CHARACTERISATION OF THE HEALTH, HABITAT USE AND MOVEMENT OF ADULT LOWVELD LARGESCALE YELLOWFISH (*Labeobarbus marequensis* Smith, 1841) AND OTHER FISHES IN THE CROCODILE RIVER, KRUGER NATIONAL PARK.

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"But ask the animals, and they will teach you, or the birds of the air, and they will tell you; or speak to the earth, and it will teach you, or let the fish of the sea inform you. Which of all these does not know that the hand of the LORD has done this? In his hand is the life of every creature and the breath of all mankind."

Job 12:7-10
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SUMMARY

Yellowfish and specifically *Labeobarbus marequensis* are a charismatic species targeted by anglers throughout South Africa. Their population are limited to the north-western parts of the country including the lower reaches of the Crocodile River that flows through the Kruger National Park (KNP). Despite conservation efforts the Crocodile River in the KNP is still highly impacted. The effect of these impacts on the ecosystem is largely unknown.

The main aim of the study was to determine the influence of changing water quantity and quality in the Crocodile River on adult *L. marequensis*. This was achieved by evaluating altered flows (discharge) on the behaviour of adult *L. marequensis* in the Crocodile River using biotelemetry over a two year period. The influence of altered water quality was assessed using metal bioaccumulation as an indicator of metal exposure in *L. marequensis*, *Clarias gariepinus* and *Hydrocynus vittatus* in the Crocodile and Sabie Rivers during a high and low flow season.

Biotelemetry was used on 16 *L. marequensis* and 12 *H. vittatus* to determine the habitat use and movement responses of the species. Fish were tagged with Advanced Telemetry Systems (ATS) and Wireless Wildlife (WW) tags and tracked remotely and manually. Home ranges were determined using Arc GIS®, Habitat uses were analyzed using Windows Excel (© 2011, Microsoft Inc.). Environment variables recorded were scored as primary and secondary and then combined with a weighting variable 2:1 ratio (primary variable: secondary variable). A mixed-model analysis of variance (ANOVA) approach with a random co-efficients model and Akaike’s information criteria (AIC) were used to test for significance. Analyses were conducted using SAS version 9 (SAS institute, Cary, NC).

The habitat use of *L. marequensis* included cobble and boulder dominated flowing habitat biotopes. A strong affiliation for cover features including rocky outcrops, undercut banks and submerged woody and rock structures were also observed. Foraging behaviour took place predominantly within in these habitat types. A single spawning event was observed within a fast flowing, boulder dominated and 1-2m deep run. Seasonal habitat utilization differed significantly (*p*=0.04). Changes in discharge significantly (*p*=0.001) effected behaviour. During high flow periods the movement of fish decreased. Significant (*p*=0.05) changes in movement were observed during rapid moderate (increase of 11m³/s) and high (61 m³/s) changes in
discharge. The study indicates that habitat available for *L. marequensis* is low within KNP and the focal area is important to the species. Reduction in habitat diversity could impact the behaviour of the species. The management of the timing, duration and frequency of flows are important for the biology and ecology of *L. marequensis*.

For the metal bioaccumulations *L. marequensis*, *C gariepinus* and *H. vittatus* were caught in two rivers, the Sabie and Crocodile River during a high flow and low flow period. In total 19 *L. marequensis*, 23 *C gariepinus* and 30 *H. vittatus* were used. The fishes spinal cords were severed and then dissected and muscle tissue removed and frozen for analysis. Tissue were then dried at 60°C for 48 hours, then after weighed and diluted in 1% HNO₃ (AR) with Milli-Q water before digestion using HNO₃/H₂O₂ and using an Ethos microwave digestion system. Metal concentrations were determined using a Thermo inductively coupled plasma optical emission spectrophotometer (ICP-OES) and an inductively coupled plasma mass spectrophotometer (ICP-MS). Metals analysed were As, Cd, Cr, Cu, Mn, Ni, Pb, Se and Zn. Quality control of metal measurements in sediment and muscle tissue was verified by including process blanks and certified reference material (CRM 278, muscle tissue Community Bureau of Reference, Geel, Belgium). Statistical analyses of significant differences, between sites and species were undertaken using one-way analyses of variance (ANOVA). Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene’s tests (Zar, 1996), respectively, prior to applying post-hoc comparisons. Post-hoc comparisons were made using the Scheffe test for homogeneous or Dunnett’s-T3 test for non-homogenous data. The use of either one of the two tests resulted in the determination of significant differences (p<0.05) between variables.

The metal bioaccumulations showed to have significant (p<0.05) differences between the Crocodile River and Sabie River, high flow and low flow and between the three species. Aluminium, Fe and Se were significantly (p<0.05) higher during the high flow in the Crocodile River for *L. marequensis* while Cr, Fe and Se was significantly (p<0.05) higher during high flows in the Sabie River for *L. marequensis*. Zinc was significantly (p<0.05) higher in *L. marequensis* than in *C gariepinus* during Crocodile River high flows. Arsenic was significantly (p<0.05) higher in *L. marequensis* than *H. vittatus* during the Crocodile River low flows. Within the Sabie River Al and Pb was significantly (p<0.05) higher in *L. marequensis* than in *C gariepinus* and significantly (p<0.05) higher in *L. marequensis* than in *H. vittatus* respectively during high flows. During low flows for the Sabie River Mn and Se were significantly (p<0.05) higher in
than in *C. gariepinus*, Se in *L. marequensis* was significantly (p<0.05) higher than in *H. vittatus*. Differences between the two rivers during high flows showed significantly (p<0.05) higher levels of Cd in *L. marequensis* and Co in *H. vittatus* in the Crocodile River. During low flow spatial differences between the two rivers, the Crocodile River showed *L. marequensis* to have significantly (p<0.05) higher levels for Al, Cr and Cd bioaccumulation. Cadmium in *H. vittatus* and As in *C. gariepinus* had significantly (p<0.05) higher metal bioaccumulations for the Crocodile River than in the Sabie River.

The hypothesis set were accepted and able to indicate the importance of flow (discharge), season and time of day on the behavioural response of *L. marequensis* and the importance of maintaining the preferred habitat for the species to ensure its survival. Discharge in river management is always of importance as it alters downstream available habitat. Constant flow allows the river to stabilize creating preferred habitat to be around for longer, this is not to distract from natural flows yet to enhance natural flows and not allow the release of water at irregular intervals. The importance of spring flows to *L. marequensis* behaviour indicates the value of natural flows during that period to allow for availability of spawning events. Further minimizing the effluent of metal wastes into the Crocodile River system is also of importance as metal concentrations were found to be higher within the Crocodile River than in the Sabie River. Therefore fish in the Crocodile River are potentially at a greater risk than that in the Sabie River. Thus minimizing or reducing the release of metals into the Crocodile River would reduce the risks.

Further studies are needed to better understand the spawning habits of *L. marequensis* and the associated cues in particular to the day length, flows and associated spawning habitat. Since *L. marequensis* bio-accumulated the highest levels of metals it is recommended that the species be used as an indicator species. Future studies should be aimed at linking the metal exposure (in the form of bioaccumulation) with the effects, e.g. using biomarkers of which behaviour could form a part.
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South Africa is regarded as a semi-arid country; that on average receives a low (500 mm) rainfall, with 61 % of the country receiving less than the average (Heath and Claassen, 1999). Due to the scarcity of water it is important that a balance between water resource developments and maintaining the natural environment to ensure that these systems remain sustainable is established (DWAF, 1995). Historically however the need to meet increasing domestic, agricultural and industrial demands have necessitated the development of an extensive national water storage, abstraction and transfer infrastructure (Paxton, 2004b). Approximately 43 % of the total mean annual runoff (MAR) in South Africa is lost from rivers as storage or abstracted as an ecosystem service (DWAF, 2002). All these activities alter downstream natural hydrological, sediment and temperature and physico-chemical conditions, which impacts on freshwater biodiversity by changing habitat ecosystem conditions. The excessive abstraction, flow modifications habitat and water quality alterations have caused South African freshwater ecosystems to be the most threatened ecosystems that are experiencing the fastest loss of biodiversity and the greatest number of species extinctions (Dallas and Day, 2004; Paxton, 2004b; Nel et al., 2007; Rivers-Moore, 2011). The last national appraisal of South African freshwater ecosystems estimates that in excess of 50 % of the rivers are critically endangered, while 82 % of river ecosystems are threatened (Driver et al., 2005; Nel et al., 2007). Conservation endeavours for these systems and the species that occur within them are urgently require.

The Kruger National Park (KNP) is recognised for its conservation of the savannah ecosystem and boasts over 100 years of conservation (Mabunda et al., 2003). Kruger National Park is mandated to conserve all ecosystems within its boundaries, this is particularly difficult as the seven major rivers begin their source outside the park and are all impacted by excessive ecosystem use (O’Keefe and Rogers, 2003; Rogers and O’Keeffe, 2003; Mabunda et al., 2003). This makes the management of KNP rivers difficult to control from within KNP boundaries as the impacts on KNP Rivers come largely from the catchment, where they flow through agricultural lands, industrial developments, urban areas, and mines (O’Keeffe and Rogers, 2003; Rogers and O’Keeffe, 2003). From as early as the late 1980’s concerns were raised about the poor state of KNP rivers in that development had increased over two decades with the quality and quantity of water being affected by the developments
west of the KNP (Du Preez and Steyn, 1992). Two decades later and there is little
relief to this deterioration with the urban development in the Komatipoort Corridor for
the trade between Maputo and Nelspruit has compounded this deterioration (Rogers
and Luton, 2011). Of all the major rivers in KNP, the Sabie River is regarded as a
healthy system as it has the least impoundments and impacts in relation to the other
rivers in KNP (Heath and Claasen, 1999). This was not always the case as gold
mining activities in the 1930’s degraded the system to very little or no aquatic life,
which slowly recovered back to normal by the 1990’s (Pienaar, 1978; Hills et al.,
2001; Rogers and O’ Keeffe, 2003). The Crocodile and Olifants Rivers are the highest
impacted KNP rivers (Heath and Claasen, 1999). The impact on the Crocodile River
are compounded as it forms the southern boundary of the KNP receiving impacts from
the local farmers, factories and mines that all extract water and discharge their
effluent back into the river (O’ Keeffe and Rogers, 2003).

The Crocodile River catchment drains an area of 10 400k m$^2$ (Hills et al., 2001). The
average rainfall in its catchment ranges from 500 mm to 1600 mm (Hills et al., 2001).
The river starting at an altitude of 2150 m drains the Eastern Highveld and is 320 km
long. Twenty percent (20 %) of the river flows through the protected KNP (Hills et al.,
2001). The river is divided into four broad eco-zones by the River Health Programme
(RHP) of which this study is situated in the warm (>23 °C) lowveld region below
800m in altitude (DWAF, 1995; Kleynhans, 1997; Hills et al., 2001). Despite flowing
through the KNP water extractions still take place for land use. The land uses in the
catchment are dominated by agriculture activities including forestry, dry land and
irrigation (Hills et al., 2001; O’Keeffe and Rogers, 2003; Fouché, 2009). Due to these
land uses the integrity state of the Crocodile River is uncertain in relation to the
excessive overutilisation of the ecosystem services provided by the Crocodile River
to water resource users (Godfrey, 2002). Alien invasive aquatic vegetation (water
hyacinth) is a periodic problem and is compounded by slow flowing water and excess
nutrients (Hills et al., 2001). According to the RHP for the Crocodile River, flow
alterations are caused from the excessive water use and extraction (Hills et al.,
2001). This is concerning as the Crocodile River is one of the most productive and
indicates the variability in the flow of water to be the major agent for disturbances in
the complexity of rivers. Flow discharge changes have a more direct impact on
organisms in the river (Resh et al., 1988).
Fish in South Africa have been used as indicators of ecological health because of their response to known pollutants which cause the degradation of river ecological health (Pienaar, 1978; Kleyhans, 1997; Skelton, 2000). Fishes are also socially and economically important and contribute to the conservation of aquatic systems and the awareness of conservation practices (Skelton, 2000). To continue developing fish as ecologically indicators is important for an understanding of the variables of ecological integrity that are indicated by fishes to be determined (Schiemer, 2000). Suggestions towards the use of *Labeobarbus* spp. to indicate flow rates in a river ecosystem have been made (Fouché and Gaigher, 2001; Fouché, 2009). Vidal (2008) stated that the knowledge for each species of their biology, range of tolerance and responses towards different kinds of variables will allow the use of freshwater fish as ecological indicators. Paxton (2004b) describes the value of using fish behaviour for indications of river health as a fish's survivability is influenced by changes in the ecosystems in which they live which is reflected as changes in behaviour. This can be due to the availability of habitats affected by different flows and water quality alterations, food availability and direct health of a fish. *Labeobarbus* spp. have already been shown to respond to changes in water quality, quantity and habitat availability (Impson et al., 2008). They thrive in rivers that have a near natural flow regime and abound in man made lakes that have diverse habitat, good water quality and few or no alien fishes or plants (Impson et al., 2008). *Labeobarbus* spp. have widely been used as indicators of ecological health and as umbrella species for other aquatic species in South Africa including the Vaal River system (Ellender, 2008; De Villiers and Ellender 2008a & 2008b; O’ Brien and De Villiers, 2011).

The Yellowfish (*Labeobarbus* spp.) belong to the Cyprinidae family that include all the barbs, yellowfish and labeos in South Africa. The genus *Labeobarbus* is differentiated from other Cyprinids in being hexaploid, with around 150 chromosomes as opposed to the tetraploid and diploid state in other genus’s (Oellerman and Skelton, 1989; Skelton, 2001). In addition, morphologically their scales have parallel striae and a spinous primary dorsal fin ray (Skelton, 2001). *Labeobarbus* spp. are a targeted game fish in South Africa supporting angling industries such as the industry on the Vaal River which is valued at 133 million/annum (Brand et al., 2009). Despite the large contribution this species has to the economical growth in South Africa they are being poorly conserved. Two of the five endemic South African yellowfishes (*L. aeneus* and *L. kimberlyensis*) have conservation status (IUCN, 2007). Conservation of South African yellowfishes requires detailed knowledge on the health and behaviour of the species. Characterising behaviour of these fish is important and...
contributes greatly to the management of the river system they occur in (O’ Brien and De Villiers, 2011). By evaluating the behavioural response of yellowfish to changing environmental conditions, scientists can evaluate the ecological consequences of the changes in these variables (O’ Brien and De Villiers, 2011).

The health of the Lowveld largescale yellowfish (*Labeobarbus marequensis* (Smith, 1841)), population in the Crocodile River system in KNP has recently received attention. There has been a noticeable decline in abundance of this population without the cause being characterised during the last decade (Leslie, 2007). *Labeobarbus marequensis* are in tolerant to the water quality, habitat and flow alteration (Fouché, 2009). Various agriculture, industries, urban areas and their waste water treatment works for example all occur in the Crocodile River catchment with known impacts (Godfrey, 2001). Due to the health concerns of *L. marequensis* populations in the Crocodile River, local management authorities are interested in characterising the cause determining the decline (Leslie, 2007). Health in this study refers to the metal concentrations found within fish tested and the habitat required for *L. marequensis* to survive.

The biology and ecology of *L. marequensis*, specifically in the Crocodile River is poorly known (Impson et al., 2008). What is surprising is that *L. marequensis* populations in other severely impacted rivers in the KNP such as the Olifants River are considered to be in a better state of health (Kleynhans, 1991; Rogers and O’ Keeffe, 2003; Fouché, 2009). The decline in abundances in the Crocodile River suggests that the species may be intolerant to unknown stressors or the synergistic effect of multiple stressors associated with the change in environmental variables (Leslie, 2007). With the lack of juvenile fish abundant in the river and the very low numbers of adults, the population dynamics of *L. marequensis* in the Crocodile River has been disrupted and is concerning for the species’ long term survival (Leslie, 2007).

To address the information requirements of the *L. marequensis* for stakeholders in the KNP, test hypotheses have been established including:

- *Labeobarbus marequensis* make use of defined home ranges on a reach scale (<10 km) and behave differently during different seasons and times of day.
- The movement of *L. marequensis* decreases when flows increase rapidly in the Crocodile River.
The levels of metals in *L. marequensis* in the Crocodile River are greater than *H. vittatus* and *C. gariepinus* from the Crocodile and Sabie rivers, which negatively affects the *L. marequensis* population in the Crocodile River.

The aim of this study is to evaluate the current health, habitat use and movement of *L. marequensis* and other fishes in the Crocodile River, KNP. To reach the aims a behavioural and ecotoxicology assessment of the population of *L. marequensis* in the Crocodile River has been undertaken. The objectives of the study include:

- To characterise the home range, habitat use and movement of *L. marequensis* and other fishes in the Crocodile River, using biotelemetry methods.
- The response of *L. marequensis* changes in accordance with changing flows and natural cycles (time of day and seasons) in the Crocodile River.
- To determine the extent of metal bioaccumulation in three fish species from two rivers in the Kruger National Park.
- To provide management considerations for the conservation of the *L. marequensis* population in the Crocodile River in the Kruger National Park.

The study has been divided into two sections including a behavioural section and ecotoxicological section. In additional a general introduction, literature survey and general conclusion with management recommendations has been included as separate sections. As the structure of this thesis includes:

Chapter 1: Introduction
Presents the rationale of the study, a review of the existing knowledge and background to the species in question.

Chapter 2: *Labeobarbus marequensis* species and biotelemetry review
A review of the known biology and ecology of *L. marequensis* is projected. Relevant literature regarding its behavioural ecology and ecotoxicological studies specific to *L. marequensis* are presented.

Chapter 3: *Labeobarbus marequensis* behavioural study
This chapter presents the biotelemetry approach adapted in determining habitat use, and movement for *L. marequensis*.

Chapter 4: *Labeobarbus marequensis* ecotoxicology study
This chapter presents the bioaccumulation assessment of metals in *L. marequensis* and other species within the Crocodile and Sabie rivers, in KNP.

Chapter 5: General conclusions and management recommendations
In this chapter the results from chapter three and four are reviewed and summarized to suggest ways forward for *L. marequensis* and the management of the Crocodile River.

1.1 Study Area

The study area referred to as the Crocodile Site, includes a reach of the Crocodile River, which forms the southern boundary of the KNP. The site is situated along the lower sections of the Crocodile River, upstream from its confluence with the Inkomati River (Figure 1). On the southern bank the Mjejane Game Reserve’s Lodges are located. The area’s access is controlled because it falls under the mandate of the KNP to be conserved (Mabunda et al., 2003). Therefore disturbance to wildlife impacts on the population are minimal. The Crocodile Site is well known for large specimens of *L. marequensis* despite their notable decline. *Labeobarbus marequensis* have been found to utilize cobble, gravel and deep rocky pools or rapids (Pienaar, 1978; Russel, 1997; Fouché, 2009). The Crocodile Site is well suited for the study, in that it is the only stretch of river where these habitats occur extensively. The rest of the Crocodile River forms long sandy runs and pools (Hill et al., 2001).

For the ecotoxicological component of the study, a comparative river system was needed to evaluate the ecotoxicology of the *L. marequensis* and the other species. For this the Sabie River was chosen as it also forms part of the Inkomati catchment and is regarded as having a near-pristine integrity state (Pienaar, 1978; Hills et al., 2001; Rogers and Luton, 2011). In Figure 1 the geographical areas where the samples were taken from the Crocodile Site on the Crocodile River and the two sample sites on the Sabie River are depicted.
Figure 1: Shows the location of the Crocodile Site on the Crocodile River and the control sample sites along the Sabie River, KNP.
2 CHAPTER 2: A COMPREHENSIVE LITERATURE REVIEW ON LOWVELD LARGESCALE YELLOWFISH, LABEOBARBUS MAREQUENSIS

2.1 Introduction

South African “yellowfish” fall under the Genus Labeobarbus and lies within the family of Cyprinidae. This family composes of all the barbs, yellowfish and labeos. The genus Labeobarbus is differentiated from other Cyprinidaes in being a large barbine cyprinid (Skelton, 2001). Within southern Africa the genus Labeobarbus spp. can be split into two groups, largescale and smallscale yellowfish. The latter of the two groups are endemic to the South African rivers while L. marequeunsis are present in lowveld rivers and L. codringtonii in the upper Zambezi, Okavango and Kunene rivers (Impson et al., 2008). Oellerman and Skelton (1989) showed Labeobarbus spp. to be hexaploid, with around 150 chromosomes. Due to this the subgenus was elevated to full generic status by Skelton (2001). Further their scales are longitudinal or parallel striae and the primary dorsal fin ray is usually spinous (Skelton, 2001).

The genus Labeobarbus has a poor fossil record and is said to have come from the mid-Miocene period of East Africa (Skelton and Bills, 2008). Geologist indicate that systems were all connected at some point in earth’s history and is the cause for close relatedness between species in now separated river catchments (Skelton and Bills, 2008). The adaption of yellowfish (Labeobarbus spp.) into what we know today was derived from the nature of the species in a system when more than one species is present (Skelton and Bills, 2008). This allowed for the diversification of the genus into the different species that we know today, Labeobarbus intermedius as example has diversified less than other species, indicating large similarities in the ancestral yellowfish kind whilst others have adapted to the catchment in which they now occur (Skelton and Bills, 2008).

The African yellowfishes or Labeobarbus spp. are a well-known charismatic, indicator fish that are economically important, targeted by dedicated angling industries and harvested as important sources of protein for many Africans (De Villiers and Ellender, 2008a; Impson et al., 2008; Brand et al., 2009). Being widely distributed in Africa the lineage constitutes of roughly 80 different species (Skelton and Bills, 2008). Of these only seven species occur within southern Africa (Skelton, 2001). Angling for Labeobarbus spp. plays a role in its social and economical importance (Brand et al.,
Furthermore as an ecological indicator this genus plays an important role in the conservation of the ecosystems, as they have been shown to respond to changes in water quality, quantity and habitat availability (Impson et al., 2008). They thrive in rivers that have a near natural flow regime and abound in man made lakes that have diverse habitat, good water quality and few or no alien fishes or plants (Impson et al. 2008).

Apart from being regarded as an abundant species, it is also showing a decline in its abundance due to the current poor state of South African rivers, (Kleynhans, 1999; Leslie, 2007; Fouché 2009; Nel et al., 2007). The utilization of water resources and abuse of angling for the species has lead to concern for the survival and studies on the different species, there has been very little done for the species \textit{L. marequensis} (Impson et al., 2008). The biology and ecology of \textit{L. marequensis}, specifically in the Crocodile River is poorly known (Impson et al., 2008). However \textit{L. marequensis} populations in other more heavily impacted rivers in KNP such as the Olifants River are in a better state (Kleynhans, 1991; Seymore et al., 1995; Rogers and O’ Keeffe, 2003; Fouché, 2009). The decline in abundances in the Crocodile River suggests that the species may be sensitive to unknown stressors or the synergistic effect of multiple stressors associated with the change in environmental variables. With juvenile fish low in abundance and the very low numbers of adults in the river, the population dynamics of \textit{L. marequensis} in the Crocodile River has been disrupted and is concerning for the species’ long term survival (Leslie, 2007).

### 2.2 \textit{Labeobarbus marequensis} past and present

Genus and Species \textit{Labeobarbus marequensis} was first referred to by Smith, (1841) as \textit{Barbus marequensis}, until \textit{Labeobarbus} was elevated to full generic status (Skelton, 2001). The species is widely distributed, its range extends from the middle and lower Zambezi south to the Phongolo system, with larger specimens generally occurring below 600m altitude (Skelton, 2001). Their range within the KNP is common throughout the rivers and tributaries (Pienaar, 1978). They are separated from the similar largescale species \textit{L. condrintoni} in distribution and morphology in having a longer dorsal fin than the head (Skelton, 2001).
Conservation status of *L. marequensis* is regarded as “Least Concern” as it is still relatively abundant and widespread in its distribution (IUCN, 2007). *Labeobarbus marequensis* within the Crocodile River has had a noticeable decline in the population and has been found true to other river systems such as the Luvhuvhu River (Russel and Rogers, 1989; Fouché, 2009). In South Africa *L. marequensis* occur in six water management areas (WMA) namely: Crocodile (west)-Marico, Limpopo, luvhuvhu-letaba, Olifants, Inkomati and the Usuthu-Mhlatuze. Threats that face *L. marequensis* are changes in flow regimes, impoundments (man made lakes and weirs), illegal netting, invasive alien species and degradation of water quality (Fouché, 2009).

The Kruger National Park is regarded as the stronghold for the conservation of South Africa’s species including *L. marequensis* and incorporates a large percentage of its distribution range in South Africa. Numbers however are said to be declining due to influences upstream out of the control of the KNP (Russel and Rogers, 1989; Rogers and O’Keeffe, 2003). The importance of conserving the upper catchment of the river for the species is much needed in South Africa and has largely been neglected (Angliss et al., 2005). The declining numbers show the necessity for the establishment of conservancies (Impson et al., 2008). The fragmentation of rivers is a concern and the construction of fish-ways at newly planned and existing weirs is a priority (Impson et al., 2008). Integrated Water Management Areas (WIMA) are also being developed towards the better management of water resources in South Africa and will contribute greatly towards the conservation of all aquatic fish species including, *L. marequensis* (Rogers and Luton, 2011).
2.3 *Labeobarbus marequensis* biology and ecology

The distribution of *L. marequensis* is depicted in Figure 2.

![Distribution range of *Labeobarbus marequensis* in Africa (after Fouché, 2009).](image)

**Figure 2**: Distribution range of *Labeobarbus marequensis* in Africa (after Fouché, 2009).

Morphology

*Labeobarbus marequensis* has the typical shape of a cyprinid (Figure 3). The species have 27-33 scales in the lateral line and 12 around caudal peduncle (Skelton, 2001). The species seldom exceeds 3.5 kgs (7lbs) (Jubb, 1967). In addition the dorsal fin shows a large degree of variation with Jubb (1967) and Bell-Cross and Minshull (1988) finding the dorsal fin to decrease in height within its distribution. The dorsal fin
height can even vary within a single population (Skelton, 2001). The mouth is terminally positioned with the mouth-form and lips being variable (Jubb, 1967; Bell-Cross and Minshull, 1988). Three mouth forms are shown to exist, which according to Pienaar (1978) have no diagnostic value to the species. Pienaar (1978) further distinguished the three different mouth forms as *forma varicorhinus* (square mouth with chisel-shaped lower cutting jaw), *forma gunningi* (thick ‘rubber-lips’ form) and *forma typical* (intermediate form). The different lip forms are said to be a result of the species feeding habits and habitat biology (Jackson, 1961; Pienaar, 1978; Skelton, 2001). Colouration of the fish varies from golden yellow in clear water (Bell-Cross and Minshull, 1988; Skelton, 2001) and pale olive in turbid water (Fouché, 2009).

Figure 3: Picture showing a *Labeobarbus marequensis* caught within the lower reaches of the Crocodile River, Kruger National Park.

Growth

Skelton (2001) makes note that large specimens of the species only occur in the lowveld within its distribution range. Large *L. marequensis* specimens occur in deep slow flowing waters, while smaller individuals prefer fast flowing shallow water (Gaigher, 1969; Fouché et al., 2005). The size class differences of *L. marequensis* and the difference in habitat use within those size classes suggested the species undergoes “ontogenetic shift” (Fouché, 2009). Juveniles and large adult *L. marequensis* have the same body length and depth ratios, Fouché, 2009 suggests that the geomorphological differences influences habitat use at different ages. Smaller adults of the species are more slender and adapted better for faster flowing waters (Fouché, 2009). On the Luvhuvhu system low numbers of specimens longer than a fork length of 180mm within the population of *L. marequensis* were found (Fouché, 2009). Skelton (2001) indicates the slow growth of the species.
Population structure

Population structure varies between catchment within catchment and changes over years as discussed under natural reproduction. Within the Luvhuvhu River the male to female ratio changed over time from 1:3 in the 1960’s to 3.3:1 in 2009 (Gaigher, 1969; Fouché, 2009). Regardless of the shift in sex ratio there was an abundance of juvenile fish (Fouché, 2009). Large specimens of the species only occur in Lowveld rivers and further within deep pools in these rivers (Pienaar, 1978; Skelton, 2001). Large specimens were also only found within the lower reaches of the Luvhuvhu River with smaller or younger fish occurring throughout the river (Fouché, 2009). To maintain population structure the population is dependent on a good recruitment from mature individuals which are long lived and slow growing (Skelton, 2001; Fouché, 2009).

Natural reproduction

*Labeobarbus marequensis* are serial spawners that have a prolonged spawning period confirmed by the bi-modal distribution in egg diameter (Fouché, 2009). The species shows to have two distinct spawning periods a year in southern Africa (May and September) with the spring spawning period dominating (Fouché, 2009). The September spawn coincides with increased temperature and changes in discharge including freshet flows following the onset of major summer rain fall (Fouché, 2009). Breeding occurs at sites where water flows over boulders and cobbles, riffles and rapids biotopes or resting areas where mature specimens condition for breeding (Vlok,1992; Fouché, 2009). Maturity of individual differs within different river systems. The Luvhuvhu River male *L. marequensis* mature at fork length 90mm while in the Inkomati River the species matured at fork length 70mm (Gaigher, 1969; Fouché, 2009). Females of the species differed between the two river systems maturing at fork lengths 200mm and 280mm in the Luvhuvhu and Inkomati rivers respectively (Fouché, 2009; Gaigher, 1969). Egg size of *L. marequensis* are regarded as large ranging from 0.9mm to 1.2mm, this indicates that embryo have more yolk to feed off when incubating allowing them to hatch relatively large fish that can avoid predation (Nikolsky, 1963; Fouché, 2009). The fecundity rate of the species is 44.7 ova per gram of body mass thus, in a sexually mature female, 200mm fork length, weighing 400 grams, 17,000 mature ova are present in the ovaries (Fouché, 2009). It is still uncertain if a population will spawn every year or periodically over a number of years explaining the importance of the large number of eggs produced for the survival of the species (Fouché, 2009).
Migration

Three types of coordinated movement patterns of populations have been described including: local and seasonal movements, dispersals, and true migrations (Dodson, 1997). Some populations of *L. marequensis* are known to migrate which is seemingly associated with spawning during summer and spring (Crass 1964; Pienaar 1978; Skelton 2001). Fouché (2009) described *L. marequensis* as spawning migrants predominantly with an additional small percentage migrating for habitat selection during non-spawning periods. Possible cues for *L. marequensis* to migrate include a complexity of factors varying from flow, water quality and day length (Fouché, 2009). If the cues are absent despite the condition of fish being ripe for spawning they will not migrate (Fouché, 2009). Adult *L. marequensis* have been reported to migrate up rivers during high flows to spawn in rapids between October and April in Zimbabwe (Bell-Cross and Minshull, 1988). Although not specific to *L. marequensis* the presence of small adult specimens and lack of large adult specimens in fish ladders during times of high flows in the river suggest that only a portion of the adult population migrate (Paxton, 2004a).

Food and feeding

*Labeobarbus marequensis* are opportunistic omnivores that feed primarily on algae and aquatic insect larvae (midge and mayfly larvae) and small fishes, snails, freshwater mussels and drifting organisms such as beetles and ants (Crass, 1964; Skelton, 2001). The relative gut intestinal length of *L. marequensis* is longer than the fork length suggesting the fish are herbivorous (Fouché, 2009; Fouché and Gaigher, 2001). *Labeobarbus marequensis* stomach is straight-tubed and monogastric, it is funnelled wide at the anterior tapering towards the posterior (Fouché, 2009). There is no distinct external change-over from the stomach to the intestine and internally there is no sphincter present at this point, there is a distinct difference between the rugae of the stomach and the intestines with structural changeover indicating the posterior boundary of the stomach (Fouché, 2009). The stomach wall had thicker muscular layers than the rest of the intestines (Fouché and Gaigher, 2001). The length of the intestinal tract is longer than the fork length with the exception of the smaller classes of *L. marequensis* and suggests that small classes may feed off the benthic and planktonic invertebrates while larger fish are more herbivorous (Fouché, 2009). This is contrary to Jackson (1961), Crass (1964), Bell-Cross and Minshull (1988) whom suggest the diet is more carnivorous.
Habitat use

*Labeobarbus marequensis* make use of a variety of habitats. Pienaar (1978) indicated use of sandy reaches, reed-fringed pools and deep rocky pools below rapids where the current is swift and strong as habitat use. Russel (1997) showed preferences to swift-water seams between the rapids and stream margins, particularly where they have gravel and/or cobble beds. Fouché (2009) found *L. marequensis* in both fast-shallow and fast deep habitats. Different age classes are shown to frequent different habitat types (Gaigher, 1969; Fouché et al., 2005; Fouché, 2009). Within the Crocodile River the species was found only in the lower reaches, below 800m and in habitat types characterized by large rocky pools and runs, rapids and riffles (Kleynhans, 1999). Habitat use is clearly associated with flows and increased levels of siltation, especially at breeding sites affects habitat use (Fouché and Gaigher, 2001; Fouché, 2009). In the Crocodile River they were found within the reaches with a moderate slope indicating moderate flows characteristic of these reaches (Kleynhans, 1999).

### 2.4 *Labeobarbus marequensis* ecosystem service use

The use of riverine ecosystem service use may directly or indirectly affect the populations of *L. marequensis* by the use of the ecosystems they occur in. The relationship and importance of *L. marequensis* towards humans and the species effect from human intervention is presented as follows:

**Angling stress**

Growing interest in the species for angling purpose has brought about the awareness to practice catch-release angling methods for these species and the slow increase of private conservation areas currently being established within its natural distribution will contribute and benefit the conservation of the species (Impson et al., 2008). Angling on a catch and release basis will be more beneficial to the species as opposed to fishing for subsistence (Impson et al., 2008). Legislation for bag limits varies greatly between the provinces within the distribution of *L. marequensis*. In KwaZulu-Natal and Limpopo there are no restrictions on the number and size of fish that may be kept (Impson et al., 2008). In Mpumalanga six fish can be kept with minimum length of 200 mm and in the North West Province the equivalent figures are ten and 300 mm respectively. The Yellowfish Working Group (YWG) has
recommended a maximum daily bag limit of two fish between 30-50 cm, captured only by rod and line (Impson et al., 2008).

Ecotoxicology
Although the *L. marequensis* is considered to be a water quality tolerant species (Kleynhans, 1991; Fouché 2009) the decline in the population on the Crocodile River suggests otherwise (Fouché, 2008). Pollard et al. (1996) showed that during periods of drought in the Sabie River, specimens of *L. marequensis* were observed to survive in pools where the electrical conductivity ranged between 200 and 800 μScm⁻¹ and the dissolved oxygen saturation was low at 50% saturation. It should however be noted that these conditions probably lasted for short periods (Fouché 2009). Due to its water quality tolerance, *L. marequensis* has not received much attention regarding its importance in indicating changes in water quality related environmental integrity. Despite this it has been found to be sensitive to flow alterations and increased levels of siltation, especially at breeding sites (Fouché and Gaigher, 2001). The species has been used as a bioaccumulation indicator species for metals (Barker, 2006; Seymore et al., 1995). Although ecotoxicology studies have been done on the species non-specifically for the Crocodile River have been published. Barker’s (2006) study served as a baseline study for the Sabie, Shingwedzi, Letaba and Luvhuwhu Rivers while Seymore et al. (1995) looked at the occurrence of metals in the species and used it as indication of excessive metal contamination for the Olifants River. *Labeobarbus marequensis* metal levels of Cr, Cu, Fe, Pb, Mn, Ni and Zn specific to the Luvhuwhu and Shingwedzi Rivers were high (Barker, 2006) compared to other rivers. In the Olifants River the levels were highest in the vertebrae and the gills and it was suggested to use bony tissues, gills, liver and muscles tissue to test for Mn, St and Pb (Seymore et al., 1995). In 1994 the species was used in a study on the Olifants River (KNP) to test for pollutants. Findings were such that over-exposure from Zn, Cu, Pb and Ni could be sub-lethal (Van Vuren, et al., 1994). Many studies have used *L. marequensis* to test for metals within Lowveld Rivers since they all have similar geological sources (Rogers and O’Keeffe, 2003).
CHAPTER 3: BEHAVIOURAL STUDY OF AN ADULT POPULATION OF LOWVELD LARGESCALE YELLOWFISH (LABEOBARBUS MAREQUENSIS) IN THE CROCODILE RIVER, KRUGER NATIONAL PARK.

3.1 Introduction

Behavioural studies of freshwater fish have contributed to gaining an understanding of how fish adapt to; optimise the utilisation of the ecosystem resources, successfully recruit and survive natural and anthropogenic changes in ecosystem conditions (Godin, 1997; O’ Brien and De Villiers, 2011). A number of behaviour variables of fishes can be evaluated in a behavioural study including: migration behaviour, habitat selection, territoriality behaviour, foraging and diet, anti-predator tactics, reproduction, reproductive strategies and the response of species to changing environmental variables states (Godin, 1997). This behavioural information of freshwater fish can contribute greatly towards the understanding of their biology and ecology. This further will assist in the conservation and management of the species concerned and their aquatic ecosystems in which they occur (Paxton, 2004b; O’ Brien and De Villiers, 2011).

Telemetry or bio-telemetry has been successfully used as early as the 1950s’ in acquiring information on the behaviour and physiology of fish and was successfully used to address and mitigate the impact of stressors on ecosystems (Trefethen, 1956; Cooke et al., 2004). From its origin biotelemetry methods, used to monitor the behaviour of fish in relation to changing environmental conditions, have been used in recruitment studies, aquaculture and behavioural studies (Trefethen, 1956; Johnson, 1957; Thorstad et al., 2001; Crook, 2004; Cooke and Schreer, 2003; Cooke et al., 2004; Økland et al., 2005; Tomschi et al., 2009, O’Brien et. al, 2012). This approach is recognized as the most effective way of acquiring information of wild adult fish over extended periods and areas to meet specified management questions (Lucas and Baras, 2000; Rogers and White, 2002; Paxton, 2004a). Due to bio-telemetry’s high cost it has been limited in its use up until recently and is now becoming more readily available and affordable (Rogers and White, 2002). Despite the value of telemetry studies to behaviour of fish, very few have been conducted within freshwater ecosystems in southern Africa (Thorstad et al., 2001; Økland et al., 2003; Paxton, 2004b; Thorstad et al., 2003; Økland et al., 2005; O’Brien et al., 2012).
Biotelemetry involves the attachment and tracking of fish using radio or acoustic transmitters or transceivers (tags) and the continual monitoring of the tagged individual to acquire spatial area use and behavioural patterns (Lucas and Baras, 2000). Tag attachments include: external, stomach and implants (Rogers and White, 2002; Cooke et al., 2004). The size of the tags limits the application of the tags and should not exceed 2% of the mass of the tagged fish (Jepsen, 2002).

The aim of this portion of the study is to evaluate the habitat use and movement of *L. marequensis* in the Crocodile River, KNP. To reach the aim a biotelemetry assessment of the population of *L. marequensis* in the Crocodile River has been undertaken. The objectives of this portion of the study include:

- To characterise the home range, habitat use and movement of *L. marequensis* and *H. vittatus* fishes in the Crocodile River.
- The response of *L. marequensis* changes in accordance with changing flows and natural cycles (time of day and seasons) in the Crocodile River.

This chapter presents the approach established and the findings of the behavioural ecology assessment of *L. marequensis* in the Crocodile River.

### 3.2 Material and Methods

In this study biotelemetry has been used to characterise the home range, habitat use and the responses of the tagged individuals to select changing environmental conditions in the Crocodile River (KNP). The study was carried out on the Crocodile River within the KNP (Figure 1). The site selected is situated along the lower sections of the Crocodile River, upstream from its confluence with the Inkomati River.

#### 3.2.1 Biotelemetry systems

For this study very high frequency (VHF) radio telemetry systems developed by Advance Telemetry Systems Inc (ATS) and Wireless Wildlife (WW) systems were used. Additional anecdotal observations were made of tagged individuals and non-tagged individuals in the vicinity while manually tracking tagged individuals (Koehn, 2000; Lucas and Baras, 2000). Tags weight in relation to the body weight of the fish.
used in the study ranged between 0.6% and 1.1% of body mass, consistent with the recommended carrying capacity of fishes (<2%) (Jepsen, 2002).

Advanced Telemetry Systems Inc, (ATS) USA

Advance Telemetry Systems (ATS) was used for the first portion of this study, ATS tags were used from August 2009 to March 2011. Tags with externally attached radio transmitters obtained from ATS, three types of transmitters were used (Table 1 and Figure 4). Each tag transmits a pulse to indicate position of the tag. The tags used signals within the range of 142.017 to 142.466 MHz at least 10 kHz of each other. Tagged fish were tracked by foot using a portable ATS-R2100 receiver connected to a directional 4 element Yagi antennae (Figure 5). The tags battery life lasts from 94 to 366 days with signal transmission set to every 1 second (Table 1). In addition an ATS receiver, model R4500S (Receiver/ Datalogger with DSP), was use in a remote station to under take remote monitoring. The station had two antennae positioned 90° angle apart one facing upstream and the other downstream to indicate up and down movement of the tags in the river (Figure 5). The station needed to be as close to the river to maximise its use. The station was erected at location S 25.37880 E 031.714350 (GPS co-ords in dec.deg).

Table 1: Types of tags used by Advanced Telemetry Systems and Wireless Wildlife Systems in the study. Wireless Wildlife tags Series V included a LED light and series IV included a depth sensor.

<table>
<thead>
<tr>
<th>Models</th>
<th>Weight (g)</th>
<th>length (mm)</th>
<th>diameter/width (mm)</th>
<th>height (mm)</th>
<th>Time span (days)</th>
<th>pulse per minute (ppm)</th>
<th>Activity (counts)</th>
<th>Temperature (°C)</th>
<th>Depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATS-2030</td>
<td>10.1</td>
<td>50</td>
<td>12</td>
<td>-</td>
<td>264</td>
<td>40</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>ATS-2060b</td>
<td>22</td>
<td>53</td>
<td>17</td>
<td>-</td>
<td>366</td>
<td>35</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>ATS-1930</td>
<td>2.4</td>
<td>25</td>
<td>9</td>
<td>6</td>
<td>94</td>
<td>30</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>WW-Series III</td>
<td>20 (+/- 1.5g)</td>
<td>53</td>
<td>17</td>
<td>-</td>
<td>365 *</td>
<td>10 default*</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>WW-Series IV</td>
<td>20 (+/- 1.5g)</td>
<td>53</td>
<td>17</td>
<td>-</td>
<td>365 *</td>
<td>10 default*</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>WW-Series V</td>
<td>20 (+/- 1.5g)</td>
<td>53</td>
<td>17</td>
<td>-</td>
<td>365 *</td>
<td>10 default*</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Note: (*) Refers to tags with remote/manual monitoring pulse frequencies which can be changed.
Figure 4: Advanced Telemetry Systems’ tags, models 2030, 2060 (A) and 1930 (H) used in the study and attached to fish. *Hydrocynus vittatus* with an ATS 2060b transmitter (B), *Labeobarbus marequensis* with an ATS 2030 transmitter (E) and 1930 transmitter (I). Wireless Wildlife Systems’ tags, Series III (D), Series IV (G) and Series V (K) used in the study attached to *Hydrocynus vittatus* (C) and *Labeobarbus marequensis* (F and J).

**Wireless Wildlife (WW) RSA**

From September 2011 onwards WW systems were used. Tags with externally attached radio transmitters obtained from WW, three types of tags were used in the study (Table 1 and Figure 4). Tagged fish were tracked by foot using a portable note book connected to a directional 4 element Yagi antennae (Figure 5). The lifespan of all WW-tags have a battery lifespan of 365 days (based on battery life expectancy with an 80 % safety factor) by combining default and active tracking modes. Defaults modes include on of the digital transmission sent every ten minutes. This can be changed to transmissions every 1 sec, 2 min and 5 min depending on the type of monitoring required (remote or manual). During field monitoring, transmissions are changed to 1 sec mode in order to intensively follow and track the fish. After a monitoring session, the transmissions are switched back to 10 minutes by default in order to conserve the tags battery life.
Figure 5: Advance Telemetry System's manual monitoring (A) equipment used during the study and Wireless Wildlife's manual monitoring (D and E) used during the study with a base station and relay station (C) used for remote monitoring.
Receivers were used to track the tags; this included both a notebook (small laptop) with WW tracking software installed and an Omni-antennae or directional Yagi antennae for manual tracking with a network of station set up strategically over the Crocodile Site for remote tracking (Figure 5). Each tag has additional sensory components, including: an activity sensor, measured in counts, temperature sensors measured in degrees Celsius and depth measured in cm.

Five remote monitoring stations were constructed for the study this included 1 base station and 4 repeater stations. The repeaters were remote receivers which transmits data acquired from tags within its coverage to the base station. The base station transmits all data it receives from the repeaters and tags in its coverage and sends that data through to a survey at WW (Figure 6).

![Figure 6: Placement of the remote stations used for the Wireless Wildlife system covering the Crocodile Site. The signal range for each station is indicated by colour, yellow being the strongest to red being the weakest.](image)

### 3.2.2 Capture and tagging

Adult *L. marequensis* weighing more than two kilograms were captured using a range of sampling techniques (Figure 7). All fish caught were obtained using the following techniques: angling techniques, electro-shocker (220 V) and gills nets in deep (>1.5 m) areas. The various fishing techniques were used in order to get a good depict of the population (Rogers and White, 2002).
Figure 7: Capture techniques, Fly fishing (A and G), bait fishing with worms, corn and crickets (E and F), Netting (D), electro-fisher (220 V) (B) and Casting nets (C) were used in order to obtain suitable fish for the study.
Captured fish were immediately placed in an aerated anaesthesia container containing clove oil (0.5 ml l⁻¹) and sedated until signs of narcosis became evident (Figure 8) (O’Brien et al., 2012). This was indicated by the fish losing its ability to maintain its orientation. A transmitter was attached by inserting two anchoring wires through the musculature of the *L. marequensis* at the base of the dorsal fin. Two spinal needles were pushed through the sedated individual; anchoring wires attached to the transmitter are then threaded through the spinal needles. Plastic washers and metal sleeves were then attached to the loose end of the anchoring wires and then secured in place by crimping the metal sleeves. The excess anchoring wire was then clipped off. During the recovery phase the fish’s measurements were taken namely: weight, standard length, fork length, total length and the girth (Figure 8). The fish was then allowed to recover fully and released once it could swim away strongly on its own accord. The recovery and early behaviour of the fish is monitored after capture and tagging. This allowed for an assessment of the effect of the capture and tagging procedure on the fish. Two weeks were given to each individual before perceived natural behavioural activities were documented (Rogers and White, 2002; O’Brien and De Villiers, 2011, O’Brien et al., 2012).

A minimum of 2% body:tag ratio meant that the individuals used for the study were all above 2 kg and exceeded the minimum fork length of adults reaching sexual maturity (Fouché 2009; Gaigher, 1969; Jepson, 2002). All fish were captured, tagged and release within the Crocodile Site with the exception of one individual fish (LMAR8) which was captured, tagged and released within 5km upstream from the Crocodile Site.

In addition to *L. marequensis* suitable *Hydrocynus vittatus* individuals were also considered in the study for comparative purposes (Table 2). The same capture, tagging and tracking techniques established for *L. marequensis* were implemented for the *H. vittatus* individuals.
Figure 8: The tagging procedures included: capture fish and the equipment needed to tag the fish (A). The fish is anaesthetised before operating and then submerged back into the water once the spinal needles have been inserted to start recovery (B, C). The spinal needles are inserted through the muscle below the dorsal fin and anti-biotics applied into the needles to coat the anchoring wires from the transmitter (D, E). Plastic and metal sleeves are placed onto the coated anchoring wires and crimped (F) more anti-biotics are applied around the tag and above the head (G). Fish measurements are recorded (H) the fish recovery starts (I) and the fish once fully recovered allowed to swim away freely (J, K).
Table 2: Species, tag type, capture location, monitoring period from capture date to the last day monitored (end date) and fixes during manual and remote monitoring during the study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Tag type</th>
<th>Tag no.</th>
<th>Mass (g)</th>
<th>Capture location (GPS location)</th>
<th>Capture Date</th>
<th>End Date</th>
<th>Days tracked</th>
<th>Manual monitoring fixes</th>
<th>Remote monitoring fixes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMAR2</td>
<td>ATS-1930</td>
<td>142.232</td>
<td>1800</td>
<td>-25.37872 31.71271</td>
<td>15/08/2009</td>
<td>15/05/2010</td>
<td>273</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>LMAR3</td>
<td>ATS-1930</td>
<td>142.113</td>
<td>2200</td>
<td>-25.37804 31.70758</td>
<td>15/08/2009</td>
<td>1/12/2009</td>
<td>108</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>LMAR4</td>
<td>ATS-1930</td>
<td>142.092</td>
<td>2100</td>
<td>-25.37804 31.70758</td>
<td>16/08/2009</td>
<td>15/05/2010</td>
<td>272</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>LMAR5</td>
<td>ATS-2030</td>
<td>142.153</td>
<td>2500</td>
<td>-25.37872 31.71271</td>
<td>16/08/2009</td>
<td>17/02/2011</td>
<td>550</td>
<td>109</td>
<td>-</td>
</tr>
<tr>
<td>LMAR6</td>
<td>ATS-1930</td>
<td>142.051</td>
<td>2100</td>
<td>-25.37872 31.71271</td>
<td>16/08/2009</td>
<td>15/05/2010</td>
<td>272</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>LMAR7</td>
<td>ATS-1930</td>
<td>142.032</td>
<td>3000</td>
<td>-25.22722 31.71271</td>
<td>19/09/2009</td>
<td>15/05/2010</td>
<td>238</td>
<td>65</td>
<td>-</td>
</tr>
<tr>
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<td>ATS-1930</td>
<td>142.072</td>
<td>2500</td>
<td>-25.39905 31.6784</td>
<td>19/06/2010</td>
<td>16/02/2011</td>
<td>242</td>
<td>46</td>
<td>-</td>
</tr>
<tr>
<td>LMAR9</td>
<td>ATS-2030</td>
<td>142.014</td>
<td>2600</td>
<td>-25.37884 31.71291</td>
<td>17/09/2010</td>
<td>14/11/2010</td>
<td>58</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>LMAR10</td>
<td>WW-Series II</td>
<td>6</td>
<td>2550</td>
<td>-25.3784 31.7197</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>232</td>
<td>8</td>
<td>6602</td>
</tr>
<tr>
<td>LMAR11</td>
<td>WW-Series I</td>
<td>25</td>
<td>2875</td>
<td>-25.3784 31.7197</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>232</td>
<td>7</td>
<td>2229</td>
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<tr>
<td>LMAR12</td>
<td>WW-Series II</td>
<td>7</td>
<td>2550 est</td>
<td>-25.3784 31.7197</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>232</td>
<td>2</td>
<td>6116</td>
</tr>
<tr>
<td>LMAR13</td>
<td>WW-Series I</td>
<td>17</td>
<td>2300 est</td>
<td>-25.3784 31.7197</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>232</td>
<td>4</td>
<td>316</td>
</tr>
<tr>
<td>LMAR14</td>
<td>WW-Series I</td>
<td>12</td>
<td>2250 est</td>
<td>-25.37885 31.71478</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>232</td>
<td>-</td>
<td>3324</td>
</tr>
<tr>
<td>LMAR15</td>
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<td>14</td>
<td>2300</td>
<td>-25.3784 31.7197</td>
<td>15/10/2011</td>
<td>01/06/2012</td>
<td>206</td>
<td>-</td>
<td>479</td>
</tr>
<tr>
<td>LMAR16</td>
<td>WW-Series IV</td>
<td>42</td>
<td>3900</td>
<td>-25.3784 31.7197</td>
<td>29/11/2011</td>
<td>01/06/2012</td>
<td>161</td>
<td>-</td>
<td>1262</td>
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<tr>
<td>HVIT1</td>
<td>ATS-2060b</td>
<td>142.322</td>
<td>1300</td>
<td>-25.37884 31.71469</td>
<td>19/08/2009</td>
<td>19/09/2009</td>
<td>31</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>HVIT2</td>
<td>ATS-1930</td>
<td>142.132</td>
<td>500</td>
<td>-25.37884 31.71469</td>
<td>19/08/2009</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HVIT3</td>
<td>WW-Series I</td>
<td>21</td>
<td>2200</td>
<td>-25.37864 31.71333</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>235</td>
<td>-</td>
<td>1552</td>
</tr>
<tr>
<td>HVIT4</td>
<td>WW-Series I</td>
<td>23</td>
<td>1380</td>
<td>-25.22438 31.42529</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>232</td>
<td>-</td>
<td>58</td>
</tr>
<tr>
<td>HVIT5</td>
<td>WW-Series I</td>
<td>19</td>
<td>2580</td>
<td>-25.37871 31.71586</td>
<td>19/09/2011</td>
<td>01/06/2012</td>
<td>232</td>
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<td>1626</td>
</tr>
<tr>
<td>HVIT6</td>
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<td>9</td>
<td>1750</td>
<td>-22.22422 31.43179</td>
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<td>-</td>
<td>3702</td>
</tr>
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<td>HVIT7</td>
<td>WW-Series I</td>
<td>13</td>
<td>2100</td>
<td>-22.22422 31.43179</td>
<td>22/09/2011</td>
<td>01/06/2012</td>
<td>229</td>
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<td>HVIT8</td>
<td>WW-Series I</td>
<td>15</td>
<td>4000</td>
<td>-22.22422 31.43179</td>
<td>20/10/2011</td>
<td>01/06/2012</td>
<td>201</td>
<td>-</td>
<td>1834</td>
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<td>HVIT9</td>
<td>WW-Series I</td>
<td>16</td>
<td>2000</td>
<td>-25.37885 31.71478</td>
<td>04/11/2011</td>
<td>01/06/2012</td>
<td>186</td>
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<td>2272</td>
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<td>HVIT10</td>
<td>WW-Series IV</td>
<td>41</td>
<td>3000</td>
<td>-25.22394 31.44291</td>
<td>23/11/2011</td>
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<td>167</td>
<td>-</td>
<td>899</td>
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<td>26/11/2011</td>
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<td>-</td>
<td>236</td>
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<td>HVIT12</td>
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<td>2000</td>
<td>-25.2249 31.42282</td>
<td>28/11/2011</td>
<td>01/06/2012</td>
<td>162</td>
<td>-</td>
<td>224</td>
</tr>
</tbody>
</table>

Note: LMAR = Labeobarbus marequnesis; HVIT = Hydrocynus vittatus
3.2.3 Monitoring techniques

The manual monitoring approaches involved the tracking or homing of the tagged fish during the study using the ATS or WW receivers and directional Yagi antennae. Manual monitoring methods used were based on techniques established by O’Brien and De Villiers (2011) (Figure 9). Tagged individuals were monitored from the banks of the Crocodile River, the narrow width of the river allowed for easy access to accurately (<1m from the observer) find fish. Weekly manual monitoring surveys were scheduled on a monthly basis throughout the year. This allow for a seasonal bio-telemetry evaluation. Habitat preferences and movement variables of the tagged fish were monitored and captured using data sheets and a field diary of anecdotal events. These variables included intra- and inter-species variation in the area where the fish were monitored (Godin, 1997).

The remote monitoring approaches involved the setting up of a coverage station allowing the continual tracking of individuals within the Crocodile Site. Data were then downloaded to a data management system (DMS) that could be accessed using an internet interface WW system or downloaded directly onto a personal computer. The ATS remote monitoring system made of one remote monitoring station and could obtain signals from tags up to 1000 m from the station. Two directional antennae were used to locate tagged fish up or down stream from the remote monitoring station. The WW remote monitoring network consisted of a base station and four relay stations that received data from the tag between 500 m to 1000 m from the remote monitoring stations. This included additional monitoring components which allowed for the continuous (every 10 minutes) monitoring of the tag individual. These data could be retrieved in real time from the DMS.
Figure 9: Schematic diagram presenting the monitoring methods followed out during monitoring surveys adopted from O'Brien and De Villiers (2011)
3.2.4 Habitat preferences

This included the consideration of the spatial behaviour (location and associated movement) in relation to available cover features and habitat biotopes measured using manual monitoring techniques. To determine the spatial behaviour of the fish the location of the tags within the Crocodile Site were used.

Spatial behaviour
The home range has been defined as the spatial range measured between the maximum upstream and downstream movement of a tagged individual over a certain time frame (Hodder et al., 2007). This is the area of a river that an organism requires to acquire food, shelter and breed successfully (Hodder et al., 2007). The home ranges of individuals monitored were evaluated using the known locations of tagged individual and the area in which they occurred whilst monitoring. Geographical Information Systems (GIS) were implemented to demarcate the home ranges utilised during the study by *L. marequensis*.

Cover features
Kramer et al. (1997) argues that the behaviour of the fish is orientated around the particular habitat most suitable for (maximising) the survival and reproductive success of an individual. Environmental variables were documented in an attempt to evaluate any existing relationships between the environmental variables and *L. marequensis* movement in the study. Table 3 shows the habitat variables measured within the study, these were adapted from O’ Brien and De Villers (2011). Habitat cover features specifically for fish included the consideration of the use of and or availability of undercut banks or root wads (UBRW), dead and or submerged trees (S-trees), complex substrate types such as boulder beds (S-boulders), rocky outcrops or emerging boulders (E-boulders) and submerged ridges (S-ridge), marginal aquatic (M-veg) and emergent vegetation (E-veg), island, water column and the top of or tail out of pools. Primary and secondary habitat types associated with tagged fish locations were considered to ensure that the area where the *L. marequensis* was found was descriptive of the area.
Table 3: Spatial habitat data associated with the observed locations and movement of the tagged L. marequensis monitored during the study.

<table>
<thead>
<tr>
<th>Behaviourable Variable</th>
<th>Substrate</th>
<th>Surface types</th>
<th>Habitat availability</th>
<th>Colour</th>
<th>Weather</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum displacement per Minute</td>
<td>Silt</td>
<td>No flow</td>
<td>Undercut bank/roots</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>Sand</td>
<td>Barely perceptible flow</td>
<td>Dead/submerged trees</td>
<td>Opaque</td>
<td>overcast</td>
<td></td>
</tr>
<tr>
<td>Gravel</td>
<td>Smooth and turbulent</td>
<td>Submerged-Boulders</td>
<td>Light brown</td>
<td>cloudy</td>
<td></td>
</tr>
<tr>
<td>Cobble</td>
<td>Ripple surface</td>
<td>Emerging-Boulders</td>
<td>Dark chocolate</td>
<td>lightrain</td>
<td></td>
</tr>
<tr>
<td>Boulder</td>
<td>Undular/broken standing waves</td>
<td>Submerged-Ridge</td>
<td></td>
<td>heavy rain</td>
<td></td>
</tr>
<tr>
<td>Bed Rock</td>
<td>Free falling</td>
<td>Marginal Vegetation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Chaotic flow</td>
<td>Aquatic Vegetation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wind</td>
<td>Water Colum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ripple Surface</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Habitat biotopes

Habitat biotopes included the availability and use of surface flow types and substrate type combinations or geomorphological habitat units as defined in Hirschowitz et al. (2007). Table 3 presents the surface flows and substrate types recorded in order to determine biotopes where tagged individuals were frequently found, such as runs, riffles rapids backwater areas, pools and glides (Hirschowitz et al., 2007).

3.2.5 Behavioural variables

A behavioural variable used in this portion of the study was movement measured either in activity counts (remote monitoring) or as maximum displacement per minute (MDPM) in metres per minute (m.min⁻¹). Depth was included as measurable behavioural variable. The MDPM includes the recorded displacement (m) of the fish during a five minute manual monitoring period spaced over ten minute intervals. The MDPM was recorded by estimating the distance (in metres) a fish moved during a five minute observation period and then calculated into metres per minute. Activity for remote data was measured in counts per minute while depth was measured in mm.

Natural cycles (time of day and seasons) and discharge

The behavioural variables were compared within six time of day (TOD) intervals, season intervals and established discharge classes according to flows observed during the study. Time of day intervals considered included: 00h00-04h00, 04h00-08h00, 08h00-12h00, 12h00-16h00, 16h00-20h00 and 20h00-24h00. Seasons were determined by three month periods starting from December to February (summer), March to May (autumn), June to
August (winter) and September to November (spring). Flow classes were determined using existing flows over the duration of the study obtained from Department of Water Affairs (DWA) (Station number: X2H046Q01 Crocodile River at Riverside/Kruger National Park) and then divided into three flow classes namely: low flows (0 m$^3$.s$^{-1}$ to 10 m$^3$.s$^{-1}$), moderate flow (10 m$^3$.s$^{-1}$ to 60 m$^3$.s$^{-1}$) and high flows (>60 m$^3$.s$^{-1}$).

### 3.2.6 Data analysis

Behavioural and environmental variables were broken down into seasons and then analysed using statistical and descriptive statistical techniques. Location and movement data were analysed using spatial analysis software (ARC GIS®), this approach allows for the assessment of spatial behaviour of individuals, the high use, preferred areas and some relationships between the locations of the tagged fish and the state of general environmental variables as shown in Hodder et al. (2007). Descriptive statistical analyses included the use of Windows Excel, (©2011, Microsoft Corporation). Ecosystem variable data associated with the behaviour of *L. marequensis* was recorded using primary variables (scored first) and secondary variables (scored second) and then combined with a weighting variable of a 2:1 ratio (primary variables: secondary variables). This indicated the importance of the tagged individual’s immediate variables associated with the tagged fish as well as any nearby important other variables. To determine movement patterns for cover features and biotopes, previously described time intervals, seasonal intervals and flow classes (see material and methods). Remote and manual behavioural data were analysed separately using MDPM and activity counts as the behavioural variable (movement) respectively (O’ Brien et al., 2012). This was done using a mixed-model analysis of variance (ANOVA) approach with a random coefficients model (Littell et al., 1996) and Akaike’s information Criteria (AIC) model selection (Burnham and Anderson, 1998) were used. Analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC).

### 3.3 Results

Of the 16 *L. marequensis* and 12 *H. vittatus* monitored in the behavioural study, nine of the *L. marequensis* and two *H. vittatus* were attached with ATS tags and the remainder with WW tags. *Labeobarbus marequensis* mean weight was 2464.1 g (SD=±476.6 g) with the
mean standard length 453 mm (SD=±33.8 mm). *Hydrocynus vittatus* mean weight was 1874.8 g (SD=±879.7 g) with the mean standard length 45 mm (SD=±81.2 mm) (Table 2).

Advance Telemetry Systems and WW manual monitoring observations totalled 479 fixes from 15 individuals (Table 2). Wireless Wildlife remote monitoring observations totalled 39505 strings of data from 17 individuals; two were tagged with WW-series ν (depth censor) tags (Table 2). Remote monitoring from the ATS system recorded over 44171 strings of data over a period from the 21 September 2010 to 12 November 2010. Wireless Wildlife tags obtained 20327 *L. marequensis* and 19130 *H. vittatus* strings of data for movement and 1262 *L. marequensis* and 899 *H. vittatus* strings for depth. Wireless wildlife tags were only able to obtain remote seasonal data for spring, summer and autumn for activity counts and spring and summer for depth.

### 3.3.1 Habitat Preference

**Spatial behaviour**

The location of individuals LMAR 1-9 was plotted within in the Crocodile Site and mapped to depict the home range of each individual could be depicted (Figure 10, 11 and 12). These individuals were selected as there were sufficient data to graphically depict their movements along the Crocodile Site. This showed that *L. marequensis* preferred the reach of river around the Crocodile Site. The remote data from the ATS remote station showed that LMAR5 and LMAR9 were found constantly in the Crocodile site for a period of two months before the station was destroyed during floods. Key areas were identified within the Crocodile Site which was used frequently by tagged individuals (Figure 13).
Figure 10: The home ranges of *Labeobarbus marequensis* for individuals LMAR 1 (A), LMAR 2 (B) and LMAR 3 (C).
Figure 11: The home ranges of *Labeobarbus marequensis* for individuals LMAR 4 (A), LMAR 5 (B) and LMAR 6 (C).
Figure 12: The home ranges of *Labeobarbus marequensis* for individuals LMAR 7 (A), LMAR 8 (B) and LMAR 9 (C).
Fish caught and tagged in a location had a tendency to return to and frequently be found within its tagged location (Table 2). This was evident with LMAR3 and LMAR4 individuals. All individuals show clear home ranges with high frequency to key areas with distinct cover features and habitat biotopes (Table 4). Fish LMAR3 and LMAR4 were frequently associated with key area 1 (Figure 13 and 14). Two other distinct channel types were key area 3 and key area 4 (Figure 13 and 14). The key area 3 had a deep run (through bed rock) and opened up into a reeded southern bank and bedrock northern bank with back flow, while key area 4 had a pool flowing into a run with a reeded southern bank and bedrock northern bank. This location was well utilized by tagged individuals LMAR 1, LMAR 6, LMAR 7 and LMAR 4. Individuals LMAR2 and LMAR5 were frequently found together in the reach of river named area 4. It must be noted that at the key area 4 in the channel produced five of the *L. marequensis* of tagging criteria for the WW component of the study. Where as the other locations were not nearly as active with *L. marequensis* in 2011 than in 2009 (Table 2).

Table 4: Cover Features and Habitat Biotopes for the key area’s within the Crocodile Site

<table>
<thead>
<tr>
<th>Area</th>
<th>Dominant cover feature</th>
<th>Habitat biotopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Emerging Boulders, Marginal Vegetation</td>
<td>Run</td>
</tr>
<tr>
<td>2</td>
<td>Submerged Boulders, Emerging Boulders</td>
<td>Glide</td>
</tr>
<tr>
<td>3</td>
<td>Submerged Boulders, Marginal Vegetation</td>
<td>Glide</td>
</tr>
<tr>
<td>4</td>
<td>Submerged Boulders, Marginal Vegetation</td>
<td>Glide and Run</td>
</tr>
</tbody>
</table>
Figure 14: Pictures depicting the key areas in the Crocodile Site and main habitats where tagged individuals were found most frequently: Key area 4 (A); A channel (B); Key area 3 (C); the location where LMAR2, LMAR5, LMAR6 and LMAR9 were tagged (D and E); Key area 2 (G, H) where LMAR1 was tagged; Key area 1 where LMAR3 and LMAR4 were tagged and found regularly (F)
Cover features
Differences in the cover features associations between *L. marequensis* and *H. vittatus* were found. *Hydrocynus vittatus* was more commonly (33 %) associated with aquatic vegetation than *L. marequensis* (1.4 %). Submerged boulders were used by both species frequently, 32.4 % and 66.7 % for *L. marequensis* and *H. vittatus* respectively. There was a significant increase in movement for *L. marequensis* when associated with S-boulder cover than any other cover feature tested (p=0.0001). The most frequently visited cover feature was S-boulders (32.38 %). The other habitats that took preference were habitats of similar description being UBRRW (22.92 %), E-boulders (13.37 %) and M-veg (12.76 %) all having some permanent structure with the exception of M-veg. A-veg (1.39 %) along with substrate ridge (1.39 %) was the least presented cover feature (Figure 15).

![Diagram showing cover features and biotopes preference for L. marequensis and H. vittatus](image.png)

Figure 15: Cover features (A and B) and Biotopes preference *vittatus* (B and D).

The movement of the *L. marequensis* and *H. vittatus* differed between seasons and flow discharge categories, Seasonal differences were associated with day length and
temperature fluxes while changes in movement between flow (discharge) categories occurred despite the seasonal variations. Although flow discharges varied between seasons with spring having a mix of high flows and extreme low flows. Average discharges per season included: 86.53 m$^3$.s$^{-1}$ (summer), 35.22 m$^3$.s$^{-1}$ (autumn), 8 m$^3$.s$^{-1}$ (winter) and 14.54 m$^3$.s$^{-1}$ (spring)(Figure 16).

![Graph showing discharges over time](image)

**Figure 16**: Discharges (m$^3$.s$^{-1}$) for the Crocodile River and season correlation for the duration of the study.

Cover features used differed seasonally (Figure 17). During autumn and winter only three cover features were utilised. In spring and summer a higher diversity of cover features were used. Submerged boulders were utilised the greatest through all seasons, summer (26.35 %), autumn (39.39 %), winter (51.04 %) and spring (33.55 %). Marginal vegetation featured in three of the four seasons namely: summer (12.57 %), winter (17.71 %) and spring (14.69 %). Aquatic vegetation and substrate ridge only featured in summer (both at 3.19 %). Cover features used in different flow discharges showed that despite different discharges S-boulders was used more frequently than any other cover feature (low flow, 35.86 % and moderate flow, 44.94 % and high flow, 22 %) (Figure 18). High flow cover features used were UBRW (25.27 %) and S-boulders (22 %). Undercut bank/root wad featured with S-boulder as used cover feature in moderate flows at 28.46 %. Marginal vegetation was utilised with S-boulder cover in low flows (19.72 %) while it did not feature in moderate flows. Note that aquatic vegetation was only 3.49 % in high flows while non-selected in low and moderate flows. High and low flow showed high use for various cover features where moderate flow had high use of S-boulders.
Figure 17: Cover features (A-D) and Biotopes (E-H) of *Labeobarbus marequensis* for different seasons, Summer (A and E), Autumn (B and F), Winter (C and G) and Spring (D and H).
Figure 18: Cover features (A-C) and Biotopes (D-F) of *Labeobarbus marequensis* for different flows (discharge), low flow (A and D), moderate flow (B and E), high flow (C and E).
Habitat biotopes

Habitat biotopes used by *L. marequensis* were glides 55 % of the time. Run and pool biotopes had high preferences with 21.2 % and 11.9 % respectively. *Hydrocynus vittatus* were associated with different habitat biotopes than *L. marequensis* frequenting the riffle biotopes. This showed *L. marequensis* used deeper biotopes than that of *H. vittatus*.

Biotopes used in different season varied in a manner similar to that of cover features (Figure 17). Autumn and winter had only three frequented biotopes whilst summer and spring had a high diversity of biotopes frequented. Riffle biotopes being frequented in autumn more than in the other seasons. Glide biotope types were dominated throughout all season for *L. marequensis*. In winter pools, glides and runs were highly utilised. Habitat biotopes during moderate and high flows showed similar uses for glide, pool and run. Low flows had the most diverse biotope use.

3.3.2 Behaviour variables

The influences of the natural cycles on the movement of *L. marequensis* showed to change within the time of day, season and discharges. Further depth preferences were found across these natural cycles.

Time of day

*Labeobarbus marequensis* exhibited diurnal activity with heightened movements during mid-day. While *H. vittatus* displayed crepuscular movements that were heightened during the early mornings (Figure 19). Increases in movement were also associated with depths of *L. marequensis* over the day period increasing during midday while *H. vittatus* depth increased during early morning periods (Figure 19).
Figure 19: The movement (activity counts) (A and B) and depth (mm) (C and D) relationship between time of day for *Labeobarbus marequensis* (A and C) and *Hydrocynus vittatus* (B and D).

**Seasons**

*Labeobarbus marequensis* movement increased during spring while decreasing from spring through to autumn (Figure 20). Depth from spring to winter also change in that *L. marequensis* took up shallower depths in summer than in spring. *Hydrocynus vittatus* had insufficient data over seasons to determine difference between seasons.
Flow (discharge)
Flows showed to have an effect on the movement of *L. marequensis* in that during low and moderate flows movement was higher than in high flows where almost no movement was found (Figure 21). Depth preferences were more variable during low flows than moderate flows. *Hydrocynu vittatus* had insufficient data over seasons to determine difference between seasons.
Figure 21: The movement (activity counts) (A) and depth (mm) (B) relationship between flows (discharges) for *Labeobarbus marequensis*.

3.4 Discussion

3.4.1 Habitat preferences

The GIS data showed that *L. marequensis* establish home ranges within the study area with some individuals establishing focal areas. No observations of fish migrating out of the Crocodile Site were observed indicating the use of the Crocodile Site as there home range. Within these home ranges fish frequented specific locations over other locations. This suggests that the population sample is specific to the Crocodile Site showing the importance of these biotopes and cover feature found within the reach, unlike the rest of the Crocodile River (Hill et al., 2001). Furthermore this suggests that adult *L. marequensis* in the Crocodile River have relatively small home ranges and do not use reaches (>5 km) of the river extensively. The sessile behaviour of the adult *L. marequensis* (>2 kg) observed in the study suggests after maturity home ranges are established in small reaches that meet their biological functioning. The extent of these home ranges appears to be dependent on the availability of habitats and will possibly be affected by population pressure. Fouché (2009) showed that *L. marequensis* exhibit reach scale migrations that may exceed 50 km. These migrations may involve smaller sub-adult *L. marequensis*.

Cover features and habitat biotopes used by *L. marequensis* were found to be similar to that described by Pienaar (1978) apart from sandy reaches which appeared to be avoided by the tagged individuals. Contrary to what Fouché (2009) described this study showed that large *L. marequensis* frequented slow, deep habitat types as apposed to fast, deep and fast, shallow habitats depending on the age of the fish. Further, this study showed that *L. marequensis* frequent different habitats during different seasons and flows. Habitat use during different seasons and flows has not previously been described for adult *L. marequensis*. Seasonal changes determine the daylight length and its effects on an organism. In this study adult *L. marequensis* showed to frequent various habitat biotopes and cover features during summer and spring, differing to winter and autumn. This indicates some form of change in their biology during those seasons which may cause various habitat biotopes and cover feature required for those functions. Spawning during early spring and late summer may affect these habitat changes during the seasons (Fouche, 2009). Under
changing conditions habitat use varies, with fish changing location in response to
to changes in season and flow seeking out preferred habitat types (Kramer et al., 1997).
This was found within this study in that deeper biotopes were preferred during both
moderate and high flows. The diverse cover features in high flows and summer and
spring periods could be due to the availability of different habitat types that come with
changes in flow. However this is contradicted in that the low flow also show diversity
in cover features frequented although not as diverse as in high flows. Habitat
biotopes show distinct differences between low flow use and moderate to high flow
use indicating more flow related changes than to seasons. During low flows adult L. 
marequensis are more inclined to seek out cover features related to feeding and
protection from predation in good water clarity, and used certain cover features in
high flows to shelter from the high flows.

*Labeobarbus marequensis* were shown to use habitats that differed to *H. vittatus.*
*Labeobarbubs marequensis* frequented glides and runs while *H. vittatus* took
preference to riffles and backwaters. This was similarly to Thorstad et al (2003) in
that backwaters featured in *H. vittatus* as a habitat used. Aquatic vegetation played a
large role in the cover feature use of *H. vittatus*. The species is known to hunt small
fish within and adjacent to aquatic vegetation (O’Brien et al., 2012). Small fish can be
seen using aquatic vegetation as cover. *Labeobarbus marequensis* show very little
preference for aquatic vegetation as they are not strictly piscivorous and need not
chase the fish using the aquatic vegetation as cover. These findings demonstrate the
differences in the feeding behaviour of the species considered including the
predatory nature of *H. vittatus* and the foraging behaviour of *L. marequensis."

**3.4.2 Behaviour variables**

Findings of the behavioural experiments showed that natural ecological cycles affect
the movement of *L. marequensis*. Movement over the TOD show *L. marequensis* to
be active during mid-day periods. Movement during nocturnal periods have low
counts and in case no counts this indicates *L. marequensis* to be resting during those
periods. The movement of *L. marequensis* over TOD show them to be diurnal in
habit. Depth showed *L. marequensis* to move into deeper waters during the mid-day
periods this could be due to increased visibility allowing them to feed deeper. The
deeper they feed the better their evasion from terrestrial predators, such as the
African fish eagle (*Haliaeetus vocifer*) (Steyn, 1982). The increase in movement with
the increase in depth over the same time of day indicates that the species forages in deeper waters during the day. *Hydrocynus vittatus* showed crepuscular movements that increased early mornings until mid-day before decreasing. This shows that heightened feeding periods occur during different times of the day between *L. marequensis* and *H. vittatus*. This was also evident in depth between the two species in *L. marequensis* occurring in a different depth profile to *H vittatus* with *H. vittatus* deeper during early mornings.

Seasonal changes showed *L. marequensis* movement decreases in summer. Summer periods have variable flows related to rainfall and discharge, *L. marequensis* during these periods move into cover features and wait out unfavourable conditions. This co-related the same way with the habitat preferences for *L. marequensis* during different seasons. High movements during spring co-relate to the spawning periods of *L. marequensis* (Fouché, 2009), therefore indicating the change in day length to be a cue for heightened activity during spring (Fouché, 2009). Other yellowfishes such as *Labeobarbus aeneus* and *Labeobarbus kimberleyensis* have also been shown to make use of different habitat types and demonstrate reductions in movements in the Vaal River due to changes in variable states in summer (O’Brien et al., 2011). Water fluxes are more erratic and clarity is poor during high fluxes in the River during summer, thus the summer period may not be a suitable period for *L. marequensis*. These conditions are drastically different to the spring period, where flow is constant and clarity is good.

Flow changes can be regarded as the most important influence on a river system, habitats are subject to strong temporal variation in flow discharge, with the rise and fall of water level conditions change at each specific location (Rogers and O’keeffe, 2003). Townsend (1989) indicates the variability in the flow of water to be the major agent for disturbances in the complexity of rivers. *Labeobarbus marequensis* showed decrease in movement with the increase in flow to a point where no movement occurred during high flows. This shows the increased flow reduces the movement of *L. marequensis* indicating that low and moderate flows are better suited for *L. marequensis*. High flows are turbid and effect the clarity, if energy conservation is the primary goal of an organism there may come a point when changes in visibility due to high flows may be no longer be beneficial to continue feeding (Stephens and Krebs, 1986). This indicates that *L. marequensis* will use constant flows which do not affect the visibility of the water allowing the species to feed normally. Therefore high flows appear to be a difficult occurrence for *L. marequensis* in the Crocodile River due to
reduction in habitat suitability and time available for foraging. These findings indicate that careful attention should be afforded to the timing and duration of flow releases during summer in the Crocodile River to ensure that they do not occur consistently and that unnatural changes in flow during autumn, winter and spring should be avoided. Should consistent changes in flows in the Crocodile River during these unnatural periods persist, disruptions in the behaviour of *L. marequensis* similarly to those observed during high flows may occur. This may negatively impact on the biology of the species and ultimately affect the survivability of the population.

The data acquired from the *H. vittatus* showed large differences with *L. marequensis*. A huge difference was found between the two species MDPM. *Hydrocynus vittatus* was found to have an average of 4.08 m.min\(^{-1}\) and *L. marequensis* 1.17 m.min\(^{-1}\). This shows a big difference in feeding behaviour for the two species. *Labeobarbus marequensis* being omnivorous and *H. vittatus* piscivorous could determine displacement effort needed to find food (Skelton, 2001; Fouché, 2009). During resting periods *H. vittatus* was found to rest for the night underneath a mat of water hyacinth in still backwater. *Hydrocynus vittatus* showed the same active periods as *L. marequensis* yet took up different resting positions. Another difference observed was the feeding depth of the *H. vittatus* in comparison to the *L. marequensis*. *Labeobarbus marequensis* was often found in depths >1m while *H. vittatus* would chase small fingerlings up into shallows exposing their dorsal fin before retreating back into deeper waters. This showed that *H. vittatus* made more use of the water column than *L. marequensis*, which would stay closer to the river bed. Although *H. vittatus* are said to be piscivorous (Skelton, 2001), they were observed to take other organism such as, nymphs and water monitor lizard as observed in this study.

### 3.5 Conclusion

This study successfully demonstrated that adult *L. marequensis* established defined home ranges that are limited to small reaches (<5 km) of the Crocodile River in the KNP. Within these home ranges the *L. marequensis* utilised focal areas (key areas) suggesting that specific habitat types that are relatively uncommon in the study area were used by the species. Despite the various documented habitat requirements for *L. marequensis*, this study showed the species utilises different habitats during different flow conditions and seasons. Furthermore the findings indicate that the
habitat uses for *L. marequensis* include glides and runs with preferences for submerged boulders and undercut bank or root wads with other cover features such as emerging boulders and submerge trees.

The behavioural experiment revealed that *L. marequensis* respond to seasonal, flow and other natural cycle changes. Time of day showed that *L. marequensis* was active during mid-days periods and move into deeper water during those periods. Seasonal variation in movement showed that during spring periods movement was higher than that in summer. Flows affect the movement of fish in that when flows become more erratic and turbid fish movement decreases. Low constant flow favours the movement of *L. marequensis*. Flows have the ability to change habitats and thus the changes in flow were shown to effect the change in habitat use by *L. marequensis*. Findings showed that the normal behaviour of *L. marequensis* was disrupted by moderate (10 m$^3$.s$^{-1}$ to 60 m$^3$.s$^{-1}$) to high (>60 m$^3$.s$^{-1}$) increases in flows which currently predominantly occur during summer. Should the frequency of these changes in flow increase noticeably or frequently occur in other seasons the survivability of the local *L. marequensis* population may be threatened. These findings do not suggest that all flows negatively impact the survivability of the local population, but due to the behavioural response of the individuals considered in the study, extensive, frequent elevations in moderate and high flow and disruptions to the timing of the flows may affect the population.
4 CHAPTER 4: METAL BIOACCUMULATION IN LOWVELD LARGESCALE YELLOWFISH (LABEOBARBUS MAREQUENSIS), TIGERFISH (HYDROCYNUS VITTATUS) AND SHARPTOOTH CATFISH (CLARIAS GARIEPINUS) FROM THE CROCODILE AND SABIE RIVERS, KRUGER NATIONAL PARK.

4.1 Introduction

Rivers are uni-directional, serving as drains for the landscape, accumulating metals brought in by wind, run-off water and humans from their surroundings (Dallas and Day, 2004). These pollutants are then exposed to aquatic organisms which live within rivers. Discharges of metals into the aquatic ecosystem may result in numerous physical, chemical and biological responses, which can be separated into two categories: (1) the effect of the environment on the metal, and (2) the effect of the metal on the environment (Marx and Avenant-Odelwage, 1998). The latter is of importance as organisms cannot break down chemical elements like metals and store excess metals, showing over exposure of those metals found in storage tissues (Dallas and Day, 2004). Further affecting the biological response to a change in density, diversity, community structure and species composition of populations (Marx and Avenant-Odelwage, 1998).

Measurements of chemicals such as metals by direct chemical analysis in water and sediment are limited in reliability. Chapman (1997) and Rainbow (2007) stress that at present bioaccumulation studies are used to provide information on contaminant-specific bioavailability; assist in identifying possible causative agent(s) of toxicity; and relate body burdens to food chain accumulation values relative to secondary poisoning or bio-magnification. These authors caution against the application of bioaccumulation to identify potential toxicity caused by metals, as toxic reactions are related to a threshold concentration of metabolically available metal and not to total accumulated metal concentration. Therefore the bioaccumulation results that are presented should be seen as a biological measure of metal bioavailability within the study area.

The Kruger National Park (KNP) is orientated north-south and has five major river systems flowing from east to west through it. Implications of this geographic arrangement are that the management of the rivers in the park and the adjacent terrestrial systems are fundamentally affected by the management of the catchment
upstream of the park and are largely out of the district's control (O'Keeffe and Rogers, 2003). Land uses outside KNP which effect quality and quantity of water flowing into KNP include; mining, irrigation, agriculture and forestry (O’Keeffe and Rogers, 2003; DWAF, 2004; Fouché, 2009; Rogers and Luton, 2011). Despite negative impacts outside its boundaries, KNP is still mandated to conserve that which is within its boundaries (Mabunda et. al., 2003). For this reason the KNP is largely dependent on the ecological reserve to help maintain its water needs in both quality and quantity (DWAF, 2004).

The Crocodile River is the third largest river system flowing through KNP, with 115 km of its 320 km length flowing through the KNP boundary, its catchment area is the third largest of KNP rivers measured at 10,420 km² (O’Keeffe and Rogers, 2003). Unlike the other two larger rivers, i.e. the Olifants and Letaba River, the Crocodile River forms the southern boundary of the KNP and is exposed to water extractions for irrigation. Impoundments in the upper reaches release abnormally high and stable amounts of winter flows to support irrigation on the southern banks opposite KNP and is heavily infested with water hyacinth (O’Keeffe and Rogers, 2003). Kleynhans et al. (1992) recorded the loss of *Chiloglanis bifurcus* and other fish species during a papermill spill in the upper reaches of the Crocodile River. The spill did not reach as far downstream as the KNP. Kleynhans et al. (1992) does mention the possibility of more pollution spillages and that the increase in the forestry industry will have an increase in the pollutants, affecting the water quality of the Crocodile River. Water usage for mining purposes in the Crocodile River amounts to 3% and makes up the entire amount for the Inkomati WMA (Rogers and Luton, 2011). Mining activity consists mainly of coal mines in the Highveld regions, gold mines near Barbeton and a magnetite mine near Malelane (Rogers and Luton, 2011). Recent concerns for *L. marequensis* on the Crocodile River have risen as a decline in the population has been observed in surveys KNP management (pers. comm. A. Deacon, 2009).

Despite the Sabie River having been reduced to a putrid, sterile stream by the slimes of a series of small gold mines along its head waters in the 1920’s and 1930’s, it has recovered to be regarded as a pristine river in comparison to the other 4 major rivers flowing through the park, namely: Luvhuvhu, Letaba, Olifants, Sabie and Crocodile Rivers (Plenaar, 1978; O’Keeffe and Rogers, 2003). Of its natural mean annual run-off (MAR) 70% is retained and 30% being used for forestry and irrigations of fruit

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1 Dr A Deacon, Scientific Service KNP (October, 2009).
farms along the upper catchment (O’Keeffe and Rogers, 2003). With over half the length of the Sabie River situated in the park and the tributaries north of the KNP boundary being in rural or game reserve areas, the rivers impacts are low and thus can be regarded as a pristine river (Hills et al., 2001).

*Labeobarbus* spp. are regarded as good indicators of aquatic ecosystem health, as they require rivers and man made lakes that have diverse habitat, good water quality and few or no alien fishes and plants (Impson et al., 2007). Considering the decline in the *L. marequensis* population on the Crocodile River it is of importance in understanding the presence of stressors in the aquatic environment on this population. Biomarkers of exposure in the form of bioaccumulation of metals indicate the extent of stressors on organisms (Seymore et al., 1995; Van der Oost et al., 2002). *Labeobarbus marequensis* in past studies have been used to determine the extent of the effects of pollutants on the species and river systems (Seymore et al., 1995; Barker, 2006).

The aim of this chapter is to determine the extent of metal bioaccumulation in three fish species from two rivers in the KNP. To achieve the aim three objectives were set:

- To determine the differences in metal bioaccumulation in muscle tissue of *L. marequensis*, *H. vittatus* and *C. gariepinus* from the Crocodile and Sabie Rivers;
- To determine if there are differences in bioaccumulation patterns between the two major river systems and,
- To determine if there are differences in bioaccumulation patterns between high flow and low flow periods.

4.2 Materials and Methods

The bioaccumulation of metals in fish from the Crocodile and Sabie Rivers was determined during a high flow season (June 2010) and low flow season (September 2010). Surveys for the two flow periods were conducted towards the end of each seasonal flow period in order to allow for the accumulation of metals within the fish to reflect the respective flow period. Fish samples were collected from the focal area along the Crocodile River at the Lwakhale confluence and at two sites along the Sabie River (Figure 1) Fish were sacrificed by severing the spinal cord before removing the muscle tissue for analyses. Tissue samples were dissected using pre-
cleaned dissecting equipment and care was taken not to contaminate the samples and then placed in a sterile zip-lock bag and frozen at -20°C prior to analysis.

Tissue samples were dried at 60 °C for 48 hours and then weighed and diluted in 1% HNO₃ (AR) with Milli-Q water before digestion using HNO₃/H₂O₂ and using an Ethos microwave digestion system. Metal concentrations were determined using a Thermo inductively coupled plasma optical emission spectrophotometer (ICP-OES) and an inductively coupled plasma mass spectrophotometer (ICP-MS). All samples were analysed for arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn). Yttrium was used as an internal standard to correct for the interference from high-dissolved solids in the different matrices. Concentrations are expressed on a dry weight basis. Quality control of metal measurements in sediment and muscle tissue was verified by including process blanks and certified reference material (CRM 278, muscle tissue Community Bureau of Reference, Geel, Belgium). All samples were processed and analysed in the University of Johannesburg (UJ) laboratory.

Statistical analyses of significant differences, between sites and species were undertaken using one-way analyses of variance (ANOVA). Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests (Zar, 1996), respectively, prior to applying post-hoc comparisons. Post-hoc comparisons were made using the Scheffe test for homogeneous or Dunnett’s-T3 test for non-homogenous data. The use of either one of the two testes resulted in the determination of significant differences (p<0.05) between variables.

4.3 Results

Data were analysed and compared between species, river and flows. There were significant temporal differences for Al, Fe and Se bioaccumulation in muscle tissue of *L. marequensis* in the Crocodile River, concentrations of these metals being significantly higher in high flows (Figure 22 and 23). There were significant differences (p<0.05) in Fe bioaccumulation between *C. gariepinus* and *H. vittatus* and Zn bioaccumulation between *L. marequensis* and *C. gariepinus* in the Crocodile River during the high flow survey, with Zn and Fe concentration being significantly higher in *L. marequensis* and *C. gariepinus* respectively (Figure 22 and 23). During the Crocodile River low flow survey, significant differences in Co, As and Se
bioaccumulation between *L. marequensis* and *H. vittatus* was recorded with Co and Se being significantly higher in *H. vittatus* (Figure 22 and 23).

There were significant temporal differences for Cr, Fe and Se bioaccumulation in muscle tissue of *L. marequensis* in the Sabie River, concentrations of these metals being significantly higher in high flows (Figure 24 and 25). There were significant differences (p<0.05) in Al and Pb bioaccumulations between *L. marequensis* and *C. gariepinus* and Pb bioaccumulations between *L. marequensis* and *H. vittatus* in the Sabie River during the high flow survey, with *L. marequensis* having significantly higher concentrations between the species (Figure 24 and 25). During the Sabie River low flow survey, significant differences (p<0.05) in Mn and Se were found between *L. marequensis* and *H. vittatus* and Se between *L. marequensis* and *C. gariepinus* with *L. marequensis* being significantly higher than *H. vittatus* and *C. gariepinus*. No significant differences between *H. vittatus* and *C. gariepinus* was recorded for the Sabie River (Figure 24 and 25).

There were significant spatial differences for Cd bioaccumulation in muscle tissue of *L. marequensis* during high flows, with the Crocodile River population having significantly higher levels for Cd bioaccumulation (Figure 26 and 27). There were significant differences (p<0.05) in Co bioaccumulations in *H. vittatus* with the Crocodile River population having significantly higher levels for Co bioaccumulation (Figure 26 and 27). No significant differences were found in *C. gariepinus* (Figure 26 and 27).

There were significant spatial differences for Al, Cr and Cd bioaccumulations in muscle tissue of *L. marequensis* during low flows, with the Crocodile River population having significantly higher levels for Al, Cr and Cd bioaccumulation (Figure 28 and 29). There were significant differences (p<0.05) in Co and Cd bioaccumulations in *H. vittatus* and As in *C. gariepinus*, with the Crocodile River population having significantly higher levels for Cd and As bioaccumulation (Figure 28 and 29).
Figure 22: Metal bioaccumulation in muscle tissue (mean ± standard error in µg/g dw) of *Labeobarbus marequensis* (n\(^{HF}\)=4 & n\(^{LF}\)=6), *C. gariepinus* (n\(^{HF}\)=1 & n\(^{LF}\)=9) and *H. vittatus* (n\(^{HF}\)=10 & n\(^{LF}\)=3) during the high and low flow sampling surveys in the Crocodile River. Within surveys, means with common alphabetical superscript indicate significant differences between species (p<0.05), while asterisks indicate significant differences between surveys for a particular species.
Figure 23: Metal bioaccumulation in muscle tissue (mean ± standard error in μg/g dw) of *Labeobarbus marequensis* (n\(^{HF}\)=4 & n\(^{LF}\)=6), *C. gariepinus* (n\(^{HF}\)=1 & n\(^{LF}\)=9) and *H. vittatus* (n\(^{HF}\)=10 & n\(^{LF}\)=3) during the high and low flow sampling surveys in the Crocodile River. Within surveys, means with common alphabetical superscript indicate significant differences between species (p<0.05), while asterisks indicate significant differences between surveys for a particular species.
Figure 24: Metal bioaccumulation in muscle tissue (mean ± standard error in μg/g dw) of *Labeobarbus marequensis* (n^HF^=3 & n^LF^=6), *C. gariepinus* (n^HF^=9 & n^LF^=4) and *H. vittatus* (n^HF^=8 & n^LF^=9) during the high and low flow sampling surveys in the Sabie River. Within surveys, means with common alphabetical superscript indicate significant differences between species (p<0.05), while asterisks indicate significant differences between surveys for a particular species. Below detectable limits is indicated by BDL.
Figure 25: Metal bioaccumulation in muscle tissue (mean ± standard error in μg/g dw) of *Labeobarbus marequensis* \( (n_{HF}^{HF}=3 & n_{LF}^{LF}=6) \), *C. gariepinus* \( (n_{HF}^{HF}=9 & n_{LF}^{LF}=4) \) and *H. vittatus* \( (n_{HF}^{HF}=8 & n_{LF}^{LF}=9) \) during the high and low flow sampling surveys in the Sabie River. Within surveys, means with common alphabetical superscript indicate significant differences between species \( (p<0.05) \), while asterisks indicate significant differences between surveys for a particular species.
Figure 26: Metal bioaccumulation in muscle tissue (mean ± standard error in µg/g dw) of *Labeobarbus marequensis* (n<sub>CR</sub>=6 & n<sub>SR</sub>=6), *C. gariepinus* (n<sub>CR</sub>=9 & n<sub>SR</sub>=4) and *H. vittatus* (n<sub>CR</sub>=3 & n<sub>SR</sub>=9) between the Crocodile River and Sabie River during low flows. Within surveys, means with common alphabetical superscript indicate significant differences between species (p<0.05), while asterisks indicate significant differences between surveys for a particular species. Below detectable limits is indicated by BDL.
Figure 27: Metal bioaccumulation in muscle tissue (mean ± standard error in μg/g dw) of Labeobarbus marequensis ($n^{CR}=6$ & $n^{SR}=6$), C. gariepinus ($n^{CR}=9$ & $n^{SR}=4$) and H. vittatus ($n^{CR}=3$ & $n^{SR}=9$) between the Crocodile River and Sabie River during low flows. Within surveys, means with common alphabetical superscript indicate significant differences between species ($p<0.05$), while asterisks indicate significant differences between surveys for a particular species. Below detectable limits is indicated by BDL.
Figure 28: Metal bioaccumulation in muscle tissue (mean ± standard error in μg/g dw) of *Labeobarbus marequensis* (n^CR=4 & n^SR=3), *C. gariepinus* (n^CR=1 & n^SR=9) and *H. vittatus* (n^CR=10 & n^SR=8) between the Crocodile River and Sabie River during high flows. Within surveys, means with common alphabetical superscript indicate significant differences between species (p<0.05), while asterisks indicate significant differences between surveys for a particular species. Below detectable limits is indicated by BDL.
Figure 29: Metal bioaccumulation in muscle tissue (mean ± standard error in µg/g dw) of *Labeobarbus marequensis* (n<sub>CR</sub>=4 & n<sub>SR</sub>=3), *C. gariepinus* (n<sub>CR</sub>=1 & n<sub>SR</sub>=9) and *H. vittatus* (n<sub>CR</sub>=10 & n<sub>SR</sub>=8) between the Crocodile River and Sabie River during high flows. Within surveys, means with common alphabetical superscript indicate significant differences between species (p<0.05), while asterisks indicate significant differences between surveys for a particular species.
4.4 Discussion

It is well documented that metal bioaccumulation is affected by many factors including species specific differences (van der Oost et al., 2003). *Labeobarbus marequensis*, *C. gariepinus* and *H. vittatus* are all at different trophic levels and therefore exposed to different routes of metal uptake. *Labeobarbus marequensis* and *C. gariepinus* are regarded as omnivorous where *H. vittatus* are primarily carnivorous, even said to be piscivorous in adults (Bell-Cross, 1965-66; Skelton, 2001). *Labeobarbus marequensis* have intestines long enough to characterise them as herbivorous but are known to eat small invertebrates and fish, although during surveys stomach content consisted primarily of filamentous algae, they are also regarded as bottom feeders shifting through cobble and bottom substrate for food (Bell-cross and Minshull, 1988; Fouché, 2009). This could be the reason for the significantly different concentrations of Co, As, Se and Zn in *L. marequensis* from the Crocodile River and Al, Mn, Se and Pb in *L. marequensis* from the Sabie River when compared to *H. vittatus* and *C. gariepinus*.

Metals occur in suspended water and the sediments transported and deposited by water (Dallas and Day, 2004). Therefore fish exposed to sediment when feeding expose themselves to the metals within the sediment over and above the metals in the water. Being bottom feeders *L. marequensis* could possibly ingest more metals and possibly be exposed to them when feeding in sediment in relation to *C. gariepinus* and *H. vittatus*. *Hydrocynus vittatus*, which do not feed near sediment, feed primarily off other fish (Bell-Cross, 1965-66; Skelton, 2001). *Clarias gariepinus* are known to be closely related to sediment, often resting and feeding in it, their diet has high variation to certain food groups and composes primarily of fish, insect, crustaceans, even plant material (fruits) (Skelton, 2001). Although they eat plant material it is not their primary diet. *Labeobarbus marequensis* feed off similar biota yet their diet is not as varied and they feed primarily off algae, crustaceans and insect larvae found within cobble beds and bottom substrate (Bell-Cross and Minshull, 1988; Skelton, 2001; Fouché, 2009).

There were differences shown between the different flows within both the Crocodile and Sabie rivers with bioaccumulation being significantly higher in fish from the high flow surveys when compared to the low flow surveys. The Crocodile River showed greater variation in this than the Sabie River in particular to *L. marequensis*. This could be an indication that surface run-off washes down metals and exposes them into the system at higher levels. Barker (2006) found seasonal variation in metals for fish tested in lotic systems. Further physiological state of the fish may change over season due to certain metals needed for the biological functioning of the
species (Falchuk, 1989). Considering that *H. vittatus* and *L. marequensis* share similar breeding periods the difference in metal bioaccumulation could be exposure related (Skelton, 2001; Fouché, 2009).

For most metals bioaccumulation in muscle of *L. marequensis* and Cu for *H. vittatus* was higher in the Crocodile River than in the Sabie River. For *C. gariepinus* all metal bioaccumulations (except Cr) were higher in the Sabie River than in the Crocodile River. Considering that the Sabie River is regarded as a healthier river to that of the Crocodile River the metal concentrations for the all three species in the Sabie River can reflect this (O’Keeffe and Rogers, 2003; DWAF, 2004; Rogers and Luton, 2011). Despite the similar geological compositions between the two Rivers, the higher impacts on the Crocodile River can be shown in the results (O’Keeffe and Rogers, 2003; Rogers and Luton, 2011). The Crocodile River is regarded as a larger anthropogenic impacted river than the Sabie River (O’Keeffe and Rogers, 2003; Rogers and Luton, 2011). This is reflected in the results for *L. marequensis* and *H. vittatus* having higher metal exposures in the Crocodile River during high flows and low flows this was significant for metals Al, Cr and Cd and for Cd, during high and low flow conditions respectively. Of interest *H. vittatus* showed lower levels of metal concentration than both *L. marequensis* and *C. gariepinus* in both rivers.

Comparisons were made with other known metals concentrations from other systems and fish species in order to get a guideline to determine the extent of the severity metal accumulations on the Crocodile River. Prior to this study there have been no metal accumulation studies done for the Crocodile River. Concentrations for this study were thus compared to other studies carried out on fish bioaccumulation in other KNP Rivers. Concentrations in *L. marequensis* from the Olifants River (Seymore et al., 1995) were found to be lower or similar for Pb (2.9-9.1 μg/g dw) and Mn (7.35-5.3 μg/g dw). Comparisons to the results by Barker (2006) who studied metal bioaccumulation in whole species of *L. marequensis* revealed lower and in some cases similar concentrations for Al (13.0-663.0 μg/g dw), Cd (0.1-2.8 μg/g dw), Mn (5.2-75.0 μg/g dw), Ni (0.1-12 μg/g dw), Fe (0.5-754.4 μg/g dw), Pb (0.4-26.0 μg/g dw), Cr (0.1-19.9 μg/g dw), Cu (0.1-208 μg/g dw) and Zn (20.2-134.8 μg/g dw).

When results of this study are compared to metal bioaccumulation in muscle tissue of the Orange River mudfish, *Labeo capensis* from the known contaminated Vaal River (Wepener et al., 2011) concentrations were found again to be similar or higher for Pb (0.025-0.067 μg/g dw), Zn (13.1-18.46 μg/g dw), Cr (0.142-0.261 μg/g dw). The concentrations of Ni (0.187-0.517 μg/g dw) and Cu (1.78-14.3 μg/g dw) were lower than in the fish muscle from the Vaal River. Gutleb et al. (2002) conducted a baseline study for a rain forest fed river in Peru before oil mining was
started and metal bioaccumulation results from this study were higher for Fe, Co, Zn, As, Se, Cd and Pb; Fe (0.071-0.157 μg/g dw), Co (0.017-0.127 μg/g dw), Zn (23.75-27.02 μg/g dw), As (0.015-0.101 μg/g dw), Se (0.070-0.142 μg/g dw), Cd (0.010-0.071 μg/g dw), Pb (0.091-0.441 μg/g dw) with the exception of Cu (1.62-4.12 μg/g dw), Mn (2.91-20.75 μg/g dw) and Ni (0.240-0.943 μg/g dw).

Compared to regions with known high metal bioaccumulation, e.g. a study in Rwanda by Sekomo et al. (2011) concentrations were found to be higher in Zn (5.11 μg/g dw) for this study. While Cd (2.85 μg/g dw) during the high flow in the Crocodile River was higher than the respective other flows and species tested. Bervoets and Blust (2002) tested for metal concentrations in fish along a Cd and Zn pollution gradient in a Belgian River system. Results from this study in L. marequensis were similar to levels for Zn (40-45 μg/g dw, while in this study = 39-49 μg/g dw) in the highly polluted Belgian site. In a later study by Reynders et al. (2008) for the same Belgian sites metal concentrations were found above the levels in this study for Cu (2-3 μg/g dw), Zn (30-60 μg/g dw) but below for Cd, which was below detectable limits.

Zinc concentrations within the Crocodile River for the population of L. marequensis were similar or higher in concentration than in other river systems (Seymore et al., 1995; Barker, 2006; Wepener et al., 2011). Zinc however is crucial for the development of eggs and gets stored in storage tissue until eggs are fertilized (Falchuk, 1998). This is relevant for female fish; the male to female ratio in this study was 6:1, possibly indicating the moderate levels of Zn in the study. This does not indicate why all the other metals are slightly elevated in L. marequensis for the Crocodile River. Metal concentrations being similar and in cases higher for this study in relation to other studies raises concern for the population of L. marequensis in the Crocodile River as metal levels are significantly higher than that of H. vittatus and C. gariepinus and than the population within the Sabie River, showing that despite having similar to above normal metal concentration they are being more effected by metals than other species and than in other river systems.

4.5 Conclusion

With significantly (p>0.05) higher metal bioaccumulation in L. marequensis from Crocodile River than the Sabie River, it does raise concerns with regards to the potential influence it may have on this river’s population. This could possibly be compounded with other stress factors which can cause the overall decline in the L. marequensis population on the Crocodile River. Metals
that were consistently higher in *L. marequensis* were Zn and Pb. Zinc, in particular when compared to other studies, showed that levels were similar or slightly elevated in this study than to other polluted sites. However high bioaccumulation due to Zn storage for the development of eggs needs to be considered and could be the reason for the high concentration of Zn. The sample did obtain more male *L. marequensis* than females, the ratio of male to female (6:1) presenting moderate levels of Zn in the results, as females had high levels (± 90 μg/g dw) while males had low levels (± 15 μg/g dw) of Zn concentrations.

Possible sources of metal pollution in the Crocodile River can be from a number of industrial sectors in the Nelspruit area, gold mines towards Barberton and the magnetite mine near the town of Malelane (DWAF 2004; Rogers and Luton, 2011). With the increases in water usage and demand from various sectors, there is a concern as to the use and industrial effluent run-off further impacting on the Crocodile River (Rogers and Luton, 2011). This study has indicated that the population of *L. marequensis* in the Crocodile River has elevated levels of metals Pb, Zn and Cd although the metal bioaccumulations are not at concerning levels yet.
5 CHAPTER 5: GENERAL CONCLUSION AND MANAGEMENT RECOMMENDATIONS.

5.1 General conclusions

The aims of the study were to evaluate the current health, habitat use and movement of *L. marequensis* and other fishes in the Crocodile River, KNP. To reach the aims a behavioural and ecotoxicology assessment of the population of *L. marequensis* in the Crocodile River was undertaken and the hypothesis then accepted or rejected.

In consideration to the first hypothesis which states; *Labeobarbus marequensis* make use of defined home ranges on a reach scale (<10 km) and behave differently during different seasons and times of day, the outcomes of the study allow for the hypothesis to be accepted. Large *L. marequensis* do make use of defined home ranges on a reach scale which is restricted to less than 10 km it was further found that the home ranges are smaller and found to be <5 km. Large *L. marequensis* do behave differently during different seasons, during the summer showing the greater difference in behaviour. Behaviour of *Labeobarbus marequensis* change during the course of the day, TOD showed the species to a diurnal species. The study showed that these large *L. marequensis* do not take part in seasonal migrations, instead remained in a home range surrounding the focal area of the study. This shows the importance of these habitats within the Crocodile River. These habitat preferences are unique to only a few stretches of the Crocodile River. Therefore it is of importance to the conservation of the species that these habitats are maintained and protected.

Furthermore in consideration of the second hypothesis which states; the movement of *L. marequensis* decreases when flows increase rapidly in the Crocodile River. This hypothesis was accepted, the movement of *L. marequensis* decreases significantly when flows in the Crocodile River increase rapidly from 10 m$^3$.s$^{-1}$ to 60 m$^3$.s$^{-1}$. Flows play an important role in change and availability of habitat types. The response to flow changes indicated that during extreme high and erratic flows *L. marequensis* would decrease their movements and wait for low to moderate and consistent flows. Combining flow and seasonal response showed that flows are important during the spring period and the two together can be some form of cue to the heightened activity during consistent low to moderate flows and spring. The importance of more natural flows should be fed into management policies and programs to regulate the flow (discharge) from dam releases and extractions to better suite *L. marequensis*. Considering *L. marequensis* do better in constant, stable flows the irregular discharges caused by impoundments may cause a river to be in a high unstable flux longer than it should be naturally. Therefore a more natural discharge will bring back stability into the aquatic river system minimising high extended unfavourable flows.
In considering the third hypothesis which states; the levels of metals in *L. marequensis* in the Crocodile River are greater than *H. vittatus* and *C. gariepinus* from the Crocodile and Sabie Rivers was accepted. Metal bioaccumulations in the study showed significant differences in concentration between the Crocodile River and the Sabie River populations of *L. marequensis*. The same spatial differences were also found for *H. vittatus* and *C. gariepinus*. Further significant differences were found between the different species indicating different routes of metal exposure and/or ability to metabolize following uptake between the three species. Overall however *L. marequensis* had higher metal concentrations than the other two species. The higher metal bioaccumulation in the fish from the Crocodile River than in the Sabie River was expected due to levels of activity in the respective catchments. This could indicate that the population within the Crocodile River is under more stress than that in the Sabie River. The second part to the hypotheses stating that the metal concentrations negatively affect the *L. marequensis* population in the Crocodile River is inconclusive. The comparisons made with bioaccumulation in fish from known heavily polluted rivers and this study is still of no concern as yet, however the increased pressure from human activity on the Crocodile River may cause these concentrations to worsen over time.

### 5.2 Management recommendations

There are management plans and policies in place to better manage the greater Nkomati River systems, of which the Crocodile River falls under. The Interim IncoMaputo Agreement (IIMA) between Swaziland, South Africa and Mozambique is internationally important in providing Mozambique with enough water to sustain the urban growth of Maputo (DWAF, 2004; Rogers and Luton, 2011). These plans and policies are managed using existing models and information which may not always be true indications to what is happening in the environment and because of this it is crucial to continue with findings to better these management plans and policies as in the adaptive management approach by KNP (Roger and O’Keefe, 2003). Therefore the evaluation of implementing management plans and policies can be re- incorporated into management ending in a better use of the river system in the long term. This study was able to indicate the importance of flow (discharge) and time of day on the behavioural response of *L. marequensis* and the importance of maintaining the preferred habitat for the species to ensure its survival. Discharge in river management is always of importance as it alters downstream available habitats (Dallas and Day, 2004). Constant flow allows the river to stabilize creating preferred habitats to be around for longer periods, this is not to distract from natural flows yet to enforce natural flows and not allow the release of water at irregular intervals outside the natural
discharge caused by seasonal rains. The importance of spring to *L. marequensis* behaviour indicates the value of natural flows during that period to allow for availability of spawning events. The bio-accumulation of metals in tested fish is concerning and minimizing the effluent of heavy metal wastes into the Crocodile River system is important to the survival of *L. marequensis* and should be mitigated where ever possible.

Further studies are needed to better understand the spawning habits of *L. marequensis* and the associated cues in particular to the day length, flows and associated spawning habitat. This study has gained more knowledge on the response of *L. marequensis* to discharge. Further studies on this topic will help fine tune the understanding that discharge has to concerned fish species, such as *L. marequensis*, assessing in the better management of impoundments to replicate natural discharges or discharges better suited the survival of fish. Current telemetry techniques can assist greatly in these studies. Since *L. marequensis* bioaccumulated the highest levels of metals it is recommended that the species be used as indicator species when testing for heavy metals. Future studies should be aimed at linking the metal exposure (in the form of bioaccumulation) with their effects on ecology and biology of organisms, e.g. using biomarkers with behaviour to find effects of metals on behaviour of fish.
CHAPTER 6: REFERENCES


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CHAPTER 7: APPENDICES
### Appendix 1: RAW data acquired during manual monitoring observations

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### Appendix 8: Continuation of RAW data acquired during manual monitoring observations

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Appendix 10: Continuation of RAW data acquired during manual monitoring observations
Appendix 11: Graphical representation of the home for the 2.5 kg *Labeobarbus marequensis* LMAR1, with ATS tag 2030 frequency 142.214. Tagged on the 15/08/2009 and monitored until the 19/09/2009, monitored on 15 occasions.
Appendix 12: Graphical representation of the home for the 1.8 kg *Labeobarbus marequensis* LMAR2, with ATS tag 1930 frequency 142.232. Tagged on the 15/08/2009 and monitored until the 15/05/2010, monitored on 30 occasions.
Appendix 13: Graphical representation of the home for the 2.2 kg *Labeobarbus marequensis* LMAR3, with ATS tag 1930 frequency 142.113. Tagged on the 15/08/2009 and monitored until the 01/12/2009, monitored on 16 occasions.
Appendix 14: Graphical representation of the home for the 2.1 kg *Labeobarbus marequensis* LMAR4, with ATS tag 1930 frequency 142.092. Tagged on the 16/08/2009 and monitored until the 15/05/2010, monitored on 42 occasions.
Appendix 15: Graphical representation of the home for the 2.5 kg *Labeobarbus marequensis* LMAR5, with ATS tag 2030 frequency 142.153. Tagged on the 16/08/2009 and monitored until the 17/02/2011, monitored on 109 occasions.
Appendix 16: Graphical representation of the home for the 2.1 kg *Labeobarbus marequensis* LMAR6, with ATS tag 1930 frequency 142.051. Tagged on the 16/08/2009 and monitored until the 15/05/2010, monitored on 42 occasions.
Appendix 17: Graphical representation of the home for the 3 kg *Labeobarbus marequensis* LMAR7, with ATS tag 1930 frequency 142.032. Tagged on the 19/09/2009 and monitored until the 15/05/2010, monitored on 65 occasions.
Appendix 18: Graphical representation of the home for the 2.5 kg *Labeobarbus marequensis* LMAR8, with ATS tag 1930 frequency 142.072. Tagged on the 19/06/2010 and monitored until the 16/02/2011, monitored on 46 occasions.
Appendix 19: Graphical representation of the home for the 2.6 kg *Labeobarbus. marequensis* LMAR9, with ATS tag 2030 frequency 142.014. Tagged on the 17/09/2010 and monitored until the 14/11/2010, monitored on 19 occasions.
Appendix 20: Graphical depiction of: The 2.5 kg *Labeobarbus marequensis* LMAR10, with WW tag Series IV no. 6, tagged on the 19/09/2011, tag active until the 01/06/2012 (A). The 2.8 kg *L. marequensis* LMAR11, with WW tag Series III no. 25, tagged on the 19/09/2011, tag active until the 01/06/2012 (B). The 2.5 kg *L. marequensis* LMAR12, with WW tag Series IV no. 7, tagged on the 19/09/2011, tag active until the 01/06/2012 (C). The 2.3 kg *L. marequensis* LMAR13, with WW tag Series III no. 17, tagged on the 19/09/2011, tag active until the 01/06/2012 (D). The 2.2 kg *L. marequensis* LMAR14, with WW tag Series III no. 12, tagged on the 19/09/2011, tag active until the 01/06/2012 (E). The 2.3 kg *L. marequensis* LMAR15, with WW tag Series III no. 14, tagged on the 15/10/2011, tag active until the 01/06/2012 (F). The 3.9 kg *L. marequensis* LMAR16, with WW tag Series V no. 42, tagged on the 29/11/2011, active until the 01/06/2012 (G).
Appendix 21: Graphical depiction of: The 1.3 kg *Hydrocynus vittatus* HVIT1, with ATS tag 2060b frequency 142.322, tagged on the 19/08/2009 and found in a fish eagle’s nest on the 19/09/2009 (A). The 500 g *H. vittatus* HVIT2, with ATS tag 1930 frequency 142.132, tagged on the 19/08/2009 and never found again (B). The 2.2 kg *H. vittatus* HVIT3, with WW tag Series III no. 21, tagged on the 16/09/2011, tag active until the 01/06/2012 (C). The 1.3 kg *H. vittatus* HVIT4, with WW tag Series III no. 23, tagged on the 19/09/2011, tag active until the 01/06/2012 (D). The 2.5 kg *H. vittatus* HVIT5, with WW tag Series III no. 19, tagged on the 19/09/2011, tag active until the 01/06/2012 (E). The 1.7 kg *H. vittatus* HVIT6, with WW tag Series III no. 9, tagged on the 19/09/2011, tag active until the 01/06/2012 (F). The 2.1 kg *H. vittatus* HVIT7, with WW tag Series III no. 13, tagged on the 22/09/2011, tag active until the 01/06/2012 (G). The 4 kg *H. vittatus* HVIT8, with WW tag Series III no. 15, tagged on the 22/10/2011, tag active until the 01/06/2012 (J). The 2 kg *H. vittatus* HVIT9, with WW tag Series III no. 16, tagged on the 04/11/2011, tag active until the 01/06/2012 (H). The 3 kg *H. vittatus* HVIT10, with WW tag Series V no. 41, tagged on the 23/11/2011, tag active until the 01/06/2012 (I). The 2.1 kg *H. vittatus* HVIT11, with WW tag Series III no. 31, tagged on the 26/11/2011, tag active until the 01/06/2011 (K). The 2.1 kg *H. vittatus* HVIT12, with WW tag Series III no. 35 tagged on the 28/11/2011, tag active until the 01/06/2012 (L).