CHAPTER 6

Species diversity, general health and condition of fish from the Klip River and bioaccumulation of selected metals in fish from two impoundments in this system.

6.1 INTRODUCTION

Water is one of the most precious natural resources on earth, and creates a wide range of benefits to humans, including fisheries, wildlife, agriculture, urban, industrial and social development (Allan and Flecker, 1993). The activities in the catchment, however, generally result in changes of the physical, chemical and biological components of the aquatic system, which often threatens the sustainability of these valuable resources (Adams *et al*., 1993). The unfortunate effect of anthropogenic activities is therefore often detrimental to the watercourses, and usually results in a degradation of the natural aquatic ecosystem (Karr *et al*., 1986; Schleiger, 2000).

The composition of the native fish communities is often changed due to anthropogenic activities in the catchment. Changes in water quality, flows and habitat can result in the disappearance or addition of species, changing the biotic integrity of the system (Karr *et al.*, 1995; Loeb & Spacie, 1994; Karr *et al*., 1996; Lyons *et al*., 1996). Apart from water quality, quantity and habitat related impacts, the introduction of introduced fish species (exotic and indigenous) is a major cause for degradation of ecological integrity (Allan and Flecker, 1993). The most common reasons for the deliberate introduction of exotic species into a region are associated with aquaculture, aquarium value and recreational (angling) purposes (Skelton, 2001). Furthermore, water-transfer schemes are often responsible for the introduction of species into a river system (Davies & Day, 1998). River systems of southern Africa were, by 1988, already invaded by 54 alien and translocated and fish species (de Moor & Bruton, 1988). The major mechanisms whereby exotics affect native species include predation, habitat alterations, hybridisations, the introduction of disease or parasites (Taylor *et al*., 1984) and competition for food and habitat (Skelton, 2001). As a consequence of increased human activities, the problems associated with introduced species are likely to intensify, and managers of aquatic ecosystems will increasingly be confronted with a shifting mix of native and non-native, rendering the assessment of the impact of introduced species on aquatic ecosystems very important.

In some cases human-induced stresses are not extreme enough to change species diversity and structure, but it does have obvious sub-lethal and chronic effects on the aquatic biota (Karr *et al*., 1987; Adams *et al*., 1992; Heath, 1999; du Preez 2000). High pollutant levels could lead to bioaccumulation (Kotze, 1997; Heath, 1999; du Preez, 2000), while habitat and water quality changes could result in deterioration of fish condition (van der Bank, 1984), health impairment (Adams, 1990; Adams *et al*., 1992; Adams *et al*., 1993; Avenant-Oldewage, 2001; Watson, 2001) and changes in species diversity (Karr et al., 1985; Lyons *et al*., 1996).

The state and health of fish communities has been noted to give a reliable indication of short and long-term stress on aquatic systems (Karr et al., 1986; Adams, 1990; Adams *et al*., 1992; Adams *et al*., 1993; Allan and Flecker, 1993; Barbour *et al*. 1999). Fish communities possess various characteristics that render them important in the assessment of river health. They occupy positions throughout the aquatic food web, and are typically present in all but the most polluted of waters. Because fish often range over considerable distances, they have the potential to integrate diverse aspects of relatively large-scale habitats. Fish can therefore provide an integrative view of watershed conditions. Compared to other aquatic organisms, fish are furthermore relatively long-lived, and are therefore useful in providing a temporal dimension of conditions. They are also relatively easy to identify, and after data is gathered, they can be released again. For the general public, fish are also the most well known of aquatic organisms, and they are more likely to understand information about the condition of the fish community than of other taxa such as invertebrates. There are, however, some difficulties in using fish as biomonitors. Among these problems is the selective nature of some sampling gear for specific biotopes and for certain sizes and species of fish, the mobility of fish on spatial and temporal time scales, and the labour intensity of fish sampling (Karr *et al*., 1986).

An essential component of biological assessments and criteria is how to determine whether a water body is healthy or unhealthy (Hughes, 1995). In an attempt to answer this commonly asked question, the current ecological status of a river must be compared to an expected or best attainable scenario, generally based on reference conditions. An ecological reference condition is defined as the condition that is representative of a group of “least-impacted” or minimally disturbed (compared to the natural condition) sites, organised by selected physical, chemical and biological characteristics (Dallas, 2000), and in general equates with biological integrity (Karr & Chu, 1997). Reference conditions enable the degree of degradation or deviation from natural conditions to be ascertained, and thereby serve as a foundation for developing biological criteria for the protection of aquatic ecosystems. When using biotic communities to detect the impact of one or more environmental stresses on a site, it is thus necessary to know the fauna expected at the site in the absence of environmental stress (Dallas 2000). In the determination of the ecological integrity of the Klip River, it was therefore essential to determine reference conditions against which the current status of the Klip River could be compared.

This chapter gives an overview of selected aspects of the fish communities of the Klip River and of a locality in the Suikerbosrand River. The main objectives of this part of the study were to:

- Assess the current fish species diversity of the Klip River system.
- Determine the extent of bioaccumulation of metals in fish from two impoundments of the Klip River.
- Assess the health of fish by the application of an external health assessment index.
- Assess the general condition of fish by the application of condition factors.
- Develop a method to quantify the potential impact that exotic and introduced fish species may have on the indigenous species.
- Describe the methodology applied to estimate the expected fish assemblage at each site.

6.2 MATERIALS AND METHODS

6.2.1 Defining and estimating the expected scenarios of the fish assemblages.

In terms of *ecological health*, a biological system can be considered healthy when "its inherent potential is realized, its condition is stable, its capacity for self-repair when perturbed is preserved, and minimal external support for management is needed" (Karr *et al.*, 1986). This scenario would thus refer to an ecological reference condition, and in multimetric biological assessments equate to biological integrity (Karr & Chu, 1997; Dallas 2000). For the purpose of determining the present health of a system, a criterion must be set with which the current state can be compared (Dallas, 2000).

Hughes (1995) noted a few important and interrelated aspects that must be considered when looking for a reference condition, namely:

- The reference condition must be politically palatable and reasonable.
- The reference condition for a general type of river should represent larger numbers of defined populations of rivers, although we should not expect that a single reference condition would be satisfactory for all rivers. Some sort of regional or water body classification is needed.
- A reference condition, or process for determining it, must represent important aspects of natural conditions.
- Given the difficulty of empirically determining what conditions would be like in the absence of humans, and the fact that humans have resided here for thousands of years, we are forced to use minimal disturbance as a reference condition.

The following aspects could be used when determining reference conditions (Hughes, 1995), namely:

- Regional reference sites
- Historical data
- Paloecological data (from lake sediments and wetlands)

- Experimental laboratory data
- Quantitative models and
- Best professional judgement.

The approach with the greatest potential for success combines reference sites and historical data, interpreted through the use of linear models and professional judgement (Hughes, 1995). The first method applied in the estimation of reference conditions for the Klip River was the reference to *historical data (*Mulder (1971), Skelton (2001) and personal communication with conservationists and residents in the area). This would give the best indication of what the conditions could be expected to be like, especially if data sets are complete and are from periods prior to human activities.

Historic data on the Klip River system was, however, very limited. The second approach followed in the setting of reference criterion was the *reference to a minimally disturbed (least-impacted) site* in the Suikerbosrand River. This approach is also incorporated in the assessment of biotic integrity, defined as "the ability of an ecosystem to support and maintain a balanced, integrated, adaptive community of organisms, *having a species composition, diversity and functional organization comparable to that of the natural habitat of the region*" (Karr and Dudley, 1981). Due to the general lack of historic data on the Klip River, more emphasis was placed on this (reference site). The reference site was, however, not directly comparable to all sites in the Klip River, due to variation in stream order, biotope diversity and other habitat components. The third approach incorporated in the setting of reference conditions was the use of estimates made by a group of fish *biologists and specialists*. In this approach, professional judgment by these persons was used at each site to estimate what the fish assemblage should be at a site if human-induced stressors were limited and/or impacts reversed.

Based on this approach, it is believed that the estimated reference/expected conditions are best attainable in the system, based on the current information and expertise available. It is therefore the most accurate depiction of the fish assemblage expected at a site and depicts the scenario that should prevail if the catchment is managed effectively and human activities are minimal.

The expected scenario/reference condition for the fish assemblage of each site was therefore done in an attempt to derive reference conditions of the fish component for each site. A detailed assessment was made for each site investigated in the study area, based on the three approaches mentioned above. The following questions and considerations guided the estimations made on the expected fish assemblage of each site:

- Is the observed fish habitat diversity, amount of cover and habitat condition at the site attributed to current human impacts? (e.g. increased or decreased flows).
- If human impacts were removed from the site, what would the habitat diversity, amount of cover and condition for fish be? (e.g. after establishing natural flow regimes by regulating upstream dam releases).

The expected fish species diversity was firstly estimated based on the expected habitat diversity attainable at each site, if human impacts were minimal. Based on the expected amount of habitat available at a site, an estimation of the number of individuals that should be sampled during one unit of sampling (20 minutes electro-fishing) was then made.

6.2.2 Fish species diversity in the Klip and Suikerbosrand Rivers

Sampling techniques

Fish surveys were conducted twice a year from August 1997 until February 1999, during the winter and summer seasons. Nine sites were sampled in the Klip River, and one in the Suikerbosrand River as a reference site (Figure 2.1, Chapter 2). The primary method used in the sampling of fish was electronarcosis (Meador *et al*., 1993; Barbour *et al*., 1999). This method was found to be the most effective way for sampling the habitat types present in the Klip River, being primarily runs, riffles, rapids and deep pools. In the shallow habitats (riffles and rapids) electronarcosis was conducted by wading in the water (Plate 6.1), while a boat was used (plate 6.2) for electronarcosis of the deeper areas (runs and pools). A small fibreglass boat with an outboard engine was used (Plate 6.2) and electro-fishing was then applied up and downstream for a certain period of time, while fish were collected from the boat with handheld nets. The relatively slow flow within the deep pools at locality 10 (Suikerbosrand River) permitted the use of gill nets, and due to the fact that this was the reference site and it was of cardinal importance to determine the complete fish composition at this site, the decision was made to apply this technique. This method (gill netting) can, however, be destructive and should, as far as possible, be excluded from biomonitoring techniques. All fish sampled were kept in an aerated holding tank to optimise survival rate. Each fish was identified (species level), weighed, and its total and fork length measured and recorded (Meador *et al*., 1993; Barbour *et al*., 1999).

Plate 6.1: Electronarcosis by wading.

Plate 6.2: Electronarcosis by boat.

6.2.3 Measure of impact by introduced (exotic and indigenous) species

A method was developed to quantify the expected impact that introduced species may have on the biotic integrity, by considering the type of introduced species and their relative abundance at a site. Any species other than those that are naturally indigenous to the system should be regarded as introduced (includes trans-located species indigenous to other systems in the region).

In general, it can be expected that, in an aquatic ecosystem, some exotic species may have a greater impact than others, depending on their characteristics and abundance. It is expected that in the Klip River, the impact of the large predacious alien *Micropterus salmoides*

(Largemouth Bass) is greater than the impact of the small alien ornamental fish species *Gambusia affinis* (mosquito fish), due to the fortunate scarcity of the last mentioned species in this system. Each exotic species was then given an impact rating (IR's), based on the expected impact that an introduced species might have on the native fish assemblage of a system where it occurs (Table 6.1). The impacts could be direct, such as predation or competition, or indirect, such as habitat destruction or habitat alteration. The impact ratings were 1, 3 or 5 with the following description connected to each score:

Impact rating of **1:** The species has no obvious, or only slightly visible impacts on a few of the native fish species (e.g. limited competition for habitat and food with small proportion of native species).

Impact rating of **3**: The species has a moderate impact on some of the native fish species at a site (e.g. competition for habitat and food, as well as moderate habitat and/or water quality alteration)

Impact rating of **5***:* This species is expected to largely impact all native species in some or other way (especially top predators which feed on all indigenous species present).

The following exotic species were observed in the Klip/Suikerbosrand systems during this study:

- *Cyprinus carpio*
- *Micropterus salmoides*
- *Gambusia affinis*

The expected impact by introduced species on the native fish at a site was then estimated as follows:

Introduced Species Impact score $(ISI)_{(site\ 1)}$ = [(relative abundance of introduced $species_A$) x (Impact Rating of $Species_A$)] + [(relative abundance of introduced $species_B$) x (Impact Rating of $Species_B$)]…

Where: Relative abundance of species_A = ($\text{CPUE}_{\text{Spesies A}}$/ $\sum$ CPUE of all species at site) x 100.

and Catch per unit Effort (CPUE) refers to number of individuals caught per sampling effort of 20 minutes electro-fishing)

Table 6.1: Impact ratings (IR) of exotic species as calculated for the Klip and Suikerbosrand Rivers.

SPECIES	Impact Rating (IR)	Expected impact
Micropterus salmoides	5	• Predation (piscivorous behaviour[1]) • Competition (especially feeding[1,2])
Cyprinus carpio	3	• Habitat destruction (bottom feeding habits[1, 2]) • Water quality change (increased turbidity)[2] • Competition (especially feeding)[2] • Preying on spawn of indigenous fish[2]
Gambusia affinis	1	• Predation of fish eggs and larvae[2]. • Competition (especially feeding by reducing zooplankton & insect larvae)[1, 2]

[1] – Skelton (2001) [2] – de Moor & Bruton (1988)

6.2.4 External Fish Health Assessment Index (FHAIe)

The health of adult fish was evaluated according to the External Fish Health Assessment Index (FHAIe) which is a simplified approach based on the Fish Health Assessment Index (FHAI) as described by Kilian *et al*. (1997). This investigation was only conducted on the external morphology, due to the fact that non-destructive sampling was applied. Where possible, a minimum of 20 adult individuals was assessed to increase accuracy of the method, and to meet minimum statistical requirements. The skin, fins, eyes, opercula and gills of each fish were examined and according to its condition, a score of 1 (poor), 3 (moderate) or 5 (normal) was given. The scoring criteria were, in general, similar to those used by Kilian *et al.* (1997), with an exception being made to the scoring of gills. The original index only had two scoring possibilities, namely 5 or 1. It was, however, decided to also include a middle option of 3, to score slight to moderate changes in the gills. Ecto-parasites were also not used in this study, due to the choice of sampling techniques, which often results in the expulsion of parasites from the fish. Furthermore, this protocol was developed to be a rapid assessment, and therefore each individual fish could not be surveyed extensively for the presence of parasites. Crafford (2000) also stated that some ectoparasites might be negatively affected by poor water quality (*Lamproglena* sp., *Achtheress percarum*, *Ergassilus sieboli*, *Gyrodactylus* sp.), while others may be favoured or unaffected (*Trichodinid ciliates*, *Argulus* sp.). Crafford (2000) furthermore concluded that total parasite numbers (endo- and ectoparasites) could not distinguish between two sites with differing water quality in the Vaal River (Crafford, 2000). The scores assigned to each organ were based on the following description of the observed condition:

SKIN

5 - (N): Skin appears intact (normal) with no active aberrations. This would include previous scars on the skin, which were completely healed over (Appendix 6.1, Plate A6.6).

3 - (M): Current skin damage but relative mild degradation, scale loss and/or with scars, with possible slight haemorrhaging (Appendix 6.1, Plate A6.8).

1 - (S): Extensive active tissue degradation, and/or with severe manifestation of scars, haemorrhaging, tumours, cysts and possibly accompanied by secondary infection (Appendix 6.1, Plate A6.1, A6.2, A6.3, A6.4, A6.5, and A6.7).

FINS

5 - (N): Normal appearing fins with no active erosion. This would include previously eroded fins, which were completely healed over (Appendix 6.1, Plate A6.6).

3 - (M): Active fin erosion process, with slight haemorrhaging but no secondary infection in evidence.

1 - (S): Severe active fin erosion with many haemorrhaging and possibly accompanied by tumors and/or secondary infection (Appendix 6.1, Plate A6.4, A6.9 and A6.10).

EYES

5 - (N): Normal, no aberration in eyes (Appendix 6.1, Plate A6.1).

1 - (E): Exopthalmic eyes: swollen, protruding eyes, more commonly referred to as popeye (E1 -one or E2-both); or

(B): Blind eyes: refers to opaque (dull) eyes and this condition includes cataracts (B1/B2).

(H): Haemorrhagic eyes: heamorrhaging or bleeding in the eyes (H1/H2).

(M): Missing: an eye actually missing from the fish (M1/M2)

(OT): Any other manifestation not fitting the above.

OPERCULA

5 - (N): Appears normal with no shortening of the opercula, gills completely covered (Appendix 6.1, Plate A6.1).

3 - (M): Slight shortening of opercula with very small portion of gill exposed.

1 - (S): Severe shortening of opercula with a considerable portion of gill exposed (Appendix 6.1, Plate A6.14).

GILLS

5 - (N): Gills with red colour are considered normal, no aberration in gill(s) (Appendix 6.1, Plate A6.11).

3 – (SF): Slightly frayed, erosion of tips of gill lamellae resulting in 'ragged' or frayed appearing gills (Appendix 6.1, Plate A6.12).

(CY): Few cysts visible on gill filaments.

1 - (F): Extensively Frayed:

(C): Clubbed: swelling of the tips of the gill lamellae. It can often appear bulbous or 'club-like'.

(D): Marginate: gills with light, discoloured margin along the distal ends or tips of the lamellae or filaments, often associated with 'clubbing'.

(P): Pale: gills are definitely very light in colour.

(M): Mucus: increasing mucus production on the gills

(OT): Any other observation that does not fit the above (Appendix 6.1, Plate A6.13).

Calculation of the health, according to the scores given to the external organs, was calculated as follows:

- The FHAIe score for each individual investigated was calculated as follows:

$$\text{FHAIe}_{(\text{fish 1, Species A})} = S + F + E + O + G$$

where S = skin, F = fins, E = eyes, O = opercula and G = gills.

- The FHAIe score for specific tissues of a species was calculated as follows (e.g. skin=S):

$$\text{FHAIe}(_{\text{species A, skin}}) = \text{average } (S_{(\text{fish 1, species A})}; S_{(\text{fish 2, species A})} ; ....S_{(\text{fish n, species A})}).$$

where n is the number of fish sampled of a specific species.

- The FHAIe score for a specific species was calculated as follows:

$$FHAIe_{(species\ A)} = \text{average}\ (FHAIe_{(fish\ 1,\ species\ A)};\ FHAIe_{(fish\ 2,\ species\ A)}\ ;\ ....FHAIe_{(fish\ n,\ species\ A)}).$$

where n is the number of fish sampled of a specific species.

- The FHAIe score for a site was calculated as follows:

$$FHAIe_{(site)} = \text{average}\ (FHAIe_{(species\ A)},\ FHAIe_{(species\ B)}..........\ FHAIe_{(species\ n)}).$$

where n is the number of species sampled at a site.

6.2.5 Fish Condition Factors

Condition factors were only determined when the sample size was large enough (minimum 20) to achieve a more reliable assessment and to meet statistical requirements. Condition factors were calculated for the two most abundant species in the Klip River, namely *Labeo capensis* and *Labeobarbus aeneus*, and an average factor was also determined for each site. Condition factors were calculated for the August 1997 (winter), August 1998 (winter) and February 1998 (summer) surveys. Consequently the length-weight relationship was applied to the following formulae as used by Le Cren (1951), Du Toit (1971), Schutte (1973) and Van der Bank (1984) to determine the condition factor:

$$M = c\,L^n$$

$$\therefore \text{Log } M = \log c + n \log L$$

$$\text{Log } c = \frac{\Sigma \log M\ \Sigma (\log L)^2 - \Sigma \log L\ \Sigma (\log L \log M)}{N\ \Sigma (\log L)^2 - (\Sigma \log L)^2}$$

$$\text{and} \quad n = \frac{\Sigma \log M - N \log c}{\Sigma \log L}$$

where M = mass, L = length, N = amount of fish and $K = \frac{\text{(average mass)}}{c\ (\text{average length})^n}$

6.2.6 Bioaccumulation of Metals

The bioaccumulation of metals was investigated during May 1998 at two sites in the Klip River. This investigation focused on two impoundments, one in the middle and one in the lower section of the river. The Olifantsvlei weir (middle section) is situated directly upstream of biomonitoring locality 4 (See chapter 2, figure 2.1). It is a shallow impoundment (± 1,5m deep) with a highly organic rich bottom substrate. Reeds cover most of its banks and scattered emergent aquatic vegetation occurs throughout the dam. The second site selected for the bioaccumulation investigation was the Henley-on-Klip weir in the lower section of the river (See chapter 2: Figure 2.1). This site is surrounded by small-scale agricultural holdings. The bottom substrate at this site is also highly organic, primarily due to suspended solids originating from upstream activities, such as water care works, agriculture and informal settlements.

Fish were collected by means of gill nets and electro fishing (Meador *et al*., 1993; Barbour *et al*., 1999). After capture, the mass and length of each fish was recorded. The fish were then killed with a blow to the head and by cutting of the spinal cord behind the head. Fish were dissected on a polyethylene work-surface, using stainless steel tools, while taking care to prevent any contamination of the samples (Heit & Klusek, 1982; Kotze, 1997; du Preez, 2000). Muscle, gill, liver and skin tissues were removed from each fish and frozen until metal analyses could be performed.

Laboratory procedures

The samples were thawed in the laboratory and dried in an oven at 60°C for a period of 48 hours. In order to calculate the moisture content of each sample, the dry and wet mass of each sample was recorded. Twenty ml of concentrated nitric acid (55%) and 10 ml of perchloric acid (70%) were added to approximately 1g tissue (dry mass) in a 100 ml Erlynmeyer flask. Digestion was performed on a hotplate (200 to 250°C) until the solutions were clear (Van Loon, 1980). The solutions were then filtered through acid-resistant 0.45µm filter paper and made up to 50 ml each with doubly distilled water. The samples were stored in clean glass bottles until the metal concentration could be determined using Ion Chromatographic Procedures.

6.3 RESULTS

6.3.1 Expected fish species diversity and abundances of the selected sites in the Klip and Suikerbosrand Rivers.

The fish habitat available at *locality 1* (See chapter 4, Figure 4.1 and Appendix 4.5) was estimated to be generally similar to that expected under unimpacted conditions. Minor changes in the habitats at this site were attributed to the following (Chapter 4):

- Decreased flows due to upstream damming result in loss of habitat diversity.
- Increased embeddedness of pool substrates due to sedimentation and suspended solid input.
- Lack of marginal and aquatic vegetation cover for fish, due to the impact of exotic Black wattle trees (blocks sun from instream channel, thereby inhibiting growth of aquatic plants).

At *locality 2* (See chapter 4, Figure 4.1 and Appendix 4.6) the fish habitats were also expected to resemble those expected under natural conditions. The following changes were however considered during the estimations of expected fish assemblage composition for this site (Chapter 4):

- Increased flows due to mining effluent reduced slow flowing habitat types.
- Extensive embeddedness of pool substrates occurred, mainly due to mining and erosion.
- Lack of marginal and aquatic vegetation as cover for fish due to the impact of exotic Black wattle trees (blocks sun from instream channel, thereby inhibiting growth of aquatic plants).

At *locality 3* (See chapter 4, Figure 4.1 and Appendix 4.7) habitat alterations due to human activities became more evident and the following changes were considered in the estimation of the expected habitat composition at this site (Chapter 4):

- Inundation and unnaturally created rapids (below dam walls) due to damming for irrigation purposes changed habitat diversity.
- Increased flow due to urban runoff flooded shallow habitats and eliminated many slow flowing habitats (e.g. backwaters, shallow runs, rapids and riffles).

The most prominent cause of habitat alteration from *locality 4 to 9* (See chapter 4, Figure 4.1 and Appendix 4.8 to 4.13) in the Klip River was increased flows that were primarily attributed to Water Care Works (WCW) effluents present along this section of the river. The primary changes in the expected fish habitats considered in the determination of the expected fish assemblage composition were (Chapter 4):

- Inundation of natural habitats such as shallow pools, riffles and rapids being transformed into runs, thereby decreasing habitat diversity. There was a general lack of slow habitat types at these sites.
- Increased flow resulted in extensive scouring and bank erosion, eliminating natural growth and stability of marginal and aquatic vegetation. Increased flows were also responsible for the uprooting of most aquatic vegetation.

The scenario observed at locality 10 (reference site) in the Suikerbosrand River was the opposite of most sites in the Klip River, where a reduction in flow was seen as the most obvious impact in the habitat components for fish. The decrease in flow was primarily attributed to an upstream dam (Balfour) and water abstraction for irrigation and livestock watering. Considerations for estimating the fish community at this site included (Chapter 4):

- Flow modification causing reduced habitat diversity due to loss of fast flowing habitats (rapids, riffles and runs). Domination by slow habitat types, namely shallow and deep pools.
- Lack of and reduction in natural marginal and aquatic vegetation due, to unnatural low water level, and unnatural fluctuations in the water levels.

The following procedures were then followed in the estimation of the number of individuals expected at a site:

A group of fish specialists (Prof. G. Steyn - Rand Afrikaans University, Dr. Hein du Preez – Rand Water, Mr. B. Niehaus – Rand Afrikaans University) visited each site. The above-mentioned aspects were discussed and considered during estimations, and reference was also made to the results gained at the reference site. Professional judgement was then applied and the group estimated the number of individuals of each species that should be expected at the site during the application of one sampling unit (20 minutes of electro-fishing) (Table 6.2 and Appendix 6.2).

Table 6.2: Expected relative composition of each species (%) estimated for each site (also see Appendix 6.2).

SPECIES	LOCALITY									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Labeobarbus aeneus [O-RRR]	0	17.9	8.8	16.7	10.3	18.2	22.3	6.9	13	13.9
Labeobarbus kimberleyensis [RRR]	0	0	29.4	0	5.2	9.1	4.5	6.9	6.5	6.9
Barbus anoplus [T-O-VH-PS]	26.3	17.9	14.7	8.3	15.5	9.1	13.4	13.9	6.5	6.9
Barbus pallidus [VH-PS]	15.8	10.7	14.7	8.3	10.3	9.1	8.9	6.9	6.5	6.9
Barbus paludinosis [T-VH-PS]	15.8	10.7	0	8.3	10.3	9.1	8.9	6.9	6.5	6.9
Labeo capensis [RRR]	0	0	5.9	16.7	10.3	9.1	13.4	27.8	26	27.8
Labeo umbratus [T-PS]	0	0	0	8.3	10.3	9.1	4.5	6.9	13	6.9
Clarias gariepinus [T-O-PS]	10.5	7.1	8.8	8.3	5.2	5.5	4.5	6.9	6.5	6.9
Austroglanis sclateri [RRR]	0	0	0	0	2.1	3.6	1.8	2.8	2.6	2.8
Tilapia sparrmanii [T-O-VH-PS]	15.8	17.9	8.8	8.3	10.3	9.1	8.9	6.9	6.5	6.9
Pseudocrenilabrus philander [T-VH-PS]	15.8	17.9	8.8	16.7	10.3	9.1	8.9	6.9	6.5	6.9

6.3.2 Observed fish species diversity in the Klip and Suikerbosrand Rivers

Ten indigenous fish species (*Austroglanis sclateri*, *Labeobarbus aeneus*, *Barbus anoplus, Barbus pallidus, Barbus paludinosus, Clarias gariepinus*, *Labeo capensis*, *Labeo umbratus*, *Pseudocrenilabrus philander and Tilapia sparrmanii*) and three exotic species (*Cyprinus carpio, Gambusia affinis* and *Micropterus salmoides*) were observed in the Klip River during the study period. Except for *Barbus paludinosus*, all of the above-mentioned indigenous species were also observed at the reference site in the Suikerbosrand River, with another species, *Labeobarbus kimberleyensis*, also being present (Table 6.3).

Table 6.3: Number of individuals of each fish species sampled at the selected sites over the total study period (Reference site shaded).

Species	LOCALITY										Total for Klip River
	1	2	3	4	5	6	7	8	9	10	
Austroglanis sclateri	0	0	0	0	0	0	1	5	0	6	6
Labeobarbus aeneus	0	0	0	48	76	37	194	65	57	88	477
Barbus kimberleyensis	0	0	0	0	0	0	0	0	0	4	0
Barbus anoplus	0	0	301	1	35	13	126	18	0	5	494
Barbus pallidus	0	0	0	0	1	0	0	0	0	5	1
Barbus paludinosus	0	0	2	0	28	0	52	2	0	0	84
Clarias gariepinus	0	0	2	5	21	2	4	1	4	48	39
Labeo capensis	0	0	0	0	13	10	70	256	116	245	465
Labeo umbratus	0	0	0	15	46	3	13	2	3	58	82
Pseudocrenilabrus philander	0	0	2	65	41	5	55	30	1	16	199
Tilapia sparrmanii	0	4	0	6	34	2	81	57	0	1	184
Cyprinus carpio (E)	0	0	0	13	13	2	3	5	38	12	74
Micropterus salmoides (E)	0	0	4	0	1	0	0	1	5	0	11
Gambusia affinis (E)	0	0	0	13	13	0	0	0	0	0	26
Number of indigenous species	0	1	4	6	9	7	9	9	5	10	10
Number of exotic species	0	0	1	2	3	1	1	2	2	1	3
Number of indigenous individuals	0	4	307	141	295	72	596	436	181	476	2031
Number of exotic individuals	0	0	4	26	27	2	3	6	43	12	111
Total number of sampling units applied.	6 [e]	5 [e]	14.5 [e]	9.35 [e]	15 [e]	18.5 [e]	13.9 [e]	15.65 [e]	12.5 [e]	17.25 [e] + 12 [g]	110.4 [e]

E - Exotic. [e] - Electrofishing sampling units (20 minutes) [g] – gill netting sampling units (60 minutes)

The rock catfish (*Austroglanis sclateri*) was only sampled at localities 7 and 8 in the Klip River, and at the reference site in the Suikerbosrand River (Table 6.3). The smallmouth yellowfish (*Labeobarbus aeneus*) was observed at localities 4 to 9 in the Klip River, as well as in the Suikerbosrand River at locality 10, and was the second most abundant species in the Klip River (Table 6.3). *Labeobarbus kimberleyensis* (Largemouth yellowfish) was only sampled at the reference site in the Suikerbosrand River. The Chubbyhead barb (*Barbus anoplus*) was sampled at sites 3 to 8 in the Klip River, and at site 10 in the Suikerbosrand

River, and was the most abundant species observed during this study (Table 6.3). The Goldie barb (*Barbus pallidus*) was only sampled once at locality 5 in the Klip River and also at the reference site in the Suikerbosrand River and was the most scarce of all indigenous barb species in the Klip River (Table 6.3). The Straightfin barb (*Barbus paludinosus*) was sampled at localities 3, 5, 7 and 8 in the Klip River.

The Sharptooth catfish (*Clarias gariepinus*) was sampled at localities 3 to 10. The Orange River mudfish (*Labeo capensis*) was sampled at sites 5 to 9 in the Klip River catchment, and at the reference site in the Suikerbosrand River, being the third most abundant species in the Klip River. Moggels (*Labeo umbratus*) were sampled at localities 4 to 10. The fourth most abundant indigenous species in the Klip River was *Pseudocrenilabrus philander* (Southern mouthbrooder), being present at sites 3 to 10. Banded tilapias (*Tilapia sparrmanii*) were the only fish species to be sampled at locality 2, and were also present at sites 4 to 8 in the Klip River. The Largemouth bass (*Micropterus salmoides*) was sampled at localities 3, 5, 8 and 9 in the Klip River System. The exotic species, *Gambusia affinis* (mosquito fish), was sampled at localities 4 and 5 in the Klip River system.

6.3.3 Impact by introduced (exotic and indigenous) species.

Three introduced species were observed in the Klip River during the current study, namely *Cyprinus carpio*, *Gambusia affinis* and *Micropterus salmoides*. The expected impact of introduced species on the indigenous fish, as indicated by the Introduced Species Impact (ISI) scores was the most prominent at locality 9, where very high scores were attained for all four surveys (Figure 6.1). Locality 4 also had a very high ISI of 71 during August 1998 (Table 6.4). Expected impacts by introduced species were very low at localities 7, 8 and 10 (Figure 6.1). A moderate impact by introduced species can also be expected at locality 5, where a continuous presence of introduced species was observed over the entire study period (Figure 6.1).

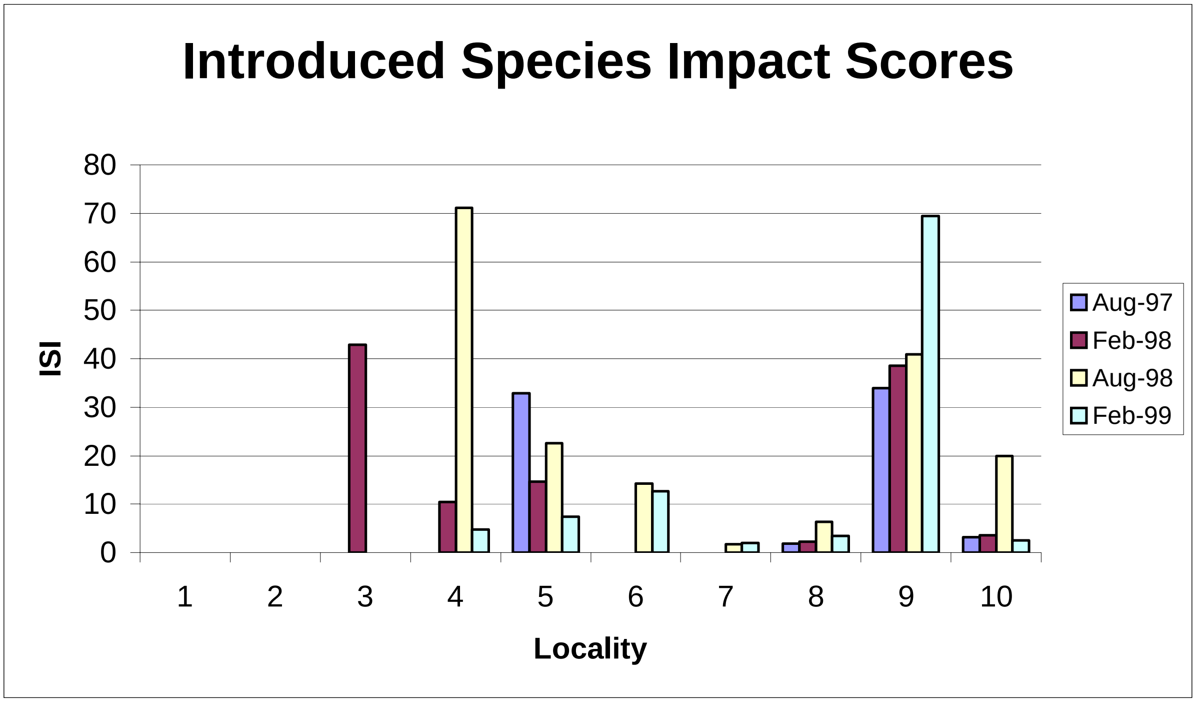

Figure 6.1: Introduced Species Impact (ISI) scores calculated for each site and survey.

Table 6.4: Introduced Species Impact (ISI) scores calculated for each site and survey in the Klip and Suikerbosrand Rivers.

	LOCALITY									
SURVEY	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Aug-97	0	0	0	0	33	0	0	2	34	3
Feb-98	0	0	43	10	15	0	0	2	39	4
Aug-98	0	0	0	71	23	14	2	6	41	20
Feb-99	0	0	0	5	7	13	2	3	69	2

6.3.4 External Fish Health Assessment Index (FHAIe)

Organ/tissue differences

The results from all surveys and localities were grouped in an attempt to identify differences in tissues and organs to indicate differences in fish health. The median FHAIe score calculated for the gills (3.92) was the lowest of all organs assessed by this method (Table 6.5).

Skin and fin tissue had the second and third lowest median FHAIe scores, being 4.24 and 4.27, respectively. Very few individuals had anomalies in the eyes (4.88), and even fewer on the opercula (5.00). In general, median FHAIe scores for tissue types were lower for the winter surveys than the summer surveys (Table 6.5).

Table 6.5: Median External Fish Health Assessment Index (FHAIe) scores each external fish organ/tissue of all fish examined (Klip & Suikerbosrand River).

Survey	Sample size (n)	Skin	Fins	Eyes	Opercula	Gills
Aug-97	364	4.23	4.17	5.00	5.00	3.67
Feb-98	141	4.25	4.37	4.89	4.84	4.17
Aug-98	115	4.00	3.74	4.57	5.00	3.60
Feb-99	496	4.48	4.81	4.88	5.00	4.70
Median	**1116**	**4.24**	**4.27**	**4.88**	**5.00**	**3.92**

<u>Species differences</u>

The external Fish Health Assessment Index (FHAIe) was only applied to specimens above a total length of 200mm, and an attempt was made to assess at least ten individuals at a site. The preferred sample size for more accurate and conclusive results would be twenty individuals per site. It is also advised to assess different species separately. The smallmouth yellowfish *(Lb. aeneus*) had the lowest median FHAIe score (21.43) of the three most common fish species used for the FHAIe during this survey (Table 6.6). The median FHAIe score calculated for *Labeo umbratus* was 22.93 and for *Labeo capensis*, 23.13. FHAIe scores for the different species also indicated that the scores attained during the winter surveys were generally lower than those of the summer surveys (Table 6.6).

Table 6.6: External Fish Health Assessment Index (FHAIe) scores determined for selected fish species examined during each survey in the Klip and Suikerbosrand River.

Species	Aug-97	Feb-98	Aug-98	Feb-99	Median
L. capensis	22.11	23.71	22.55	23.94	**23.13**
L. umbratus	23.00	22.86	19.57	23.24	**22.93**
Lb. aeneus	19.48	21.54	21.31	22.88	**21.43**

Spatial and temporal differences

The lowest median FHAIe percentage (86.7) was calculated for locality 10 (Table 6.7). This was primarily attributed to the very low percentage of 68.5 observed during the August 1998 survey. In the Klip River localities 5 and 8 had the lowest median FHAIe percentage of 89.9. The highest median FHAIe percentage in the Klip River was calculated for locality 9 (97.3). The general trend indicated that FHAIe percentages were lower during the winter surveys than the summer surveys, being especially evident at localities 5, 6 and 9 (Figure 6.2). Most sites in the Klip River also indicated a general increase in FHAIe percentages over the study period, while locality 10 had a clear decrease in FHAIe scores (Figure 6.2).

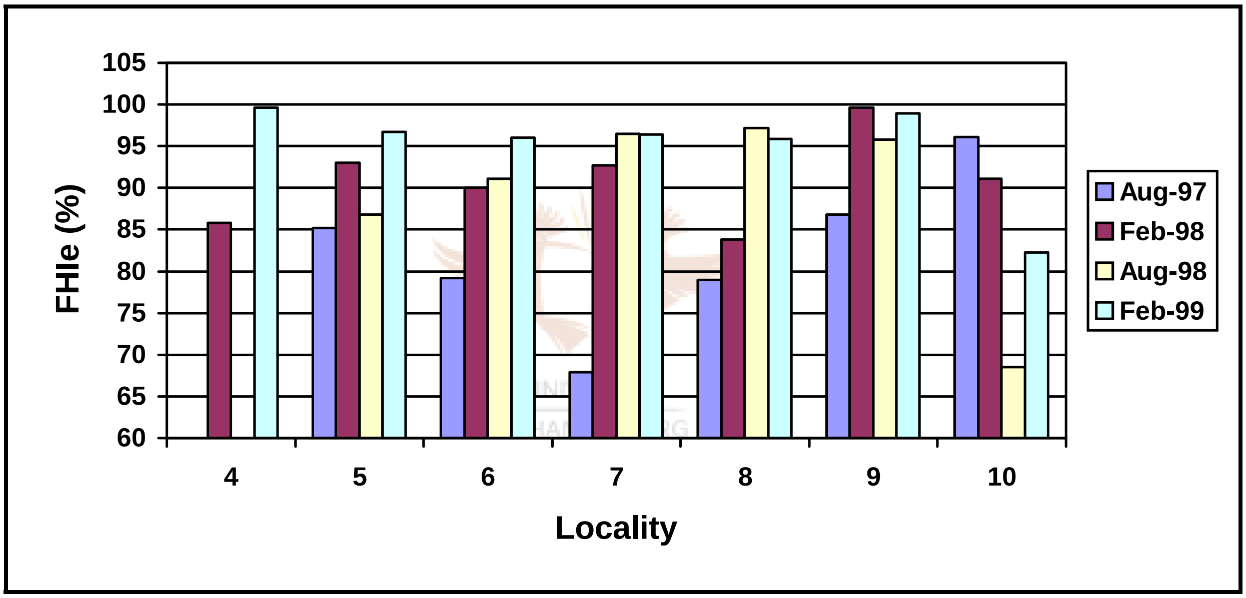

Figure 6.2: External Fish Health Assessment Index (FHAIe) percentages determined for selected sites in the study area.

Table 6.7: External Fish Health Assessment Index (FHAIe) percentages determined for each locality and survey.

Survey	Locality						
	4	**5**	**6**	**7**	**8**	**9**	**10**
Aug-97		85.2	79.2	67.9	79.0	86.8	96.1
Feb-98	85.8	93.0	90.0	92.7	83.8	99.6	91.1
Aug-98		86.8	91.1	96.5	97.2	95.8	68.5
Feb-99	99.6	96.7	96.0	96.4	95.9	98.9	82.3
Median	**92.7**	**89.9**	**90.6**	**94.6**	**89.9**	**97.3**	**86.7**

6.3.5 Condition factors

Condition factors were calculated for the most ubiquitous and numerous species in the Klip River, namely *Labeo capensis* and *Labeobarbus aeneus* (Table 6.8). In the Klip River, condition factors for *L. capensis* ranged from 1.07 (locality 5, February 1998) to 2.46 (Locality 7, February 1998). Condition factors calculated for *Lb. aeneus* in the Klip River ranged from 1.1 (Locality 5, August 1997) to 3.94 (Locality 7, February 1998). Condition factors were generally higher during the summer season than during the winter in both the Klip and Suikerbosrand Rivers. The average winter condition factors calculated for the Klip River (1.64 and 1.55) were slightly higher than that of the reference site in the Suikerbosrand River (1.48 and 1.06). The opposite phenomenon was observed for the summer survey, where the fish at the reference site (2.63 and 3.6) were in better condition than the average of the Klip River species (1.82 and 2.62) (Table 6.8).

Table 6.8: Condition factors (CF's) calculated for *Labeo capensis* and *Labeobarbus aeneus* at selected sites during the August 1997 & 1998 (winter) and February 1998 (summer) surveys.

Locality	Date	**CF*(L.capensis)***	Sample size (n)	**CF*(Lb.aeneus)***	Sample size (n)
5	Aug-97			1.1	17
	Feb-98	1.07	9	2.03	25
	Aug-98			1.12	26
6	Feb-98			2.34	11
	Aug-98			1.8	9
7	Aug-97	1.87	6		
	Feb-98	2.46	25	3.94	67
8	Aug-97	1.66	48	2.09	16
	Feb-98	1.57	116	1.99	28
9	Aug-97			1.7	22
	Feb-98	1.39	55	1.85	24
Klip River (average)	Aug-97	1.64	54	1.55	55
	Feb-98	1.82	205	2.62	155
10	Aug-97	1.48	33	1.06	3
	Feb-98	2.63	68	3.6	65

6.3.6 Bioaccumulation of Metals

In the Olifantsvlei weir, 25 specimens of *Clarias gariepinus* (13 female, 12 male) and 20 *Labeobarbus aeneus* individuals (15 female, 5male) were assessed. In the Henley-on-Klip weir, five *C. gariepinus* (4 female, 1 male) and 25 *Labeo umbratus* (8 females, 17 males) were investigated (Table 6.9).

In general, most metals were higher in tissues of fish from Henley-on-Klip (close to locality 7) than at Olifantsvlei (close to locality 4) in the Klip River. The only obvious exception was iron, which was generally higher in fish from Olifantsvlei. The general trend in bioaccumulation from high to low concentrations were as follows:

Olifantsvlei: Fe > Al > Zn > Sr > Mn > Pb > Cu > Ni

Henley-on-Klip: Fe > Al > Sr > Zn > Mn > Pb > Ni > Cu

Table 6.9: Statistics of fish investigated to determine the extent of bioaccumulation of metals at Olifantsvlei and Henley-on-Klip weirs in the Klip River.

Locality	Species	Statistic	Mass (g)	Total length (mm)	Fork length (mm)
Olifantsvlei	*Clarias gariepinus*	Average	2200	661	576
		Median	2104	650	572
		Minimum	1248	535	460
		Maximum	3809	821	726
		SD	566	63	54
	Labeobarbus aeneus	Average	1066	472	423
		Median	959	460	419
		Minimum	587	393	350
		Maximum	1875	550	492
		SD	350	46	40
Henley-on-Klip	*Clarias gariepinus*	Average	1900	619	554
		Median	1750	610	550
		Minimum	1250	540	470
		Maximum	2750	730	660
		SD	547	71	72
	Labeo umbratus	Average	1252	490	436
		Median	1250	490	440
		Minimum	1000	450	400
		Maximum	2000	526	477
		SD	268	23	22

Table 6.10: Metal concentrations (median±SD in µg/g dry mass) observed in the different fish tissue assessed at the selected sites on the Klip River during May 1998 (highest level of two sites indicated in red).

	Olifantsvlei				**Henley-on-Klip**			
	Gill	**Liver**	**Muscle**	**Skin**	**Gill**	**Liver**	**Muscle**	**Skin**
Sr	56±38	Bdl	1+2	1+1	382+375	0	4±17	1±7
Cu	3±1	17±9	1+1	1+1	3+1	29±11	3±1	1±1
Al	177±39	20±31	14+18	12+5	382±269	11±9	19±11	12±5
Ni	Bdl	Bdl	Bdl	Bdl	3±2	Bdl	Bdl	Bdl
Mn	23±37	2±5	1+3	Bdl	90±38	2±1	1±2	Bdl
Fe	306±82	4986±6976	63+409	49+31	397±294	366±1277	49±49	20±19
Zn	71±16	87+21	20+10	61+67	110±52	68±57	35±18	94±19
Pb	4±1	1+3	Bdl	3+1	5±3	3±1	2±1	2±2

Bdl – Below detection limit (see Chapter 3 for detection limits of different metals)

Median levels of strontium, aluminium, manganese and lead were always the highest in the gill tissue, while copper was higher in the liver tissue at both sites (Table 7.8). Iron and zinc were the highest in the liver of fish at Olifantsvlei, and highest in the gills of fish from Henley-on-Klip. Skin generally had the lowest metal concentrations of all tissues sampled (Table 6.10 and Appendix 6.3 to 6.6).

6.4 DISCUSSION

Expected fish species composition of the Klip and Suikerbosrand Rivers.

When using biotic communities to detect the impact of one or more environmental stresses on a site, it is necessary to know the fauna expected at the site in the absence of environmental stress (Dallas 2000). It was therefore essential in the determination of the ecological integrity of the Klip River to determine an expected or reference condition, against which the current status of the Klip River could be compared. An ecological reference condition is defined as the condition that is representative of a group of "least-impacted" or minimally disturbed sites organised by selected physical, chemical and biological characteristics (Dallas, 2000) and, in general, equates with biological integrity (Karr & Chu, 1997). Reference conditions enable the degree of degradation or deviation from natural conditions to be ascertained, and thereby serve as a foundation for developing biological criteria for protection of aquatic ecosystems.

Due to the fact that very little historic data on fish was available for the Klip River catchment, the estimation of reference conditions was primarily based on the scenarios observed at a less impacted reference site in the Suikerbosrand River, as well as professional judgment by specialists. If a purist approach was followed, which might be idealistic but safe, the reference/expected condition at a site would be taken as its state under unimpacted conditions, before human activities have altered the system. In some rivers, especially those draining highly populated areas, alterations of the rivers have reached irreversible dimensions, and this approach would be unrealistic. The Klip River catchment has been impacted by humans for more than a century (KKVO, 1910; Du Preez, 2000) and it can therefore be expected that this river system has been changed irreversibly. The expected condition of the fish communities of the Klip River was therefore taken as the best attainable scenario, should the current human impacts be reduced and limited. Although they may be unrealistic, and unattainable in some cases, they are at least a step in the right direction, towards the improvement of the system. These estimations are of utmost importance for the application of biotic indices that quantifies the observed condition in relation to the expected or reference conditions (Karr and Chu, 1997). It thus provided the necessary yardstick to measure future improvement or deterioration and the guideline for managers to be able to set goals for the future of the system.

The Klip River can be classified as a cool water system, with mean summer temperatures ranging between 17°C in the upper reaches, and 26°C in the lower reaches. Fish species diversity is generally lower in cold and cool water rivers than warmer tropical rivers (Skelton, 2001). The Klip River, with its cool waters had an expected diversity of only 11 fish species, which is low when compared with, for instance, the warm water Mpumalanga lowveld rivers with up to 35 fish species (Pienaar, 1978). Similar scenarios in temperature to species diversity ratios have also been observed in other aquatic systems around the world (Paller, 1994; Kleynhans, 1999).

Observed diversity and general description of native fish species of the Klip and Suikerbosrand River

Ten of the eleven expected native fish species were observed in the Klip River during this study, namely *Austroglanis sclateri, Labeobarbus aeneus, Barbus anoplus, Barbus pallidus, Barbus paludinosus, Clarias gariepinus, Labeo capensis, Labeo umbratus, Pseudocrenilabrus philander and Tilapia sparrmanii*. The only expected species not observed in the Klip River was *Labeobarbus kimberleyensis*. Three exotic species (*Cyprinus carpio, Gambusia affinis* and *Micropterus salmoides*) were also sampled in the Klip River during the study period.

Changes in fish species diversity in rivers, due to anthropogenic activities have been observed throughout the world (Karr *et al*., 1986; Hay *et al*., 1996; Lyons *et al*., 1996; Niehaus, 1996; Kleynhans, 1999). It is often difficult to identify a single cause, due either to inadequacy of data or, often because multiple factors play a role. Allan and Flecker (1993) stated that the most critical factors causing loss of species in rivers are generally habitat loss and degradation, the spread of exotic species (animal & plant), overexploitation, secondary extinctions, chemical and organic pollution, and also climate change.

All the available habitats at each site were sampled thoroughly, and all sampling methods applicable at the sites were always used. It is therefore expected that all fish species present at a site at the time of sampling would have been included in the sample, and the results are therefore a reasonable reflection of the current fish diversity of the Klip River System. There is however, no information available on the number of passes (repeat sampling in same area), or the distance of a reach that should be sampled in order to collect all the species at a site in the Klip River. Future studies should give attention to this aspect as it could determine the accuracy of results and conclusions based on these results. It is therefore assumed that the absence of an expected fish species from a site in the Klip River was attributable to anthropogenic impacts such as changes in water quality (Chapter 3) and/or habitat condition (Chapter 4).

Many water quality variables exceeded water quality guidelines, set for the protection of aquatic ecosystems, during the current survey. The human impacts responsible for water quality degradation in the upper Klip River were primarily mining and industrial activities, and formal and informal settlement runoff (Chapter 2 & 3). Water quality variables of special concern to the well being of the fish, in the vicinity of locality 1, 2 and 3 in the upper Klip River, were electrical conductivity and dissolved solids, sulphates, calcium, total alkalinity, magnesium, sodium, manganese. These variables are known to affect the metabolism of fish, decrease aquatic species diversity, change community structure and ecological processes (Alabaster & Lloyd, 1980). It has also been known to decrease growth rate and life expectancy and to influence osmotic balance in fish (Kotze, 1997).

The middle and lower reaches of the Klip River also suffered from the activities in the upper catchment, and were further impacted on especially by WWTW effluents and agricultural activities. The highly polluted Rietspruit (Schoonbee *et al.*, 1995) also joined the Klip River in this section and contributed greatly to pollution loads (see Chapter 3). Guidelines for the protection of aquatic ecosystems were exceeded in the middle and lower reaches of the river for suspended solids, chloride and nitrate. Phosphates exceeded guideline values throughout the Klip River (Chapter 3). The high turbidity and suspended solids in the middle and lower sections of the Klip River may affect the fish community, especially by the clogging of their gills, reducing visibility and thus feeding success, and also other sub-lethal effects (Mulder, 1973; Berkman *et al.*, 1987; Buerman *et al.*, 1995; Ardjosodiro & Ramnarine, 2001).

The species diversity of the upstream sites was lower than at the downstream sites in the Klip River. Similar scenarios have been observed in other aquatic systems around the world (Paller, 1994; Kleynhans, 1999). These differences are primarily related to longitudinal changes in habitat structure, climate and gradient. In some coldwater systems of the USA, the fish species diversity has increased with environmental degradation (Lyons *et al.*, 1996). This scenario was, however, not observed in the Klip River system, where species diversity decreased with environmental degradation.

The distribution and general characteristics, based on existing literature, and observations made during the study on each of the observed species are discussed below. Aspects on feeding (Appendix 6.7), breeding (Appendix 6.8), habitat requirements (Appendix 6.9) and general characteristics (Appendix 6.10) are also summarised as appendix tables.

Austroglanis sclateri (Rock catfish)

The rock catfish (*Austroglanis sclateri*) was sampled at localities 7 and 8 in the Klip River, and at the reference site in the Suikerbosrand (Plate 6.1). This species occurs naturally in the major tributaries and mainstream of the Orange-Vaal system. It usually lives in rocky habitats in flowing water, favouring rapids. During this survey, this species was sampled in riffle habitats (slow to moderate flows over cobbles and boulders) and in slow flowing areas of deep pools along rocky cliffs. It feeds on invertebrates, especially insects, taken from rock surfaces, explaining its occurrence in the rocky habitats. Larger specimens also take small fish. It attains a standard length of 300mm (Niehaus, 1996; Skelton, 2001). The conservation status of *Austroglanis sclateri* (Rock catfish) was previously stated as rare to indeterminate (Skelton, 1993). Recent assessments, however, showed this species to be more common and widespread than previously thought (Skelton, 2001).

Plate 6.1: *Austroglanis sclateri* - Rock catfish

This species is classified as moderately intolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b), and sensitivity is primarily attributed to habitat degradation. During this study, *A. sclateri* was observed in a relatively wide range of most water quality variables (Appendix 6.11). This species was abundant at the reference site during the initial survey, but their numbers decreased over the study period. This could be attributed to flow modification by an upstream dam, which resulted in loss of riffle/rapid habitats (see Chapter 4), which are their optimal habitats. Their scarcity in the Klip River can most probably also be related to habitat changes due to flow modification, but the opposite scenario occurred here. Increased flows primarily due to waste water treatment works (WWTW) effluents flooded riffle/rapid habitats in a large section of the Klip River, and especially in the lower reaches. This reduced their optimal habitat requirements, decreasing their population in the Klip River drastically.

Labeobarbus aeneus (Smallmouth Yellowfish)

The smallmouth yellowfish (Plate 6.2) was sampled at localities 4 to 9 in the Klip River, as well as in the Suikerbosrand River at locality 10, and was the second most abundant species in the Klip River (Table 7.2). This species occurs naturally in the Orange-Vaal River system and was also translocated to larger Cape coastal rivers including the Gourits, Great Fish and the Kei, as well as the Limpopo system and the Kyle Dam in Zimbabwe. It prefers clear flowing water of large rivers with sandy or rocky substrates, and is also found in large dams. This probably explains its success in the Klip River, where these habitats were abundant. Small individuals of this species were generally sampled in the white waters below rapids, while larger individuals favoured runs. *Labeobarbus aeneus* breeds from spring through to midsummer after the first substantial rains of the season. It migrates upstream to spawn over suitable gravel beds. Larger fishes are broadly omnivorous depending on the available food, with benthic invertebrates, vegetation, algae and detritus forming the major food sources of this species. It attains a total length of around 500mm (Skelton, 2001).

Plate 6.2: *Labeobarbus aeneus* - Smallmouth yellowfish

Labeobarbus aeneus is generally seen as being tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study, *Lb. aeneus* was observed at a wide range of most water quality variables, often present at the extreme values detected for a certain water quality variable (Appendix 6.11). This species was present throughout most of the middle and lower Klip River, and occurred in relatively large numbers at sites with poor water quality (see Chapter 3). Its absence from the upper reaches is mainly attributed to habitat restrictions, with the river being shallower and very uniform, although water quality could not be ruled out as a possible explanation for their absence. *Labeobarbus aeneus* was the second most abundant fish species in the Klip River, and occurred at localities 4 to 9. The fast habitats created by the flow modification increased the favorable areas for the existence of this species, which resulted in its wide distribution in the Klip River. The omnivorous feeding behavior of this species furthermore increased its ability to be present in viable populations in a highly altered system such as the Klip River. Results obtained in this study also suggest that this species may be relatively tolerant to poor water quality.

Labeobarbus kimberleyensis (Largemouth yellowfish)

Labeobarbus kimberleyensis (Plate 6.3) was only sampled at the reference site in the Suikerbosrand River. This species occurs in the Orange-Vaal system where it is generally only found in the larger tributaries and dams. It is absent from the higher reaches in Lesotho and the southern tributaries of the Cape. Adults prefer flowing water in deep channels or below rapids, but the species does well in dams. *Labeobarbus kimberleyensis* is primarily a predator, initially taking insects and small crustaceans, however, it is piscivorous above a 300mm fork length. Breeding takes place during mid- to late summer over gravel beds in running water. A maximum total length of 825mm has been recorded for this species (Skelton, 2001). This species is classified as being intolerant to changes in the aquatic ecosystem (Kleynhans, 1999b) and is especially noted to be sensitive to changes in flow (see Chapter 7). During this study, *Lb. kimberleyensis* was generally observed within a narrow range for most water quality variables (Appendix 6.11).

Plate 6.3: *Labeobarbus kimberleyensis* – Largemouth yellowfish

Labeobarbus kimberleyensis is becoming scarce in most rivers that it previously occurred in, and its conservation status is presently stated as being vulnerable (Skelton, 2001). Concern has been raised over the past years about the decrease in numbers of this species, and restocking programmes have been implemented in some areas of the Orange-Vaal System to correct this issue (Skelton, 2001). The absence of *Labeobarbus kimberleyensis* from the Klip River is of special concern. There is much value to the presence of this fish, due to it being a valued angling species. Adults prefer flowing water in deep channels or below rapids, habitat types common in the Klip River, as a result of flow modification. The relocation of this species to the Klip River could be considered if the extremely high flows persist in future. Restocking should however be preceded by studies to determine this species' tolerance to the water quality prevalent in the Klip River System. An assessment should, however be done to determine if the Klip River water quality meets the requirements of this species. Mulder (1973) noted that the increase in turbidity levels in the Vaal River restricted the ability of piscivorous species such as *Lb. kimberleyensis* to see its prey, and proposed that this was partly the reason for the decline of this species in the Vaal system.

Barbus anoplus (Chubbyhead barb)

The Chubbyhead barb (Plate 6.4) was sampled at sites 3 to 8 in the Klip River and at site 10 in the Suikerbosrand River, and was the most abundant species observed during this study (Table 6.3). This species has a wide distribution from the highveld tributaries of the Limpopo, to the highlands of Kwa-Zulu Natal, Transkei and the middle- and upper Orange River basins, including the Karoo. It is absent from the lower Orange. It also occurs in the larger coastal rivers of the Southern and Western Cape. It prefers cold waters and occurs in a wide range of habitats. Breeding occurs in the summer, when rivers are laden by rain. *Barbus anoplus* is omnivorous, and is preyed upon by larger fish and birds. It usually attains standard lengths between 100 and 120mm (Skelton, 2001).

Plate 6.4: *Barbus anoplus* - Chubbyhead barb

This species is classified as being tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study, *B. anoplus* was observed at a wide range of most water quality variables, often present at the extreme values detected for a certain water quality variable (Appendix 6.11). The presence of this species throughout the Klip River emphasizes its tolerance to environmental stress, being present at sites with poor water quality and degraded habitats. The high abundance of this species at locality 3 was attributed to the presence of large areas of its favorable habitat type, namely slow flowing areas with abundant cover. Reduction in this habitat type downstream as flows increased, decreased the favorable habitats for this species, which resulted in less individuals being observed. The tolerance of this species to changes in the environment was however reflected, as it still managed to be present at most sites. It was also observed that this was the only barb species in the system that was content with either vegetation or substrate as cover.

<u>*Barbus pallidus* (Goldie barb)</u>

Another species that raised concern during this study was *Barbus pallidus* (Plate 6.5). Only one individual of this species was sampled, at only one locality (locality 5) in the Klip River (Table 6.2). This species has a scattered distribution from coastal streams of the eastern Cape, from the Great Fish to the Krom River, and also tributaries of the Vaal- and Limpopo River in "Transvaal", and in the Pongolo and the Tugela in Kwa-Zulu Natal. It prefers pools in clear, rocky streams often with protruding vegetation. The few individuals sampled during this survey were found under overhanging vegetation in backwaters and pools. *Barbus pallidus* breeds in summer when pairs lay eggs between plants. It attains a standard length of approximately 70mm (Skelton, 2001).

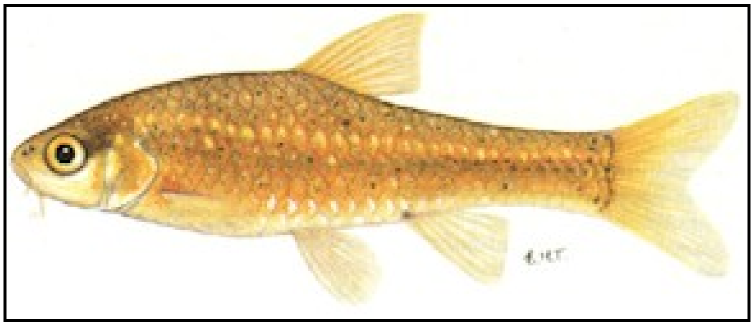

Plate 6.5: *Barbus pallidus* – Goldie barb (From: Skelton, 2001).

This species is expected to be moderately intolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study *B. pallidus* was generally observed within a narrow range for most water quality variables (Appendix 6.11). Very little is known about the ecology of this species, and their scarceness in the Klip and Suikerbosrand River indicates that it is sensitive to changes in the environment. Although water quality could have contributed to their absence, habitat degradation may have been the main reason for their general absence from the Klip River. Their requirement for pools with vegetated fringes was generally not met, especially in the middle and lower sections of the Klip River, due to flow modifications by WWTW effluents. Water quality restraints were most probably the main cause for its absence from the upper Klip River.

Barbus paludinosus (Straightfin barb)

The Straightfin barb (Plate 6.6) was sampled at localities 3, 5, 7 and 8 in the Klip River. This species has a widespread distribution in East Coast rivers from East Africa southwards to the Vungu River in Kwa-Zulu Natal and from southern tributaries, from Zaire- and Quanga Rivers in Angola southwards to the Orange system. *Barbus paludinosus* is a hardy species preferring quiet, well-vegetated water in lakes, marshes and wetlands, or near banks of large rivers and slow-flowing streams. Individuals were always associated with vegetation during this study.

It feeds on a large variety of small organisms, including insects, small snails, algae, diatoms and organic matter. They breed in summer amongst vegetation and attain a standard length of approximately 150mm.

Plate 6.6: *Barbus paludinosus* - Straightfin barb

Barbus paludinosus is a species most often preyed upon by predatory fish and birds (Skelton, 2001). This species is seen as being relatively tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study, *B. paludinosus* was observed at a wide range of most water quality variables, often present at the extreme values detected for a certain water quality variable (Appendix 6.11). It was present at sites with poor water quality (see Chapter 3), stressing its tolerance towards water quality changes. Their absence at some sites is probably attributed to lack of slow habitats and vegetation.

Clarias gariepinus (Sharptooth catfish)

The Sharptooth catfish (Plate 6.7) was sampled at localities 3 to 10. This is probably the most widely distributed of fish in Africa, found throughout woodland-savanna zones of the Afrotropical region from the Nile, as far south as the Orange system and the Umtamvunu (east coast). It was translocated to the eastern Cape (Sundays, Fish and Keiskamma) and to the southwest Cape (Eerste River and the Cape Flats). *Clarias gariepinus* also occurs in Israel, Lebanon and Turkey. This species frequents almost any habitat but favours flood plains, large sluggish rivers, lakes and dams. During this study it was primarily sampled in slower flowing areas of the river, especially the quiet zones on the edges of the river. It favoured undercut banks and rootwads of, exotic Willow trees on the banks of the Klip River.

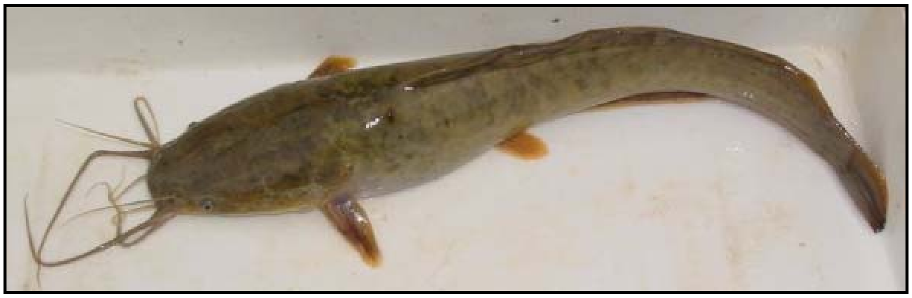

Plate 6.7: *Clarias gariepinus* – Sharptooth catfish

They can endure harsh conditions such as high turbidity or desiccation, and are frequently the last or only inhabitant of diminishing pools of drying rivers or lakes, where it may form burrows to survive in. This species can move over land in damp conditions, by using extended pectoral spines and by crawling. It is completely omnivorous and preys, scavenges or grubs on virtually any organic food source. They may hunt in packs, herding and trapping smaller fishes but are preyed upon by a wide variety of predators including man, leopards, crocodiles and marabou storks. *Clarias gariepinus* breeds in summer after the rains, when large numbers of mature fish migrate to flooded grassy edges of rivers and lakes to lay eggs on the vegetation. This species attains a standard length of approximately 1400mm (Skelton, 2001).

This species is tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study *C. gariepinus* was observed at a wide range of most water quality variables, often present at the extreme values detected for a certain water quality variable (Appendix 6.11). Their presence throughout the Klip River indicates their ability to withstand changes in water and habitat quality. Species such as *Clarias gariepinus* (Sharptooth catfish) and *Labeo umbratus* (Moggel) which favours slow flowing waters were, however, also affected by the increased water velocities due to flow modifications. Although present in low numbers, *C. gariepinus* was observed at most sites in the Klip River. The observed high abundance of this species in the weirs of the Klip River furthermore indicate its preference for lenthic conditions, although the high level of organic material suspended in these impoundments also provided ultimate feeding grounds for them.

Labeo capensis (Orange River Mudfish)

The Orange River mudfish (Plate 6.8) was sampled at sites 5 to 9 in the Klip River catchment, and at the reference site in the Suikerbosrand River, being the third most abundant species in the Klip River. This species occurs throughout the Orange-Vaal system. It prefers running waters of large rivers, and also does well in large impoundments. During this study, this species was generally sampled below rapids and the larger specimens favoured runs. It breeds in summer when large numbers of mature adults gather in shallow rocky rapids where the eggs are laid. It attains a total length of approximately 500mm (Skelton, 2001).

Plate 6.8: *Labeo capensis* – Orange River mudfish

Labeo capensis is moderately tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study, *L. capensis* was observed at a relatively wide range of most water quality variables (Appendix 6.11). The relative success of this species in the Klip River may be ascribed to their optimal habitat requirement of running water being met in the system. Another factor that could have contributed to their success was the addition of nutrients to the system (see Chapter 3), which resulted in eutrophic conditions and increased algal growth. This species favourite feeding ground are the firm surfaces of rocks and plants covered with algae.

Labeo umbratus (Moggel)

Moggels (Plate 6.9) were sampled at localities 4 to 10. This species has a distribution in the Orange- and Vaal River systems as well as the Gourits-, Gamtoos-, Sondags-, Great Fish- and

Boesmans Rivers in the southern and south-eastern coastal areas of the "Cape Province". *Labeo umbratus* has been translocated to the Keiskamma-, Buffels-, Olifants-, and Limpopo system. It feeds on soft mud and organic bottom matter and breeds after the rains in the summer. It attains a total length of 500mm (Skelton, 2001). *Labeo umbratus* is tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study, *L. umbratus* was observed at a relatively wide range of most water quality variables (Appendix 6.11). It prefers standing or slow flowing water and flourishes in shallow- and farm dams (Skelton, 2001), explaining its relative scarceness in the Klip River, where slow flowing habitats were generally scarce.

Plate 6.9: ***Labeo umbratus*** **- Moggel**

*Pseudocrenilabrus philander(*Southern Mouthbrooder*)*

The fourth most abundant of the indigenous species in the Klip River was *Pseudocrenilabrus philander* (Plate 6.10), being present at sites 3 to 10. This species is distributed from the Orange River and southern Kwa-Zulu Natal northwards, throughout the area. The distribution reaches into southern Zaire tributaries and Lake Malawi. It occurs in a wide range of habitats, from flowing water to lakes and isolated potholes. They usually prefer vegetated areas and were also during this study mostly sampled in vegetated areas and under large boulders that provided cover. *Pseudocrenilabrus philander* feeds on insects, shrimps and even small fish. They breed from early spring to late summer. They attain a standard length of about 130mm (Skelton, 2001).

Plate 6.10: *Pseudocrenilabrus philander* – Southern mouthbrooder

Pseudocrenilabrus philander is tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study, *P. philander* was observed at a wide range of most water quality variables, often present at the extreme values detected for a certain water quality variable (Appendix 6.11). Their presence throughout the Klip River is indicative of their tolerance towards changes in habitat and water quality. It was, however envisaged that their relative abundances have also been altered by the increased flows, which eradicated their favorable slow vegetated habitat types.

Tilapia sparrmanii (Banded tilapia)

Banded tilapias (Plate 6.11) were the only fish species to be sampled at locality 2, and were also present at sites 4 to 8 in the Klip River. One specimen of this species was also sampled at locality 10 in the Suikerbosrand River. This species occurs from the Orange River and Kwa-Zulu Natal southcoast northwards, to the upper reaches of southern Zaire tributaries, Lake Malawi and the Zambezi system. It is largely translocated to the Cape, south of the Orange River. This species tolerates a wide range of habitats but prefers slow flowing water with submerged or protruding vegetation. It was mostly associated with overhanging and aquatic vegetation in slow flowing or stagnant waters. *Tilapia sparrmanii* is omnivorous, feeding on algae, soft plants, small invertebrates and even small fish. It attains a standard length of about 230mm (Skelton, 2001).

Plate 6.11: *Tilapia sparrmanii* - Banded tilapia

This species is tolerant (see Chapter 7) to changes in the aquatic ecosystem (Kleynhans, 1999b). During this study, *T. sparrmanii* was observed at a wide range of most water quality variables, often present at the extreme values detected for a certain water quality variable (Appendix 6.11). This species was found highest upstream in the Klip River, being the only species sampled at locality 2 (No fish present at locality 1 – most upstream site). Locality 2 also had highly degraded habitat (chapter 4) and water quality (Chapter 2), indicating this species tolerance towards environmental degradation.

Introduced species and their relative impact on the native species

Reasons for the introduction of fishes, plants and invertebrates in alien freshwater environments are many. The most common reasons for the deliberate introduction of exotic species into a region are for aquaculture, aquarium and recreational (angling) purposes. In other instances, fish are accidentally introduced to natural water, such as in the case of flooding of aquaculture ponds (Allan & Flecker, 1993). Furthermore, water-transfer schemes are often responsible for the introduction of species into a river system (Davies & Day, 1998). The introduction of alien biota to any system generally leads to a degradation of the integrity of the system. A loss of native species diversity is the most common symptom of exotic invasions of aquatic ecosystems. The major mechanisms whereby exotics affect native species include predation, habitat alterations, hybridizations, the introduction of disease or parasites (Taylor *et al*., 1984) and competition for food and habitat (Skelton, 2001). Of the 58 alien and translocated fish species in southern African waters, 37 are considered to be detrimental, 3 beneficial and 18 equivocal i.e. beneficial under some circumstances and detrimental under others (de Moor & Bruton, 1988).

During this study, three exotic species (*Cyprinus carpio*, *Gambusia affinis* and *Micropterus salmoides*) were observed in the Klip River:

Cyprinus carpio (Common carp)

The Common carp (Plate 6.12) was the most common of all exotic species observed in this study, and occurred at sites 4 to 9 in the Klip River system. It was also the only exotic species present at locality 10 in the Suikerbosrand River. *Cyprinus carpio* was introduced to South Africa for the first time in the 18th century for both aquaculture and sport fishing purposes. It has a wide distribution in southern Africa but is absent from mountainous regions, and rare in tropical areas. It is a hardy species, tolerating a wide range of conditions, and usually prefers large water areas with slow flowing or stagnant water and a soft, muddy bottom. In the Klip River, this species was generally sampled in the quiet zones between aquatic vegetation, especially in reeds. They are omnivorous and breed in spring and summer in response to high flows. The South African angling record for this species is 21.98 kg (Skelton, 2001).

Plate 6.12: *Cyprinus carpio* – Common carp

Common carp can be seen as equivocal, having a negative impact on the environment they occur in, but being valued by certain interest groups such as sport fishermen. They are widely regarded as a pest, and are held responsible for the introduction of numerous fish parasites. They compete with other fish for food, they eat the spawn of other fish and disrupt nestbuilding activities of some fish. Furthermore, they cause habitat degradation by their feeding behaviour of grubbing in the mud for food, which causes the destruction of vegetation, rooting up of marginal vegetation and disturbing of the bottom sediments which increases turbidity (de Moor & Bruton, 1988).

Micropterus salmoides (Largemouth bass)

The Largemouth bass (Plate 6.13) was sampled at localities 3, 5, 8 and 9 in the Klip River System. It is a widely distributed species throughout the Cape coastal drainages, Kwa-Zulu Natal midlands and the "Transvaal". It also occurs in Malawi, Namibia and Zimbabwe. Its natural distribution is in central and eastern North America, from the Gulf of Mexico to southern Canada. This species favours clear, standing or slow flowing waters, with submerged or floating vegetation and does well in farm dams. *Micropterus salmoides* can tolerate temperatures below 10° C. It attains a total length of approximately 600mm (Skelton, 2001).

Plate 6.13: *Micropterus salmoides* – Largemouth bass

Micropterus salmoides have been introduced for sport fishing purposes and can be seen as equivocal, having a negative impact on the environment they occur in, but are valued by certain interest groups such as sport fishermen. This species have, however had an extremely detrimental impact on the environment (de Moor & Bruton, 1988). The primary negative impact of this species is its predation on the indigenous fish, especially small species and fingerlings of larger native species. This species has been blamed for the extinction of indigenous species from many rivers in South Africa (de Moor & Bruton, 1988). This species was especially abundant in the lower reaches of the Klip River, probably related to their movement from the Vaal River Barrage, where the habitat is more favourable for them.

Gambusia affinis (Mosquito fish)

This exotic species, *Gambusia affinis* (Plate 6.14), was sampled at localities 4 and 5 in the Klip River system. *Gambusia affinis* has been introduced to aquatic systems as a biological control method for mosquitoes (de Moor, 1988). This species consists of isolated populations in southwest- and southern Cape, "Transvaal" and Zimbabwe. The natural distribution is in rivers of North America flowing into the Gulf of Mexico, and from Mexico to Alabama. It requires slow flowing water, usually with abundant vegetation. It tolerates a wide range of temperatures (4-38°C) and salinity (fresh to more saline than seawater). It feeds on living animals, including mosquito- and fish larvae. Their eggs are fertilized and develop internally. Males attain a total length of 35mm and females 60mm (Skelton, 2001).

Plate 6.14: *Gambusia affinis* – Mosquito fish (from Skelton 2001).

Gambusia affinis do not appear to have had a major impact on indigenous species, but their impacts have not been studied quantitatively in southern Africa (de Moor & Bruton, 1988). They may alter the ecosystem by reducing the population of zooplankton and aquatic insect larvae. They also feed on the eggs and larvae of other fish and have been known to nip at the fins of larger fish (de Moor & Bruton, 1988). They prefer lakes and slow shallow habitats, and it is therefore expected that the present lack of stagnant waters and slow habitats is a major limiting factor for this species to establish itself in the Klip River. Thus, this species is thus not expected to be a major threat to the native fish species of the Klip River system.

The fact that all three introduced species observed in the Klip River favors slow flowing and stagnant water could be to the advantage of this river. Due to the flow modification resulting in extremely high flows, all year round, these introduced species do not have their optimal habitats, and should not be able to sustain large populations that could become a threat to the indigenous species. The increased flow is, however, also a threat to many indigenous species favoring these stagnant or slow flowing areas, which is of concern.

The expected impact of introduced species on the indigenous fish, as indicated by the Introduced Species Impact (ISI) scores was the most prominent at locality 9, where very high scores were attained for all four surveys. This is due to the usual presence of both *C. carpio* and *M. salmoides*, with *C. carpio* representing a relatively large proportion of the fish population observed at this site. Locality 4 also had a very high ISI score of 71 during August 1998, attributable to the high relative proportion of *C. carpio* and *G. affinis* observed at this site during this survey. A moderate impact by introduced species can also be expected at locality 5, where a continuous presence of introduced species was observed over the entire study period.

External Fish Health Assessment

Increasing environmental degradation often yields a high number of individuals in poor health (Kilian *et al*., 1997). Environmental stressors such as poor water quality can impose considerable stress on physiological systems of fish, and can impair their health. A study by Schleiger (2000) positively related the proportion of species with deformities, eroded fins, lesions or tumors, to increased levels of turbidity and suspended solids. The most common indicators of poor health in fish include tumors, haemorrhage, necrosis, fin and skin damage, or deformities, discolorations of organs or tissues, excessive mucous and heavy infestations of parasites (Karr *et al*., 1986).

Researchers have developed a number of fish assessment methods that are being used to evaluate the response of fish when exposed to environmental contaminants (Adams, 1993; Blazer *et al.*, 1995; Kilian, 1996; Kilian *et al.*, 1997). Of the methods developed, the necropsy-based assessment protocols are most often used for field assessments, and are advantageous as they are rapid, they require minimal training and equipment, and are less expensive than many more sophisticated methods (Blazer *et al.*, 1995). Kilian *et al.* (1997) developed the Fish Health Assessment Index (FHAI) as a technique of biological monitoring, to be applied within the South African Department of Water Affairs and Forestry (DWAF). The method applied in the present study was based on the FHAI, as developed by Kilian *et al.* (1996), but due to the non-destructive approach followed in biomonitoring protocols, and to make it more rapid, thus more cost-effective, it was only applied to the external morphology. The results may therefore be less informative than when both internal and external organs are assessed, but it still complies with the requirements of a rapid biomonitoring tool.

As gills have the lowest FHAIe scores of all the organs assessed, it gives an indication of the important value of this organ in indicating environmental degradation. Its sensitivity lies in the fact that it is in constant contact with the water, and it plays an essential role in the survival of the fish. Increased turbidity is a major cause of gill deterioration (Buermann *et al.*, 1995; Schleiger, 2000). Gills can be seen as the easiest assessable organ that would indicate water quality deterioration.

Organs such as the skin and fins, had the second lowest scores, and were valuable in indicating physical habitat degradation, together with water quality deterioration. In many cases, skin and fin damage were attributed to solid waste disposal and concrete structures at bridges (see Chapter 4). FHAIe scores at locality 10 (reference site) in the Suikerbosrand were generally very low, especially during August 1998 (see Appendix 6.1). The flow at this locality was generally very low, especially during August 1998 when there was almost a total cessation of flow. Damaged skin and fins were especially evident in fish during these surveys. The reference site (locality 10) also had the lowest median FHAIe for all sites assessed. It therefore seems that very low flows can cause deterioration of fish health, as reflected by the condition of the skin and fins. This may partly be attributed to fish attempting to negotiate very shallow areas, such as riffles, with most substrates exposed, in an attempt to get to deeper pools. This site generally had very good water quality, and it is therefore evident that the FHAIe is a good indicator of the total degradation of a system, giving a comprehensive assessment. It is also evident that this index is especially valuable for indicating physical habitat deterioration. In this study it proved to be very valuable in indicating the impact of reduced flows on a system.

Crafford (2000) recorded very few abnormal conditions for eyes, opercula and fins of two impoundments in the Vaal River. In the current study, very few abnormal conditions were recorded for opercula, with eyes being the second least affected organ. Fins however gave good indication of abnormalities at many of the sites investigated. The difference between these two studies may be the comparison between rivers and impoundments. Fish in rivers may be stressed more due to feeding in shallower, rocky, fast flowing water, where fins and skin can be damaged more easily than in stagnant, deep waters with mainly sandy substrates. The opercula variable could possibly be omitted from future inclusion in this index, as it may mask the extent of the impact on the other organs. Future application of a health assessment index for biomonitoring purposes could also consider the inclusion of blood variables (haematocrit %, leukocrit count and plasma protein). Blood could be drawn from larger species without major negative effects, therefore still complying with a non-destructive sampling approach. These variables have been indicated to depict accurately the differences between polluted and less polluted sites in the Olifants River (Watson, 2001). This method would, however be more time consuming and could therefore jeopardise the cost effectiveness of biomonitoring surveys.

Condition Factors

The use of condition factors (length-mass relationship) is a generalized indicator of overall fitness or "plumpness", and can reflect the integrated effect of both nutrition and metabolic cost induced stress (van der Bank, 1984; McCarthy and Shugart, 1990). Condition factors exceeding a value of one (>1) are usually indicative of fish in good condition, while factors less than one would indicate poor conditions based on length-weight relationships of fish (van der Bank, 1984). All condition factors calculated during this study were above one (1), and it can therefore be concluded that the general condition of the fish, based on condition factors, was good. Condition factors calculated for the summer surveys were generally slightly higher than those of the winter surveys. This can possibly be attributed to the fact that more food is available during the summer season, and an increase in condition can be expected with increased feeding. Another factor contributing to this scenario is the fact that gonad development during the summer may cause an increase in weight, especially in the female specimens (Mulder, 1971).

Condition factors have been determined previously for fish from the Klipspruit tributary in the upper Klip River (Van der Bank, 1984). These recorded condition factors of 1.1 were determined for *Oreochromis mossambicus* in the Orlando Dam, Soweto, which receives effluent from the Orlando Power Station. If species differences are ignored, the fish of the Klip and Suikerbosrand Rivers were in better condition than those of the upper reaches of the Klipspruit.

Condition factors are, however, generally fairly insensitive to changes in body conditions. This could be due to a process where a decline in body mass of a fish, resulting from a reduction in energy reserves, may be counteracted by an increase in body water (McCarthy and Shugart, 1990). The result from condition factors should thus be handled with circumspection, and this method is not recommended for biomonitoring studies.

Bioaccumulation

It is a well-known fact that fish have the ability to, over time, accumulate a range of components from the environment they live in, a process commonly known as bioaccumulation (Seymore *et al*., 1995, Seymore *et al*., 1996, Kotze *et al*., 1999, Du Preez, 2000). The uptake of organic or inorganic chemicals by the fish can occur directly from the ambient water (by the gills and skin), or from contaminated food/non-food particles, and drinking water (by the digestive tract). After uptake, these components are transported in the blood stream, and are therefore brought into contact with the organs, where they bind to amino acids and other binding proteins. The tissues and/or organs act as permanent or temporary dumping sites for these chemicals. The total intake concentrations are, however, not accumulated, as levels are regulated to a certain extent by the organism. Furthermore, the process and extent of bioaccumulation is also influenced by various other factors, including environmental variables, interactions between substances, uptake and excretion rates, trophic status, physiological condition, age and size of the bioaccumulator.

Accumulation of toxins by fish can have deleterious effects on both the organisms themselves, as well as other biota, and humans consuming the fish (Heath, 1987; du Preez, 2000). This is especially of great concern if human health is at risk, due to the exposure to contaminated fish. The contaminants bioaccumulated by fish could pose carcinogenic, genotoxic and non-carcinogenic health risks to consumers, if present in excessive quantities (du Preez, 2000).

Metal compounds initially occur in the environment in the chemical forms in which they were released by natural or anthropogenic processes. However, they are quickly converted into other metal species depending upon the properties of the receiving environment. This conversion happens rapidly for ionic species. Depending upon environmental conditions, metal compounds react with dissolved and solid organic matter and with clay minerals to form complexes that may reduce their bioavailability and, consequently, bioaccumulation and toxicity (Hellawell, 1986; Heath, 1987; Kotze, 1997; du Preez, 2000).

Of the two sites assessed in the Klip River, levels of metals were generally higher in fish from the Henley-on-Klip site (locality 7). Mining and industrial activities in the upper Klip River catchment are sources of metal pollution in this section of the river (see Chapters 2 and 3). It seems however that the impacts from mines in the area have been reduced with improved water quality being evident over the study period (Chapter 3). The Rietspruit is seen as the main source of metals in the middle and lower Klip River, due to it draining a catchment with high mining and industrial activity (see Chapters 2 and 3). The generally higher level of metal bioaccumulation at the downstream site (Henley-on-klip) therefore may indicate that this site is exposed to higher levels of metals over long periods.

In general the gill tissue had the highest median metal levels, with copper being the only exception, always having higher concentrations in the liver tissue. Spatial bioaccumulation trends for metal concentrations in the water were generally similar to those indicated by the gill tissue. Gills are in direct contact with the contaminated water and have the thinnest epithelium of all organs, due to their respiratory function. Metals therefore penetrate the epithelium cells of the gills easily (Part, 1987). This could result in the elevated uptake of these metals by the gills, when exposed to high levels of metals in their environment. The extensive vascular network in the gills furthermore ensures that blood-borne metals were in contact with the gill tissue. Mucous covering the gills of the fish could also have indicated the high levels of metals in the gill. The mucous layer on the gills plays a role in the excretion of metals by fish (Heath, 1987). Increased mucous production by the gills is a common reaction of fish when stressed. The mucous is produced by goblet cells in the epithelium, and is known to readily bind to metals (Heath, 1987; Kotze, 1997). Similar to previous studies (Van Hoof & Van San, 1981), gills were again the most valuable organ to indicate on acute exposure of fish to metals in the Klip River.

High levels of some metals, such as copper at both localities, and iron and zinc at Olifantsvlei, were detected in the liver of the fish. This could possibly be ascribed to the liver's function in the detoxification of metals. After exposure of fish to elevated levels of metals, the metallothionein levels in the liver increases. It is commonly accepted that metallothionein acts to detoxify metals through competitive binding and transport for excretion. The trends observed in the Klip River, where iron was the metal occurring in the highest concentration in all fish tissue, were also observed in the Olifants River study. The high levels of iron in fish are especially attributable to iron-containing enzymes, haemoglobin and the vascular system, and not necessarily to increased levels of pollution (Verob'yev & Zaytsev, 1975).

Levels of Sr, Al, Ni, Mn and Pb in muscle tissue of *L. umbratus* from the Vaal Dam (Groenewald, 2000), upstream of the Klip River confluence with the Vaal River, were generally higher than those detected in the same species within the Klip River. Levels of copper, iron and zinc were however higher in fish from the Klip River when compared to those from the Vaal Dam. The concentrations of all the metals determined in this study, were lower in the fish of the Klip River, when compared to fish from the Olifants River in Mpumalanga (Seymore, 1994; Seymore *et al*., 1995; Seymore *et al*., 1996; Kotze, 1997; Kotze *et al*, 1999). It therefore seems that the Klip River can be seen as less polluted by effluents containing metals in comparison to the Olifants River. When metal concentrations were compared to those detected in a previous study of Florida Lake, in the upper Klip River catchment (Schoonbee, 1995), the general trend, and especially for muscle tissue, were higher in fish from Florida lake than Olifantsvlei and Henley-on-Klip. When only gill tissues were considered, Cu, Ni and Pb were the highest in Florida Lake, while Mn, Fe and Zn were the highest in fish from Henley-on-Klip.

It is possible to link environmental exposure to associated human health effects by performing a *human health risk assessment*. Human health risk assessment is a qualitative and/or quantitative process conducted to characterize the nature and magnitude of risks to public health from exposure to hazardous substances released from point and/or diffuses sources (US EPA, 1991). Du Preez (2000) conducted a study on the potential health risk associated to the consumption of fish from the Klip River, and indicated that there were in general not a risk if fish were consumed from the Klip River system. Future studies should however include assessments of toxins other than metals, which may be detrimental to human health. The high level of agricultural activity in the middle and lower section of the Klip River may be sources op pollutants such as pesticides and herbicides and accumulation of these toxins in fish should be assessed in future. Due to the fact that humans consume fish from this system on a regular basis, it is also proposed that a human health risk assessment, based on the procedures stipulated by du Preez (2001) be considered in the near future.

6.5 CONCLUSIONS

This study indicated that the current species diversity in the Klip River deviate from the expected scenarios. The absence of the Largemouth yellowfish from the Klip River system was of special concern. The changes and absence of fish from certain sections of the Klip River was attributed to poor water quality and/or habitat degradations and changes. Human activities in the form of mining and industry, formal and informal settlements, WWTW effluents and agriculture were identified as being responsible for decreasing water and habitat quality. Flow modifications, especially due to WWTW effluents, were identified as a major cause for changes in habitat diversity and condition and contributed greatly to changes in the species diversity of some sites.

Three introduced species were observed in the Klip River, but they were in general not seen as a major threat to the biotic integrity of the system. This was mainly attributed to their preference for slow flowing habitats, which was not common in the Klip River. Locality 9 was identified to be the most impacted by introduced species, while high probable impacts were also identified at locality 4 and 5.

The external Fish Health Assessment Index (FHAIe) was identified to be a useful tool to apply during rapid biomonitoring procedures. The most important organ used in this protocol to indicate on environmental degradation was the gills. Skin and fins were also important indicators while eyes and opercula were identified as the least indicative of all organs used. This index proved to be especially valuable to indicate on the impact of decreased flows, as observed in the Suikerbosrand over the study period. Future studies could consider the exclusion of opercula, and the possible inclusion of blood variables and blood parasites in the assessment. It is recommended that the FHAIe scores could be used as an additional metric, or replaces the current metric of fish health, used in fish indices such as the Fish Assemblage Integrity Index (FAII) and Index of Biotic Integrity (IBI).

The general condition, calculated as condition factors (length-weight relationship) indicated that the fish of the Klip River were generally in good health. The application of this method can be time consuming due to the measuring and weighing of large sample sizes. Inaccurate results can furthermore be gained by this procedure as it is envisaged that fish may have the ability to counter weight loss via physiological processes. This method was therefore identified to be less valuable for the assessment of river health, and for biomonitoring purposes, could be excluded.

The levels of most metals accumulated by the fish of the Klip River were generally lower than those detected in fish from other river systems. Of the two areas investigated, the fish indicated that the Henley-on-Klip section of the Klip River generally had higher levels of metals accumulated in their organs. The accumulation of metals in the Klip River is probably related to the impact of mining and industrial activities, especially in the Rietspruit catchment. Future bioaccumulation studies should also consider the assessment of other chemicals than metals in fish and a human health risk assessment should be conducted in the near future.

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