
OPS 31	Mac Mac	1		2	1	2		1	1		2	1		1		1	1				2	1		2	1
OPS 32	Sabane	1		2				2				1													
OPS 33	Sabie	2		5	5	1			1	1	1	1									1		1	2	
OPS 34	Sabie	2	3	5	3					3	4	2											1	1	
OPS 35	Mac Mac		1	3	2						4	2	1									1	1	2	
OPS 36	Marite														1			1			2			2	
OPS 37	Sand	4	1					3		1		4		1				1		3				2	
OPS 38	Sand	2			1			2		3		2								1				1	
OPS 40	Groot-Sand	1		1	1	1					1	2	1	1						2		1		1	

Table 3.9.4.6: The fish species collected in the biotopes at the sites in the Sabie-Sand River system

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 7	Sabie	BMAR, CPAR, CSWI, GGUI, LCYL	CGAR, CSWI, GGUI, MACU, PCAT,	CPAR, LCYL	LCYL, PCAT, PPHI
OPS 8	Sabie	CPAR,LCYL	CGAR,BMAR, BVIV, LCYL, OMOS, PPHI, TREN,	BMAR, BTRI, BVIV, PPHI	
OPS 9	Sabie	BTRI, LCYL, LRUD, MMAC, TREN	BMAR, BVIV, CGAR, MACU, OMOS, PPHI	BVIV, MMAC, PPHI	
OPS 10	Sabie	PPHI		BVIV, CGAR, CPAR, CSWI, MMAC,	
OPS 11	Sabie	BMAR, BTRI, BVIV, CSWI, LCYL, OMOS	OMOS	BVIV, CGAR, CPAR, CSWI, LCYL, MMAC, OMOS, PPHI,TREN,	CGAR, LCYL, MMAC, OMOS, PPHI,TREN,
OPS 12	Sabie	BTRI, CPAR, LCYL	OMOS, PPHI	BMAR, CPAR, LCYL, OMOS,	BTRI, BUNI, BVIV, OMOS, PPHI
OPS 14	Sabie	BEUT, BMAR, BTRI, BVIV, CPAR, CSWI, GGUI, LCYL, MACU, MMAC, OMOS, PPHI,	CGAR, OMOS, PPHI	BMAR, BTRI, BVIV, CGAR, CPAR, CSWI, LCYL, MACU, MMAC, OMOS, SMER,	
OPS 15	Sabie	BMAR, CGAR, CPAR, LCYL		BANN, BTRI, BVIV, CANO, CPAR, CGAR, LCYL, LMOL, MMAC, PPHI,	
OPS 16	Sabie	BEUT, BTRI, BVIV, CPAR, CSWI, LCYL,	CPAR, CSWI, LCYL	BEUT, BMAR, BTRI, BVIV, CANO, CPAR, CSWI, GGUI, LCYL, LMOL, MBRE,	
OPS 17	Sabie	BEUT, BMAR, BTRI, BVIV, CANO, CPAR, LCYL, MACU, MMAC,	BVIV, BTRI, CPAR, LCYL, MACU, MBRE, PPHI, TREN,	CANO, CSWI, LCYL	
OPS 31	Mac Mac	AURA, BEUT, BLIN, BMAR, CANO, CPRE, OPER	BEUT, BLIN, MACU,OPER, PCAT, VNEL	AURA, BEUT, CANO, CPRE	BEUT, BMAR, MACU, OPER, VNEL
OPS 32	Sabane	BEUT, BMAR, MACU, OMOS, OPER, PPHI, TSPA	AURA, BEUT, BMAR, MACU, PPHI, TSPA,		BMAR, MACU
OPS 33	Sabie	AURA, BMAR, CANO, CPRE, OPER, PPHI, VNEL	MACU		BMAR, MACU, OPER, PPHI,
OPS 34	Sabie	BEUT, BMAR, CANO, CPRE, LCYL, PPHI,	BMAR, BVIV, CANO, CPRE, LCYL,OMOS, OPER, PPHI, TSPA,		
OPS 35	Mac Mac	AURA, CPRE, VNEL	AURA, CPRE, TSPA, VNEL		
OPS 36	Marite	CSWI	BMAR, CANO, OPER		BEUT, BMAR, CSWI
OPS 37	Sand	BMAR, BTRI, BVIV, CPAR, MACU	BMAR, BRAD, BTRI, BVIV, CGAR, CPAR, LMOL, MACU, TREN, TSPA,	BVIV, CSWI, LCYL, LMOL, OMOS, TREN	BMAR, BTRI, BVIV, CPAR, MACU, MBRE, OMOS,
OPS 38	Sand	CPAR, GGUI, MMAC,OMOS, PPHI	OMOS, TSPA		
OPS 40	Groot-Sand	CANO	BLIN, BMAR,	CANO	BLIN, BMAR, BTRI, BVIV, MMAC,

Table 3.9.4.7: Summary of fish biodiversity at the sites in the Sabie-Sand River system

Species	7	8	9	10	11	12	13	14	15	16	17	18	31	32	33	34	35	36	37	38	40
AURA													X	X	X		X				
BEUT										X	X		X	X		X		X			
BLIN													X								X
BMAR	X		X		X	X		X	X	X	X		X	X		X		X	X		X
BNEE																					
BPAU																					
BPOL																					
BRAD																			X		
BTRI		X	X		X	X		X	X	X	X								X		X
BUNI						X															
BVIV		X	X	X	X	X		X	X	X	X					X			X		X
CANO									X	X	X		X		X	X		X			X
CGAR	X	X	X	X	X			X	X										X		
CPAR	X	X		X	X	X		X	X	X	X								X	X	
CPRE													X			X	X				
CSWI	X			X	X			X		X	X				X			X	X		
GGUI	X									X										X	
LCYL	X	X	X		X	X		X	X	X	X					X			X		
LMOL										X									X	X	
LRUD			X																		
MACU			X					X			X		X	X	X				X		
MBRE										X	X								X		
MMAC	X		X	X	X			X	X		X									X	X
OMOS		X	X		X	X		X						X		X			X	X	
OPER													X	X	X	X		X			
PCAT	X												X								
PPHI	X	X	X	X	X	X		X	X		X			X	X	X				X	
SINT		X																			
SMER								X													
TREN		X	X		X						X								X		
TSPA														X		X	X		X	X	
VNEL													X		X		X				

3.9.7 THE CROCODILE AND KOMATI RIVERS

3.9.7.1 River description

The catchment covers an area of approximate 10 440 square km. The river rises at 2150 m.a.s.l on the Steenkamps berg in Mpumalanga (Figure 3.9.5.1) and then flows east through the Eastern Escarpment and mountains before it enters the Lowveld, after which it traverses the Lebombo mountains into Mozambique. The Komati River flows into Swaziland and reenters South Africa before flowing into Mozambique. Precipitation varies from 1200 mm in the mountainous areas and

600 mm in the Lowveld and the mean annual precipitation for the WMA is 880 mm. The Crocodile River is severely affected by various pollutants and land use factors.

3.9.7.2 Geomorphology description

Table 2.5.1 shows the specific results for this river system.

Table 3.9.5.1 shows the specific results for this river system.

3.9.7.3 Potential threats

In the Crocodile River, erosion is a problem linked to agriculture and some forestry activities in the catchment. The huge sugarcane farms have the largest negative impact in this regard. Habitat modification is a problem and thermal heating near Malelane, resulting from the outflow of the sugar mills, is of particular concern. The mines in the Kaap River system as well as sewerage problems and return flows from agriculture throughout the whole catchment contribute to the poor water quality. Downstream, lodges and golf estates increase pressure on the system. The Komati River has similar problems as the Crocodile River. Erosion is a huge problem and sewerage, run-off from settlements and agricultural return-flow increase the pollutants leading to the poor water quality. In the lower reaches of the Crocodile and Komati rivers intensive sugar cane farming with associated pollution, abstraction and weirs have a high impact of the river system.

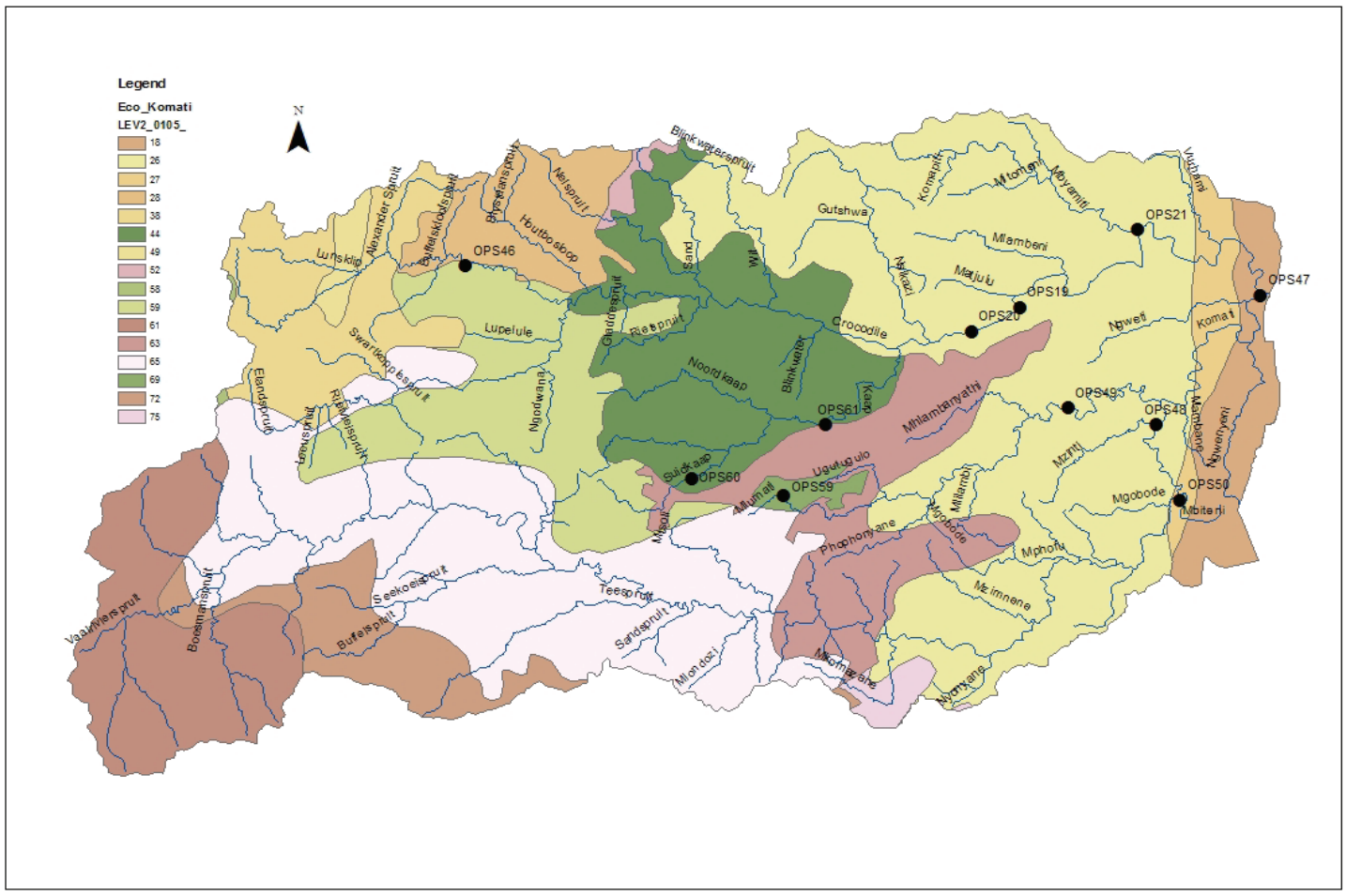


Figure 3.9.5.1: The project sample sites in the Crocodile and Komati rivers. Tributaries and Level 2 Ecoregions are also indicated.

3.9.7.4 Biotopes and site heterogeneity

In table 3.9.3 the heterogeneity, based on velocity and depth, of the sites is shown. In table 3.9.5.4 the dominant substrate in each of biotopes sampled is shown while the actual substrate of these biotopes are shown in table 3.9.4.5

3.9.7.5 Fish

The results are shown in tables 3.9.5.6 and 3.9.5.7.

Table 3.9.5.1: Some geomorphological aspects for the sites in the Crocodile River

Site number	Flow regime	Valley form	Channel dimension	Channel type	Morphological units	Reach type
46	Low	Foothill floodplain	15-30 m	Alluvial	Riffle, run, deep pool	Pool-riffle
60	Medium	Incised plain	15-30 m	Bedrock	Rock steps, bedrock pool, bedrock pavement, bedrock run, Bedrock backwater, shallow pool, deep pool, backwater	Flat bedrock, flat bed
61						
20	Medium	V-shaped valley	100-150 m	Alluvial	Bedrock pavement, step, plane bed, riffle, run, shallow pool, deep pool, flat sand bed, backwater	Flat bedrock, pool-riffle
19	Medium	Incised plain	150-300 m	Mixed	Bedrock pavement, riffle, rapid, run, shallow pool, deep pool, flat sand bed, backwater	Flat bedrock, pool-riffle
21	Medium	Incised plain	150-300 m	Mixed	Bedrock pavement, riffle, run, shallow pool, deep pool, flat sand bed, backwater	Flat bedrock, pool-riffle
59	Low	Ravine	15-30 m	Mixed	Rock step, Bedrock backwater, step, riffle, shallow pool, deep pool, backwater	Cascade, pool-riffle
49	Medium	Incised plain	150-300 m	Alluvial	Step, rapid, run, shallow pool, deep pool	Pool-riffle
50	Medium	Incised plain	150-300 m	Mixed	Rock step, bedrock pavement, step, riffle, rapid, run, shallow pool, backwater	Cascade, pool-rapid
48	Low	Alternating slopes	>300 m	Bedrock	Rock steps, bedrock rapid, bedrock pool, bedrock pavement, bedrock run, bedrock backwater	Cascade
47	Low	Incised plain	150-300 m	Bedrock	Rock steps, bedrock pool, bedrock pavement, bedrock run, bedrock backwater	Cascade

Table 3.9.5.2: The impacts observed between the different sites in the Crocodile and Komati Rivers.

Site number	Erosion and siltation	Flow reduction and impoundments	Habitat modification	WQ	Abstraction	Vegetation clearance	Land use
46	High – agriculture, roads and crossings	High – Kwena Dam	High	High – agriculture, sewerage	High	Moderate	Agriculture, residences
60	High – settlements, grazing	Moderate	High – bridges, weirs, crossings	High – agriculture, settlements, sewerage	High – sugar cane	High	Agriculture, settlements
61	High - high sediment loads	Moderate	Moderate – crossings and bridges, low weirs	Very high – mines, sewerage, settlements	High	Moderate	Mines, agriculture
20	Moderate to high	High - agriculture	Moderate – weirs, bridges and crossings	High – sewerage, agriculture	Very high	High	Agriculture, settlements
19	High – sand mining, erosion	Moderate	High – thermal pollution, crossings and bridges, sand mining	High – return flows from industry and town, agriculture, sewerage	High – agriculture, industry, town	High	Agriculture, industries, town and lodges, sand mining, conservation
21	High	High	High	High – sewerage, agriculture	High	High	Agriculture, conservation, lodges
59	High - sedimentation	High – forestry and impoundment	Moderate	Moderate	Low to moderate	High	Forestry
49	Moderate	High - weirs	High – roads, crossings, weirs	High – return flows	High	High	Agriculture, settlements
50	High – agriculture, vegetation clearance	High – weirs, agriculture	High – impoundments, crossings and bridges	High – return flows, run-off and sewerage	High	High	Agriculture, settlements
48	High – settlements, agriculture	High – water treatments works, agriculture	High – bridges and crossings, weirs	High – return flows, sewerage, run-off	High	High	Agriculture, settlements
47	High	High – weirs and abstraction	Moderate to high	High – agriculture, run-off sewerage	High	High	Agriculture, settlements and town

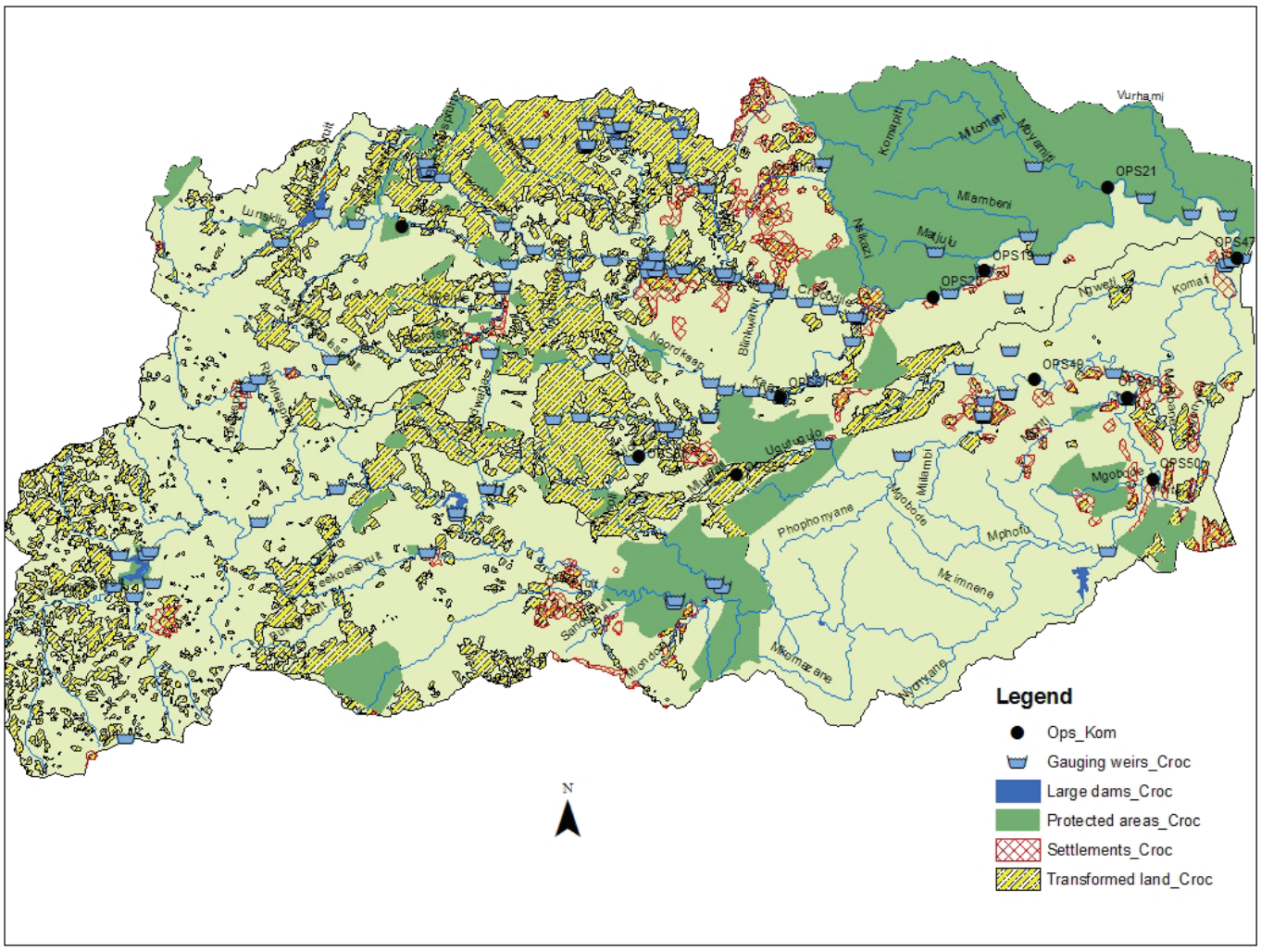


Figure 3.9.5.2: The Crocodile/Komati catchments with associated land-use impacts

Table 3.9.5.3: Summary of the diversity of the available fish habitat at the sites in the Crocodile River system. (The numbers represent the specific biotope as a percentage of the total biotopes at the site).

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 19	Crocodile	100			
OPS 20	Crocodile	80			20
OPS 21	Crocodile	40	20	40	
OPS 46	Crocodile		75		25
OPS 47	Crocodile	34	33	33	
OPS 48	Komati	38	38	24	
OPS 49	Nlomati	40		40	20
OPS 50	Komati	17	33	18	32
OPS 59	Nlomati	57	28		15
OPS 60	Suid Kaap	25		37	38

Table 3.9.5.4: Summary of the dominant substrate in the biotopes at the sites in the Crocodile River system. (Where A=bedrock, B =boulder, C = cobble, D = gravel, E = sand and F = silt)

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 19	Crocodile	BCDDD			
OPS 20	Crocodile	DEE			E
OPS 21	Crocodile	EE	E	CE	
OPS 46	Crocodile		BCC		F
OPS 47	Crocodile	A	A	A	
OPS 48	Komati	AAA	AAC	AA	AE
OPS 49	Nlomati	BC		BC	C
OPS 50	Komati	A	AAA	B	AE
OPS 59	Nlomati	CCC	CF		C
OPS 60	Suid Kaap	BA		AAA	AAE

Table 3.9.5.5: Summary of the substrate diversity in the biotopes at the sites in the Crocodile River system.

Site no.	River/Tributary	Shallow												Deep											
		Fast						Slow						Fast						Slow					
		BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si
OPS 19	Crocodile		2	1	4	2																			
OPS 20	Crocodile	4		1	3	1														1					
OPS 21	Crocodile				2	1						1				1	1	1							
OPS 46	Crocodile								2	3		1	2											1	1
OPS 47	Crocodile	1						1		1				1											
OPS 48	Komati	3		1		1		2	1	2	1	2		2	1	1									
OPS 49	Nlomati		2	2		1									2	2	1								
OPS 50	Komati	1	1					3			1				1	1				1			2	2	
OPS 59	Nlomati		1	4	1	1	1		1	1		1	2								1	1		1	1
OPS 60	Suid Kaap	1	2	1										4		1				1				2	

Table 3.9.5.6: The fish species collected in the biotopes at the sites in the Crocodile River system.

		Shallow		Deep	
Site No.	River/Tributary	Fast	Slow	Fast	Slow
OPS 19	Crocodile	BMAR, BTRI, BVIV, CPAR, LCYL, OMOS, PPHI			
OPS 20	Crocodile	BMAR, CANO, CPAR, LCYL,			BVIV, CGAR
OPS 21	Crocodile	BRAD, BTRI, BVIV		BVIV, CPAR, LCYL	
OPS 46	Crocodile	AURA, PPHI, TSPA,	AURA, CPRE, TSPA		TSPA
OPS 47	Crocodile		TSPA	GGUI, LCYL	
OPS 48	Komati	LCYL	BTRI, OMOS		
OPS 49	Nlomati	CGAR, CPRE, LMOL, PCAT		BMAR, LCYL	
OPS 50	Komati	BMAR, CPAR, LCYL	BPAU		LCYL, MACU
OPS 59	Nlomati	AURA, BAFR, CPRE, TSPA	TSPA		BAFR, BLIN, TSPA
OPS 60	Suid Kaap			BMAR	BMAR, BVIV, TSPA

Table 3.9.5.7: Summary of fish biodiversity at the sites in the Crocodile River system.

Species	19	20	21	46	47	48	49	50	59	60
AURA				X					X	
BAFR									X	
BEUT										
BLIN									X	
BMAR	X	X					X	X		X
BNEE										
BPAU								X		
BPOL										
BRAD			X							
BTRI	X		X			X				
BVIV	X	X	X							X
CANO		X							X	
CGAR		X					X			
CPAR	X	X	X					X		
CPRE				X			X			X
CSWI	X									
GGUI					X					
LCYL	X	X	X		X	X	X	X		
LMOL							X			
LRUD										
MACU								X		
MBRE										
MMAC										
OMOS	X					X				
OPER										
PCAT							X			
PPHI	X			X						
SINT										
TSPA			X	X	X				X	X

3.9.7.6 Discussion

Economic activity is mainly centred on irrigation-agriculture and forestry, with related industries and commerce, and a strong eco-tourism industry. The rivers generally show signs of stress and little pristine rivers remain. The low fish diversity at some of the sites (OPS 19, OPS 48) and observed evidence of pollution in the Suid-Kaap and Queens rivers are reasons for concern.

3.9.8 THE USUTU RIVER

3.9.8.1 River description

The upper reaches of the Usuthu River are predominantly within southern Mpumalanga with some in KwaZulu-Natal and the bordering Swaziland and Mozambique (Figure 3.9.6.1). After its origin in South Africa the river flows into Swaziland and Mozambique. The climate in the region can be described as sub-humid to humid, but varies considerably and the mean annual rainfall ranges between 600 mm and 1500 mm. Economic activity is diverse and includes subsistence agriculture, irrigation, forestry, ecotourism.

3.9.8.2 Geomorphology description

The results are shown in table 3.9.6.1.

3.9.8.3 Potential threats

The results are shown in table 3.9.6.1. Threats in the Usutu River are high and are mostly related to siltation and water quality. The high number of forestry areas and agriculture leads to high levels of erosion and the results in silt loads in the river. Poor infrastructure and return flows are responsible for the poor water quality and saw mills, agriculture and sewerage are the main culprits.

3.9.8.4 Biotopes and site heterogeneity

In table 3.9.6.3 the heterogeneity, based on velocity and depth, of the sites is shown. In table to 3.9.6.4 the dominant substrate in each of biotopes sampled is shown while the actual substrate of these biotopes are shown in table 3.9.6.5

3.9.8.5 Fish

In each biotope fish were collected using the most appropriate method or methods for the particular biotope type. These methods included electro-fishing as well as seine and cast net sampling. The fish collected in each of the habitat classes is shown in table 2.6.6 while the actual fish diversity observed at the site is summarized in table 2.6.7.

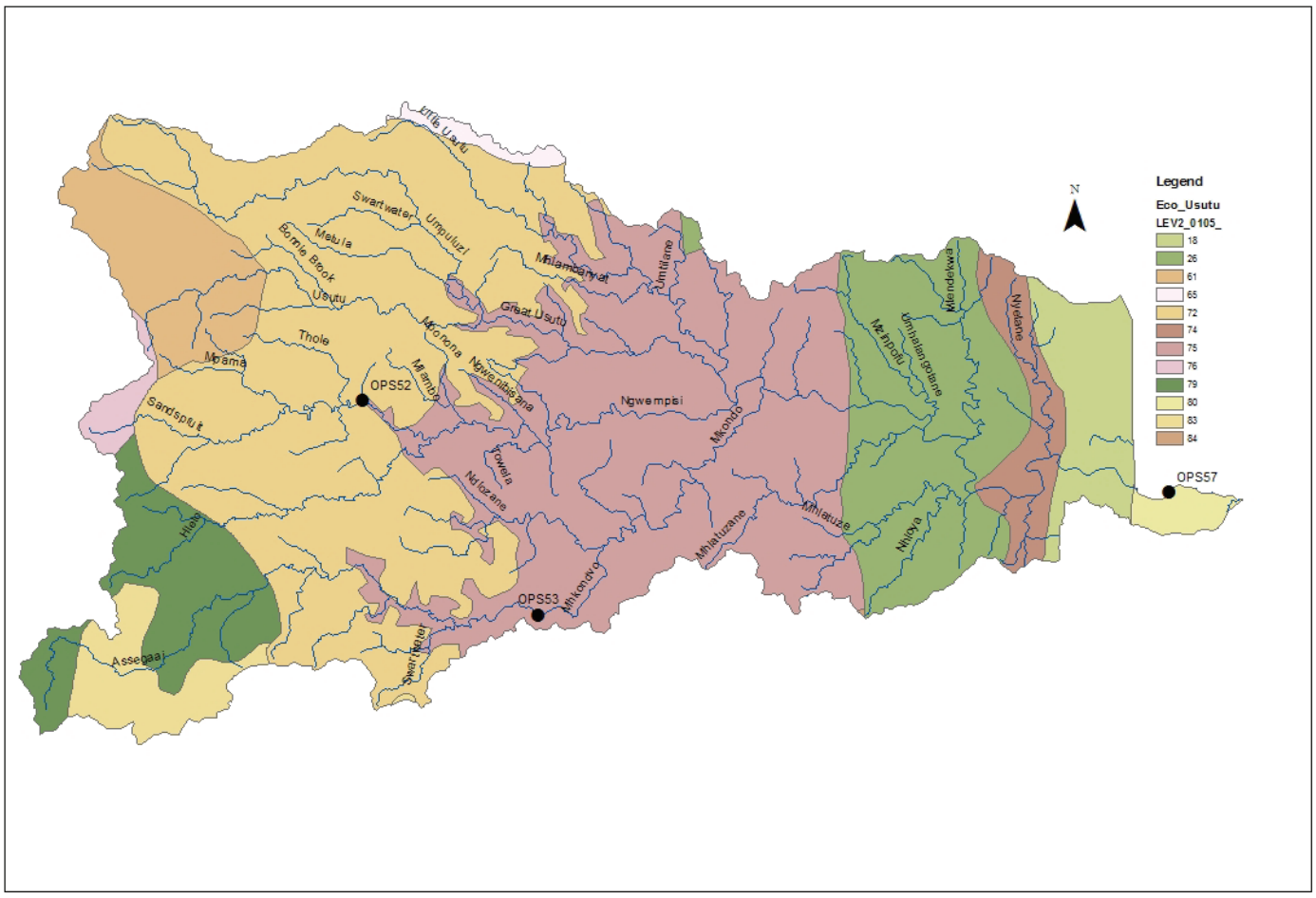


Figure 3.9.6.1: The project sample sites in the Usutu river. Tributaries and Level 2 Ecoregions are also indicated

Table 3.9.6.1: Some geomorphological aspects for the sites in the Usutu River.

Site number	Flow regime	Valley form	Channel dimension	Channels type	Morphological units	Reach type
52	Medium	Alternating slopes	100-150 m	Bedrock	Rock steps, bedrock rapid, bedrock pavement	Cascade
53	High	Alternating slopes	30-50 m	Alluvial	Step, riffle, rapid, deep pool, backwater	Pool-riffle
57	High	Extensive floodplain	150-300 m	Alluvial	Run, flat sand bed	Flat bed

Table 3.9.6.2: The impacts observed between the different sites in the Usutu River.

Site number	Erosion and siltation	Flow reduction and impoundments	Habitat modification	WQ	Abstraction	Vegetation clearance	Land use
52	High erosion – forestry, agriculture	High – dam, weirs, crossings, bridges	High	High – nutrient enrichment, factory return flows, forestry	Moderate - high	High – forestry, agriculture	Forestry, towns, agriculture
53	High – erosion and siltation from forestry and agriculture	Moderate – high – dams, crossings, bridges	High	High – sewerage and run-off from town and grazing areas	Moderate	High – forestry and agriculture	Forestry, agriculture, town
57	High – forestry, agriculture, vegetation clearing	Moderate - high	High	High – siltation, return flows, sewerage	Moderate	High	Agriculture, forestry, settlements, industries

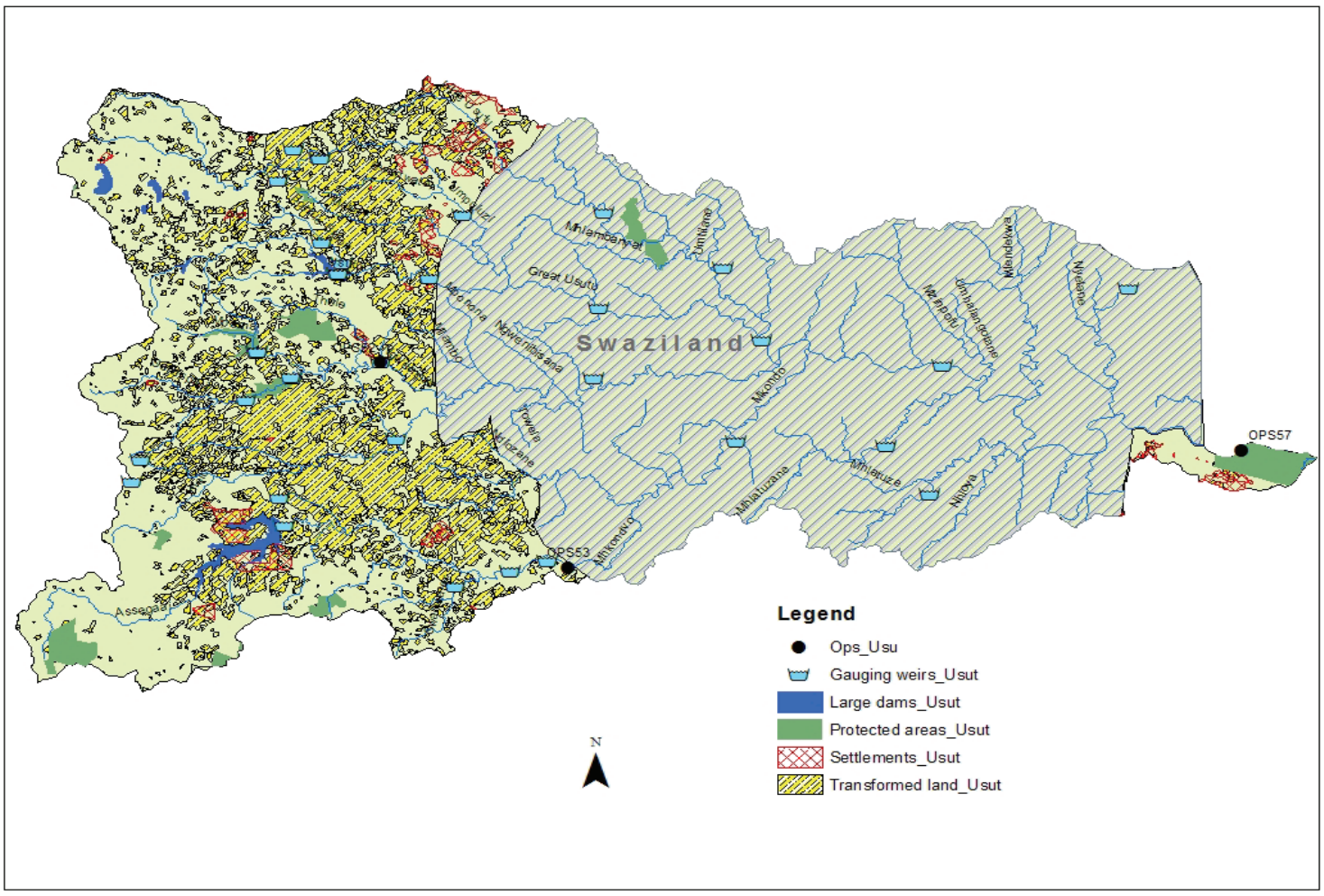


Figure 3.9.6.2: The Usutu catchment with associated land-use impacts

Table 3.9.6.3: Summary of the diversity of the available fish habitat at the sites in the Usuthu River system. (The numbers represent the specific biotope as a percentage of the total biotopes at the site).

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 52	Ngwempisi	40	20	30	10
OPS 53	Assegaai		33	50	16
OPS 57	Usuthu		87		13

Table 3.9.6.4: Summary of the substrate diversity and the substrate dominance in the biotopes at the sites in the Usuthu River system. (Where A=bedrock, B = boulder, C = cobble, D = gravel, E = sand and F = silt)

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 52	Ngwempisi	ABBD	AE	ADD	A
OPS 53	Assegaai		DC	BBC	C
OPS 57	Usuthu		DEEEEEE		E

Table 2.6.5: Summary of the substrate diversity in the biotopes at the sites in the Usuthu River system.

		Shallow												Deep											
Site number	**River/Tributary**	**Fast**						**Slow**						**Fast**						**Slow**					
		BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si
OPS 52	Ngwempisi	1	2	2	1			3				1		2	2	1				1			1	1	
OPS 53	Assegaai									1	1	2	1	1	3	2	2					1			2
OPS 57	Usuthu										1	5	1											1	

Table 3.9.6.6: The fish species collected in the biotopes at the sites in the Usuthu River system.

		Shallow		Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
OPS 52	Ngwempisi	AURA, BAFR, BEUT, BMAR, CANO		AURA, BMAR, CANO	BMAR
OPS 53	Assegaai		BMAR, PPHI, TSPA	AURA, BMAR, CANO, TSPA	BMAR, PPHI, TSPA
OPS 57	Usuthu		BAFR, CGAR, OMOS,		

Table 3.9.6.7: Summary of fish biodiversity at the sites in the Sabie-Sand River system

Species	**52**	**53**	**57**
AURA	X	X	
BAFR	X		X
BEUT	X		
BLIN			
BMAR	X	X	
BNEE			
BPAU			
BPOL			
BRAD			
BTRI			
BVIV			
CANO	X		
CGAR			X
CPAR			
CPRE			
CSWI			
GGUI			
LCYL			
LMOL			
LRUD			
MACU			
MBRE			
MMAC			
OMOS			X
OPER			
PCAT			
PPHI		X	
SINT			
TSPA		X	

3.9.8.6 Discussion

During the survey the Assegaai River was in flood, which could have influenced the survey so that no *O. peringueyi* were collected. However, based on the available habitat, this site (OPS 53) shows most potential for the species to be present. Lower downstream the river is dominated by sand with very little other material.

3.9.9 THE PHONGOLO RIVER

3.9.9.1 River description

The Phongolo River falls predominantly within northern KwaZulu-Natal with a part of the upper reach tributaries in Mpumalanga, and bordering on Swaziland and Mozambique (Figure 3.9.7.1). Two rivers are shared with these countries. The Phongolo and Usutu rivers flow together in South Africa just before entering Mozambique as the Maputo River. Climate in the region can be described as sub humid to humid, but varies considerably. Mean annual rainfall ranges between 600 mm and 1 500 mm. Economic activity is diverse and includes subsistence agriculture, irrigation, forestry and ecotourism. The Phongolopoort Dam is large dam in the middle reaches of the Phongolo River.

3.9.9.2 Geomorphology description

The results obtained are shown in table 3.9.7.1.

3.9.9.3 Potential threats

The impacts in the Phongolo River were assessed in the same way as other rivers and the results are shown in table 3.9.7.2. Spatial data, as described in paragraph 3.9.2.4., was used to investigate the impacts of land transformation and land use.

Threats in the Phongolo River are high and are mostly related to siltation and water quality. The high number of forestry areas and agriculture leads to high levels of erosion and the results in silt loads in the river. Poor infrastructure and return flows are responsible for the poor water quality and saw mills, agriculture and sewerage are the main culprits. A similar situation is present in the Usutu River with forestry, sewerage and siltation being the main threats in the system.

3.9.9.4 Biotopes and site heterogeneity

This was the first river system surveyed and after the first survey, in May 2005, methods were refined. This implied that at sites OPS 1 to OPS 7 biotopes and habitat classes were not identified. Because the South African Scoring system (SASS) method of habitat description was employed at these site certain habitat aspects could be extracted. Table 3.9.7.3 shows some of the characteristics that were extracted from the SASS form at three of the sites were *O. peringueyi* specimens were collected.

At sites OPS 54, 55 56 and 58 the same methods used in all the other river systems were used. In tables 3.9.7.3 and 3.9.7.4 the heterogeneity, based on velocity and depth, of the sites is shown. In table 3.9.7.5 the dominant substrate in each of biotopes sampled is shown while the actual substrate of these biotopes are shown in table 3.9.7.6.

3.9.9.5 Fish

At all the sites fish were collected using the most appropriate method or methods for the particular biotope or habitat type. These methods included electro-fishing as well as seine and cast net sampling. In the case of the second survey, May 2008, the fish collected in each of the habitat classes, in the sites where biotopes were identified and demarcated, is shown in table 3.9.7.7 while the actual fish diversity observed all the sites is summarized in table 3.9.7.8.

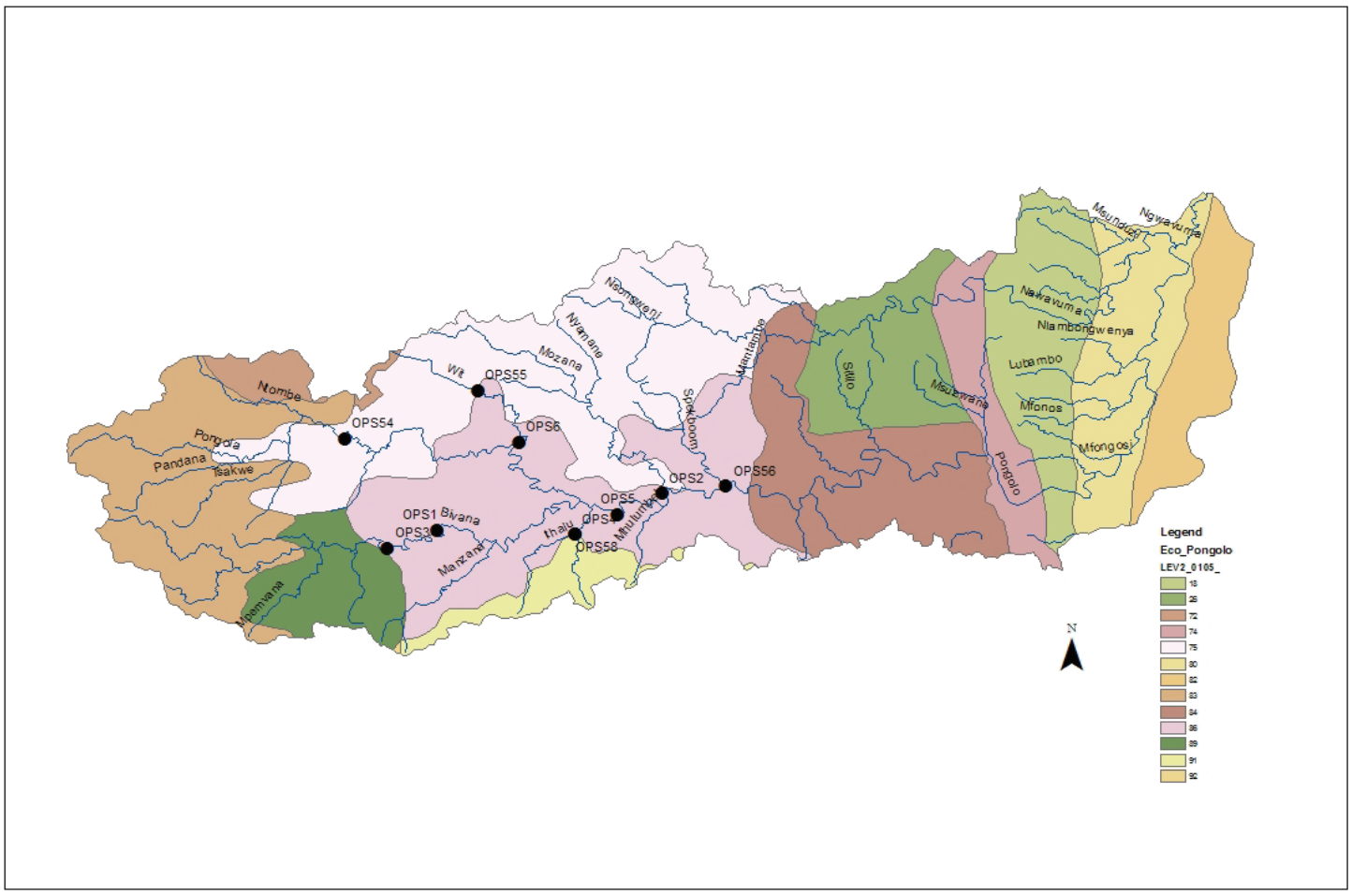


Figure 3.9.7.1: The project sample sites in the Phongolo River. Tributaries and Level 2 Ecoregions are also indicated

Table 3.9.7.1: Some geomorphological aspects for the sites in the Phongolo River.

Site number	Flow regime	Valley form	Channel dimension	Channels type	Morphological units	Reach type
55	Medium	Incised plain	50-75 m	Alluvial	Step, riffle, rapid, shallow pool, backwater	Pool-riffle
54	High	Incised plain	75-100 m	Mixed	Step, riffle, rapid, run, shallow pool, flat sand bed	Pool-riffle
6	Low	Incised plain	50-75 m	Fixed cobble bed	Rifle, shallow pool, deep pool, backwater	Pool-riffle
3	Low	V-shaped valley	15-30 m	Mixed	Bedrock rapid, bedrock pool, bedrock run, step, riffle, run, shallow pool, deep pool, flat sand bed, back water	Cascade, step-pool
1	Low	V-shaped valley	15-30 m	Bedrock	Bedrock step, bedrock rapid, bedrock pool, bedrock run, plane bed, riffle, shallow pool, deep pool	Flat bedrock, pool-riffle
58	Medium	Incised plain	75-100 m	Alluvial	Step, run, shallow pool, flat sand bed, backwater	Pool-riffle
4	Low	V-shaped valley	50-75 m	Mixed	Rock steps, bedrock pool, bedrock run, riffle, run, shallow pool, deep pool, flat sand bed, backwater	Cascade, pool-riffle
5	Low	Alternating slopes	30-50 m	Alluvial	Step, riffle, shallow pool, deep pool,	Step-pool, pool riffle
2	Medium	Incised plain	50-75 m	Mixed	Rock steps, bedrock rapid, bedrock run, bedrock backwater, step, riffle, run, shallow pool, deep pool, flat sand bed, backwater	Cascade, pool-riffle
56	High	Foothill flood plain	100-150 m	Mixed	Riffle, rapid, shallow pool, backwater	Pool-riffle, pool-rapid

Table 3.9.7.2: The impacts observed between the different sites in the Phongolo River.

Site number	Erosion and siltation	Flow reduction and impoundments	Habitat modification	WQ	Abstraction	Vegetation clearance	Land use	Other
55	Moderate - high	High - weirs	High – weirs, roads, crossings	High – nutrients, towns and settlements	Moderate – high	High	Forestry, towns, settlements	
54	High – forestry, grazing, vegetation clearing	Low - moderate	Moderate	Moderate	Low	High	Forestry, agriculture	Alien species
6	High – agriculture, forestry	Moderate	Moderate	High – sewerage, run-off	Moderate	High – subsistence farming, wood collection, forestry	Settlements, forestry, agriculture (commercial and subsistence)	
3	High – settlement, roads and crossings, forestry, agriculture	Moderate	High – bridges and crossings	High – return flows from sawmills, sewerage, run-off from settlements, lodges	High	Moderate to high	Lodges, settlements, industries, forestry	
1	Moderate to high – settlements, forestry,	Moderate – bridges and crossings	Moderate to high	High – siltation, sewerage, run-off	Moderate to high	High – grass and wood collection,	Forestry, settlements, agriculture	
58	High – settlements, agriculture	High – dams	Moderate – bridges, crossings, weirs	High – settlements and villages, sewerage	High	High – grass and wood collection, forestry	Forestry, settlements, conservation, town, offices and houses, commercial agriculture	
4	High – settlements, agriculture	High -dams	Moderate – bridges, crossings, weirs	High – settlements and villages, sewerage	High	High – grass and wood collection, forestry	Forestry, settlements, conservation, town, offices and houses, commercial agriculture	
5	High – roads, grazing, settlements	Low	Moderate	High – settlements, agriculture, run-off	Moderate	Moderate	Agriculture, settlements	
2	Low	Moderate – bridges and crossings	Moderate	High - agriculture, town, settlements, run-off sewerage	High	High	Agriculture, town, settlements	
56	High – agriculture, settlements, roads	High - canals	High – roads and crossings, weir, abstraction	Moderate – agriculture, settlements,	High - canals	High – wood and grass collection, lands	Agriculture, settlements	

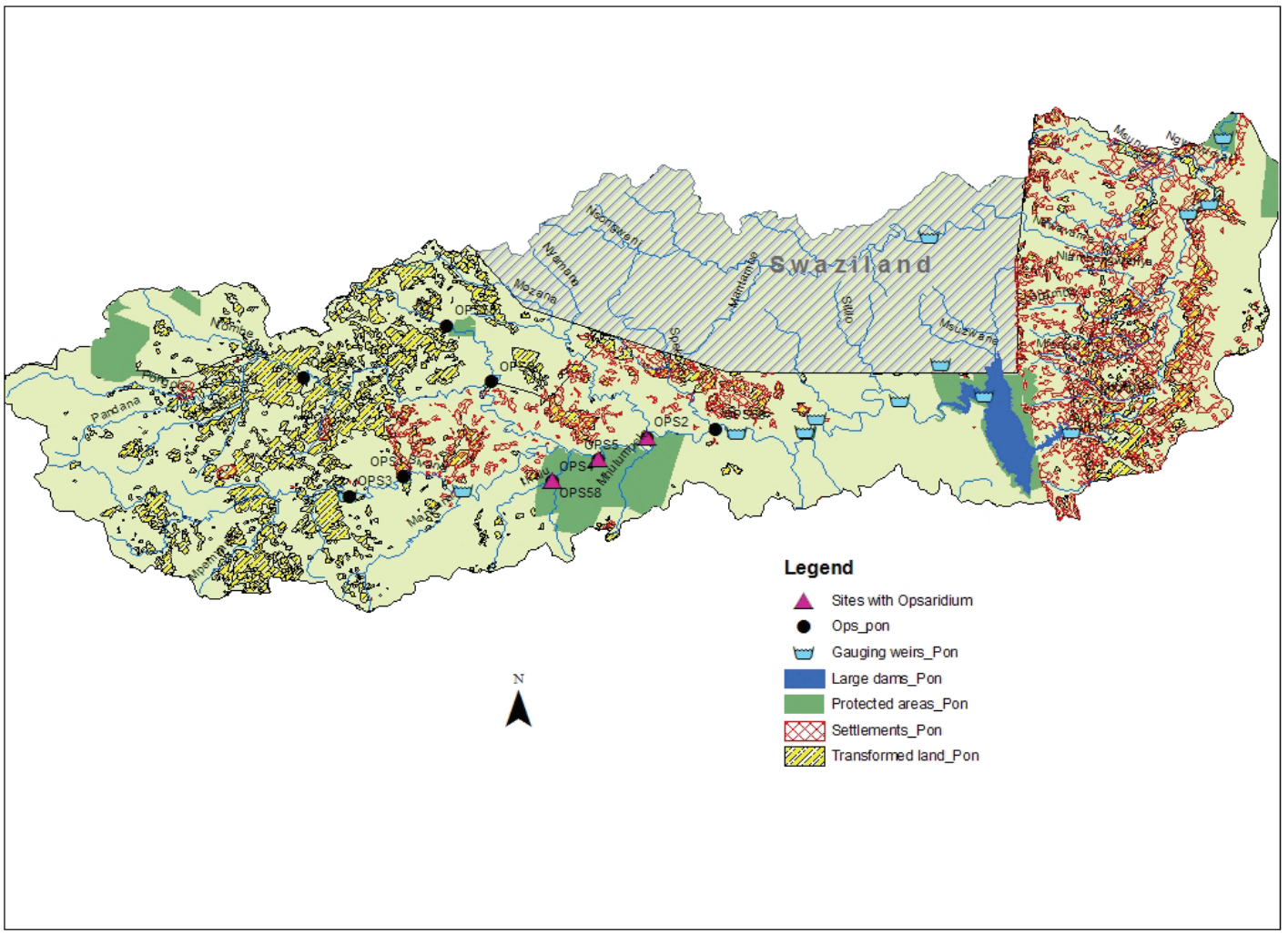


Figure 3.9.7.2: The Phongolo catchment with associated land-use impacts

Table 3.9.7.3: Summary of the diversity of the available fish habitat at the sites in the Phongola River system surveyed in May 2008.

Site number	Length of riffles (m)	Length of runs (m)	Size of larger alluvial material (cm)	Silt	Sand	Gravel	Depth	Pool –riffle
OPS 2	3-5	2-5	11-20 Boulder	No	Yes	No	<0,5	Yes
OPS 4	> 5	> 10	11-20 Boulder	No	Yes	No	> 0,5	Yes
OPS 5	> 5	> 10	2-10 Cobble	No\	Yes	No	0,5	Yes

Table 3.9.7.4: Summary of the diversity of the available fish habitat at the remainder of the sites in the Phongola River system surveyed in May 2008. (The numbers represent the specific biotope as a percentage of the total biotopes at the site).

		Shallow		Deep	
Site number	River/Tributary	Fast	Slow	Fast	Slow
OPS 54	Nlambe	63	25	12	
OPS 55	Wit	22	78		
OPS 56	Phongola		43		57
OPS 58	Thala	57	36	7	

Table 3.9.7.5: Summary of the dominant substrate in the biotopes at the sites, surveyed in May 2008, in the Phongolo River system. (Where A=bedrock, B = boulder, C = cobble, D = gravel, E = sand and F = silt)

		Shallow		Deep	
Site number	River/Tributary	Fast	Slow	Fast	Slow
OPS 54	Nlambe	BBCCC	CC	B	
OPS 55	Wit	B	AABCCCC		
OPS 56	Phongola		BCE		BBBF
OPS 58	Thala	BCCCDEEE	BCEEE	B	

Table 3.9.7.6: Summary of the substrate diversity in the biotopes at the sites, surveyed in May 2008, in the Phongola River system.

		Shallow												Deep											
Site number	**River/Tributary**	**Fast**						**Slow**						**Fast**						**Slow**					
		BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si	BR	B	C	G	S	Si
OPS 54	Nlambe		4	4	1					2					1	1									
OPS 55	Wit		2	2				3	5	7	4														
OPS 56	Phongola								3	3		1	1								3	3		1	1
OPS 58	Thala	2	5	5	7	5			3	4	3	4			1	1									

Table 3.9.7.7: The fish species collected in the biotopes at the sites, surveyed in May 2008, in the Phongolo River system during the May 2008 survey.

	Shallow			Deep	
Site number	**River/Tributary**	**Fast**	**Slow**	**Fast**	**Slow**
Site number	River/Tributary	Fast	Slow	Fast	Slow
OPS 54	Nlambe	AURA CANO LMAR	LMAR CANO	CANO LMAR AURA	
OPS 55	Wit	LMAR BANN	LMAR BANN CANO AURA BEUT TSPA		
OPS 56	Phongola	LMAR	OMOS LMAR PPHI		MACU LMAR BTRI MBRE OMOS
OPS 58	Thala	OPER LMAR BTRI AURA CANO	LMAR BTRI OMOS OPER	AURA LMOL	

Table 3.9.7.8: Summary of fish biodiversity at the sites in the Phongola River system

Species	1	2	3	4	5	6	54	55	56	58
AURA							X	X		X
BANN								X		
BEUT								X		
BLIN					X	X				
BMAR	X	X	X	X	X	X	X	X	X	X
BNEE										
BPAU										
BPOL			X	X		X				
BRAD										
BTRI		X		X	X	X			X	X
BVIV				X						
CANO	X			X	X	X		X		X
CEMA		X		X	X					
CGAR		X			X	X				
CPAR				X						
CPRE	X	X	X	X	X	X				
CSWI										
GGUI										
LCYL		X		X	X					
LMOL	X	X	X	X	X	X				X
LRUD										
MACU		X		X					X	
MBRE		X							X	
MMAC		X			X	X				
OMOS		X		X	X				X	
OPER		X		X	X					X
PCAT										
PPHI									X	
SINT		X								
TSPA			X			X		X		

3.9.9.6 Discussion

The Phongolo River is impacted by several different factors with pollution, siltation and water abstraction the most prominent.

In this river system the site in the Ithala Reserve (OPS 58) exhibits the most conservation potential of all the sites surveyed. Besides the fact that this site is within a conservation area it also displays what can be regarded as the best possible habitat heterogeneity needed for the survival of the species. Interestingly all the sites where the species were found were within the Ithala nature Reserve. The Ithala River has a small, relatively intact catchment with little negative impacts.

3.9.10 WATER QUALITY

3.9.10.1 Methods

In addition to the *in situ* physico-chemical determinations that included electrical conductivity, pH, dissolved oxygen and total dissolved solids, water samples were collected at the sites for chemical analyses. These subsurface samples were collected in pre-treated 1\one liter water sample bottles, placed on ice and transported to the laboratory where they were frozen until analyses. Two batches of water samples were submitted to an accredited laboratory in Pretoria Waterlab (Pty) Ltd (Reg no 83/09165/07) was requested a) to do a ICP MS scan for the elements and b) determine the turbidity and hardness of the water sample and establish the concentration of the nutrients.

3.9.10.2 Results

The results of the ICP scan are shown in appendices 1 and 2 and the results of the chemical tests are shown in tables 3.10.1 and 3.10.2. Due to a break in communication only the phosphate and nitrate content of the second batch was determined.

3.9.10.3 Discussion

3.9.10.3.1 Water quality and the effect on biota

Limiting factors are important role players in ecosystems and in freshwater environment, water temperature, transparency and light penetration, and chemical composition form part of these factors. Although temperature varies less in water than in air it is still important as aquatic organisms are temperature sensitive and often stenothermic. Light penetration in water is determined by the suspended substances that limit photosynthesis in deeper water. Turbidity is therefore a limiting factor. Oxygen and carbon dioxide as well as nitrates and phosphates can also be limiting factors. The physical attributes of the freshwater environment inter alia include: water, temperature, hydraulics, light, sediments with the resultant aspects of turbidity and total suspended solids (TSS) and dissolved gases. The chemical constituents include the major ions (Na+, K+, Ca2+, Mg2+ Cl-, SO42- , HCO-), hydrogen (and therefore pH), nutrients (especially N, P, K, Si), minor ions (e.g. F-, Fex+), and numerous organics (natural and human origin). Not all these aspects are of equal importance and only which are regarded to have most influence is discussed.

Table 3.10.1: The results of chemical analyses of the first batch of water samples.

Site number	River	Turbidity (NTU)	Hardness	NH_3-N (mg/l)	Nitrate – N (mg/l)	SO_4 (mg/l)	COD (mg/l)	BOD (mg/l)
1	Phongola	1.9	36	<0.2	0.3	8	40	<10
2	Phongola	0.9	36	<0.2	0.2	10	20	<10
3	Phongola	0.4	32	<0.2	0.5	11	32	<10
4	Phongola	8.1	96	<0.2	0.2	8	24	<10
5	Phongola	0.7	40	<0.2	0.2	8	24	<10
6	Phongola	3.3	36	<0.2	0.3	7	16	<10
7	Sabie	3.0	38	<0.2	0.3	6	30	<10
8	Sabie	3.0	35	<0.2	0.3	7	25	<10
9	Sabie	3.0	40	<0.2	0.3	7	28	<10
10	Sabie	2.3	44	<0.2	0.3	6	32	<10
11	Sabie	1.7	44	<0.2	0.2	7	20	<10
12	Sabie	1	40	<0.2	0.3	5	24	<10
13	Sabie	1.9	32	0.2	0.3	6	28	<10
14	Sabie	1.3	44	<0.2	0.3	11	28	<10
15	Sabie	1.5	52	<0.2	0.2	8	40	<10
16	Sabie	1.3	48	<0.2	0.3	6	44	<10
17	Sabie	1.1	48	<0.2	0.3	8	<10	<10
18	Sabie	0.9	64	<0.2	0.3	5	<10	<10
19	Crocodile	3.7	188	0.3	0.8	50	<10	<10
20	Crocodile	1.9	136	<0.2	0.9	42	<10	<10
21	Crocodile	0.4	20	0.2	0.4	5	<10	<10
22	Luvuvhu	0.7	44	<0.2	0.6	7	36	<10
23	Luvuvhu	0.9	8	<0.2	0.3	6	16	<10
24	Luvuvhu	3.7	188	0.3	0.8	50	<10	<10
25	Luvuvhu	1.3	44	<0.2	0.7	6	28	<10
26	Luvuvhu	1.1	32	0.2	0.4	5	<10	<10
27	Luvuvhu	0.4	20	<0.2	0.4	5	<10	<10
28	Luvuvhu	0.9	16	<0.2	0.3	5	<10	<10
31	Sabie	1.0	48	<0.2	<0.2	6	20	<10
32	Sabane	1.0	55	0.2	0.2	8	40	<10
33	Sabie	1.0	50	<0.2	<0.2	6	22	<10
34	Sabie	1.2	43	<0.2	<0.2	6	26	<10
35	Mac Mac	0.9	33	<0.2	0.3	8	10	<10
36	Marite	1.2	60	0.3	<0.2	12	36	<10

Table 3.10.2: The results of chemical analyses of the second batch of water samples.

Site number	River	NH_3-N (mg/l)	Ortho-Phosphate as P (mg/l)
31	Sabie	<0.2	<0.2
32	Sabane	0.2	0.2
33	Sabie	0.2	0.2
34	Sabie	<0.2	<0.2
35	Mac Mac	0.3	<0.2
36	Marite	<0.2	<0.2
39	Klaserie	<0.2	<0.2
40	Grootsand	<0.2	<0.2
44	Steelpoort	6.6	<0.2
45	Watervals	<0.2	<0.2
46	Sterkspruit	<0.2	<0.2
52	Ngwempisi	0.3	<0.2
53	Assegaai	0.6	<0.2
54	Phongola	<0.2	<0.2
55	Witrivier	0.5	0.2
56	Phongola	0.2	<0.2
57	Usuthu	0.5	<0.2
58	Ithaka	<0.2	<0.2
60	Queens	<0.2	<0.2
61	Kaap	0.2	<0.2
62	Blyde	0.5	<0.2

In water bodies the water temperature affects the availability of oxygen with less available oxygen in warmer water and vice versa. Due to the nature of the photosynthesizing component of the biota, the dissolved oxygen level is usually lowest near dawn; increases during the day; peaks in the afternoon and decreases during the night. In addition oxidizable organic waste, such as detritus, can lead to the reduction of oxygen. This reduction is due to the aerobic decomposition of detritus and is referred to as the Biological Oxygen Demand (BOD). The breakdown of chemicals on the other hand can lead to a similar situation known as the Chemical Oxygen demand (COD). The oxygen requirements of fish vary between species, with life stages and with different life processes. If possible, many species will avoid anoxic or oxygen depleted zones. Juvenile stages are more sensitive than adults to physiological stress, caused by oxygen depletion, and in particular to secondary effects such as increased vulnerability to predation and disease. According to Dallas and Day (2004) continuous exposure to depleted oxygen concentrations is most harmful and is likely to have acute effects with repeated exposure to reduced concentrations that can lead to behavioural

and physiological stress effects. The values in table 3.10.3 provide limits and targets that will ensure protection of aquatic biota from the adverse effects of oxygen depletion. The Minimum Allowable Values (MAV) aims to protect sensitive life stages but takes the resilience of biota to short term oxygen completion in account.

Table 3.10.3: Target Water Quality Range (TWQR) and Minimum Allowable Values (MAV) for dissolved oxygen concentrations in water bodies (adapted from Dallas and Day, 2004).

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

Table 3.10.4 shows some of the criteria listed in Kempster *et al.* (1980) for the protection of aquatic life. The median value can be regarded as the recommended limit for the constituent and the maximum value reportedly the upper end of the scale of tolerance. The median value is also the most reported value. It should be noted that only ammonium is toxic and according the Kempster *et al.* (1980) is it moderately toxic.

Table 3.10.4: Quality criteria for the protection of aquatic life in dams (Kempster *et al.*, 1980)

Criteria	Unit	Minimum value	Median value	Maximum value
pH		6	6-8	9
TSS	*mg/l*	25		80
Alkalinity (as $CaCO_3$)	*mg/l*	>20	>20	>20
Dissolved oxygen	*mg/l*	4,0	5,0	5,8
Ammonium	*mg/l*	0,016	0,016	124

The term suspensoids refers to the particles that are suspended in water as part of a dynamic situation where a continuous exchange between suspension and sedimentation occur. This dynamism is directly related to the turbulence and velocity of water. The turbidity of water describes the amount of suspensoids with the turbidity increasing as the amount of suspensoids increase. Turbidity is also responsible for physical interference with the passage of light with a decrease in passage as turbidity increases. Turbidity is measured in NTU with a nephelometer and readings reflect the turbidity in relation to double distilled water. The immediate effect of a change in turbidity is a change in water clarity. An increase in turbidity or suspended solids affects light penetration. As light penetration is reduced, primary production decreases and this negatively affects organisms higher in the food chain.

Most of the nitrogen in aquatic ecosystems is present as the gas N_2. Nitrate (NO_3^-), ammonia (NH_4^+), nitrite (NO_2^-) are less abundant but usually of more biological interest. In some aquatic ecosystems nitrogen is the nutrient element that most limits plant growth and the major forms of nitrogen available to bacteria, fungi, and plants are nitrate and ammonia. The nitrate concentration normally does not exceed 0,1 mg/l and is non-toxic. Nitrite is more toxic than nitrate and specifically toxic to fish. Ammonia, present in aquatic systems mainly as the dissociated ion NH_4^+ (ammonium), is much more reactive than nitrate and has a high toxicity (Table 3.10.4).

Phosphorus is a common growth-limiting factor for phytoplankton because it is often present in low concentrations seldomly exceeding 0,01 mg/liter. Phosphorus in nature can occur in inorganic form as phosphate salts such as $Ca_3(PO4)_2$ or PO_4^{3-} ions, or in organic forms. At normal pH ranges most soluble phosphate is present in three ionic forms: orthophosphate (PO_4^{3-}) is most common, with lesser amounts as monophosphate (HPO_4^{2-}) and dihydrogen phosphate ions ($H_2PO_4^-$).

Since both nitrogen and phosphorous are growth limiting, and in particular for the plant and algal communities, changes in their concentrations are reflected in the trophic status of the lentic water body (Table 3.10.5). The availability of food then indirectly affects fish and other biota via the food web. As stated previously only nitrite and ammonium are toxic to fish.

The results of the chemical analyses show the following:

That some sites in the Phongola, the lower Sabie, the Crocodile and the Luvuvhu rivers have a high that in most cases is accompanied by values of nitrogen, hardness and sulfates that are higher than the rest of the sites. With regard to the nitrogen and phosphates levels the water in the rivers are Oligotrophic. The only exceptions are sites 19, 20, 24 and 25 that tend to be slightly mesotrophic.

Table 3.10.5: Symptoms or effects associated with the ranges of inorganic phosphorous and nitrogen concentrations (Adapted from Dallas and Day, 2004)

Average summer concentration (mg/l)		
Inorganic phosphorous	**Inorganic nitrogen**	**Symptoms or effects**
<0,005	<0,5	Oligotrophic conditions, no water quality problems, low productivity, no nuisance growth of aquatic plants or algal blooms.
0,005-0,025	0,5-2,5	Mesotrophic conditions, moderate/occasional water quality problems, productive system, occasional nuisance growth of aquatic plants or algal blooms.
0,025-0,25	2,5-10,0	Eutrophic conditions, frequent water quality problems, highly productive system, frequent nuisance growth of aquatic plants or algal blooms.
> 0,25	> 10,0	Hypotrophic conditions, continuous water quality problems, very highly productive system, frequent nuisance growth of aquatic plants or algal blooms.

Sites 2, 23, 27, 28, 31 and 36 are of the sites listed in table 3.10.1 where *Opsaridium peringueyi* was found. Although these sites vary considerably with regard to their chemical aspects it is of note that the turbidity of the water at these sites was in the region of 1. It is postulated that the clarity of the water allows a carnivore such as *O. peringueyi* to visually detect their prey.

3.9.11 REFERENCES

ANONYMOS (2007) Final Scoping Report for the proposed pumped storage generation in the Steelpoort area, Limpopo and Mpumalanga Province.

ASAEDA T, PRIYAADARSHANA T and MANATUNGE, T (2001) Effects of satiation on the feeding and swimming behaviour of planktivores. Kluwer Academic Publishers. Netherlands. *Hydrobiologia*. **443** 147-157.

BALOK I, KARASAHIN B, OZKOK R, CUBUK, H and UYSAL R (2003) Diet of Silver crucian carp Crassus gableo in Lake Egirdir, Turkish. *Journal of Fisheries and Aquatic Sciences* **3:** 87-91.

BATCHELOR GR (1978) Aspects of the biology of Tilapia rendalii in Doorndraai Dam, Transvaal. *J. Limno. Soc. Sth. Afr*. **4** 65-68.

BELL-CROSS G and MINSHULL JL (1988)*The Fishes of Zimbabwe*. National Museums and Monuments of Zimbabwe, Harare. 294 pp.

BILLS R, ENGELBRECHT J and MARSHALL BE (2007) *Opsaridium peringueyi*. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.1. <www.iucnredlist.org>.

BLOOMER P and IMPSON ND (2000). Mitochondrial DNA differentiation in the critically endangered Berg River redfin (*Pseudobarbus burgi*). *The Journal of Heredity*. **91** 122-127.

BOWERS N, STAUFFER JR and KOCHLER TD (1994). Intra- and interspecific mitochondrial DNA sequence variation within two species of rock-dwelling cichlids (Teleostei: Cichlidae) from Lake Malawi, Africa. *Molecular Phylogenetics and Evolution* **3** 75-82.

BROWN WM (1985).The mitochondrial genome of animals. In: *Evolutionary genetics* (McIntyre, R.J. ed). New York, Plenum. 95-130.

CRASS RS (1964) Notes on the freshwater fishes of Natal with descriptions of four new species. *Ann. Natal. Mus*. **14** 405-458.

CRASS RS (1964) A hydrobiological study of the Mwenda River and its mouth, Lake Kariba. Unpublished PhD thesis, University of the Witwatersrand, Johannesburg.

CUI Y and WOOTON J (1988) The metabolic rate of the minnow*, Phoxinus* (L), in relation to ration, body size and temperature. *Functional Ecology* **2** (2) 157-161.

DARWALL WRT, SMITH KG, TWEDDLE D and SKELTON P (eds) (2009). The Status and Distribution of Freshwater Biodiversity in Southern Africa. Gland, Switzerland: IUCN and Grahamstown, South Africa: SAIAB. viii+120pp.

DAVIES BR and DAY J (1998) *Vanishing waters.* UCT Press, Cape Town. 487pp.

Department of Environment and Tourism (2006). Draft Norms and Standards for Biodiversity Management Plans for species (BMP-S) produced under the auspices of the National Environmental Management Biodiversity Act (NEMBA) No. 10 of 2004 – Pretoria : Department of Environment and Tourism.

Department of Environment and Tourism (2005). South Africa's National Biodiversity Strategy and Action Plan [Report] – Pretoria : Department of Environment and Tourism.

DE VILLIERS P (1991). The Ecology and Culture of the rock catlet, *Chiloglanis pretoriae* (Pisces: Mochokidae). Unpublished M.Sc. Thesis, Rhodes University. 148 pp.

DUARTE CM and ALCARAZ M (1989) To produce many small or few large eggs: a size-independent reproductive tactic of fish. *Oecologia.* **80** (3) 401-404.

DUDGEON D, ARTHINGTON AH, GESSNER MO, KAWABATA z-I, KNOWLER DJ, LéVêQUE C, NAIMAN RJ, PRIEUR-RICHARD AH, SOTO D, STIASSNY MLJ and SULLIVAN CA. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Review* **81** 163-182.

DUVERNALL DD and TURNER BJ (1998). Evolutionary genetics of DWEAh Valley pupfish populations: mitochondrial DNA sequence variation and population structure. *Molecular Ecology* **7** 279-288.

EMERY AJ, LOTTER M and WILLIAMSON SD (2002) Determining the Conservation Value of Land in Mpumalanga. DWAF/DFID Strategic Environmental Assessment. Mpumalanga parks Board, Nelspruit. 168 pp.

FACEY DE and GROSSMAN GD (1992) The relationship between water velocity, energetic costs, and microhabitat use in four North American stream fishes. *Hydrobiologia* **239** 1-6.

FELLEY JD and HILL LG (1983) Multivariate assessment of Environmental Preferences of Cyprinid Fishes of the Illinois River, Oklahoma. *The American Midland Naturalist* **109** (2) 209-221.

FOUCHé PSO (1995). An investigation of the scale morphology and scale annulus formation and an evaluation of the scale method for age determination of *Labeo umbratus* (Smith) (Pisces, Cyprinidae). Unpublished M.Sc. Thesis, University of Venda. 102 pp.

FOUCHé PSO, FOORD SH, POTGIETER N, VAN DER WAAL BCW and VAN REE T (2005). Towards an understanding of factors affecting the biotic integrity of rivers in the Limpopo Province: Niche partitioning, habitat preference and microbiological status in rheophillic biotopes of the Luvuvhu and Mutale rivers. WRC Report no.1197/05.

FOUCHé PSO and GAIGHER IG (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 report 714/3/01*.

FRANKMAN R, BALLOU JD and BRISCOE DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

GERALD MV (1976) The effect of starvation on energy turnover and protein metabolism in Opiocephalus punctatus bloch.Springer.Netherlands. *Hydrobiologia* **49** (3)191-201.

GRATWICKE B, MARSHALL BE and NHIWATIWA T (2003) The distribution and relative abundance of stream fish in the upper Manyame River, Zimbabwe, in relation to land use, pollution and exotic predator. *African Journal of Aquatic Science* **28**(1) 25-34.

GIUFFRA E, BERNATCHEZ L, and GUYOMARD R (1994) Mitochondrial control region and protein coding sequence variation among phenotypic forms of brown trout *Salmo trutta* from northern Italy. *Molecular Ecoloy* **3** 161-171.

GAIGHER IG (1969) Aspekte met betrekking tot die Ekologie, Geografie en Taksonomie van Varswatervisse in die Limpopo- en Incomatiriviersisteem. Unpublished Ph.D. Thesis, Randse Afrikaanse Universiteit. 261 pp.

GAIGHER IG (1973) The habitat preferences of fishes from the Limpopo River system, Transvaal and Mozambique. *Koedoe* **16** 103-116.

GAIGHER IG (1976) The reproduction of Barbus cf. kimberleyensis (Pisces, Cyprinidae) in the Hardap Dam, South West Africa. *Zoologica Africana* **11** (1) 97-110.

GLAZIER JR, and TABER CA (1980). Reproductive biology and age and growth of the Ozark minnow, *Dionda nubila*. *Copeia* **3** 547-550.

HAMMAN KCD (1974) 'n Ondersoek na die lengte, massa ouderdom en gonade ontwikkeling van die groter visspesies in die H.F. Verwoerddam. Unpublished M.Sc. Thesis, Randse Afrikaanse Universiteit. 78 pp.

HELFMAN GS, COLETTEE BB and FACEY DF (2000) *The diversity of fishes*. Blackwell Science Inc., Massachusetts. 528pp.

HIRZEL AH, HAUSSER J, CHESSEL D and PERRIN N (2002) Ecological niche factor analysis: how to compute habitat suitability maps without absence data. *Ecology* **83** 2027-2036

HYSLOP EJ (1980) Stomach content analysis a review of methods and their application. *J. Fish Biol.* **17** 411-429.

INGRAM BA and De SILVA SS (2007) Diet composition and preference of Murray cod, Trout cod and Macquarie perch (Percichthyidae) reared in fertilized earthen ponds. *Aquaculture Research* **271** 260-270.

KADYE WT and MOYO NAG (2007). Stream fish assemblage and habitat structure in a tropical African river basin (Nyagui River, Zimbabwe). *African Journal of Ecology*.

KAISER H, ENDEMANN F and PAULET TG (2003). A comparison of artificial and natural foods and their combinations in the rearing of Goldfish, *Carassius auratus*. *Aquaculture Research* **34** 943-950.

KAPOOR BG, SMIT H and VERIGHINA IA (1975) The alimentary canal and digestion in teleosts. *Adv. Mar. Biol.* **13** 109-309

KEAST A and WEBB D (1966) Mouth and body form relative to feeding ecology in fish fauna of a small lake, Lake Opinicon, *Ontario. J. Fish. Res. Board Can* **23** 1845-1873

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

KLEYNHANS CJ (2007) Module D: Fish Response Assessment Index in River EcoClassification: Manual
for EcoStatus Determination (version 2. Joint Water Research Commission and Department of Water
Affairs and Forestry report. *WRC Report No. TT330/08*

KLEYNHANS CJ and HOFFMAN (1992). New distribution records for *Clarias theodorae* (Weber 1897), *Barbus eutaenia* (Boulenger 1907) and *Opsaridium zambezense* (Peters 1852) from the Waterberg, Transvaal, South Africa. *Southern African Journal of Aquatic Sciences* **18** 107-111.

KLEYNHANS CJ and LOUW (2007) Module A: EcoClassification and EcoStatus determination in River EcoClassification: Manual for EcoStatus DeterminatioN. Joint Water Research Commission and Department of Water Affairs and Forestry report. *WRC Report No. TT 329/08.*

KLEYNHANS CJ, LOUW MD and GRAHAM M (2008) Module G: EcoClassification and EcoStatus determination in River EcoClassification: Index of Habitat Integrity (Section 1, Technical manual) Joint Water Research Commission and Department of Water Affairs and Forestry report. *WRC Report No. TT 377/08.*

LE ROUX PJ (1961). Growth of *Tilapia mossambica* in some Transvaal impoundments. *Hydrobiologica* **18** 165-175.

MARSHALL BE and GRATWICKE B (1999) The barred minnows (Teleostei: Cyprinidae) of Zimbabwe: is there a concern? *Sth. Afr. J. Aquat. Sci*. **24**: 157-161.

MIYA M, SAITOH K, WOOD R, NISHIDA M and MAYDEN RL (2005) New primers for amplifying and sequencing the mitochondrial ND4/ND5 gene region of the Cypriniformes (Actinopterygii: Ostariophysi). *Ichthyological Research* **53** 75-81.

MORITZ C (1994) Defining "evolutionarily significant units" for conservation. *Trends in Ecology and Evolution* **9** 373-375.

MORITZ C (2002). Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology* **51** 238-254.

MULVEY J, LYDEARD C , PYER DL, HICKS KM, BRIMBOX J, WILLIAMS JD and BUTLER RS (1997) Conservation genetics of North American freshwater mussels Amblema and Megalonaias. *Conservation Biology* **11** 868-878.

MUNRO AD, SCOTT AP and LAM TJ (1990) *Reproductive seasonality in teleosts: Environmental influences*. CRC Publishers, New York 54 pp.

NIKOLSKY GV (1963) *The Ecology of Fishes.* Academic Press, New York. 351pp.

NIELSEN JL (1995) Evolution and the aquatic ecosystem: Defining unique units in population conservation. American Fisheries Society Symposium 17, American Fisheries Society, Bethesda, MD.

O'HARA J (1968) The influence of weight and temperature on the metabolic rate of sunfish. *Ecology.* **49** (1) 159-161.

PARAMESHWARAN K, EDISHIRIGHE U, DEMATAWEWA CMB, NANDESENA KG (2001) Effect of live and formulated feeds on the larval growth and survival of guppy (*Poecilia reticulata*) reared in indoor tanks. *Aquaculture Research* **123** 421-430.

PHILLIPS SJ, ANDERSON RP and SCHAPIRE RE (2006).Maximum entropy modeling of species geographic distributions. *Ecological Modeling* **190** 231-259.

PIENAAR U de V (1978) The *Freshwater fishes of the Kruger National Park*. National parks Board of Trustees. Pretoria.

PRIYADARSHANA T, ASAEDA T and MANATUNGE J (2006) Hunger induced foraging behaviours of two cyprinid fish: *Pseudorasbora parva* and *Rasbora daniconius*. *Hydrobiologia* **568** 341-352

ROE KJ and LYDEARD C (1998). Molecular systematics of the freshwater mussel genus Potamilus (Bivalvia: Unionidae). *Malacologia* **39** 195-205.

ROTTTMANN RW, FRANCIS-FLOYD R and DRBOROW R (1992). The role of stress in fish disease. *SRAC Publication* No. 474.

ROUX F (2008) Reproduction strategy of the smallscale yellowfish (*Labeobarbus polylepis*) and breeding behaviour in the Blyde and Spekboom Rivers. Unpublished MSc. Thesis. University of Johannesburg, Johannesburg.

ROWNTREE K and WADESON R (1998) A geomorphological framework for the assessment of instream flow requirements. *Aquatic Ecosystem Health and Management.* **1** 125-141.

ROWNTREE K and WADESON R (2000) *Field manual for channel classification and condition assessment.* 62 pp.

RUSSEL IA (1997) Monitoring the conservation status and diversity of fish assemblages in the major rivers of the Kruger National Park. Unpublished PhD Thesis, University of the Witwatersrand, Johannesburg.

SCHLOSSER JI and EBEL KK (1989) Effects of flow regime and cyprinid predation on a headwater stream. *Ecological monographs* **59** (1) 41-57.

SCHROEDER GL (1980) Fish farming in manure loaded ponds. In: R.S.V. Pullin and Z.H. Shehadeh (eds). Integrated Agriculture-Aquaculture farming systems. Proc. ICLARMSEARCA Conf. Manila, Philippines, 6-9 August, 1979. *ICLARM Conf. Proc*. **4** 73-86.

SETTLES WH and HOYT RD (1978) The reproductive biology of the Southern Redbelly Dace, *Chrosomus erythrogaster* Rafinesque, in spring-fed stream in Kentucky. *Am. Midl. Nat*. **99** (2) 290-298.

SCHULZ GWC (1992). 'n Ekologiese studie van *Barbus brevipinnis* en *Opsaridium zambezense* in the Incomatiriviersisteem, Oos Transvaal. Unpublished M.Sc. Thesis, Randse Afrikaanse Universiteit. 143 pp.

SCHULZ GWC and SCHOONBEE HJ (1999). Aspects of the length, mass, fecundity, feeding habits and some parasites of the shortfin minnow, *Barbus brevipinnis* (Cyprinidae) from the Marite River, Mpumalanga Province, South Africa. *Water SA* **25** (2) 257-264.

SKELTON PH (1987) South African Red Data book – Fishes. *South African National Scientific Programmes Report* 137: 1-199.

SKELTON PH (1996) A review *of Opsaridium zambezense* (Pisces: Cyprinidae) from southern Africa with description of a new species from Malawi. *Icthyol. Explor. Freshwaters* **7** (1) 59-84.

SKELTON PH (1993) South African red data book – fishes. *South African National Scientific Programs Report* No. 137. CSIR Foundation for research and development, Pretoria.

SKELTON PH (2001) *A Complete Guide to the Freshwater Fishes of Southern Africa*. (2nd Edition). Southern Book Publishers, Halfway House. 395pp.

SIBBING FA (1991) Food Capture and oral processing: In: WINFIELD, I.J and NELSON, J.S. (Eds*) Cyprinid fishes: Systematic, Biology and exploitation*. Chapman and Hall Publishers, London.

SUNDARABARATHY TV, EDRIISHINGE U and DEMATAWEWA CMB (2004) Captive breeding and rearing of fry and juveniles of Cherry barb (*Puntius titteya*), a highly threatened endemic fish of Sri Lanka. *Tropical Agriculture Research* **16** 137-149.

TAMATAMAH R (2007) Environmental Flow Assessment (EFA), WAMI River Basin, Tanzania: Aquatic Ecology Component of the Wami River EFA Study. Department of fisheries and Aquatic Sciences. *Starter document for BBM worksHop*.

THIRION C (2008) Module E: Volume 1 Macro-invertebrate Response Assessment Index (MIRAI). Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC report TT 332/08.

VENTER JA (2007). The development of a conservation framework for threatened African fish using Opsaridium peringueyi as areference species: *Progress report: 2006/2007, WRC Project K5/1677*. Water Research Commision, Pretoria.

VILLELLA RF, KING TL and STARLIPER CE (1998) Ecological and evolutionary concerns in freshwater bivalve relocation programs. *Journal of Shellfish Research* **17** 1407-1413.

Water Research Commision. (2001). State of the Rivers Report- Letaba and Levuvhu River systems. Water Research Commision, Pretoria. WRC Report No. TT 165/01. pp 24

WAPLES RS (1991) Pacific salmon, Oncorhynchus spp., and the definition of "species" under the Endangered Species Act. U.S. *National Marine Fisheries Service Marine Fisheries Review* **53** 11-22.

WAPLES RS (1995) Evolutionary significant units and the conservation of biological diversity under the endangered species act. *American Fisheries Society Symposium* **17** 8-27.

WEEKS DC, O'KEEFE JH., FOURIE A and DAVIES BR (1996). A pre-impoundment study of the Sabie-Sand river system, Mpumalanga with special reference to predicted impacts on the Kruger National Park, Volume 1: The ecological status of the Sabie-Sand River System. *WRC Report.*

WERNER EE, MITTELBACH GG HALL DJ and GILLIAM JF (1983) Experimental tests of optimal habitat use in fish: the role of relative habitat profitability. *Ecology* **64** (6) 1525-1539.

WIKRAMANAYAKE ED and MOYLE PB (1989). Ecological structure of tropical fish assemblages in wet-zone streams of Sri Lanka. *J. Zool., London.* **218** 503 -526.

WILLERS B (1991) *Trout Biology*. Lyons and Burford, New York. 212pp.

WOOD BM and BAIN M(1995). Morphology and microhabitat use in stream fish. *Can. J. Fish. Aquat. Sc.* **52** 1487-1498.

WOYNAROVICH E and HORVATH L (1980). The artificial propagation of warm-water fin fishes: A manual for extension. *FAO Fish. Techn. Paper* **201.**

Addendum 1

Fish name abbreviations for South African fish species

ABBREVIATION	SCIENTIFIC NAME	ENGLISH COMMON NAME
AAEN	*AWAOUS AENEOFUSCUS* (PETERS 1852)	FRESHWATER GOBY (M)
ABAR	*AUSTROGLANIS BARNARDI* (SKELTON, 1981)	BARNARD'S ROCK CATFISH
ABER	*ACANTHOPAGRUS BERDA* (FORSSKÅL, 1775)	RIVERBREAM (MS)
ABIC	*ANGUILLA BICOLOR BICOLOR* MCCLELLAND, 1844	SHORTFIN EEL
ABRE	*ATHERINA BREVICEPS* VALENCIENNES, 1835	CAPE SILVERSIDE
AGIL	*AUSTROGLANIS GILLI* (BARNARD, 1943)	CLANWILLIAM ROCK-CATFISH
AJOH	*APLOCHEILICHTHYS JOHNSTONI* (GÜNTHER, 1893)	JOHNSTON'S TOPMINNOW
AKAT	*APLOCHEILICHTHYS KATANGAE* (BOULENGER, 1912)	STRIPED TOPMINNOW
ALAB	*ANGUILLA BENGALENSIS LABIATA* PETERS, 1852	AFRICAN MOTTLED EEL
AMAR	*ANGUILLA MARMORATA* QUOY & GAIMARD 1824	GIANT MOTTLED EEL
AMOS	*ANGUILLA MOSSAMBICA* PETERS 1852	LONGFIN EEL
AMYA	*APLOCHEILICHTHYS MYAPOSAE* (BOULENGER, 1908)	NATAL TOPMINNOW
ANAT	*AMPHILIUS NATALENSIS* BOULENGER, 1917	NATAL MOUNTAIN CATFISH
ASCL	*AUSTROGLANIS SCLATERI* (BOULENGER, 1901)	ROCK-CATFISH
AURA	*AMPHILIUS URANOSCOPUS* (PFEFFER, 1889)	STARGAZER (MOUNTAIN CATFISH)
BAEN	*LABEOBARBUS AENEUS* (BURCHELL, 1822)	SMALLMOUTH YELLOWFISH
BAFR	*BARBUS AFROHAMILTONI* CRASS, 1960	HAMILTON'S BARB
BAMA	*BARBUS AMATOLICUS* SKELTON, 1990	AMATOLA BARB
BAND	*BARBUS ANDREWI* BARNARD, 1937	WHITEFISH
BANN	*BARBUS ANNECTENS* GILCHRIST & THOMPSON, 1917	BROADSTRIPED BARB
BANO	*BARBUS ANOPLUS* WEBER, 1897	CHUBBYHEAD BARB
BARG	*BARBUS ARGENTEUS* GÜNTHER, 1868	ROSEFIN BARB
BBIF	*BARBUS BIFRENATUS* FOWLER, 1935	HYPHEN BARB
BBRI	*BARBUS BREVIPINNIS* JUBB, 1966	SHORTFIN BARB
BCAL	*BARBUS CALIDUS* BARNARD, 1938	CLANWILLIAM REDFIN
BCAP	*BARBUS CAPENSIS* SMITH, 1841	CLANWILLIAM YELLOWFISH
BERU	*BARBUS ERUBESCENS* SKELTON, 1974	TWEE RIVER REDFIN
BEUT	*BARBUS EUTAENIA* BOULENGER, 1904	ORANGEFIN BARB
BGUR	*BARBUS GURNEYI* GÜNTHER, 1868	REDTAIL BARB
BHOS	*BARBUS HOSPES* BARNARD, 1938	NAMAQUA BARB
BIMB	*BRYCINUS IMBERI* (PETERS, 1852)	IMBERI
BKIM	*LABEOBARBUS KIMBERLEYENSIS* GILCHRIST & THOMPSON, 1913	LARGEMOUTH YELLOWFISH
BLAT	*BRYCINUS LATERALIS* (BOULENGER, 1900)	STRIPED ROBBER
BLIN	*BARBUS LINEOMACULATUS* BOULENGER, 1903	LINE-SPOTTED BARB
BMAR	*LABEOBARBUS MAREQUENSIS* SMITH, 1841	LARGESCALE YELLOWFISH
BMAT	*BARBUS MATTOZI* GUIMARAES, 1884	PAPERMOUTH
BMOT	*BARBUS MOTEBENSIS* STEINDACHNER, 1894	MARICO BARB
BNAT	*BARBUS NATALENSIS* CASTELNAU, 1861	SCALY

BNEE	*BARBUS NEEFI* GREENWOOD, 1962	SIDESPOT BARB
BPAL	*BARBUS PALLIDUS* SMITH, 1841	GOLDIE BARB
BPAU	*BARBUS PALUDINOSUS* PETERS, 1852	STRAIGHTFIN BARB
BPOL	*LABEOBARBUS POLYLEPIS* BOULENGER, 1907	SMALLSCALE YELLOWFISH
BRAD	*BARBUS RADIATUS* PETERS, 1853	BEIRA BARB
BSER	*BARBUS SERRA* PETERS, 1864	SAWFIN
BTOP	*BARBUS TOPPINI* BOULENGER, 1916	
BTRE	*BARBUS TREURENSIS* GROENEWALD, 1958	TREUR RIVER BARB
BTRI	*BARBUS TRIMACULATUS* PETERS, 1852	THREESPOT BARB
BTRV	*BARBUS TREVELYANI* GÜNTHER, 1877	
BVIV	*BARBUS VIVIPARUS* WEBER, 1897	BOWSTRIPE BARB
CANO	*CHILOGLANIS ANOTERUS* CRASS, 1960	PENNANT-TAIL SUCKERMOUTH (OR ROCK CATLET)
CAUR	*CARASSIUS AURATUS* (LINNAEUS, 1758)	GOLDFISH (EX)
CBIF	*CHILOGLANIS BIFURCUS* JUBB & LE ROUX, 1969	INCOMATI SUCKERMOUTH (OR ROCK CATLET)
CBRE	*CHETIA BREVIS* JUBB, 1968	ORANGE-FRINGED LARGEMOUTH
CCAR	*CYPRINUS CARPIO* LINNAEUS, 1758	CARP (EX)
CEMA	*CHILOGLANIS EMARGINATUS* JUBB & LE ROUX, 1969	PONGOLO SUCKERMOUTH (OR ROCK CATLET)
CFLA	*CHETIA FLAVIVENTRIS* TREWAVAS, 1961	CANARY KURPER
CGAR	*CLARIAS GARIEPINUS* (BURCHELL, 1822)	SHARPTOOTH CATFISH
CIDE	*CTENOPHARYNGODON IDELLA* (VALENCIENNES, 1844)	GRASS CARP (EX)
CMUL	*CTENOPOMA MULTISPINE* PETERS, 1844	MANYSPINED CLIMBING PERCH
CPAR	*CHILOGLANIS PARATUS* CRASS, 1960	SAWFIN SUCKERMOUTH (OR ROCK CATLET)
CPRE	*CHILOGLANIS PRETORIAE* VAN DER HORST, 1931	SHORTSPINE SUCKERMOUTH (OR ROCK CATLET)
CSWI	*CHILOGLANIS SWIERSTRAI* VAN DER HORST, 1931	LOWVELD SUCKERMOUTH (OR ROCK CATLET)
CTHE	*CLARIAS THEODORAE* WEBER, 1897	SNAKE CATFISH
GAES	*GILCHRISTELLA AESTUARIA* (GILCHRIST, 1913)	ESTUARINE ROUND-HERRING
GAFF	*GAMBUSIA AFFINIS* (BAIRD & GIRARD, 1853)	MOSQUITOFISH (EX)
GCAL	*GLOSSOGOBIUS CALLIDUS* SMITH, 1937	RIVER GOBY (M)
GGIU	*GLOSSOGOBIUS GIURIS* (HAMILTON-BUCHANAN, 1822)	TANK GOBY (M)
GZEB	*GALAXIAS ZEBRATUS* CASTELNAU, 1861	CAPE GALAXIAS
HANS	*HIPPOPOTAMYRUS ANSORGII* (BOULENGER,1905)	SLENDER STONEBASHER
HCAP	*HYPORHAMPHUS CAPENSIS* (THOMINOT, 1886)	CAPE HALFBEAK (MS)
HMOL	*HYPOPHTHALMICHTHYS MOLITRIX* (VALENCIENNES, 1844)	SILVER CARP (EX)
HVIT	*HYDROCYNUS VITTATUS* CASTELNAU, 1861	TIGERFISH
KAUR	*KNERIA AURICULATA* (PELLEGRIN, 1905)	SOUTHERN KNERIA
LCAP	*LABEO CAPENSIS* (SMITH, 1841)	ORANGE RIVER LABEO
LCON	*LABEO CONGORO* PETERS, 1852	PURPLE LABEO
LCYL	*LABEO CYLINDRICUS* PETERS, 1852	REDEYE LABEO
LMAC	*LEPOMIS MACROCHIRUS* RAFINESQUE, 1819	BLUEGILL SUNFISH (EX)
LMCR	*LIZA MACROLEPIS* (SMITH, 1846)	LARGE-SCALE MULLET (MS)
LMOL	*LABEO MOLYBDINUS* DU PLESSIS, 1963	LEADEN LABEO
LRIC	*LIZA RICHARDSONII* (SMITH, 1846)	SOUTHERN MULLET (MS)
LROS	*LABEO ROSAE* STEINDACHNER, 1894	REDNOSE LABEO

LRUB	*LABEO RUBROMACULATUS* GILCHRIST & THOMPSON, 1913	TUGELA LABEO
LRUD	*LABEO RUDDI* BOULENGER, 1907	SILVER LABEO
LSEE	*LABEO SEEBERI* GILCHRIST & THOMPSON, 1911	CLANWILLIAM SANDFISH
LUMB	*LABEO UMBRATUS* (SMITH, 1841)	MOGGEL
MACU	*MICRALESTES ACUTIDENS* (PETERS, 1852)	SILVER ROBBER
MARG	*MONODACTYLUS ARGENTEUS* (LINNAEUS, 1758)	NATAL MOONY (MS)
MBRA	*MICROPHIS BRACHYURUS* BLEEKER, 1853	OPOSSUM PIPEFISH (M)
MBRE	*MESOBOLA BREVIANALIS* (BOULENGER, 1908)	RIVER SARDINE
MCAP	*MYXUS CAPENSIS* (VALENCIENNES, 1836)	FRESHWATER MULLET (M)
MCEP	*MUGIL CEPHALUS* LINNAEUS, 1758	FLATHEAD MULLET (M)
MCYP	*MEGALOPS CYPRINOIDES* (BROUSSONET, 1782)	OXEYE TARPON
MDOL	*MICROPTERUS DOLOMIEU* LACEPÈDE, 1802	SMALLMOUTH BASS (EX)
MFAL	*MONODACTYLUS FALCIFORMIS* LACEPÈDE, 1801	CAPE MOONY (MS)
MFLU	*MICROPHIS FLUVIATILIS* (PETERS, 1852)	FRESHWATER PIPEFISH (M)
MMAC	*MARCUSENIUS MACROLEPIDOTUS* (PETERS, 1852)	BULLDOG
MPUN	*MICROPTERUS PUNCTULATUS* (RAFINESQUE, 1819)	SPOTTED BASS (EX)
MSAL	*MICROPTERUS SALMOIDES* (LACEPÈDE, 1802)	LARGEMOUTH BASS (EX)
NORT	*NOTHOBRANCHIUS ORTHONOTUS* (PETERS, 1844)	SPOTTED KILLIFISH
NRAC	*NOTHOBRANCHIUS RACHOVII* AHL, 1926	RAINBOW KILLIFISH
OAUR	*OREOCHROMIS AUREUS* (STEINDACHNER, 1864)	ISRAELI TILAPIA (EX)
OMAC	*OREOCHROMIS (NYASALAPIA) MACROCHIR* (BOULENGER, 1912)	GREENHEAD TILAPIA
OMOS	*OREOCHROMIS MOSSAMBICUS* (PETERS, 1852)	MOZAMBIQUE TILAPIA
OMYK	*ONCORHYNCHUS MYKISS* (WALBAUM, 1792)	RAINBOW TROUT (EX)
ONIL	*OREOCHROMIS NILOTICUS (LINNAEUS, 1758)*	NILE TILAPIA (EX)
OPER	*OPSARIDIUM PERINGUEYI* (GILCHRIST & THOMPSON, 1913)	SOUTHERN BARRED MINNOW
OPLA	*OREOCHROMIS PLACIDUS* (TREWAVAS, 1941)	BLACK TILAPIA
PAFE	*PSEUDOBARBUS AFER* (PETERS, 1864)	EASTERN CAPE REDFIN
PAMP	*PROTOPTERUS AMPHIBIUS* (PETERS, 1844)	EAST COAST LUNGFISH
PANN	*PROTOPTERUS ANNECTENS BRIENI* POLL,1961	LUNGFISH
PASP	*PSEUDOBARBUS ASPER* (BOULENGER, 1911)	SMALLSCALE REDFIN
PBUG	*PSEUDOBARBUS BURGI* (BOULENGER, 1911)	BERG RIVER REDFIN
PBUR	*PSEUDOBARBUS BURCHELLI* SMITH, 1841	BURCHELL'S REDFIN
PCAT	*PETROCEPHALUS CATOSTOMA* (GÜNTHER, 1866)	CHURCHILL
PFLU	*PERCA FLUVIATILIS* LINNAEUS, 1758	EUROPEAN PERCH (EX)
PPHI	*PSEUDOCRENILABRUS PHILANDER* (WEBER, 1897)	SOUTHERN MOUTHBROODER
PPHL	*PSEUDOBARBUS PHLEGETHON* (BARNARD, 1938)	FIERY REDFIN
PQUA	*PSEUDOBARBUS QUATHLAMBAE* (BARNARD, 1938)	DRAKENSBERG MINNOW
PRET	*POECILIA RETICULATA* PETERS, 1859	GUPPY (EX)
PTEN	*PSEUDOBARBUS TENUIS* (BARNARD, 1938)	SLENDER REDFIN
RDEW	*REDIGOBIUS DEWAALI* (WEBER, 1897)	CHECKED GOBY (M)
SBAI	*SANDELIA BAINSII* CASTELNAU, 1861	EASTERN CAPE ROCKY
SCAP	*SANDELIA CAPENSIS* (CUVIER, 1831)	CAPE KURPER
SFON	*SALVELINUS FONTINALIS* (MITCHILL, 1815)	BROOK CHARR (EX)
SINT	*SCHILBE INTERMEDIUS* RÜPPELL, 1832	SILVER CATFISH
SMER	*SERRANOCHROMIS MERIDIANUS* JUBB, 1967	LOWVELD LARGEMOUTH
SSIB	*SILHOUETTEA SIBAYI* FARQUHARSON, 1970	SIBAYI GOBY (M)
STRU	*SALMO TRUTTA* LINNAEUS, 1758	BROWN TROUT (EX)
SZAM	*SYNODONTIS ZAMBEZENSIS* PETERS, 1852	BROWN SQUEAKER
TREN	*TILAPIA RENDALLI* (BOULENGER, 1896)	REDBREAST TILAPIA
TSPA	*TILAPIA SPARRMANII* SMITH, 1840	BANDED TILAPIA

TTIN	*TINCA TINCA* (LINNAEUS, 1758)	TENCH (EX)
VNEL	*VARICORHINUS NELSPRUITENSIS* GILCHRIST & THOMPSON, 1911	INCOMATI CHISELMOUTH
XHEL	*XIPHOPHORUS HELLERI* HECKEL, 1848	SWORDTAIL (EX)