# Comprehensive fish health assessment and parasitological investigation of alien and indigenous fishes from the Amatola region, South Africa

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## Abstract

The conservation of biodiversity and endemism in South Africa's freshwater aquatic ecosystems is a high priority, particularly in the Cape Floristic Region. However, the perception that South Africa lacks suitable fish species for recreational angling, aquaculture and biological control, led to the widespread introduction and use of alien fish species. As a result, formal stocking programs have seen the introduction of five of the world's top 100 invasive species into South Africa (Dudgeon *et al.* 2006). According to Dudgeon *et al.* (2006) freshwater ecosystems are the most endangered ecosystem in the world. The threats to freshwater biodiversity, according to Dudgeon *et al*. (2006), can be grouped into five categories that interact with one another: overexploitation, water pollution, flow modifications, destruction of habitat and invasion by exotic species.

This PhD study took place in the Amatola region of the Eastern Cape Province, South Africa. The Amatola region is a rural area with no large-scale mining or industrial developments, only localised settlements. These developments are mainly situated around impoundments, because of the resources such as water and food that they provide. Thus the dams within the Amatola region should theoretically have no major industrial stressors on them. The three impoundments studied were Binfield Park, Sandile and Wriggleswade Dams. Binfield Park Dam is a 260ha impoundment. It impounds the Tyume River and is used by both subsistence anglers from the local communities and occasionally by recreational bass anglers. Sandile Dam is a 146ha impoundment and is the smallest of the three dams in this study. It impounds the Wolf and Keiskamma Rivers. Wriggleswade Dam is a 1000ha impoundment used extensively by recreational bass and carp anglers, and impounds the Kubusi River.

There is a paucity of information regarding the health of the indigenous and alien fish species from the study region, as well as on the parasite diversity of these various fish species. In order to fill the gaps in the information the following hypothesis was proposed. That the necropsy based and histology based fish health assessment can be successfully implemented as tools to assess the effects of heavy metal pollution and alien fish parasites in freshwater fish from selected impoundments in the Amatola region, Eastern Cape Province, South Africa. In order to achieve this

hypothesis the main aim of this study will be to use the necropsy- and histology-based fish health assessment to determine the health status of the fish species in these impoundments as well as to understand the potential threat of water pollution and fish parasites.

Fish were sampled with the aid of gill nets, fyke nets and by angling from each of the three impoundments over three surveys in July 2011, and March and August 2012. Following capture fish were transported to a field laboratory in aerated containers. At the field laboratory the fish were examined and dissected using the methods recommended by Adams *et al*. (1993) for a necropsy-based fish health assessment. Gills, livers, kidneys and gonads samples were also collected for histological analysis.

Macroscopic and histology-based fish health assessment index was used, as well the analysis of muscle tissue of *Micropterus salmoides* and surface water and sediment from Binfield Park, Sandile and Wriggleswade Dams. It was shown that, according to the macroscopic fish health assessment index, *M. salmoides* in Wriggleswade Dam had a higher FHAI score compared to those in Binfield Park and Sandile Dam, there were no significant differences between the FHAI scores. However, the cause of the higher FHAI in the Wriggleswade Dam was because of the external skin damage caused by the presence of the alien parasite *Lernaea cyprinacea*. The histology-based fish health assessment index, however, showed that *M. salmoides* from Binfield Park had significantly higher histology Fish Index ($I_{FISH}$) scores compared to those in Sandile and Wriggleswade Dams. The main contributors to the high $I_{FISH}$ score of Binfield Park were the significantly high Liver Index ($I_L$) and Kidney Index ($I_K$). The increased severity of the alterations observed in the liver and kidney tissue of the Binfield Park *M. salmoides* samples may have been as a result of the high concentration of mercury found in the muscle tissue of *M. salmoides*. The water quality and metals detected in the water of Binfield Park, Sandile and Wriggleswade Dams were all below the target water quality guideline values, as well as below those of previous research into the nutrients and presence of metals in these impoundments. The sediment metal analysis showed that the levels of Co, Mn and Ni were above the target guideline levels for Binfield Park, Sandile and Wriggleswade Dams, Cu was above target guidelines levels for Sandile Dam, and uranium was above the target guideline concentrations for Wriggleswade

Dam. Binfield Park Dam had significantly high levels of mercury in the muscle tissue of *M. salmoides*, while Sandile Dam had significantly high levels of zinc in the muscle tissue of *M. salmoides*. It was shown that *M. salmoides* from each of the three impoundments are in a healthy state according to the parameters assessed. However, the presence of heavy metals, particularly mercury, uranium and zinc, do indicate the presence of human activities.

The indigenous parasites of *Anguilla mossambica* have been well documented including the gastrointestinal nematode *Paraquimperia africana*, and the stomach nematode *Heliconema africanum*. Indigenous parasites such as the swimbladder nematode *Anguillicola papernai* had no effect on the condition factor of infected and uninfected eels. However, the damage caused by the alien parasites were evident, including the first documented effects of the alien gill monogenean *Pseudodactylogyrus anguillae* on indigenous wild populations of the longin fin eel *A. mossambica* from the Eastern Cape, South Africa. Histological observations indicated that an alien gill monogenean caused hyperplasia, increase in mucous cells, rupture of pillar cells as well as telangiectasia. This alien parasite has invaded the Keiskamma and Kei River systems in the Eastern Cape, South Africa. According to the macroscopic fish health assessment index, *A. mossambica* from Binfield Park, Sandile and Wriggleswade Dams are in a healthy state. However, the histology-based health assessment highlighted that the effects on *P. anguillae* have a severe negative impact on the health of *A. mossambica.*

Using the macroscopic and histology-based fish health assessment, a comprehensive investigation into the fish health status of *Mugil cephalus* and *Myxus capensis* from Binfield Park Dam revealed that human effects and parasites are not the only threats to freshwater fish. Nephrocalcinosis is a non-infectious kidney disease which is characterised by abnormal calcium deposition in the kidneys of humans and some fish species. According to the macroscopic and histology-based fish health assessment, the *M. cephalus* and *M. capensis* are not in a healthy condition. The macroscopic and histology-based fish assessment indices are not stressor-specific, and therefore the cause of the poor health state of these two fish species could not be determined. A possible suggestion for the poor health of these two species is the age of the species. Because the two mullet species were stocked

into Binfield Park Dam, Ellender *et al.* (2012) could successfully age them accurately to ten years of age, which is the upper limit of the life span for these species.

Macroscopic and histology-based fish heath assessments were conducted on *Labeo umbratus* from Sandile Dam in order to determine the health of this species. Macroscopic and histology-based fish health assessment indicated that its *L. umbratus* are in a healthy state. The March 2012 survey specimens had a significantly higher macroscopic FHAI score than those from the July 2011 survey. The increased FHAI score was because of parasite infections, as well as discoloured livers and increased total blood plasma protein levels, which are indicators of nutritional state. However, the presence of the anchor worm parasite *Lernaea barnimiana* in low numbers had no significant impact on the health of *L. umbratus*.

The effect of the alien anchor worm parasite *Lernaea cyprinacea* was shown on the transloacted small mouth yellowfish *Labeobarbus aeneus.* It was also shown that *L. aeneus* are, according the macroscopic FHAI and the histology-based fish health assessment index, in a healthy state. However, the high scores observed in the macroscopic fish health assessment index were primarily as a result of the presence of the alien parasite *L. cyprinacea* and its associated affects on the fish host. Because of the significant impact of this alien parasite species on the translocated host species, it can be assumed that this alien parasite species will have a negative effect on the health of indigenous fish species in the Great Kei River.

It is clear from the results presented in this study that the necropsy based and histology based fish health assessment can be successfully implemented as tools to assess the effects of heavy metal pollution and alien fish parasites in freshwater fish from selected impoundments in the Amatola region, Eastern Cape Province, South Africa, thus the original hypothesis of this thesis is accepted.

Based on work done in this research the gaps in research have been identified. Due to the high levels of mercury indentified in the muscle tissue of *M. salmoides* from Binfield Park Dam. A human health assessment and edibility should be conducted in order to determine if the fish from Binfield Park Dam is safe for human consumption. In order to conserve South Africa's Freshwater fish biodiversity, country wide surveys of indigenous fish species must be undertaken so that the health and the parasite diversity can be evaluated.

## Key words

Alien species

Ecotoxicology

Fish Health Assessments

Histology

Indigenous spiecies

Parasitology

Translocated species

# Chapter 1 : General introduction

## 1.1. Background to study

South Africa has many unique and diverse freshwater ecosystems such as the Cape Floristic Region which is recognised as a global biodiversity hotspot (Cowling *et al*. 2003). In order to conserve South Africa's freshwater biodiversity, threats need to be identified to guide management strategies. According to Dudgeon *et al.* (2006) freshwater ecosystems are among the most endangered ecosystems in the world. The threats to freshwater biodiversity can be grouped into five categories, all of which interact with one another: overexploitation, water pollution, flow modification, destruction of habitat and invasion by alien species (Dudgeon *et al*. 2006). Protection of freshwater areas is becoming increasingly important around the world because of the loss of freshwater biodiversity caused by human-induced habitat loss or degradation, habitat alteration, over harvesting, water pollution and invasion by alien species (Abell *et al.* 2007).

In recognition of the growing threats to freshwater ecosystems, South Africa recently assessed the status of and threats to all its catchment areas. This process culminated in the identification of National Freshwater Ecosystem Protected Areas (FEPA) based on the threats to rivers, wetlands and estuary ecosystems. The FEPA project was aimed at developing a basis for enabling effective implementation of measures to protect freshwater priority areas. The National FEPA is a response to the growing need for the conservation of South Africa's freshwater ecosystems (Nel *et al*. 2011). It does this by providing strategic special priorities for the conservation of freshwater ecosystems and supporting sustainable use of water resources (Nel *et al*. 2011). The FEPA is intended for researchers, consultants and managers who are tasked with developing spatial prioritisations especially in freshwater ecosystems and designing programs that integrate science, policy and society by using the FEPA products (Nel *et al.* 2011). The key findings of the FEPA report were that tributaries are in a better condition than main rivers, and estuaries are highly threatened ecosystems. Twenty two percent of South Africa's river lengths have been identified as FEPAs and, by protecting 15% of South Africa's river length, all of South Africa's

fish species that are critically endangered, endangered and vulnerable are protected (Nel *et al.* 2011). The Department of Water Affairs and the Water Research Commission supported the development and refinement of basic methods for freshwater biodiversity planning (Nel *et al.* 2011). This led to the development of the 19 Water Management Areas (WMAs), of which six where used in case studies in order to determine which freshwater biodiversity plans could be implemented for achieving the goals of regional freshwater conservation (Nel *et al.* 2011). The Amatola region, the focus area for this PhD study, falls into the FEPA listed as Water Management Area 12 (WMA 12 Mzimvubu to Keiskamma).

The Amatola region is a rural area with no large-scale mining or industrial developments, only localised settlements. These developments are mainly situated around man-made impoundments because of the resources such as water and food that they provide. The Keiskamma and Kubusi Rivers are important fish sanctuary rivers for critically endangered and endangered fish species. Fish sanctuaries, according to the FEPA, are sub-quaternary catchments that are essential for protecting threatened and near threatened fish that are indigenous to South Africa. The upper Keiskamma River is a high priority conservation river because of the presence of endangered *Barbus trevelyani* and *Sandelia bainsii*. River systems in the Amatola contain six indigenous species, namely two small minnows, *B. trevelyani* and chubbyhead barb *B. anoplus*, the Eastern Cape rocky *S. bainsii*, the moggel *Labeo umbratus*, the freshwater goby *Glossogobius callidus* and the longfin eel *Anguilla mossambica* (Kleynhans *et al*. 2007; Ellender 2013). According to Jubb (1961), the giant mottled eel *A. marmorata* does not occur above 250 metres in altitude and therefore is unlikely to be present in the upper reaches of the Keiskamma and Kubusi Rivers (Ellender 2013). The known alien fish species in the Amatola region are rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta,* common carp *Cyprinus carpio*, bluegill sunfish *Lepomis macrochirus*, and largemouth bass *Micropterus salmoides* (Kleynhans *et al*. 2007; Ellender 2013). The Amatola region also has several translocated species including Mozambique tilapia *Oreochromis mossambicus*, banded tilapia *Tilapia sparrmanii*, sharptooth catfish *Clarias gariepinus* and smallmouth yellowfish *Labeobarbus aeneus* (Ellender 2013). Two mullet species were also translocated into Binfield Park Dam in the Tyume River to enhance its fisheries potential (Ellender *et al*. 2012). The two mullet species

are the flathead mullet *Mugil cephalus* and the freshwater mullet *Myxus capensis* (Ellender *et al*. 2012). The fish species that have been sampled in the Amatola region are presented in **Table 1.1**.

Streams of the Amatola region are, from a conservation point of view, very important as they harbour various indigenous fish species that include three IUCN red-listed species, *B. amatolicus* (Vulnerable), *B. trevelyani* (Endangered), and *S. bainsii* (Endangered) (Scott *et al*. 2006; Tweddle *et al*. 2009). According to Skelton (2001), the primary threats to stream fishes are habitat destruction, water extraction and the introduction of predators such as largemouth bass (*M. salmoides*) and sharptooth catfish (*C. gariepinus*). Upper reaches of rivers, according to Nel *et al.* (2011), are important conservation areas and need to be protected. According to Ellender *et al.* (2011), Amatola headwater streams have a low biodiversity but a high endemicity. Ellender *et al.* (2011) showed the potential of *C. gariepinus* to penetrate into these smaller headwater streams and, although they do not establish there, the threat posed by predation on the smaller naive species is still a serious conservation issue.

**Table 1.1:** The expected species list for the Amatola region, Eastern Cape, South Africa with distribution, and the 2014 IUCN red list of threatened species. CE-critically endangered, E – endangered, NT – near threatened, LC – least concern and NA – not assessed. Fish fauna records from Kleynhans *et al*. (2007) and Ellender (2013)

| Fish Species | Distribution | IUCN status |
|---|---|---|
| Indigenous species | | |
| *Anguilla bicolor bicolor* | Keiskamma and Great Kei River | NT |
| *Anguilla marmorata* | Keiskamma and Great Kei River | LC |
| *Anguilla mossambica* | Keiskamma and Great Kei River | LC |
| *Barbus anoplus* | Keiskamma and Great Kei River | LC |
| *Barbus trevelyani* | Keiskamma River | CE |
| *Glossogobius callidus* | Keiskamma and Great Kei River | LC |
| *Labeo umbratus* | Keiskamma River | LC |
| *Liza macrolepis* | Keiskamma and Great Kei River | LC |
| *Mugil cephalus* | Keiskamma and Great Kei River | LC |
| *Myxus capensis* | Keiskamma and Great Kei River | LC |
| *Sandelia bainsii* | Keiskamma River | E |
| Extralimital species | | |
| *Clarias gariepinus* | Keiskamma River | LC |
| *Labeobarbus aeneus* | Great Kei River | LC |
| *Oreochromis mossambicus* | Keiskamma River | NT |
| *Tilapia sparrmanii* | Keiskamma River | LC |
| Alien species | | |
| *Cyprinus carpio* | Keiskamma and Great Kei River | LC |
| *Lepomis macrochirus* | Keiskamma River | LC |
| *Micropterus salmoides* | Keiskamma and Great Kei River | LC |
| *Oncorhynchus mykiss* | Keiskamma River | NA |
| *Salmo trutta* | Keiskamma River | LC |

The direct impacts of alien fishes, such as predation of smaller endemic species by *M. salmoides*, are well understood (Ellender & Weyl 2014). However, the indirect impacts by their associated parasites on the endemic fishes are generally not fully understood. In addition the potential effect of metal pollution on the Amatola's freshwater ecosystem has received very little attention. Water pollution influences all forms of freshwater biodiversity from microbes to freshwater mega-fauna (Dudgeon *et al*. 2006). Fish are useful tools to assess water pollution because the pollution may cause pathological changes in fish (Bernet *et al.* 1999).

The aim of this chapter is therefore to provide background information on alien and invasive species as well as on water pollution and the tools that will be used in this thesis to increase our understanding of the threats posed by alien fish parasites and water pollution to freshwater ecosystems in the Amatola region.

### 1.2. Alien and Invasive fishes and their associated parasites

In South Africa the perceived lack of suitable indigenous fish species for recreational angling, aquaculture and biological control, has led to the widespread use of alien fish species (Ellender & Weyl 2014). As a result formal stocking programs, beginning with the introduction of brown trout *S. trutta* in 1892 (de Moor & Bruton 1988), have resulted in the introduction of five of the world's worst (having successfully invaded and having a negative impact on its new environment) 100 invasive species (Lowe *et al*. 2000); largemouth bass (*M. salmoides*), smallmouth bass (*M. dolomieu*), common carp (*C. carpio*), and rainbow trout (*O. mykiss*) into the Amatola region in the Eastern Cape province of South Africa (Ellender 2013).

In South Africa the main documented impacts associated with the introduction of alien species are competition and/or predation (Wilcove *et al*. 1998; Pimentel *et al*. 2005). Woodford & Impson's (2004) investigation of the impacts of rainbow trout (*O. mykiss*) on indigenous fishes of the Berg River indicated that the trout's diet was mainly dominated by smaller aquatic invertebrates. However, the trout stomachs did contain fish, indicating that they do feed on fish. Woodford & Impson (2004) also noticed avoidance behaviour by the cape galaxias (*Galaxias zebratus*), thus

concluding that trout have a negative effect on all aquatic communities. Woodford *et al*. (2005) showed that smallmouth bass (*M. dolomieu*) had greater a predatory impact on native fish species, indicating that four of the five native fish species were absent from a stretch of river where smallmouth bass had invaded, and that the fifth species had lost all its post-spawning recruits. Other impacts of the introduction of alien fish species have been the co-introduction and transfer of their associated parasites. As mentioned previously, while the direct impacts of alien fishes on native fishes are fairly well documented (see Ellender & Weyl 2014 for Review), indirect impacts are not. Invasive species may affect native species indirectly by altering the habitat or changing the dynamics of diseases (Lymbery *et al*. in press). Common carp (*C. carpio*) for example, have been linked to habitat alterations brought about by increased turbidity, which results from its bottom-grubbing feeding behaviour (de Moor & Bruton 1988, Koehn 2004). *Cyprinus carpio* has also been linked as the primary vector for the introduction of several parasite species, including the parasitic flagellate *Ichthyobodo necator* Henneguy 1883, the kinetophragminorid *Chilodonella cyprini* (Moroff, 1902), the ciliated protozoans *C. hexasticha* (Kiernik, 1909) and *Apiosoma piscicola* (Blanchard 1885), and the trichodinids *Trichodina acuta* Lom, 1961, *T. nigra* Lom, 1960 and *Trichodinella epizootica* (Raabe, 1950) (Ellender & Weyl 2014).

Parasites have an important role in facilitating invasions (Lymbery *et al.* in press). This is because introduced alien species may have fewer parasites and often have a lower prevalence of parasites than the native hosts. This provides them with a competitive advantage (Mitchell & Power 2003; Torchin *et al*. 2003; Lymbery *et al*. in press). Alternatively, after introduction the alien species may be susceptible to native parasites which might increase the infection of indigenous species, through host amplification and increased transmission possibilities (Kelly *et al*. 2011; Lymbery *et al*. in press). The opposite could happen if the alien hosts decrease the infection in natives by reducing transmission (Paterson *et al*. 2011; Poulin *et al* 2011; Lymbery *et al*. in press). However, if the alien hosts have an associated parasite, this new parasite could spread to the native hosts causing the emergence of a new disease in the indigenous population (Daszak *et al*. 2000; Taraschewski 2006; Lymbery *et al*. in press). In south-western Australia 12 native and six exotic fish species were sampled from 29 different sites and screened for parasites, the results showed that 44 of the

parasites were native species, while two were alien species (the copepod, also known as an anchor worm, *Lernaea cyprinacea*, and the tapeworm, *Ligula intestinalis* (Lymbery *et al.* 2010). Lymbery *et al.* (2010) noted that the presence of these two alien parasite species on the native hosts may cause severe disease and that the native species had a higher diversity of parasites than the alien species, which may allow the alien species to be more successful in the environment.

The effects of alien parasites on native hosts are emergent diseases, producing high morbidity and mortality (Taraschewski 2006; Peeler *et al*. 2011). An example is from the economically important eel *Anguilla anguilla* which was imported into Japan to meet the demands of the growing eel aquaculture industry. The Japanese eels were infected with the swimbladder nematode *Anguillicola crassus*, occurring in 10 – 40% of the eels with an intensity of 1 – 3 worms and some up 20 worms per eel swimbladder, while in the cultivated European eel it often reached a prevalence of 100% and a high number of worms per eel (Taraschewski *et al*. 2006; Wielgoss *et al*. 2008, Lefebvre *et al*. 2012). This high number of worms in the introduced host had a noticeable pathogenicity. Japanese fish biologists noticed the potential threat to European eels and declared that measures should be put into place to prevent its spread. However, only three years later, *A. crassus* was found in Europe (Taraschewski *et al*. 2006). *Anguillicola crassus* also achieved this ability to invade new territories via the movement of live economically important species and its ability to switch host species and intermediate host species. Both of these examples have indirect life cycles, and one could postulate that this would make successful invasion more difficult. On the contrary, Lympery *et al*. (in press) has shown that a substantial portion of co-introductions are with parasites that have indirect life cycles.

The fish louse *Argulus japonicus* is an example of an alien parasite species that has gained entrance into South African waters via accidental introduction prior to 1983 (de Moor & Bruton 1988). The first records of an unidentified *Argulus* species from southern Africa in the eastern Transvaal were by du Plessis (1952) and Lombard (1968) but, according to van As (1987), were believed to be *A. japonicus* (Avenant-Oldewage 2001). Because of its feeding and attachment, this parasite causes localized damaged to the skin of its host and in severe infections the host can die due to blood loss (Avenant-Oldewage 2001). *Argulus japonicus* reproduces by a male depositing its sperm into the spemathecae of the female, which then leave the

host to oviposit the eggs in a firm substratum (de Moor & Bruton 1988). The young moult through several life stages and are capable of free swimming, but prefer to be parasitic (de Moor & Bruton 1988). *Argulus japonicus* is a conservation threat, being capable of infecting a wide variety of fish hosts (Avenant-Oldewage 2001).

The parasite diversity of South Africa's major river systems is fairly well known. The main alien parasites that pose a threat to conservation by known cases of mortalities are the ciliated protozoan *Chilodonella hexasticha,* whitespot disease *Ichthyophthirius multifiliis,* the fish louse *Argulus japonicus,* the Asian tapeworm *Bothriocephalus acheilognathi* and the protozoan *Trichodina acuta* (Bruton & van As 1986; Ellender & Weyl 2014). Although there have been no official reports of mortalities of fish infested with the alien anchorworm *L. cyprinacea,* in Africa (Barson *et al*. 2008), this parasite species still poses a conservation threat.

There is a lack of information regarding the distribution of alien parasite species in South Africa. There is also a paucity of knowledge regarding the parasite diversity for many of South Africa's smaller tributaries. Especially in the Amatola Region where little is known about fish parasite communities. The known parasite diversity of the Amatola Region's rivers is represented in **Table 1.2**. The majority of the research on fish parasites in the Eastern Cape Province has largely focused on those parasitizing *A. mossambica.* Three of the parasite species listed in **Table 1.2** are known alien parasite species, namely *Pseudodactylogyrus anguillae, Ichthyophthirius multifiliis* and *Bothriocephalus acheilognathi*. *Ichthyophthirius multifiliis* and *B. acheilognathi* were probably introduced into South Africa with the introduction of grass carp *Ctenopharyngodon idella* (de Moor & Bruton 1988; Matthews 2005). *Ichthyophthirius multifiliis* or white spot is a unicellular, ciliated ecto-parasite. According to de Moor & Bruton (1988), the origins of this species are uncertain. However, it is possible that this species originated in Asia.

Fish infected with *I. multifiliis* have the appearance of pustules on the skin of the fish which cause a rapid growth of mucous cells because of the irritation (de Moor & Bruton 1988). Severe infections with *I. multifiliis* can cause ulcers on the skin and damage the gills (de Moor & Bruton 1988). Cyprinids are considered to be the most susceptible fish species (de Moor & Bruton 1988), therefore this ecto-parasite potentialy poses a serious threat to the small cyprinid fish species in the Amatola

region. *Ichthyophthirius multifiliis* has previously been reported from the Kei River, infecting *A. mossambica*.

The Asian tapeworm *B. acheilognathi* was first described from the intestine of *Acheilognathus rhombea* from Ogura Lake, Japan (Retief *et al.* 2007). It is endemic to the Amur River in China. According to de Moor and Bruton (1988) it was introduced into South Africa from Europe in 1975. The first report of *B. acheilognathi* in South Africa was during a routine monitoring of common carp (*C. carpio*) at the Lowveld Fish Research Station, Marble Hall, Mpumalanga (Retief *et al.* 2007). The damage caused by the Asian tapeworm *B. acheilognathi* is abnormal growth and damage to the intestines by blocking of the intestines causing inflammation, and high infection rates can cause fish mortalities (Retief *et al.* 2007). Retief *et al.* (2007) reported severe infections with *B. acheilognathi* from the intestines of largemouth yellowfish *Labeobarbus kimberlyensis* from the Vaal Dam. Retief *et al.* (2007) noted that there was a 100% infection rate, and the highest mean intensity of 231.1 worms per individual fish was recorded in their autumn survey and the lowest mean intensity was 73.7 worms per individual fish was recorded during their summer survey. Stadtlander *et al*. (2011) noted that the alien fish parasite *B. acheilognathi* infected the translocated smallmouth yellowfish (*L. aeneus*) in Glen Melville Reservoir. Stadtlander *et al*. (2011) reported that the intensity of infection was higher in the impoundment that in the riverine species. However, Stadtlander *et al*. (2011) did note that this may be a potential source of invasion of the smaller indigenous fish species located further upstream.

The gill monogenean *P. anguillae* (Yin & Sproston, 1948) is a parasitic gill monogenean of the gills of *Anguilla japonica* Temminck & Schlegel, 1846, one of the indigenous species of eel of East Asia. *Pseudodactylogyrus anguillae* is a specialist eel parasite and is believed to have been introduced into Europe and America through the eel trade. The first report of *P. anguillae* outside its natural range was in 1977, when It was first noticed infecting the gills European eel *A. anguilla* Linnaeus, 1758 from an eel production plant in the western Soviet Union (Buchmann *et al*. 1978). Many authorities that believe that these regions are the within the natural range of *P. anguillae* (Nie & Kennedy 1991, Marcogliese & Cone 1993, and Cone & Marcogliese 1995), and have demonstrated this by showing regions where the parasite occurs. However, there is no eel trade here. *Pseudodactylogyrus anguillae*

has recently been discovered on the gills of South African eels. The first report of *P. anguillae* in Africa was by El Nagger *et al.* (1993). The first report of *P. anguillae* was from *A. mossambica* from the Eastern Cape, as documented by Christison & Baker (2007), and the first report from a wild population of *A. mossambica* from the Nahoon River was by Parker *et al*. (2011).

In addition to the general lack of information on alien fish parasites in the Amatola Region, there is very limited information on the effect of these alien parasites on their hosts, and especially on the threats posed to native species from this region (Ellender & Weyl 2014).

**Table 1.2:** The known parasite diversity for the freshwater fish species of the Eastern Cape.

| Fish Species | Parasite species | Indigenous / alien | Attachment site | River | Author |
|---|---|---|---|---|---|
| *Anguilla mossambica* | *Anguillicola papernai* (Moravec and Taraschewski, 1988) | Indigenous | Swim-bladder | Nahoon River | Taraschewski *et al*. 2005 |
| | *Paraquimperia africana* (Moravec, Boomker and Taraschewski 2000) | Indigenous | Gastrointestinal | Nahoon River | Taraschewski *et al*. 2005 |
| | *Heliconema africanum* (Linstow, 1899) | Indigenous | Stomach | Nahoon River | Taraschewski *et al*. 2005 |
| | *Pseudodactylogyrus anguillae* (Yin and Sproston, 1948) | Alien | Gills | Great Fish River | Parker *et al*. 2011 |
| | *Ichthyophthirius multifiliis* (Fouquet, 1876) | Alien | Gills | Kei River | Jackson 1978 |
| | *Mugicola smithae* (Jones and Hine, 1978) | Indigenous | Buccal cavity | Kei River | Jones and Hine, 1978 |
| *Labeo umbratus* | *Lernaea barnimiana* (Hartmann 1865) | Indigenous | Skin | Chubu | Viljoen 1982 |
| *Labeobarbus aeneus* | *Bothriocephalus acheilognathi* (Yamaguti, 1934) | Alien | Intestine | Glen Melville Reservoir | Stadtlander *et al.* 2011 |

### 1.3. Water pollution

Freshwater pollution in South Africa is constantly increasing because of mining activities, agriculture, and industrial and domestic releases into our freshwater environments (Heath *et al.* 2010; Jooste *et al.* 2014). Although some metals may occur naturally in an ecosystem, e.g iron and nickel, metals such as mercury, lead and cadmium are detrimental to the health of aquatic organisms when they are released into freshwater environments (Dallas & Day 2004).

Previous pollution studies done in the Amatola region reflected poor water quality in the Keiskamma River. Morrison *et al*. (2001) showed that the effluent concentration being discharged into the Keiskamma River by the Keiskammahoek Sewage Treatment Plant was higher than the South African effluent guidelines. These authors also showed that the levels of orthophosphate, chemical oxygen demand and ammonium in the Keiskamma River were also in excessive concentration levels. Morrison *et al*. (2001) indicated that these high pollution levels would be harmful to the Keiskamma River and cause eutrophication of Sandile Dam, situated downstream of the Keiskammahoek Sewage Treatment Plant. These high nutrient levels in the Keiskamma River would be harmful to livestock stock drinking the water, as well as to any recreational users of the water (Morrison *et al*. 2001). The water from Sandile Dam is treated and used to supply the Keiskammahoek and Middledrift district with piped drinking water (Awofolu & Fatoki 2003). Fatoki & Awofolu (2003) investigated the high levels of cadmium, mercury and zinc in the sediment and surface water in the Buffalo, Keiskamma, and Tyume Rivers as well as in Sandile Dam in the Eastern Cape Province of South Africa. Fatoki & Awofolu (2003) stated that the high levels are a potential risk to human health and that the probable sources of the metals in the rivers and dam are diffuse, originating from rural, urban and agricultural runoffs in the catchments. Fatoki *et al.* (2003) stated that, although the pH levels in Sandile Dam and the Keiskamma River were normal, the turbidity, electrical conductivity, dissolved oxygen and biochemical oxygen demand exceeded European Union guideline levels for the protection of aquatic ecosystems. Fatoki *et al.* (2003) identified the Keiskammahoek Sewage Treatment Plant as the source of the pollution in the Keiskamma River and Sandile Dam.

Fish are good indicators of water pollution, especially for sublethal and chronic effects (Bernet *et al.* 1999). In fish, water pollution can cause changes that range from the biochemical alteration of a single cell to changes in the entire fish population (Bernet *et al*. 1999). However, the sublethal effects of water pollution may not be immediately evident. Jooste *et al*. (2009), for example, showed that rednose labeo *Labeo rosae* contained high concentrations of lead and chromium in its muscle tissue and that consumption of these fish was a potential human health risk, although the fish itself appeared to be healthy. Pheiffer *et al*. (2014) also showed that *Clarias gariepinus* from the Vaal River were in a healthy condition with no histological alterations noted in the gills or liver of this species. However, metal analyses of their muscle tissue indicated that mercury, silver, selenium, arsenic and chromium were in high concentrations and also posed a serious health threat to potential consumers (Pheiffer *et al*. 2014).

Pollution of water by metals is known to have a negative impact on freshwater organisms (Adams *et al*. 1993; Bernet *et al.* 1999). Fish can be used as bioindicators of overall ecosystem health. Tools such as necropsy-based fish health assessment and histology-based fish health assessment have been developed in order to observe these effects and impacts.

### 1.4. Fish health assessments

The quantitative fish health assessment system (Adams *et al*. 1993) was developed to be a rapid and inexpensive means of determining the condition of fish in the field. It is based on the notion that the biotic integrity of the aquatic environment cannot be determined directly, but rather through the health of the organisms that reside in it. Fish are good representatives of overall ecosystem health because they generally occupy high trophic levels. The higher position allows the fish to integrate many of the biotic and abiotic variables that may be acting on the system and to reflect the secondary chronic symptoms that are mediated through the food chain (Adams *et al*. 1993). Fish are long-lived animals, which allows for the investigation of long-term effects on the ecosystem (Larkin 1978; Adams & McLean 1985). In their aquatic

environment, fish are continually under physiological stress from factors such as fluctuating water temperatures, high water velocities, sediment loading, low dissolved oxygen concentrations and limited food availability (Adams *et al*. 1993). When these natural stressors are combined with manmade stresses the physiological stress applied to the fish will impair their health (Adams *et al*. 1993). Energy is needed to deal with stress. When energy is used to deal with these stressors it is diverted away from other critical functions such as growth and reproduction, as well as predisposing the fish to disease (Adams *et al* 1993). The fish health assessment index (FHAI) proposed by Adams *et al*. (1993) is largely based on the necropsy method of Goede & Barton (1990) in that it is a field necropsy method that provides the health profile of the fish based on the percentages of abnormalities observed in the tissues and organs of individuals sampled from a population. The main reason for doing a necropsy is to detect any abnormalities or gross change the health of the fish populations and, if possible, to detect changes early enough for remedial actions to be implemented. The advantages of the FHAI is that it provides quantitative data and can detect trends in the health of the population over time that can be statistically compared between data sets, including variables in the health index such as biometric indices (Adams *et al*. 1993). The approach of the FHAI consists of the following categories, (1) three blood parameters (hematocrit, leukocrit and total plasma protein); (2) biometric indices (length, weight, condition factor, as well as hepatosomatic, splenosomatic and gonadosomatic indices); (3) the percentage of fish with abnormal or normal eyes, gills, spleens, livers and kidneys; and (4) index values of degree of damage to eyes, skin, fins, opercula, gills, liver, spleen, hindgut and kidney. To provide variable ranking for a quantitative statistical analysis, the FHAI assigns values of 0, 10, 20 and 30, where 0 indicates normal or no abnormalities identified and 30 indicates that a severe alteration or abnormality was identified. The calculation of the FHAI is done according to Adams *et al*. (1993) to calculate a FHAI score for each fish by summing all the variables. To get the final fish health assessment score the scores of all the individual fish are summed and divided by the sample size. A standard deviation is also calculated.

The histology-based fish health assessment is based on Bernet *et al*'s (1999) proposal to use histology in fish to assess aquatic pollution. Contaminants and pollutants in the water that fish are exposed to will induce pathological changes in

the fish (Bernet *et al*. 1999). Pathogens, toxic compounds, limited food availability and temperature fluctuations are environmental stressors that can impact on organ histopathology (Zimmerli *et al*. 2007). Additional stresses and anthropogenic effects will impair organ structure and function. Impaired organ structure and function will limit the chances of survival, growth and reproduction of an organism (Zimmerli *et al*. 2007). Therefore histology is an important tool in monitoring environmental stress. The advantage of histology is that it provides a rapid method for detecting the effects of irritants, especially if these are chronic in various tissues and organs (Bernet *et al*. 1999).

The Bernet *et al*. (1999) methodology is based on two factors; firstly that the extent of the pathological change is rated as a core value; and secondly that the importance of the pathological alteration is defined as the importance factor. The advantage of histology as a biomarker of ecosystem health is that it can show pathological changes in the environment with regard to the level of biological organization (Bernet *et al*. 1999). Zimmerli *et al*. (2007), developed a classification system based on the Bernet *et al*. (1999) scoring system, allowing for a semi-quantitative assessment of the histological alterations to the tissue structure.

In South Africa both these tools have been widely applied (van Dyk *et al*. 2009a; van Dyk *et al.* 2009b; McHugh *et al.* 2011). The first application of the necropsy-based fish health assessment index method was by Crafford & Avenant-Oldewage (2009), who showed that the method of Adams *et al*. (1993) could distinguish between samples from two localities on the Vaal River, with higher fish health assessment scores in areas with poorer chemical and water quality variables. Van Dyk *et al.* (2007) observed the histological alterations caused by two heavy metals, cadmium (Cd) and zinc (Zn), in the liver tissue of *O. mossambicus*. The histological alterations noted in the liver where hyalinization, vacuolation of the hepatocytes, congestion of the blood vessels and cellular swelling (van Dyk *et al*. 2007). Van Dyk *et al*. (2007) conducted a laboratory exposure and noted that the histological alterations observed were as a result of duration of the exposure period. Marchand *et al*. (2008) used histopathology of *C. gariepinus* liver tissue as a bio-indicator for the early warning of the threat of water pollution. Marchand *et al*. (2008) showed that *C. gariepinus* from two impoundments in a Gauteng nature reserve where polluted by heavy metals. In order to show the effects of these heavy metals of the health of *C. gariepinus*,

Marchand *et al*. (2008) used the qualitative and quantitative histology-based fish health assessment to show the adverse effects these pollutants were having on *C. gariepinus*. Marchand *et al*. (2008) showed that *C. gariepinus* were exposed to aluminium (Al), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) mercury (Hg), lead (Pb) and arsenic (As), and that the effects of the these metals on the liver of *C. gariepinus* were an increase in melano-macrophage centres, hepatocyte vacuolation, nuclear alterations and necrosis of the liver. Van Dyk *et al*. (2009a) applied both the necropsy- and histology-based fish health assessments to various fish species in the Okavango Delta in order to establish baseline data set for further toxicity studies. These authors noted that the necropsy-based fish health assessment corresponded to the histology-based fish health assessment. Van Dyk *et al*. (2009a) also found that the majority of the alterations noted in the Okavango Delta samples were as a result of parasitic infections. Van Dyk *et al*. (2009b) used the histology-based fish health assessment in order to assess semi-quantitatively the histological alterations in the gills of *C. gariepinus* from two separate polluted impoundments and to compare the results against laboratory-bred reference *C. gariepinus*. The results from that study showed that there was a significant difference in the severity of the alterations identified in the gill histology between the two impoundments and the laboratory-bred reference group. An evaluation of the fish health assessment index in the Olifants River system was done by Watson *et al*. (2012), and showed that *C. gariepinus* is a suitable candidate for the fish health assessment index and, if used in conjunction with a parasite index, can be a vital tool in assessing water quality. Sara *et al*. (2013) used the necropsy-based fish health assessment sharptooth catfish (*C. gariepinus*), Mozambique tilapia (*O. mossambicus*) and common carp (*C. carpio*) to determine that the Hout River Dam was in a good ecological state, because the low health assessment scores correlated with the recommended South African water quality guidelines. Pheiffer *et al*. (2014) showed that there were no histological alterations that could be related to the heavy metal exposure in *C. gariepinus* collected from various sites along the Vaal River. However, Pheiffer *et al*. (2014) did note that the heavy metal concentrations that had bioacccumulated in the muscle tissue of *C. gariepinus* could pose serious human health threats if the meat was consumed.

The only health assessment of an aquatic ecosystem done in the Eastern Cape Province was that by Richardson *et al*. (2011) in the East Kleinmonde, Old Woman's and Mtana estuaries. These estuaries were assessed using a prototype Estuarine Fish Health Index and the cape stumpnose *Rhabdosargus holubi* as a biological indicator (Richardson *et al.* 2011). The health of *R. holubi* from each of the estuaries was assessed using liver histopathology, acetylcholinesterase and lipid peroxidise assays, as well as condition factor (Richardson *et al.* 2011). Richardson *et al.* (2011) showed the Estuarine Fish Health Index to be a useful indicator of the overall health of the estuary. Richardson *et al.* (2011) indicated that the East Kleinemonde was in a healthy state requiring monitoring, and the Old Woman's and Mtana estuaries to be adversely affected and in need of management.

Both the necropsy- and histology-based fish health assessments have proved to be important and effective assessment tools for preserving the quality of South African waterways. It is the combination of the necropsy-based fish health assessment and the histology-based fish health assessment that will give a true reflection of the state of the fish populations. However, there is a paucity of information regarding the health of the fish populations from the Amatola region of the Eastern Cape.

### 1.5. Impoundments as sources of threats to freshwater biodiversity

The introduction of alien fish species into South Africa was largely motivated by the need to enhance recreational fishing, which is unlike most other countries which seek to develop subsistence and commercial fisheries (Ellender *et al.* 2014). According to Ellender *et al.* (2014) the commercial aspects of fisheries enhancement were only considered much later, with the development of large impoundments. According to Ellender *et al*. (2014), *C. carpio* was the first angling species to be introduced into South Africa, in 1859, followed by *S. trutta* in 1890 and *O. mykiss* in 1892. However, *S. trutta* and *O. mykiss* had to be continually restocked, because of their specific spawning requirements (Ellender *et al.* 2014). The common carp *C. carpio* does not have spawning requirements as specific as those of *S. trutta* and *O. mykiss*, and was able to establish more successfully and now occurs in most of South Africa's major catchments (Ellender *et al.* 2014). In southern Africa, large

impoundments have been the focal points for the introduction of many alien species such as of kapenta *Limnothrissa miodon* (Boulenger 1906) into Lake Kariba, Zimbabwe, and Nile tilapia *Oreochromis niloticus* (L. 1758) into Cahora Bassa Dam, Mozambique. The alien fish species *C. carpio* is also the primary target for many subsistence fishermen living around South Africa's largest impoundment, Lake Gariep (Ellender *et al*. 2009). Impoundments are sources of water for domestic and recreational use, as well as a providing a potential source of protein, and in South Africa many communities either have access to these impoundments or establish themselves around them (Heath *et al*. 2004). However, due to human impacts, many of these impoundments are becoming polluted by chemicals that can accumulate in the muscle tissue of fish (Heath *et al*. 2004). Pheiffer *et al.* (2014) noted that the metal concentrations in the sediment and muscle tissue of *C. gariepinus* were lower in the Vaal River sections below impoundments, indicating that the impoundments act as sinks and that metal concentrations build up in the impoundments. Impoundments not only allow for the build up of metal concentrations within them, but also of parasites. Stadtlander *et al.* (2011) observed that the prevalence and mean intensity of *B. acheilognathi* in *L. aeneus* was higher in Glen Melville Dam than in the Great Fish River. These authors stated that the impoundment acted as the source of infection. Retief *et al.* (2007) also reported the high prevalence and intensity of infection of *L. kimberlyensis* from the Vaal Dam. Therefore the impoundments act as the ideal sampling sites, as they are sinks that reflect the human activities taking place further up in the catchment, as well serving as reservoirs for the increase of parasite numbers.

For these reasons this thesis will focus on assessing the fish species contained within Binfield Park, Sandile and Wriggleswade Dams. The fish species will act as indicators of aquatic pollution and hosts to parasites. The fishes' health status will be semi-quantitatively assessed using the necropsy-based (FHAI) and the histology-based ($I_{FISH}$) fish health assessment protocols.

This study took place in three man-made impoundments, namely Binfield Park Dam, Sandile Dam and Wriggleswade Dam in the Amatola Region, Eastern Cape Province of South Africa. All three impoundments lie on the boundary of FEPA catchments and therefore act as indicators for all activities that take place higher up in the catchments. The Tyume River, which flows into Binfield Park Dam, and the

Keiskamma River, which flows into Sandile Dam, are both fish FEPA sanctuaries for endangered *B. trevelyani* and *S. bainsii* (Nel *et al.* 2011), while the Kubusi River, which flows into Wriggleswade Dam, is a fish sanctuary for threatened fish species such as *A. bicolor bicolor* (Nel *et al.* 2011). These three impoundments were built primarily to provide irrigation and drinking water to the local municipalities.

### 1.6. Hypothesis

This study was part of a larger SANPAD project entitled: *Assessing impacts and benefits of alien fish introductions: Do biodiversity costs outweigh economic, food security and recreational benefits derived from alien fishes in South Africa?* The main objective of the larger SANPAD project was to determine the impacts of alien fishes on the local economy, food security, employment provision and on the environment in three man-made impoundments. The present PhD project focused on the health of the fish species in these three impoundments, particularly in terms of the effects of heavy metal pollution and of alien parasite species. From the previous section it can be seen that there is a paucity of information regarding the health status of the fish species within the selected impoundments. In order to fill the gaps in the information, the following hypothesis was proposed: necropsy-based and histology-based fish health assessment can successfully be implemented as tools to assess the effects of heavy metal pollution and alien fish parasites in freshwater fish from selected impoundments in the Amatola region, Eastern Cape Province, South Africa.

#### 1.6.1. Aims and objectives

To test the hypothesis, the main aim of this thesis was to use FHAI, histology and metal analysis to determine the impact of parasites and metal pollution on the health of selected fishes from three impoundments in the Amatola Region

The thesis is structured as follows:

After the general introduction **Chapter 1**, **Chapter 2** describes the study sites with previous research conducted in the study sites as well as the materials and methods used in this study

In order to understand the potential threat of water pollution in the Amatola Region, **Chapter 3** investigates the heavy metal concentrations in the surface water, sediment and the muscle tissue of a long lived predatory fish species that is present in all three impoundments. The aim of **Chapter 3** is to assess the health status of largemouth bass (*M. salmoides*) from the three impoundments, using a histology-based fish health assessment protocol (**Chapter 3**). To achieve this aim the following objectives were set:

- To compare possible macroscopic abnormalities between the three different impoundments
- To assess semi-quantitatively the histology of selected target organs (gills, liver, kidney and gonads), and to compare the results between the different impoundments.
- To categorise the results in terms of a classification system, indicating the severity of the histological responses indentified.

Another indigenous species that occurs in all three impoundments is *A. mossambica*. Therefore, the aim of **Chapter 4** was to conduct a comprehensive fish health assessment on wild-caught longfin eel (*A. mossambica*). In order to achieve this aim, the following objectives were set:

- To identify any possible macroscopic abnormalities.
- To assess the histology of selected target organs (gills, liver, kidney and gonads).
- To screen *A. mossambica* for parasites.
- To determine if the severity of the parasitism, and to determine whether the parasites had an effect on *A. mossambica*.

In order to assess the health status and a parasite diversity of each of the three impoundments, a large-bodied, dominant species from each impoundment was selected and studied. From Binfield Park Dam the two mullet species that had been stocked in the impoundment for the enhancement of the fisheries were used. From

Sandile Dam the moggel (*L. umbratus*) was used, and from Wriggleswade Dam the smallmouth yellowfish (*L. aeneus*) was used.

The aim of **Chapter 5** was thus to assess the health status of flathead mullet (*M. cephalus*) and the freshwater mullet (*M. capensis*) from Binfield Park Dam, using the fish health assessment protocols. To achieve this aim the following objectives were applied:

- To compare possible macroscopic abnormalities between *M. cephalus* and *M. capensis*.
- To assess semi-quantitatively the histology of selected target organs (gills, liver, kidney and gonads) and to compare the results between the two different species.
- To categorise the results in terms of a classification system indicating the severity of the histological responses indentified.

In **Chapter 6**, the aim was to determine health status of the moggel (*L. umbratus*) in Sandile Dam, and to determine the effects of *L. barnimiana* on *L. umbratus*, as well as to establish whether *Lernaea barnimiana* is an alien, indigenous or translocated parasite species to this region. The following objectives were applied to achieve this aim:

- To identify any macroscopic abnormalities through necropsy.
- To assess semi-quantitatively the histology of selected target organs (gills, liver, kidney and gonads).
- To classify the results in terms of a classification system indicating the severity of the histological responses indentified.
- To screen *L. umbratus* for parasites.
- To determine the severity of the parasitism, and to determine its effect on *L. umbratus*.

The aim of **Chapter 7** was to determine if smallmouth yellowfish (*L. aeneus*) could be used as a sentinel species in the Eastern Cape of South Africa to determine the potential impacts of the alien invasive parasite *L. cyprinacea* on the indigenous cyprinid species. To achieve this aim the following objectives were set:

- To identify any macroscopic abnormalities through necropsy.
- To assess semi-quantitatively the histology of selected target organs (gills, liver, kidney and gonads).
- To classify the results in terms of a classification system indicating the severity of the histological responses indentified.
- To screen *L. aeneus* for parasites.
- To determine the severity of the parasites infecting *L. aeneus* and to determine their effect on *L. aeneus*.

Finally, the general conclusion in **Chapter 8** will examine the necropsy- and histology-based fish health assessment protocols and determine their effectiveness in assessing the threat of water pollution and alien parasites in the Amatola Region of the Eastern Cape.

### 1.7. Project outputs

Some of the research completed throughout the duration of this PhD project has been presented as oral and poster presentations at national conferences as follows:

South African Society for Aquatic Scientists conferences:

- **McHugh KJ**, Weyl OLF, Smit NJ. 2012. A fishy tale of three man-made impoundments. Cape St Francis. Poster.
- **McHugh KJ**, Weyl OLF, Smit NJ. 2013. The health status of the African longfin eel, *Anguilla mossambica*, from three Eastern Cape impoundments, South Africa. Arniston, Western Cape. Oral.
- **McHugh KJ**, Weyl OLF, Smit NJ. 2014. The crustacean fish parasite *Learnaea barnimiana* in the Eastern Cape of South Africa: indigenous or alien? Thaba Nchu. Free State. Oral.

Parasitological Society of Southern Africa

- **McHugh KJ**, Weyl OLF, Smit NJ. 2013. Alien parasite invasion in the Eastern Cape: Threat to economically important freshwater fishes? Parys, Free State. Oral.
- **McHugh KJ**, Weyl OLF, van As LL, Smit NJ. 2014 The potential threat of two alien fish parasites to cyprinids in the Eastern Cape of South Africa. Skukuza. Kruger National Park. Oral.

One international conference:

World Association for the Advancement of Veterinary Parasitology

- **McHugh KJ**, Weyl OLF, Smit NJ. 2013. The potential impact of alien invasive parasites on economically important freshwater fish species in the Eastern Cape, South Africa. Perth, Australia.

One histology-based paper covering work from this thesis (**Chapter 5**) has already been published in an international peer-reviewed journal:

- **McHugh KJ**, van Dyk JC, Weyl OLF, Smit NJ. 2013. First report of nephrocalcinosis in a wild population of *Mugil cephalus* L. and *Myxus capensis* (Valenciennes). Journal of Fish Diseases 36: 887–889.

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# Chapter 2 : Materials and methods, and general sampling results

## 2.1. Site selection

This study was carried out in three impoundments, Binfield Park, Sandile and Wriggleswade, located in the Amatola region of the Eastern Cape, South Africa. The impoundments are close to Bisho, the capital city of the Eastern Cape (**Figure 2.1**).

### 2.1.1. Binfield Park Dam

Binfield Park Dam (S32° 40' 58.38"; E26° 54' 05.12") (**Figure 2.2A**), is a 260ha impoundment. It impounds the Tyume River and is used by both subsistence anglers from the local communities and occasionally by recreational bass anglers. Binfield Park Dam was completed in 1987, primarily to provide irrigation and drinking water to the surrounding communities in particular the nearby town of Alice. Binfield Park Dam is located 220 km inland, at an altitude of 665 m above sea level. It receives approximately 400 mm of rain per year and the mean ambient temperature is 18°C (Schulze *et al*. 2008). The Tyume River is a headwater tributary of the Keiskamma River and drains into the Indian Ocean to the west of the coastal town of Hamburg.

According to the reference frequency of occurrence of fish species in South Africa (Kleynhans *et al*. 2007) Binfield Park Dam falls in eco-region 16, the South Eastern uplands. This area is characterised by a variety of vegetation types including grassland, bushveld, thicket and afromontane forests, the most prominent being moist upland grassland (Kleynhans *et al*. 2005). According to Kleynhans *et al.* (2007), the expected indigenous fish species for the Binfield Park Dam are *Anguilla marmorata, A. mossambica, Barbus anoplus, B. trevelyani, Glossogobius callidus, Labeo umbratus, Sandelia bainsii, Myxus capensis* and *Mugil cephalus*. Its translocated species are *Clarias gariepinus* and *Tilapia sparrmanii* and the alien fish species are *Micropterus salmoides* and *Oncorhynchus mykiss* (Kleynhans *et al*. 2007).

Previous research in Binfield Park Dam includes work by Ellender *et al*. (2012), who validated the age of *Mugil cephalus* and *Myxus capensis*. Validation of the age of these two mullet species could be achieved because 75 000 wild-caught young of the year were introduced into Binfield Park Dam from the Keiskamma River estuary on the 13th of September 2000 (Ellender *et al.* 2012). McHugh *et al*. (2013) were the first to report on the kidney alteration nephrocalcinosis from these same two mullet species (*Mugil cephalus* and *Myxus capensis*) in Binfield Park Dam. This was the first report of nephrocalcinosis in these two mullet species in South Africa (McHugh *et al*. 2013). A microbiological study by Zamxaka *et al*. (2004) showed that water samples from Binfield Park Dam were less contaminated than other water samples collected from dams in the surrounding areas. Zamxaka *et al*. (2004) attributed this lower contamination to the fact that Binfield Park Dam was completely fenced, preventing domestic livestock from having access to the dam. However, recent sampling has shown that livestock now have access to the dam. The sharptooth catfish (*Clarias gariepinus*) derived from fish kept by the University of Fort Hare in aquaculture ponds and which escaped into Binfield Park Dam and the Tyume River and now threaten the endangered fish species in this system (Cambay 2003; de Moor & Bruton 1988).

### 2.1.2. Sandile Dam

Sandile Dam (S32° 42' 23.57"; E27° 06' 34.54") (**Figure 2.2B**) is a 146ha impoundment and is the smallest of the three dams in this study. It impounds the Wolf and Keiskamma Rivers. The dam was completed in 1983 for the sole purpose of supplying water for irrigation to surrounding communities. It was named after King Sandile the former king of the Rharbe, a sub-group of the Xhosa nation.

Sandile Dam also falls within eco-region 16, the South Eastern uplands (Kleynhans *et al*. 2005; Kleynhans *et al*. 2007). The expected indigenous fish species for Sandile Dam are *Anguilla marmorata, A. mossambica, Barbus anoplus, B. trevelyani, Glossogobius callidus, Labeo umbratus* and *Sandelia bainsii* (Kleynhans *et al*. 2007). The expected translocated species are *Clarias gariepinus, Oreochromis mossambicus* and *Tilapia sparrmanii* and the alien fish species are *Micropterus salmoides* and *Oncorhynchus mykiss* (Kleynhans *et al*. 2007).

Sandile Dam is situated downstream of the Keiskammahoek Transitional Local Council area, to which it provides treated water, as well as to the whole Middledrift District (Morrison *et al*. 2001). The Keiskamma River which flows into Sandile Dam is used by the local communities for the watering of livestock, as a source of drinking water, and for fishing and recreational purposes (Morrison *et al.* 2001). Morrison *et al.* (2001) stated that the waste water treatment plant situated in the town of Keiskammahoek was not efficient, and that, due to improper sanitation, the water is continually being polluted and that these problems were further compounded by sewage discharges into the Keiskamma River by the development of Reconstruction and Development Program (RDP) houses. Morrison *et al*. (2001) showed that pH, electrical conductivity and nitrate levels were below guideline levels. However, the orthophosphate, chemical oxygen demand and ammonium levels were higher than suggested guidelines and could potentially encourage eutrophication in Sandile Dam. Fatoki *et al*. (2003) also did research into pollution from the Keiskamma River entering Sandile Dam. These authors found the pH levels in the river and the impoundment were normal, but the turbidity and electrical conductivity of both the river and the impoundment were high. Fatoki *et al*. (2003) also found that the dissolved oxygen and biochemical oxygen demand were above the guidelines set out by the EU for the protection of aquatic ecosystems. He also found that the nutrient levels in these systems are eutrophic. Fatoki *et al*. (2003) attributed the pollution levels the Keiskammahoek Sewage Treatment Plant stating that, because of the poor performance of the sewage plant, the electrical conductivity, orthophosphate and oxygen demanding substances were above the South African water quality guideline levels.

### 2.1.3. Wriggleswade Dam

Wriggleswade Dam (S32° 35' 49.49"; E27° 33' 51.70") (**Figure 2.2C**), 1000 ha, is the largest of the three impoundments in this study. This impoundment is used extensively by recreational bass and carp anglers. Wriggleswade Dam, on the Kubusi River, was completed in 1991 and was constructed to supply water to the local municipalities. The Kubusi River flows into the Great Kei River before flowing into the Indian Ocean at the town of Kei Mouth. Wriggleswade Dam is situated at an altitude of 723 m above mean sea level (Taylor & Weyl 2013).

Wriggleswade Dam also falls within eco-region 16, the South Eastern uplands (Kleynhans *et al*. 2005; Kleynhans *et al*. 2007). The expected indigenous fish species for Sandile Dam are *Anguilla bicolor bicolor, A. marmorata, A. mossambica, Barbus anoplus* and *Liza macrolepis* (Kleynhans *et al*. 2007). The expected translocated species are *Clarias gariepinus, Labeobarbus aeneus* and *Tilapia sparrmanii,* and the expected alien fish species are *Cyprinus carpio, Micropterus punctulatus, M. salmoides* and *Oncorhynchus mykiss* (Kleynhans *et al*. 2007).

Previous research in Wriggleswade Dam was done on largemouth bass (*Micropterus salmoides*) to determine their movements after being displaced during catch-and-release angling tournaments (Huchzermeyer *et al*. 2013). Huchzermeyer *et al*. (2013) found that the bass displaced by up to 3.5 km remained within 3 to 4 km of their release site, but that fish displaced by 4.3 km immediately returned to their capture locations. Taylor & Weyl (2013) did an age validation on the resident largemouth bass population of Wriggleswade Dam in order to compare growth zone deposition between the introduced alien species to global data. Taylor & Weyl (2013) indicated that the growth zone deposition rate is annual throughout the native and introduced range of *M. salmoides*. According to Campana (2001), knowledge of growth and aging is an essential in providing population data that can be used in fisheries management. According to Gerber *et al.* (2012), the management of many tropical and subtropical freshwater fishes has been hindered by a lack of knowledge on their age structure.

## 2.2. Sampling

The sampling was done over three surveys in July 2011, March 2012 and August 2012. Fish were sampled from the three impoundments with the aid of gill nets (**Figure 2.2D**), fyke nets (**Figure 2.2E**), and rod-and-line angling (**Figure 2.2F**). The gill and fyke nets were left overnight with surface marker bouys for their easy relocation.

### 2.2.1. Necropsy, blood parameters and biometric indices

After collection, the live fish were kept in aerated containers (**Figure 2.3A**) and brought to a field laboratory (**Figure 2.3B**), where they were weighed using a calibrated digital lip-grip scale (Berkley Fishing, United States of America) (**Figure 2.3C**) and measured to the nearest mm (Total, fork and standard length) (Figure 2.3D) for the calculation of condition factor (CF) (Carlander 1969) and gutted condition factor (GCF). Blood was drawn from the live fish using a sterile 21 gauge 1½ inch needle and 4 mL heparin vacutainers (**Figure 2.3E**). The vacutainers were centrifuged for 10 min at 3 000 $r \cdot min^{-1}$ for the determination of the total plasma protein and stored at 4 °C until samples could be returned to the laboratory. Once returned to the laboratory, the total plasma protein samples were prepared using a total protein kit (Roche) and analysed in triplicate using a universal micro-plate reader (Biotek micro-plate reader, 540 nm wave lengths). Blood was also collected in capillary tubes and centrifuged for 10 min at 3 000 r·min-1 for the determination of the hematocrit (**Figure 2.3F**). Blood smears were made and fixed in methanol for the determination of the leukocrit.

Each fish was macroscopically examined for the presence of parasites or any injuries or abnormalities. The fish were sacrificed by severing the spinal cord anterior to the dorsal fin. Fish were then internally examined for any abnormalities, parasites or injuries, and assessed according to the protocol of Adams *et al*. (1993). The whole liver and gonads were removed and weighed. These weights were used for the calculation of hepatosomatic index (HSI), spleenosomatic index (SSI) and gonadosomatic index (GSI) using the formula: organ weight/body weight x 100.

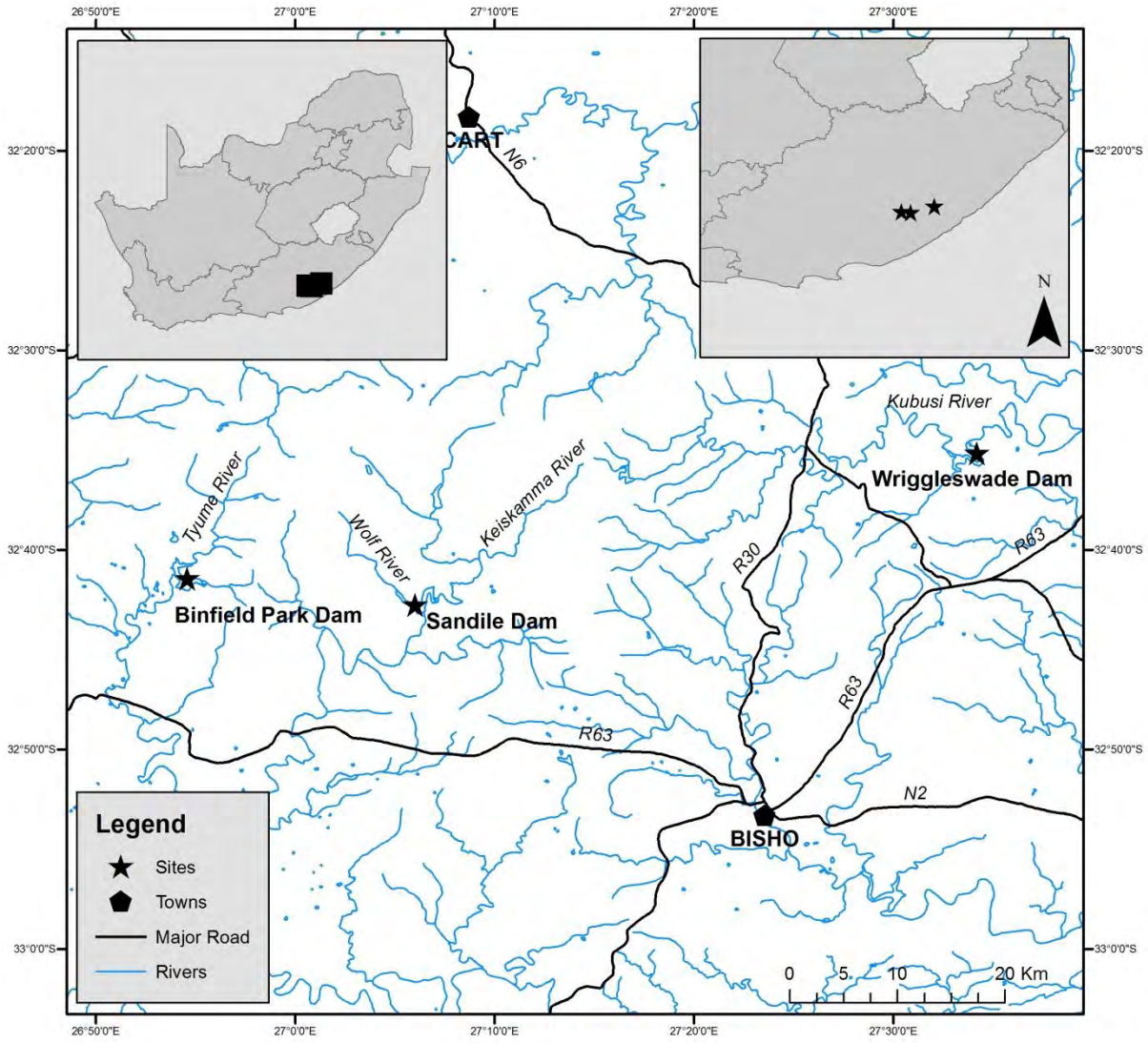


**Figure 2.1:** Location of Binfield Park, Sandile and Wriggleswade Dams near the town of Bisho, Eastern Cape Province, South Africa.

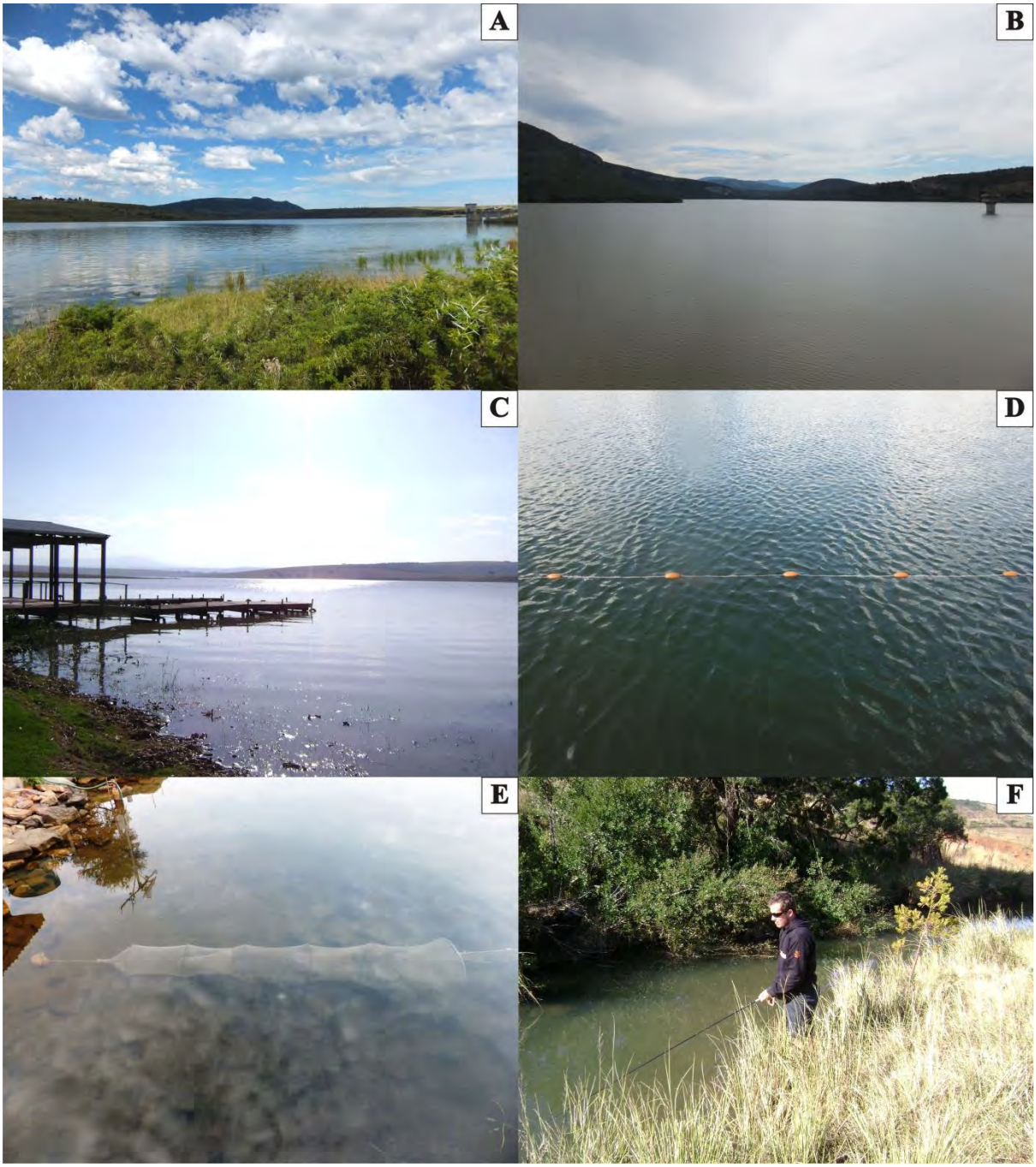


**Figure 2.2:** Research sites at each impoundment and sampling techniques used: (A) Binfield Park Dam; (B) Sandile Dam; (C) Wriggleswade Dam; (D) a section of gill net; (E) a fyke placed in the water; (F) a researcher practicing recreational fishing techniques.

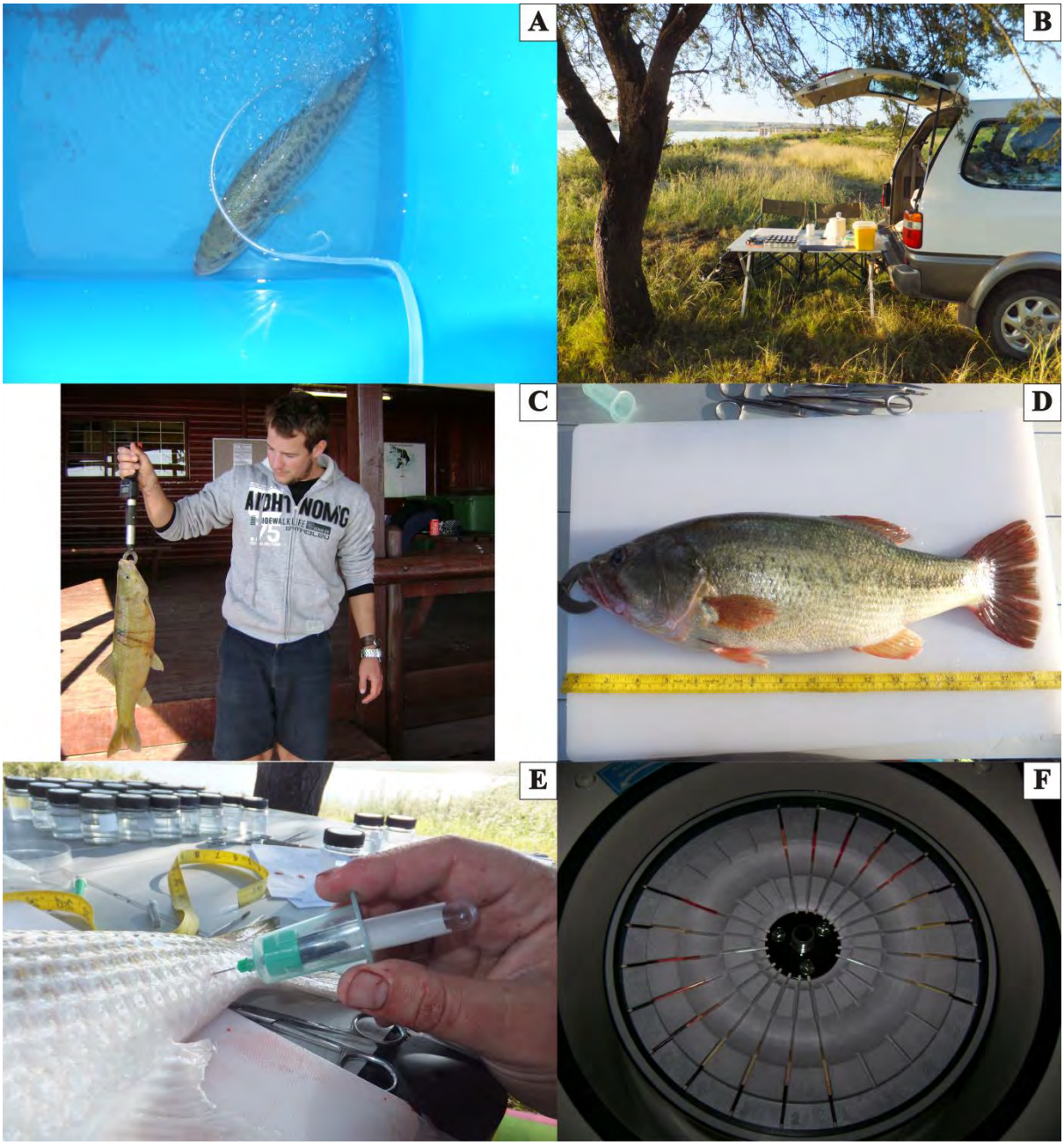


**Figure 2.3:** Sampling in the Amatola Region: (A) Fish sample in aerated plastic container; (B) Field laboratory were fish were analysed; (C) Researcher weighing fish using a lip grip scale; (D) measuring lengths; (E) Bloodletting from fish samples; (F) centrifuging hematocrit samples**.**

### 2.2.2. Tissue sampling

A section of the gills, liver, kidney and gonads were removed and fixed in 10% neutral buffered formalin for 48 hours. Following fixation, the samples were washed in tap water and dehydrated in a series of increasing concentrations of ethanol (30% – 50% – 70%). The tissue samples were prepared for histological assessment using standard procedures (Humason 1962). Samples were stained with haematoxylin and eosin (H&E). The histological samples were analysed by means of light microscopy. The samples were assessed using a semi quantitative histological assessment protocol (van Dyk *et al*. 2009) adapted from Bernet *et al*. (1999), to quantify any histological alterations observed in the selected target organs. Any observed histological alternations were assessed in terms of five reaction patterns: circulatory disturbances (CD), regressive changes (RG), progressive changes (PC), inflammation (I) and tumours (T). The semi-quantitative results were used to calculate an organ index for each of the selected target organs (gill index $I_G$, liver index $I_L$, kidney index $I_K$, testis index $I_T$ and/or ovary index $I_O$) for each specimen. These index values were used to classify the severity of the histological alterations using a scoring scheme classification system of van Dyk *et al*. (2009) adapted from Zimmerli *et al*. (2007).

- Class 1 (index < 10) normal tissue structure with slight histological alterations
- Class 2 (index 10 – 25) normal tissue structure with moderate histological alterations
- Class 3 (index 26 – 35) pronounced alterations of organ tissue
- Class 4 (index > 35) severe alterations of organ tissue

The sum of the three organ indices (gill index $I_G$, liver index $I_L$, kidney index $I_K$, testis index $I_T$ and/or ovary index $I_O$) was used to calculate an overall fish index ($I_{FISH}$) value, in order to indicate the combined histological response of the sampled organs per fish.

### 2.2.3. Parasite assessment

The fish were screened macroscopically for the presence of any external parasites. Any macroscopic parasites observed were removed and placed in sample bottles for further analysis at the North West University laboratories. The fish were then sacrificed by severing the spinal cord anterior to the dorsal fin and dissected. The gills were dissected out and placed in saline solution in petri dishes and examined for any gill parasites. Once dissected, the intestines were placed in saline solution in petri dishes for examination. Parasites were collected immediately after the death of the fish to prevent the effect of decay. The intestines were carefully opened and examined for the presence of cestodes, digeneans, nematodes and acanthocephlans. If found, all cestodes, digeneans, nematodes and acanthocephlans were removed and transferred to clean sample bottles.

In order to determine if there were any blood parasites, blood slides were made using a drop of blood collected from the caudal vein. The blood smear was air-dried, fixed in methanol and stained with phosphate-buffered Giemsa (pH 6.8) according to standard procedures (Davies *et al*. 2005).

### 2.2.4. Statistical analysis

Descriptive statistics are presented in the form of means, standard deviations and percentage prevalence. The CF, GCF, HSI, GSI and semi quantitative histology results were compared between the sampling surveys using one-way ANOVA (IBM SPSS V22). Statistical differences were considered to be significant at $p < 0.05$. Degrees of freedom are represented by *df1* =. Degrees of freedom are the number of values in the final calculation of a statistic that are free to vary.

### 2.3. General sampling results

The catch results for Binfield Park, Sandile and Wriggleswade Dams are represented in **Table 2.1**, **2.2** and **2.3,** respectively.

**Table 2.1:** Catch results for Binfield Park Dam: mean total body mass (g), fork length (mm), except for *Anguilla mossambica* which is total length, and condition factor (CF).

| Survey | Males | Females | Mass | Length | CF |
|---|---|---|---|---|---|
| *Anguilla mossambica* (n = 11)** | 0 | 11 | 820.0 ± 405.9 | 665.0 ± 111.2 | 2.6 ± 0.1 |
| *Anguilla mossambica* (n = 1)*** | 0 | 1 | 1740.0 ± 0 | 878.0 ± 0 | 2.57 ± 0 |
| *Micropterus salmoides* (n = 7)* | 0 | 7 | 1588.6 ± 534.3 | 462.3 ± 55.8 | 1.6 ± 0.1 |
| *Micropterus salmoides* (n = 9)** | 4 | 5 | 866.7 ± 647.5 | 371.9 ± 75.9 | 1.5 ± 0.1 |
| *Myxus capensis* (n = 15)* | 0 | 15 | 2233.3 ± 263.4 | 543.7 ± 17.1 | 1.4 ± 0.1 |
| *Mugil cephalus* (n = 20)** | 8 | 12 | 2263.0 ± 428.0 | 544.4 ± 37.5 | 1.4 ± 0.1 |

Denotes * = July 2011, ** = March 2012, *** = August 2012

**Table 2.2:** Catch results for Sandile Dam: mean total body mass (g), fork length (mm), except for *Anguilla mossambica* which is total length, and condition factor (CF).

| Survey | Males | Females | Mass | Length | CF |
|---|---|---|---|---|---|
| *Anguilla mossambica* (n = 2)** | 0 | 2 | 600.0 ± 367.7 | 667.0 ± 178.2 | 1.9 ± 0.1 |
| *Micropterus salmoides* (n = 4)* | 1 | 3 | 390.0 ± 315.6 | 313.5 ± 55.4 | 1.10 ± 0.3 |
| *Micropterus salmoides* (n = 15)** | 4 | 11 | 456.67 ± 352.3 | 327.8 ± 75.9 | 1.16 ± 0.2 |
| *Labeo umbratus* (n = 15)* | 9 | 6 | 344.0 ± 75.7 | 295.8 ± 21.4 | 1.16 ± 0.2 |
| *Labeo umbratus* (n = 15)** | 9 | 6 | 356.0 ± 89.2 | 313.6 ± 31.1 | 1.17 ± 0.2 |

Denotes * = July 2011, ** = March 2012

**Table 2.3:** Catch results for Wriggleswade Dam: mean total body mass (g), fork length (mm), except for *Anguilla mossambica* which is total length, and condition factor (CF).

| Survey | Males | Females | Mass | Length | CF |
|---|---|---|---|---|---|
| *Anguilla mossambica* (n = 1)** | 0 | 1 | 480.0 ± 0 | 591.0 ± 0 | 2.3 ± 0 |
| *Anguilla mossambica* (n = 4)*** | 0 | 4 | 900.0 ± 465.2 | 680.25 ± 143.6 | 2.5 ± 0.1 |
| *Micropterus salmoides* (n = 15)* | 4 | 11 | 708.53 ± 339.6 | 371.10 ± 65.2 | 1.30 ± 0.2 |
| *Micropterus salmoides* (n = 15)** | 6 | 9 | 374.67 ± 137.8 | 309.93 ± 42.1 | 1.22 ± 0.2 |
| *Labeobarbus aeneus* (n = 15)* | 5 | 10 | 1734.93 ± 484.3 | 509.8 ± 43.5 | 1.28 ± 0.1 |
| *Labeobarbus aeneus* (n = 15)** | 10 | 5 | 1796.0 ± 387.7 | 535.6 ± 33.5 | 1.15 ± 0.1 |

Denotes * = July 2011, ** = March 2012, *** = August 2012

### 2.4. General discussion of sampling results

Compared to the expected species for these systems, the catches were largely dominated by alien and translocated fish species. The only true indigenous fish species represented in all three impoundments was the longfin eel *A. mossambica*. Although *M. capensis* and *M. cephalus* are indigenous to the Keiskamma River system, they were originally stocked into Binfield Park Dam as fingerlings (Ellender *et al*. 2012). These two mullet species are estuarine species and the dam wall has prevented their movement back to the Keiskamma estuary. According to Skelton (2001), *L. umbratus* has a natural distribution in the Orange-Vaal River system, as well as in the Gourits, Gamtoos, Sundays, Great Fish and Bushmans River systems, and that it has been translocated to the Keiskamma and Buffalo River systems. However, according to Weyl (South African Institute of Aquatic Biodiversity. pers. comm.), *L. umbratus* is indigenous to the Keiskamma River system. The smallmouth yellowfish *L. aeneus* was transloacted from its natural distribution range in the Orange-Vaal River system to the Gourits, Great Fish and Kei River systems. *Labeobarbus aeneus* was accidently introduced into the Great Fish River via the Orange-Fish River tunnel, which was opened in 1975. It was first recorded in Grassridge Dam in 1976 (de Moor & Bruton 1988). Only the alien invasive fish species, the largemouth bass *M. salmoides*, was sampled from the three impoundments in sufficient numbers to use them for histological comparisons between the three impoundments.

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# Chapter 3 : Research into the heavy metal concentrations in the water and sediment from three Eastern Cape impoundments, South Africa as well as a complete health assessment and metal analysis of the muscle tissue of *Micropterus salmoides*.

## 3.1. Introduction

The largemouth bass *Micropterus salmoides* (Lacepède, 1802) is an alien invasive species that was introduced into South Africa in 1928 (de Moor & Bruton 1988). Largemouth bass were imported to the Jonkershoek Hatchery from the Surrey trout farm, England. Interestingly, these fish were originally bred in Holland (de Moor & Bruton 1988). Distribution from the Jonkershoek Hatchery to other localities began in 1930 and, in 1952, the Umgeni Hatchery opened in what is currently known as the Kwazulu-Natal province (KZN), was stocked with largemouth bass and began to distribute these species around KZN (de Moor & Bruton 1988). Largemouth bass were imported primarily as an angling species. However, recommendations were also made for the species to be used in aquaculture (de Moor & Bruton 1988). They are predatory fish. And mainly consume invertebrates when they are small and shift their diets towards fish as their size increases (Weyl & Hecht 1999; Wasserman *et al*. 2011). The introduction of largemouth bass into South Africa has had a negative impact on South Africa's aquatic biodiversity (Ellender & Weyl 2014; Ellender *et al*. 2014). Weyl *et al*. (2010) noted that the presence of largemouth bass had disrupted macro-invertebrate community structure, having a significant negative influence on Odonata, Hemiptera and Coleoptera. In a study by Gratwicke & Marshall (2001), from 42 sampling stations in streams around Harare, Zimbabwe, the authors recorded the severe predatory impact of largemouth bass on the indigenous *Barbus* species, compared to that of the other exotic predator *Serranochromis robustus*.

Originally eels were the only predator in Eastern Cape rivers (Jubb 1964). However, the introduction of largemouth bass resulted in an introduction of a new predatory species. Although the introduction of largemouth bass has had negative impacts on river systems (Woodford *et al*. 2005; Weyl & Lewis 2006; Wasserman *et al*. 2011) they do provide an additional non indigenous fish species that can be exploited for

research into the threats of human activities. According to Adams *et al.* (1993), organisms at the top of the food chain are representative indicators of overall ecosystem health. In South Africa predatory species such as the tigerfish *Hydrocynus vittatus* have successfully been used to determine the levels of metal concentrations in muscle tissue (du Preez & Steyn 1992) as well as the bioaccumulation of organohalogens (Wepener *et al.* 2012). Bouwman *et al*. (1990) compared the levels of DDT between three species of fish from the Phongolo system and showed that tigerfish had three times higher DDT levels than the other fish species. Wepener *et al.* (2012) indicated that this demonstrated that the predatory species bio-magnified the levels of DDT. The second advantage of using largemouth bass in the Eastern Cape impoundments is that they prefer slow-flowing or standing waters and are not a migratory species (Skelton 2001). This results in these fish remaining in the impoundments permanently. Internationally, largemouth bass have been used extensively as an indicator species for the determination of heavy metal contamination in fish (see below 3 references).

Murphy *et al.* (1978) analysed the muscle tissue of *M. salmoides* from an industrially contaminated lake and found that *M. salmoides* was significantly contaminated with cadmium and zinc, and reported that the consumption of these fish was not a potential human hazard, unless these fish formed a substantial part of the diet. Foster *et al.* (2000) collected *M. salmoides* from the Dorena Reservoir, Oregon, in order to determine the concentrations of total mercury in the muscle, liver and gonads. Foster *et al.* (2000) found that there were seasonal differences in the concentration levels of mercury in the liver and gonads, but not in the muscle tissue of *M. salmoides*. Foster *et al.* (2000) also noted that the concentrations of mercury were higher in the liver samples than in the muscle tissue, and stated that the liver tissue gave a more sensitive result than the muscle tissue. However, the muscle tissue concentrations provide more accurate information in areas where *M. salmoides* is consumbed. Sepúlveda *et al.* (2002) studied the effects bleached and unbleached kraft mill effluent on the reproductive fitness of *M. salmoides*. The authors showed that *M. salmoides* exposed to kraft mill effluent had a decline in sex steroids, a decline in GSI values as well as changes in organ tissue responses.

In the early part of this century, Morrison *et al.* (2001) noted that the Keiskammahoek Sewage Treatment plant, on the Keiskamma River, has a discharge point just above

Sandile Dam. Morrison *et al.* (2001) noted that the sewage treatment plant is simple and not efficient, and that often, due to overflows or malfunctioning, sewage is dumped directly into the Keiskamma River at the discharge point. This sewage discharge is a major pollution source which causes nutrient loading, contributing to higher oxygen demand in the water and can contribute to the eutrophication of Sandile Dam, as well as to algae blooms (Morrison *et al.* 2011). Other research by Awofolu & Fatoki (2003) in the Keiskamma and Tyume Rivers as well Sandile Dam indicated that there were high concentration levels of persistent organochlorines such as DDT, chlordane, hexchlorobezene, heptachlor and endosulfan in the water and sediment samples. The authors determined that the sources of the pollution were from runoff from agricultural lands and effluents from industries. Fatoki & Awofolu (2003) reported on high levels of trace metals including Cd, Hg and Zn in sediment and surface water from the Keiskamma and Tyume River systems, as well as from Sandile Dam. These authors noted the that the excessive levels of Cd found in the rivers and dam may affect the health of the aquatic ecosystem, as well as the health of the rural communities that use the rivers and dam for domestic purposes (Fatoki & Awofolu 2003). Fatoki & Awofolu (2003) noted that the possible sources the high levels of Cd, Hg and Zn may be rural and urban runoff, agricultural runoff as well as point source pollution from the Keiskammahoek Sewage Treatment plant.

Although limited research has been done on metal concentrations in the water and sediment of the Keiskamma and Tyume Rivers and Sandile Dam, these studies were unclear on whether the samples had been taken from above or below Binfield Park Dam. To date, no research has also been done on the metal concentrations in the Kubusi River above its confluence with the Great Kei River. There is also no information on the health of the fish in these systems, as well the metal concentrations in the muscle tissue of the fish species.

Therefore, the first aim of this section of the study was to assess the metal concentrations in the water, sediment and muscle tissue of *M. salmoides* from Binfield Park, Sandile and Wriggleswade Dams. The second aim was to compare the health status of *M. salmoides* between the three impoundments, using a histology-based fish health assessment protocol. Theses aims were achieved using the following objectives: to compare any possible macroscopic abnormalities between the same fish species from the three different impoundments. Too semi-

quantitatively to assess the histology of selected target organs (gills, liver, kidney, testis and ovaries), and compare the results between the different the different impoundments. The final objective was to categorise the results in terms of a classification system indicating the severity of the histological response identified.

### 3.2. Materials and methods

The study took place in Binfield Park Dam (S32° 40' 58.38"; E26° 54' 05.12"), Sandile Dam (S32° 42' 23.57"; E27° 06' 34.54") and Wriggleswade Dam (S32° 35.187; E27° 34.055) (see **Chapter 2, Section 2.1**). The materials and methods used in this chapter were discussed in detail in **Chapter 2**. *Micropterus salmoides* were collected from Binfield Park Dam (n = 16), Sandile Dam (n = 19) and Wriggleswade Dam (n = 30) in July 2011 and March 2012 by means of gill nets and by angling (see **Chapter 2, Section 2.2**). Determination of the blood parameters, necropsy, and biometric indices followed **Section 2.2.1**. Tissue preparation for histological analysis followed the method set out in **Section 2.2.2** and statistical analysis followed **Section 2.2.5**.

#### 3.2.1. Metal analysis

Muscle tissue, sediment and water were collected from Binfield Park, Sandile and Wriggleswade Dams for chemical analysis.

##### 3.2.1.1. Water quality

The water samples were collected in pre-cleaned 1 ℓ plastic bottles. The bottles were rinsed with water from the site before a sample was collected. After collection, samples were stored at -4°C until further analysis. *In situ* water quality variable were also recorded using a YSI professional plus water quality meter. The *in situ* water quality parameters collected were temperature (°C), pH and electrical conductivity (µS/cm), total dissolved solids (ppm) and turbidity.

The frozen samples were returned to the laboratory for further analysis. Once back at the North West University Laboratories, the water samples were allowed to

defrost. The water samples were separated for additional chemical analysis. The samples assigned for metal analysis (As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Ti, U and Zn) were filtered through 0.45µm filter paper before being analysed on an inductively-coupled plasma spectrometer (ICP-MS), using standard techniques.

The additional nutrients analysed were: alkalinity (mmol/l and mg/l), ammonium ($NH_4^+$-mg/l), chloride (Cl – mg/l), Nitrate ($NO_3$ – mg/l), Nitrite ($NO_2$ – mg/l), phosphate (P – mg/l), sulphate ($SO_4$ – mg/l).

#### 3.2.1.2. Sediment

Sediment samples were collected in 350 mℓ plastic jars and frozen to prevent the loss of organic material through organic decomposition. In the laboratory the samples were allowed to thaw before the moisture content was determined, using the methods described by Wepener & Vermeulen (2005). Metal (Al, As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Ti, U and Zn) concentrations in the sediment were determined using an inductively-coupled plasma mass spectrometer (ICP-MS), using standard techniques (USEPA 2001; Hassan *et al*. 2007).

#### 3.2.1.3. Muscle tissue

Approximately 15 grams of axial muscle tissue were removed from each fish and wrapped in aluminium foil before being frozen in a transport freezer, transported back to the laboratory and stored at -20°C. In the laboratory, tissue samples were thawed and dried at 60°C for 96 hours and digested using a microwave digester. Samples were then screened for metals (Al, As, Fe, Hg, Mn, Ti and Zn) using an inductively-coupled plasma mass spectrophotometer (ICP-MS), following standard techniques (Blust *et al*. 1988).

## 3.3. Results

### 3.3.1. Water quality analysis

Water quality parameters measured at each of the three impoundments are presented in **Table 3.1**. There was no significant difference ($p \geq 0.05$; $df1 = 2$) between the parameters assessed between the three impoundments. However, chloride, conductivity and sulphate in Wriggleswade Dam were slightly higher than in the other two impoundments, and Sandile Dam was more turbid.

**Table 3.1:** Water quality for Binfield Park, Sandile and Wriggleswade Dams

| Parameter | Binfield Park Dam | Sandile Dam | Wriggleswade Dam |
|---|---|---|---|
| Alkalinity (mg/l) | 48.8 | 42.7 | 36.6 |
| Alkalinity (mmol/l) | 0.8 | 0.7 | 0.6 |
| Ammonium (mg/l) | 0.19 | 0.16 | 0.14 |
| Chloride (mg/l) | 9.1 | 7.9 | 17.8 |
| Conductivity (µS) | 70.8 | 72.2 | 109.8 |
| Nitrate (mg/l) | 2.2 | 7.1 | 5.5 |
| Nitrite (mg/l) | < 0.01 | < 0.01 | < 0.01 |
| pH | 9.16 | 8.96 | 9.51 |
| Phosphate (mg/l) | 0.40 | 0.39 | 0.90 |
| Sulphate (mg/l) | 18.0 | 16.0 | 25.0 |
| Temperature (°C) | 11.9 | 9.6 | 9.4 |
| Total dissolved solids (ppm) | 35.6 | 40.8 | 56.2 |
| Turbidity (NTU) | 53 | 107.2 | 62.3 |

The metal concentrations in the water are represented in **Table 3.2,** as well as the results of previous research by Fatoki & Awofolu (2003) in the Tyume River, which flows into Binfield Park Dam, as well as in Sandile Dam. Fatoki & Awofolu (2003) only reported on the concentrations of Cd, Hg and Zn in the water from the Tyume River and Sandile Dam. The Cd levels for Binfield Park, Sandile and Wriggleswade Dams were lower than those reported by Fatoki & Awofulu (2003) from Tyume River and Sandile Dam. Mercury (Hg) was found only in water from Wriggleswade Dam, and was at a similar concentration to the levels reported by Fatoki & Awofulu (2003). However, Hg was below detection values in Binfield Park and Sandile Dams. Zinc (Zn) was also below detection level in the water of Binfield Park, Sandile and Wriggleswade Dams, although Fatoki & Awofulu (2003) did report its presence here.

When the differences in the water metal concentrations measured at each impoundment are presented as a percentage contribution (**Figure 3.1),** it is clear that Binfield Park Dam had the highest levels of Cd, Co, Cu, Mn, Ni, Pb and U, compared to the water sampled from Sandile and Wriggleswade Dams, although the levels of Cd were lower than the previously recorded levels of Fatoki & Awofolu (2003). Wriggleswade Dam had the highest concentration of Fe and Ti. Mercury (Hg) was detected only in Wriggleswade Dam. However, the levels of Hg detected in the present study were similar to those reported by Fatoki & Awofolu (2003). Zinc (Zn) was not detected during the present study in any of the three impoundments.

**Table 3.2:** Various metals recorded in the surface water from Binfield Park (BD), Sandile (SD) and Wriggleswade Dams (WD) analysed using ICP-MS and compared to previous research by Fatoki & Awofolu (2003) in the Tyume River (TYR) and Sandile Dam (SD). Values reported in µg/mℓ.

| | Sampling site | | | Fatoki & Awofolu (2003) | |
|---|---|---|---|---|---|
| Metal | BD | SD | WD | TYR | SD |
| As | 0.00056 | 0.000255 | 0.000711 | NR | NR |
| Cd | 0.000163 | 0.000126 | 0.000131 | 0.017 | 0.015 |
| Co | 0.000706 | 0.000228 | 0.000254 | NR | NR |
| Cu | 0.0005069 | ND | 0.0004042 | NR | NR |
| Fe | 0.0198 | 0.01599 | 0.02316 | NR | NR |
| Hg | ND | ND | 0.004879 | 0.003 | 0.004 |
| Mn | 0.01529 | 0.002159 | 0.003658 | NR | NR |
| Ni | 0.002578 | 0.000511 | 0.00065 | NR | NR |
| Pb | 0.000121 | 0.0000540 | 0.000057 | NR | NR |
| Ti | 0.000907 | 0.001038 | 0.001454 | NR | NR |
| U | 0.000201 | 0.000149 | 0.000161 | NR | NR |
| Zn | ND | ND | ND | 0.324 | 0.327 |

NR = not reported by Fatoki & Awofolu (2003), ND = below detection limit

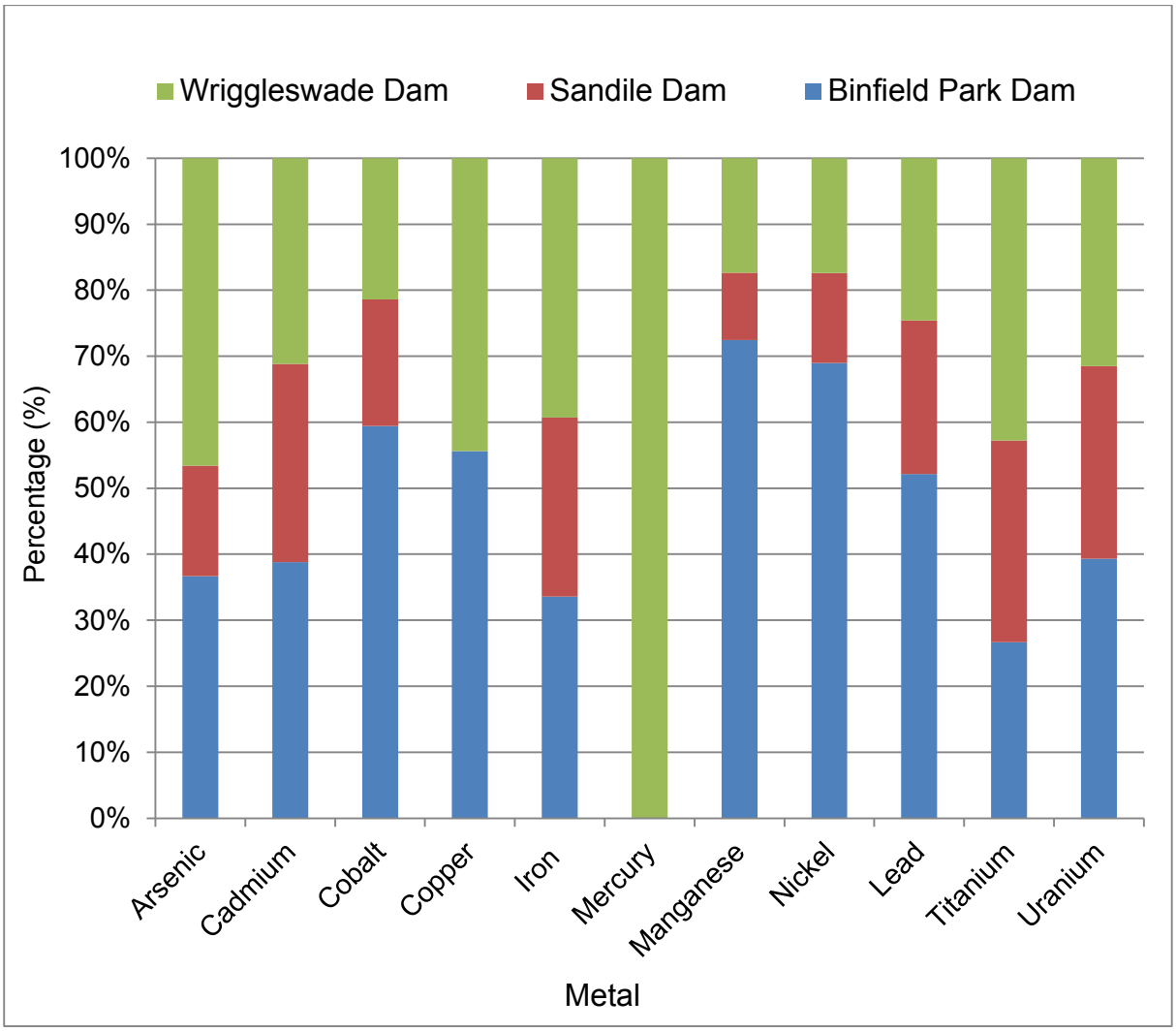


**Figure 3.1:** Differences in metal concentrations measured in surface water from Binfield Park, Sandile and Wriggleswade Dams, indicated as a percentage contribution.

### 3.3.2. Sediment analysis

The levels of the various metals that were recorded in the sediment samples from Binfield Park, Sandile and Wriggleswade Dams are summarised in **Table 3.3**. **Figures 3.2** and **3.3** show the comparison of the metals between each impoundment. Binfield Park Dam had the highest levels of Mn and Ni. However, both of these metals, as well as Co, were above the guidelines highlighted by Pheiffer *et al*. (2014) for metal concentrations in sediment. Sandile Dam had the highest concentrations of Al, As, Co, Cu, Fe, Hg, Pb, and Ti. The metal concentrations were below the guideline concentrations levels of Pheiffer *et al*. (2014). The concentration levels of Co, Cu, Mn and Ni exceeded the guideline values in Sandile Dam. Wriggleswade Dam had the highest level of U. Of the other impoundments, the concentration levels were also above the guideline levels. Cadmium (Cd) was not detected in any of the three impoundments. However, previous research by Fatoki & Awofolu (2003) found trace levels of the metal in the sediment of the Tyume River. Mercury (Hg) was not detected in the previous study by Fatoki & Awofolu (2003). However, trace amounts of the metal are present although below the guideline levels. Zinc (Zn) was found in all three impoundments. However, the levels were below the guideline levels. Previous research by Fatoki & Awofolu (2003) only found trace amounts of Zn in the Tyume River, and it was not detected in Sandile Dam. The concentrations of Zn are now present in the sediment of all three impoundments and highest in Sandile Dam.

**Table 3.3:** Metals recorded in sediment from Binfield Park Dam (BD), Sandile Dam (SD) and Wriggleswade Dam (WD) analysed using ICP-MS, compared to previous results by Fatoki & Awofolu (2003) in the Tyume River (TYR) and Sandile Dam (SD). Values reported in µg/g. Threshold values are from the guidelines of Australia–New Zealand (ANZECC 2000), Netherlands (Friday 1998), Canada (Friday 1998), Hamilton 2004, Sheppard *et al*. (2005).

| | Sampling site | | | Fatoki & Awofolu (2003) | | Guideline value |
|---|---|---|---|---|---|---|
| Metal | BD | SD | WD | TYR | SD | |
| Al | 10934.04 | 41781.32 | 3978.95 | NR | NR | |
| As | 0.81 | 2.53 | 0.72 | NR | NR | 5.90 |
| Cd | ND | ND | ND | 0.063 | ND | 0.57 |
| Co | 33.52 | 42.80 | 2.11 | NR | NR | 20.0 |
| Cu | 13.46 | 48.91 | 0.00002 | NR | NR | 16.0 |
| Fe | 22073.65 | 50950.57 | 4257.27 | NR | NR | |
| Hg | 0.0000020 | 0.0020 | 0.0018 | ND | ND | 0.17 |
| Mn | 1633.80 | 950.13 | 52.10 | NR | NR | 460.0 |
| Ni | 205.74 | 37.45 | 2.55 | NR | NR | 18.0 |
| Pb | 1.81 | 9.06 | 0.95 | NR | NR | 35.0 |
| Ti | 190.75 | 647.65 | 76.11 | NR | NR | |
| U | 0.67 | 0.72 | 2.51 | NR | NR | 2.50 |
| Zn | 16.35 | 34.43 | 12.73 | 0.225 | ND | 123.0 |

Grey indicates vales above guideline concentrations.

NR = not reported by Fatoki & Awofolu (2003), ND = below detection limit

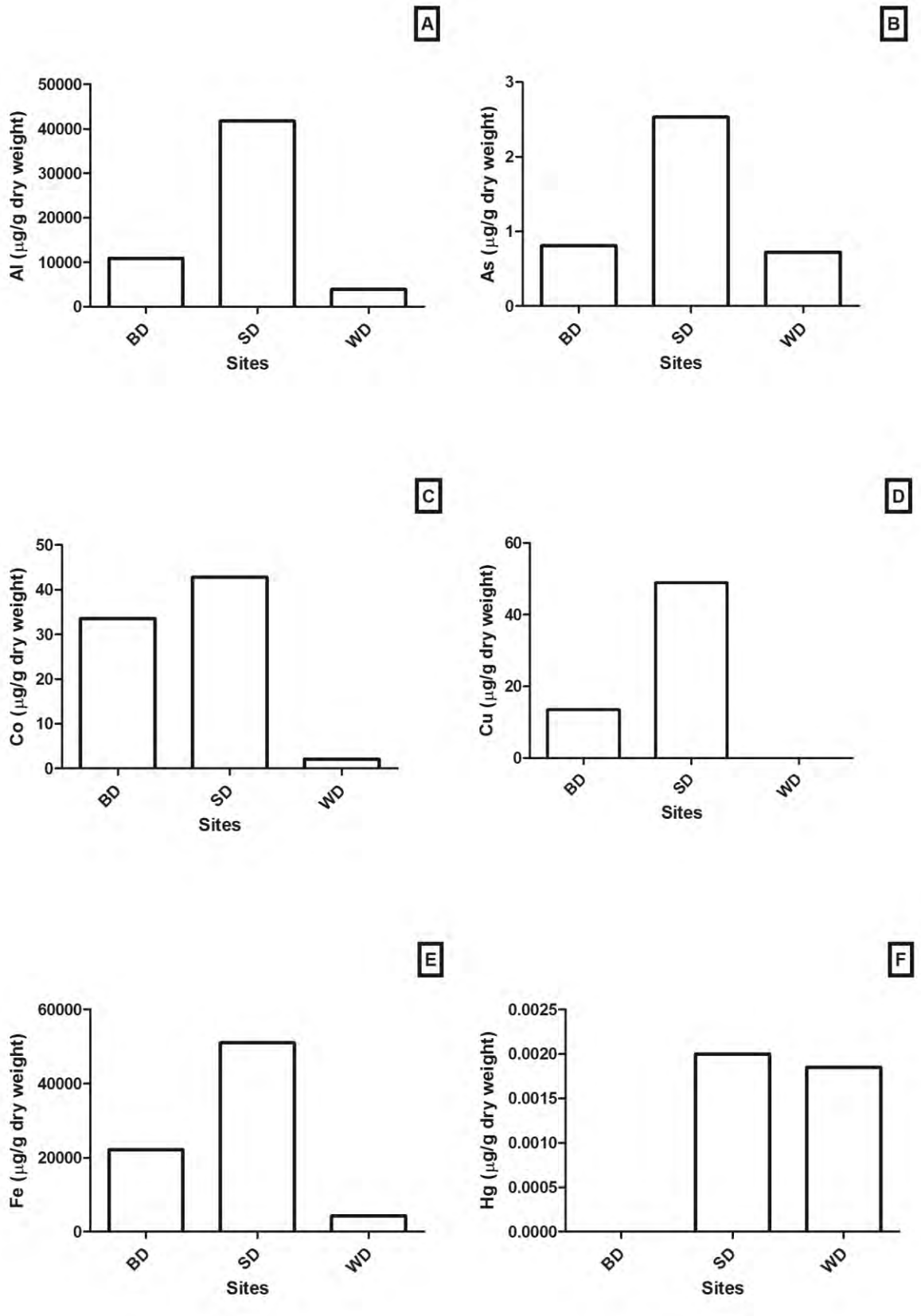


**Figure 3.2:** Mean concentrations (µg/g) of (A) alumium (Al), (B) arsenic (As), (C) cobalt (Co), (D) copper (Cu), (E) iron (Fe) and (F) mercury (Hg) in sediment from Binfield Park (BD), Sandile (SD) and Wriggleswade Dams (WD).

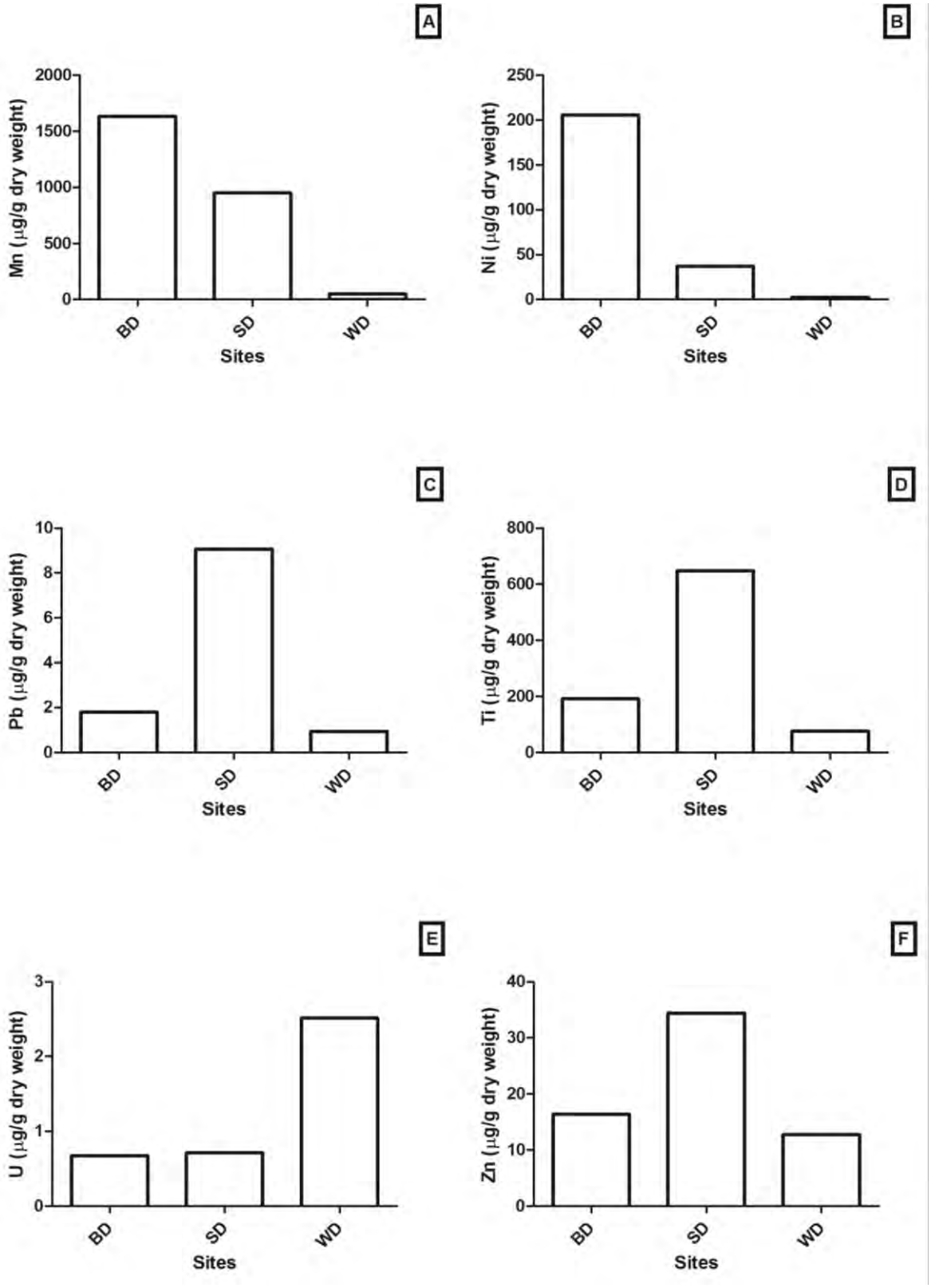


**Figure 3.3:** Mean concentrations (µg/g) of (A) manganese (Mn), (B) nickel (Ni), (C) lead (Pb), (D) titanium (Ti), (E) uranium (U) and (F) zinc (Zn) in sediment from Binfield Park (BD), Sandile (SD) and Wriggleswade Dams (WD).

### 3.3.3. Muscle tissue analysis

The levels of the different metals found in the muscle tissue of *Micropterus salmoides* are summarised in **Table 3.4**. A statistical comparison was made between the levels of the various metals detected in *M. salmoides* from Binfield Park, Sandile and Wriggleswade Dams. There was no significant ($p \geq 0.05$) difference between the levels of Al in the muscle tissue of *M. salmoides* between Binfield Park, Sandile and Wriggleswade Dams. Similarly, there was no significant ($p \geq 0.05$) difference between the levels of As (**Figure 3.4A**) and Fe (**Figure 3.4B**) in the muscle tissue of *M. salmoides* between the three impoundments. There was a significant difference ($p \leq 0.05$) in the concentration levels of Hg between the impoundments with the values from Binfield Park Dam significantly higher than those from Sandile or Wriggleswade Dam. However, there was no significant difference in the concentration levels between Sandile and Wriggleswade Dams (**Figure 3.4C**). There was no significant ($p \geq 0.05$) difference between the levels of Mn (**Figure 3.4D**) and Ti (**Figure 3.4E**) in the muscle tissue of *M. salmoides* between Binfield Park, Sandile and Wriggleswade Dams. Sandile Dam fish had significantly ($p \leq 0.05$) higher levels of Zn in the muscle tissue than *M. salmoides* from Binfield Park Dam (**Figure 3.4F**). Sandile Dam also had higher levels of Zn in the muscle tissue of *M. salmoides* from Wriggleswade Dam however, the difference was not significant ($p \geq 0.05$) (**Figure 3.4F**).

**Table 3.4:** Metals detected in the muscle tissue of *Micropterus salmoides* from Binfield Park (BD), Sandile (SD) and Wriggleswade Dams (WD) analysed using ICP-MS. Values reported in µg/g.

| | Sampling site | | |
|---|---|---|---|
| Metal | Binfield Park Dam | Sandile Dam | Wriggleswade Dam |
| Al | 3.61 (0.89) | 10.37 (4.55) | 7.09 (5.54) |
| As | 0.16 (0.003) | 0.07 (0.008) | 7.03 (1.41) |
| Fe | 7.80 (2.82) | 9.48 (3.28) | 5.31 (0.07) |
| Hg | 7.03 (1.41) | ND | 0.48 (0.30) |
| Mn | 0.09 (0.05) | 0.38 (0.74) | 0.61 (0.25) |
| Ti | 26.94 (0.95) | 29.67 (0.44) | 27.32 (0.91) |
| Zn | 12.38 (1.74) | 22.08 (2.96) | 14.56 (1.68) |

Parenthese denotes standard error, ND = below detection limit

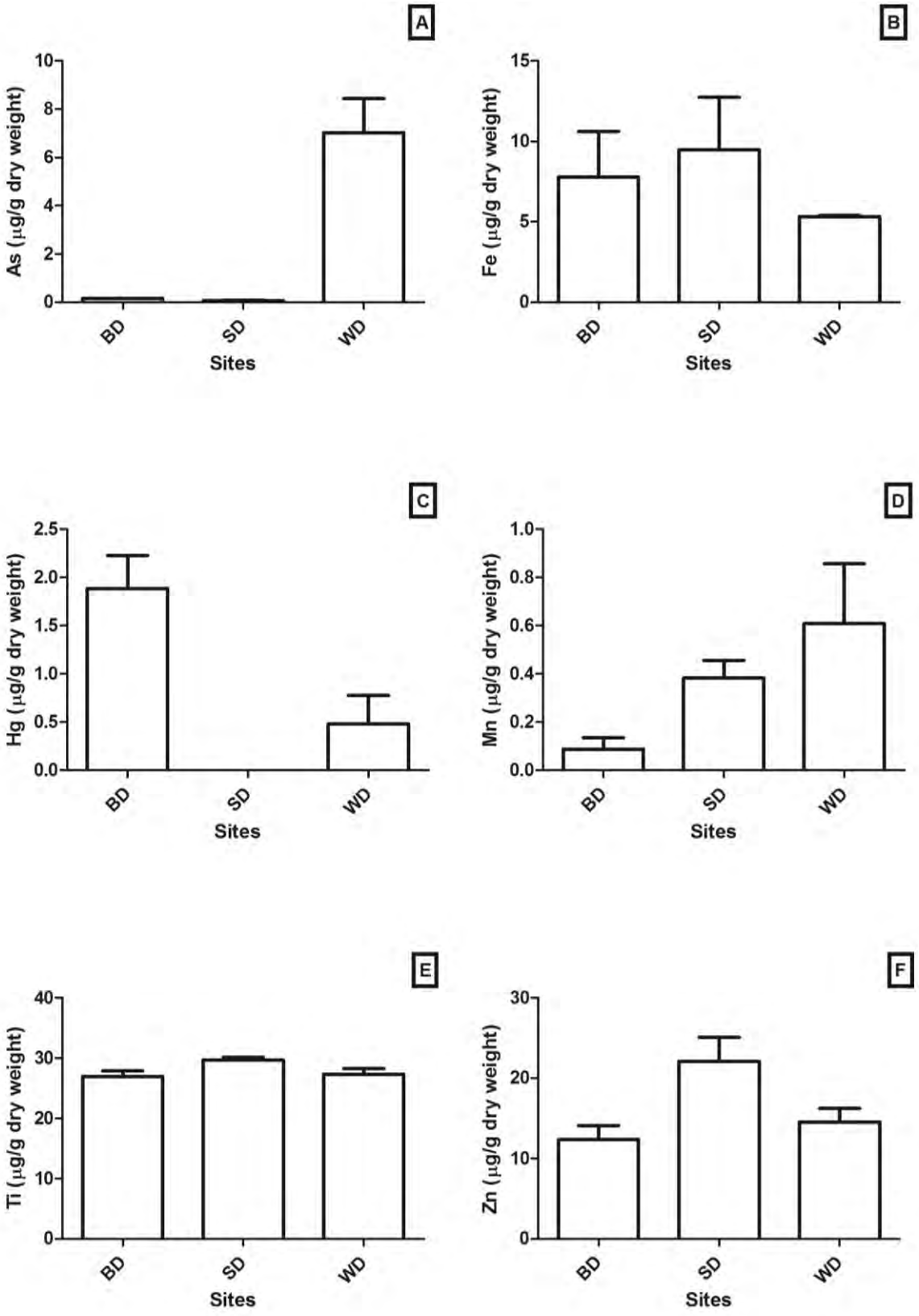


**Figure 3.4:** Mean concentrations (µg/g) of (A) arsenic (As), (B) iron (Fe), (C) mercury (Hg), (D) manganese (Mn), (E) titanium (Ti), (F) zinc (Zn) in muscle tissue of *Micropterus salmoides* from Binfield Park (BD), Sandile (SD) and Wriggleswade Dams (WD). Error bars denote SEM.

### 3.3.4. Biometric, necropsy and gross body indices

The biometric and FHAI scores are presented in **Table 3.5**. The mean mass of the sampled Binfield Park Dam bass was significantly higher than that of both the Sandile Dam ($p \leq 0.05$; $df1 = 2$) and Wriggleswade Dam ($p \leq 0.05$; $df1 = 2$) bass. However, there was no significant ($p \geq 0.05$; $df1 = 2$) difference between the masses of the sampled Sandile and Wriggleswade Dam samples. The fork lengths of the sampled Binfield Park Dam bass were also significantly longer than those of the Sandile ($p \leq 0.05$; $df1 = 2$) and Wriggleswade ($p \leq 0.05$; $df1 = 2$) Dam bass. There was no significant ($p \geq 0.05$; $df1 = 2$) difference in the fork lengths between Sandile and Wriggleswade Dam bass. The condition factor (CF) of the Binfield Park Dam bass was also significantly greater than both the Sandile ($p \leq 0.05$; $df1 = 2$) and Wriggleswade ($p \leq 0.05$; $df1 = 2$) Dam bass. However, there was no significant ($p \geq 0.05$; $df1 = 2$) difference between the CF of the Sandile and Wriggleswade Dam bass. The Binfield Park Dam gutted condition factors (GCF) of the bass samples were significantly higher than those of both Sandile ($p \leq 0.05$; $df1 = 2$) and Wriggleswade ($p \leq 0.05$; $df1 = 2$) bass. The GCF of the Wriggleswade Dam samples was also significantly ($p \leq 0.05$; $df1 = 2$) higher than the GCF values of the Sandile Dam bass. The Binfield Park Dam bass hepatosomatic index (HSI) values where not significantly different to Sandile ($p \geq 0.05$; $df1 = 2$) and Wriggleswade Dams ($p \geq 0.05$; $df1 = 2$).There was also no significant ($p \geq 0.05$; $df1 = 2$) difference between the HSI values between Sandile and Wriggleswade Dams. The spleenosomatic index (SSI) showed that there was no significant ($p \geq 0.05$; $df1 = 2$) difference in the spleen sizes between the Binfield Park and the Sandile Dam samples. However, there was a significant difference ($p \leq 0.05$; $df1 = 2$) between the Binfield Park and Wriggleswade Dam bass samples. There was also a significant ($p \leq 0.05$; $df1 = 2$) difference in the SSI value between the Sandile and Wriggleswade Dam samples. There was no significant difference in the gonadosomatic index (GSI) for the male bass from Binfield Park Dam and the males of Sandile ($p \geq 0.05$; $df1 = 2$) and Wriggleswade Dams ($p \geq 0.05$; $df1 = 2$). There was also no significant difference ($p \geq 0.05$; $df1 = 2$) between the Sandile Dam males and the Wriggleswade Dam males. The Binfield Park Dam female bass GSI values were significantly higher than those of the Sandile Dam females ($p \leq 0.05$; $df1 = 2$) There was no significant ($p \geq 0.05$; $df1 = 2$) difference between the Binfield Park females and the Wriggleswade Dam

females. There was a significant difference ($p \leq 0.05$; $df1 = 2$) between the Sandile Dam females and the Wriggleswade Dam females.

The macroscopic FHAI showed that there was no significant difference between the FHAI scores of the fish from Binfield Park Dam and Sandile Dam ($p \geq 0.05$; $df1 = 2$) or Wriggleswade Dam ($p \geq 0.05$; $df1 = 2$), nor was there a significant difference ($p \geq 0.05$; $df1 = 2$) between the Sandile Dam and Wriggleswade Dam fish. The main contributing factors to the higher Binfield Park Dam FHAI score was that 6% of the bass samples had damaged tailfins (**Figure 3.5A**), frayed gills (**Figure 3.5B**), granular spleens (**Figure 3.5C**) and were egg-bound (**Figure 3.5E**), while 38% of the samples had discoloured and fatty livers (**Figure 3.5F**). The Sandile Dam bass samples had pale and frayed gills (5%) (**Figure 3.5B**) and fatty, discoloured livers (42%) (**Figure 3.5F**). The Wriggleswade Dam bass samples had skin damage from the anchor worm *Lernaea cyprinacea* (13%), tailfin damage (7%) (**Figure 3.5A**) and fat infiltration and discolouration of the liver (57%) (**Figure 3.5F**).

The blood parameter results were evaluated in terms of the normal ranges stipulated by Adams *et al*. (1993). The mean hematocrit levels for Binfield Park Dam were 60.37 ± 11.24. 75% of the samples were above the normal range (30 – 45 %) of Adams *et al*. (1993). The Binfield Park Dam hematocrit levels were significantly different ($p \leq 0.05$; $df1 = 2$) to the Sandile Dam levels. The mean hematocrit percentage for Sandile Dam was 44.12 ± 6.70%, with 42% of the bass sample having a higher than normal hematocrit value. The Binfield Park Dam fish hematocrit levels were also significantly ($p \leq 0.05$; $df1 = 2$) higher than the Wriggleswade Dam hematocrit values. The mean hematocrit value for Wriggleswade Dam bass was 45.29 ± 10.65%. 37% of the samples were above the normal range and 3% had a value below (19 – 29%) the normal range proposed by Adams *et al*. (1993).

There was no significant difference ($p \geq 0.05$; $df1 = 2$) between the hematocrit percentages of the Sandile and Wriggleswade Dam fish. The mean total plasma protein levels for Binfield Park Dam were 61.92 ± 16.44 mg $dl^{-1}$, with 69% of the samples in the normal (30 – 69 mg $dl^{-1}$) and 31% of the samples greater (> 70 mg $dl^{-1}$) than the normal range, as proposed by Adams *et al*. (1993). The mean total plasma proteins levels from Binfield Park Dam fish were significantly ($p \leq 0.05$; $df1 = 2$) higher than the Sandile Dam samples. The mean total plasma protein levels for

Sandile Dam were 46.12 ± 14.12 mg $dl^{-1}$, 89% of the Sandile Dam total protein samples were within the normal range of Adams *et al*. (1993) and 11% of the samples were below (<39 mg $dl^{-1}$) the normal range. There was no significant ($p \geq 0.05$; $df1 = 2$) difference between the Binfield Park and Wriggleswade Dam total protein values. The mean total protein levels for Wriggleswade Dam were 45.29 ± 10.65 mg $dl^{-1}$. 74% of the Wriggleswade Dam total protein samples were in the normal range, 23% of the samples were above the normal range and 3% of the samples were below the normal range of Adams *et al*. (1993).

**Table 3.5:** Biometric indices for *Micropterus salmoides*: mean total body mass (g), fork length (mm), condition factor (CF), gutted condition factor (GCF), hepatosomatic index (HSI), spleenosomatic index (SSI), gonadosomatic index (GSI) and fish health assessment index (FHAI).

| Biometric indice | | Binfield Park Dam (n = 16) | Sandile Dam (n = 19) | Wriggleswade Dam (n = 30) |
|---|---|---|---|---|
| Mass | | 1182.5 ± 688.9 | 449.7 ± 337.8 | 541.6 ± 306.1 |
| Length | | 411.4 ± 80.4 | 324.8 ± 70.9 | 340.5 ± 62.2 |
| CF | | 1.50 ± 0.12 | 1.15 ± 0.18 | 1.26 ± 0.23 |
| GCF | | 1.33 ± 0.08 | 1.01 ± 0.15 | 1.11 ± 0.15 |
| HSI | | 0.83 ± 0.19 | 0.77 ± 0.53 | 0.92 ± 0.56 |
| SSI | | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.06 ± 0.02 |
| GSI | Male | 0.22 ± 0.45 | 0.19 ± 0.15 | 0.19 ± 0.09 |
| | Female | 2.29 ± 1.54 | 0.61 ± 0.21 | 1.48 ± 0.97 |
| FHAI | | 21.8 ± 20.4 | 17.4 ± 18.8 | 26.0 ± 19.9 |

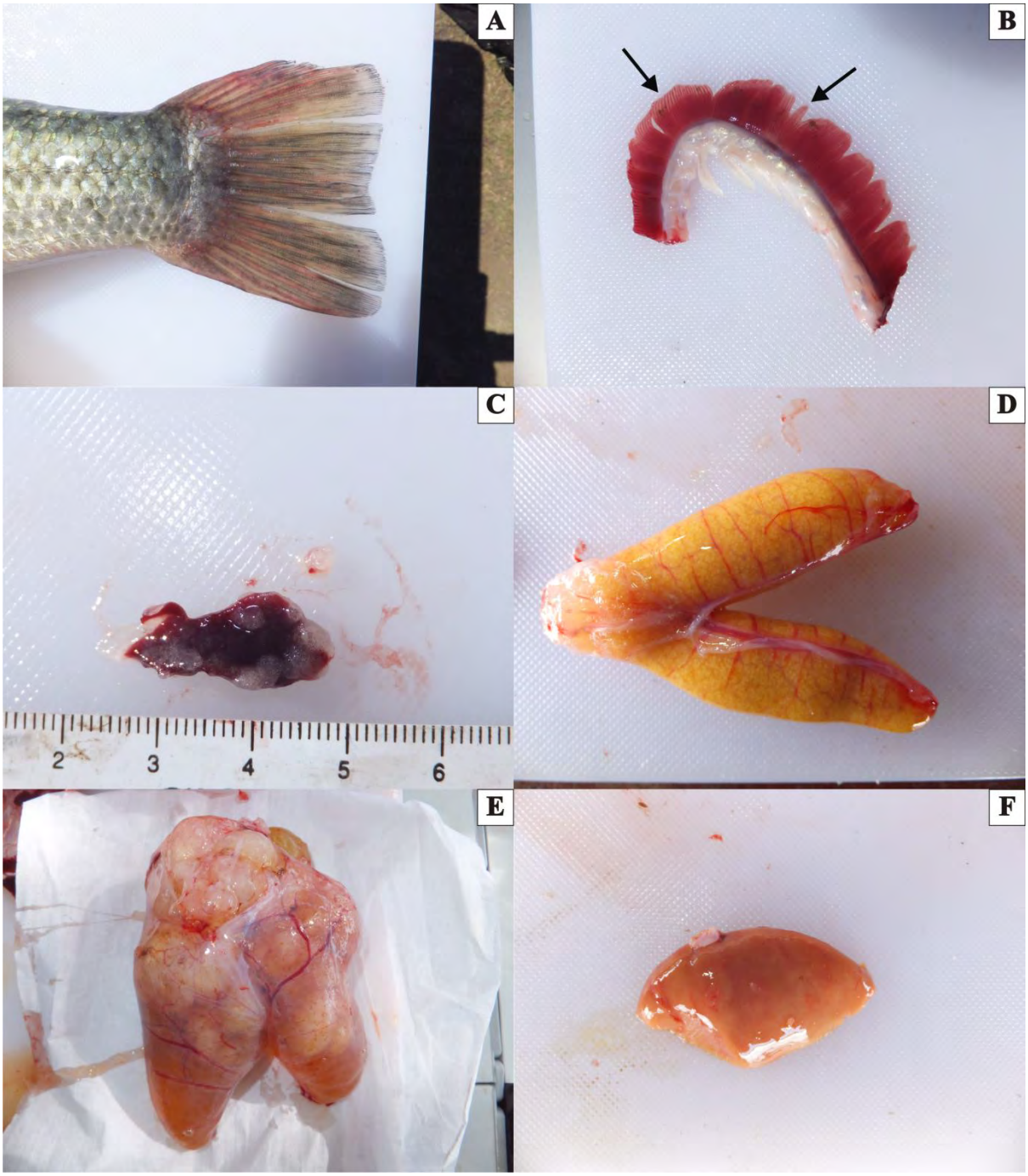


**Figure 3.5:** External and internal abnormalities indentified in *Micropterus salmoides* samples from Binfield Park, Sandile and Wriggleswade Dams; (A) Damaged tailfins; (B) frayed and pale gills (arrows); (C) granular spleen; (D) normal ovaries; (E) egg-bound ovaries; (F) fatty and discoloured livers.

### 3.3.5. Histology analyses

The prevalence of the histological alterations identified, as well as the histological index results, are presented in **Tables 3.6** and **3.7,** respectively. In terms of the number of histological alterations identified, the gills of the *M. salmoides* from the three impoundments showed the highest deviation from the norm. The main histological alterations identified in the gills of *M. salmoides* from the three impoundments were eosinophillic granular cells (**Figure 3.6A**), fusion of the secondary lamellae (**Figure 3.6B**), hyperplasia (**Figure 3.6C**), increase in mucous cells (**Figure 3.6D**), telangiectasia and rupture of pillar cells (**Figure 3.62E**). Parasitic gill monogeneans were also noted on the gills of the bass from all three impoundments (**Figure 3.6F**). The histological alterations identified are grouped together as circulatory disturbances, regressive changes and progressive changes under the reaction patterns of Bernet *et al.* (1999). In terms of the mean Gill Index ($I_G$), there was no significant difference between the Binfield Park Dam samples and those of both Sandile ($p \geq 0.05$; $df1 = 2$) and Wriggleswade Dams ($p \geq 0.05$; $df1 = 2$). There also was no significant difference ($p \geq 0.05$; $df1 = 2$) between Sandile and Wriggleswade Dams in the $I_G$ score. For Binfield Park Dam, 94% of the samples fell within Class 1, indicating normal tissue structure with slight histological alterations (Zimmerli *et al.* 2007), and 6% fell within Class 2, indicating normal tissue structures with moderate histological alterations (Zimmerli *et al.* 2007). The gills of the Sandile Dam bass fell predominately in Class 1 (84%), and the gills of a few samples were in Class 2 (16%). All the gills of the Wriggleswade bass fell into Class 1.

There were fewer histological alterations identified in the liver tissue of the three impoundments. However, in terms of percentage prevalence, the alterations were more pronounced. The main histological alterations indentified in the liver samples from the three impoundments were hepatocyte vacuolation (**Figure 3.7A**), increase in melano-macrophage centres (**Figure 3.7B**), intercellular deposits (**Figure 3.7C**) and pyknotic nuclei (**Figure 3.7D**). Binfield Park Dam had the highest Liver Index ($I_L$) when compared with the Sandile and Wriggleswade Dam bass liver samples. The $I_L$ score for Binfield Park Dam was significantly higher than both the Sandile ($p \leq 0.05$; $df1 = 2$) and Wriggleswade ($p \leq 0.05$; $df1 = 2$) Dam bass liver samples. There was no significant difference ($p \geq 0.05$; $df1 = 2$) in the $I_L$ score between Sandile Dam and Wriggleswade Dam. The Binfield Park Dam bass liver samples had 56% in Class 1

and 44% in Class 2. The Sandile Dam bass liver sections were in Class 1 (84%) and Class 2 (16%) while the Wriggleswade Dam samples were also in Class 1 (90%) and Class 2 (10%).

There were relatively few histological alterations indentified in the kidney tissue of the bass from the three impoundments. The histological alterations identified were hyaline droplet degeneration (**Figure 3.8B**), increase in melano-macrophage centres (**Figure 3.8C**) and an increase in the Bowman's space (**Figure 3.8D**). Here again the Binfield Park samples had the highest organ index score. The Binfield Park Dam Kidney Index ($I_K$) was significantly higher than those of the Sandile ($p \leq 0.05$; $df1 = 2$) and Wriggleswade ($p \leq 0.05$; $df1 = 2$) Dam bass kidney samples. There was no significant ($p \geq 0.05$; $df1 = 2$) difference between the $I_K$ for the Sandile and Wriggleswade Dam kidney samples. However, all the Binfield Park (100%), Sandile (100%) and Wriggleswade (100%) Dam samples fell into Class 1.

The gonads of *M. salmoides* from the three impoundments were the least affected in terms of histological alterations identified. The only histological abnormality identified in the gonads was an increase in the melano-macrophage centres (**Figure 3.9A** and **3.9B**). For male *M. salmoides* samples, there were histological alterations identified in the Binfield Park Dam Testis Index ($I_T$). There was no significant difference ($p \geq 0.05$; $df1 = 2$) between the Sandile and Wriggleswade Dam bass males in terms of their $I_T$. The female Binfield Park Dam bass samples were not significantly different to the Sandile ($p \geq 0.05$; $df1 = 2$) and Wriggleswade ($p \geq 0.05$; $df1 = 2$) Dam samples in terms of the Ovary Index ($I_O$). There was also no significant difference ($p \geq 0.05$; $df1 = 2$) between the Sandile Dam $I_O$ and the Wriggleswade Dam $I_O$. All *M. salmoides* testis and ovary histology samples from Binfield Park (100%), Sandile (100%) and Wriggleswade (100%) Dams fell within Class 1.

The combined mean Fish Index ($I_{FISH}$) of Binfield Park was higher than those of Sandile or Wriggleswade Dams, indicating that the Binfield Park Dam *M. salmoides* were more affected in terms of prevalence and severity of the histological alterations identified. The $I_{FISH}$ for Binfield Park Dam was not significantly ($p \geq 0.05$; $df1 = 2$) higher than the $I_{FISH}$ of the Sandile Dam samples. However, the Binfield Park Dam was significantly ($p \leq 0.05$; $df1 = 2$) higher than the $I_{FISH}$ of the Wriggleswade Dam

samples. There was no significant difference ($p \geq 0.05$; $df1 = 2$) between the Sandile Dam $I_{FISH}$ and the Wriggleswade Dam $I_{FISH}$.

### 3.3.3. Gonad development

The developmental stages of the testes and ovaries were assessed according to the criteria stipulated in the Biomonitoring of Environmental Status and Trends Program (BEST) (Schmitt & Dethloff 2000). The male to female sex ratios of *M. salmoides* collected from Binfield Park Dam was 1:3, Sandile Dam was 1:2.8 and Wriggleswade Dam was 1:2.

The developmental stages of the Binfield Park Dam bass males were all (100%) Stage 2, which is mid-spermatogenic. Here the germinal epithelium is of a moderate thickness, there is moderate proliferation and maturation of the spermatozoa and an equal mix of spermatocytes, spermatids and spermatozoa present (Schmitt & Dethloff 2000). The Sandile Dam bass males were 80% in Stage 2 of development and 20% in Stage 3 of development, which is late spermatogenic, indicated by a thick germinal epithelium with diffuse regions of proliferation and maturation of spermatozoa. Here all the stages of development are represented, but the spermatozoa dominate. The Wriggleswade Dam bass males were 60% Stage 2 and 40% Stage 3.

Twenty five percent of the females from Binfield Park Dam were in Stage 1, which is the early developmental stage. This is characterised by 90% or more of the oocytes being pre-vitellogenic, and the remainder of the oocytes being early to mid-vitellogenic, and the oocytes are slightly larger than in Stage 0, up to 300µm. The other 67% were in Stage 2, which is mid-development. Here the majority of the observed follicles are early and mid-vitellogenic, the oocytes are between 300 - 600µm in diameter and contain peripheral yolk vesicles. The remaining 8% of the Binfield Park female samples were egg-bound. Thirty five percent of the Sandile Dam females were in Stage 0, which is underdeveloped. The stage is characterised by pre-vitellogenic oocytes observed only, the oocyte diameter is smaller than 250µm and the cytoplasm stains basophilic. The remaining Binfield Park females were in Stage 1 (35%) and Stage 2 (33%). The Wriggleswade Dam female bass samples were in Stage 1 (45%) and Stage 3 (55%) of development. Stage 3 of ovary

development is late development, characterised by developing follicles that are late vitellogenic and an oocyte diameter of 600 - 1000µm, the yolk globules are eosinophilic.

**Table 3.6:** Percentage prevalence of histological alterations identified in *Micropterus salmoides* from Binfield Park, Sandile and Wriggleswade Dams.

| Target Organ/ histological alteration | Percentage prevalence | | |
|---|---|---|---|
| | Binfield Park Dam (n = 16) | Sandile Dam (n = 19) | Wriggleswade Dam (n = 30) |
| **Gills** | | | |
| Branching of primary lamellae | 6 | – | – |
| Eosinophilic granular cells | – | 26 | 70 |
| Fusion of secondary lamellae | 31 | 11 | 20 |
| Hyperplasia | 13 | 53 | 57 |
| Increase in mucous cells | 81 | 63 | 83 |
| Rupture of pillar cells | 63 | 21 | 20 |
| Telangiectasia | 63 | 21 | 20 |
| **Liver** | | | |
| Hepatocyte vacuolation | 88 | 74 | 63 |
| Increase in melano-macrophage centres | 63 | 47 | 73 |
| Intercellular deposits | 19 | – | – |
| Pyknosis | 81 | 21 | 33 |
| **Kidney** | | | |
| Hyaline droplet degeneration | 19 | 21 | 33 |
| Increase in melano-macrophage centres | 38 | 58 | 43 |
| Increase in the Bowman's spaces | 69 | 16 | 17 |
| **Testis** | **n = 4** | **n = 5** | **n = 10** |
| Increase in melano-macrophage centres | – | 40 | 30 |
| **Ovaries** | **n = 12** | **n = 14** | **n = 20** |
| Increase in melano-macrophage centres | 67 | 43 | 85 |

'-' denotes alteration not detected

**Table 3.7:** Mean organ index and fish value index for *Micropterus salmoides*. $I_L$ = Liver Index, $I_K$ = Kidney Index, $I_G$ = Gill Index, $I_T$ = Testis Index, $I_O$ = Ovary Index and $I_{FISH}$ = Fish Index. Ranges are indicated in parentheses.

| Survey | $I_G$ | $I_L$ | $I_K$ | $I_T$ | $I_O$ | $I_{FISH}$ |
|---|---|---|---|---|---|---|
| Binfield Park Dam | 4.3 (0 – 11) | 10.6 (0 – 25) | 3.00 (0 – 9) | 0 | 1.7 (0 – 3) | 19.1 (3 – 38) |
| Sandile Dam | 5.8 (0 – 17) | 4.2 (0 – 12) | 1.0 (0 – 2) | 0.8 (0 – 3) | 0.9 (0 – 3) | 11.8 (2 – 23) |
| Wriggleswade Dam | 4.4 (1 – 9) | 4.5 (0 – 12) | 1.1 (0 – 4) | 0.7 (0 – 3) | 1.7 (0 – 3) | 11.3 (2 – 24) |

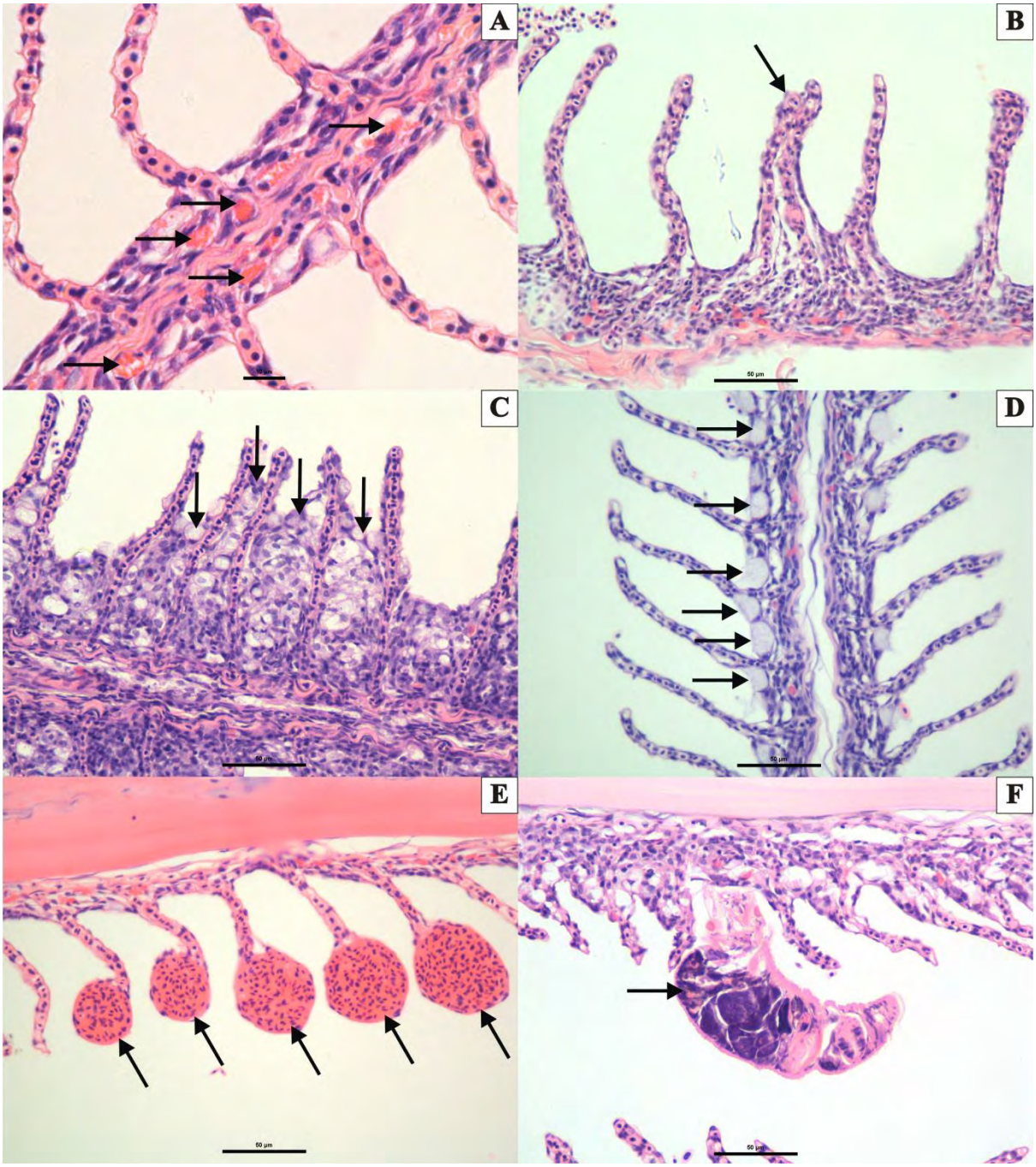


**Figure 3.6:** Micrographs of *Micropterus salmoides* gill sections stained with H&E. (A) eosinophilic granular cells (arrows); (B) fusion of secondary lamellae (arrow); (C) hyperplasia (arrows); (D) increase in mucous cells (arrows); (E) telangiectasia with rupture of pillar cells (arrows); (F) monogenean parasites between the secondary lamellae (arrow).

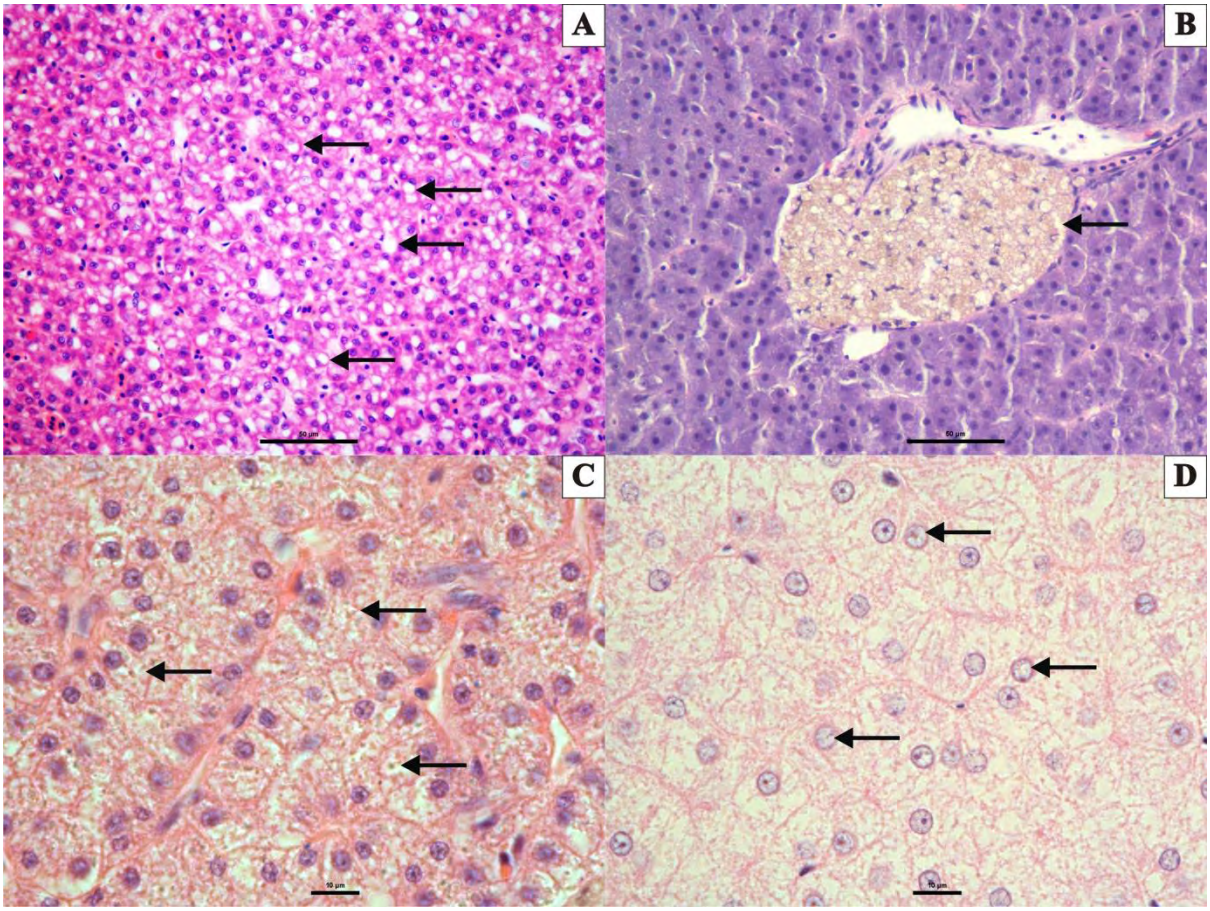


**Figure 3.7:** Micrographs of *Micropterus salmoides* liver sections (5 µm) stained with H&E. (A) Hepatocyte vacuolation (arrows); (B) increase in melano-macrophage centres (arrow); (C) intercellular deposits (arrows); (D) pyknosis of hepatocyte nuclei (arrows).

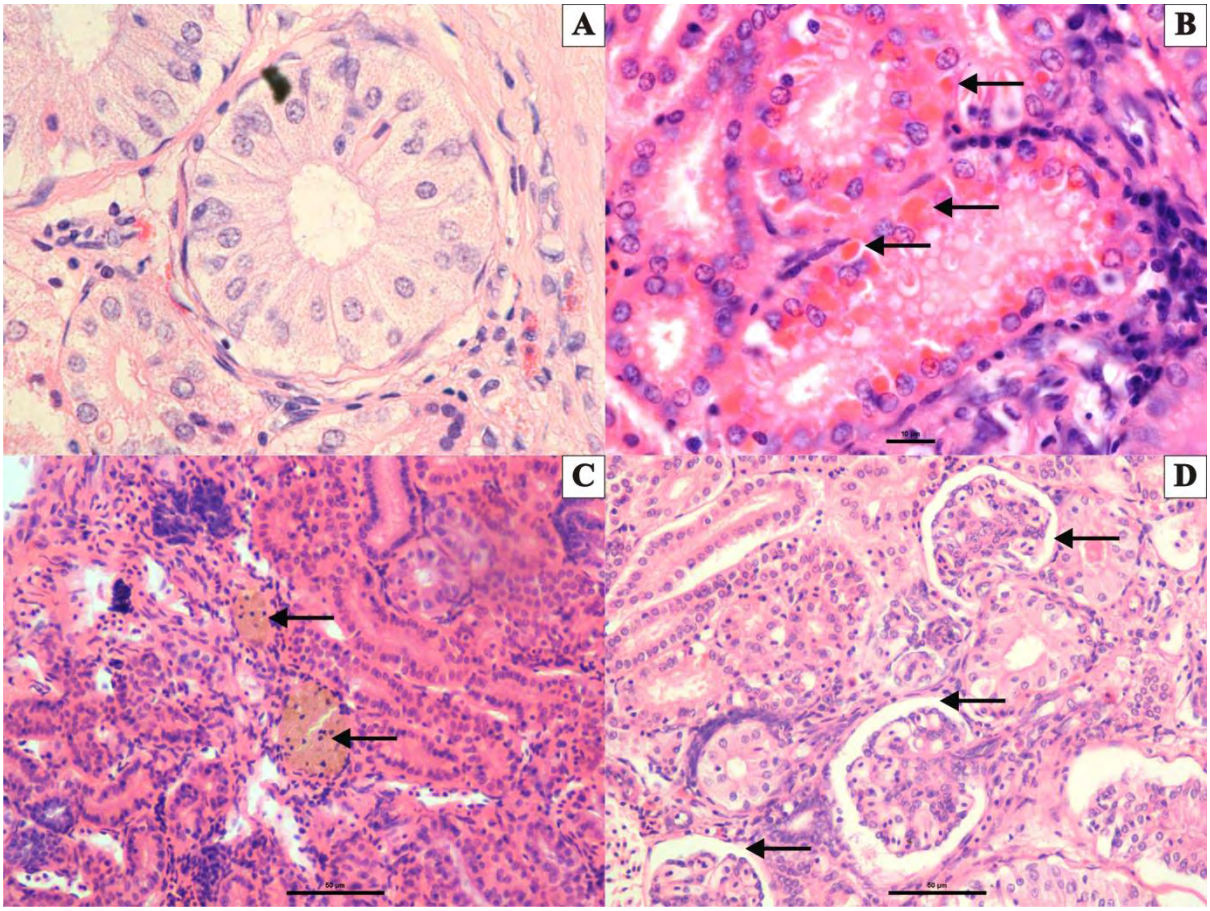


**Figure 3.8:** Micrographs of *Micropterus salmoides* kidney sections (5 µm) stained with H&E. (A) normal renal lumen; (B) hyaline droplet degeneration (arrows); (C) increase in melano-macrophage centres (arrows); (D) increase in Bowman's space (arrows).

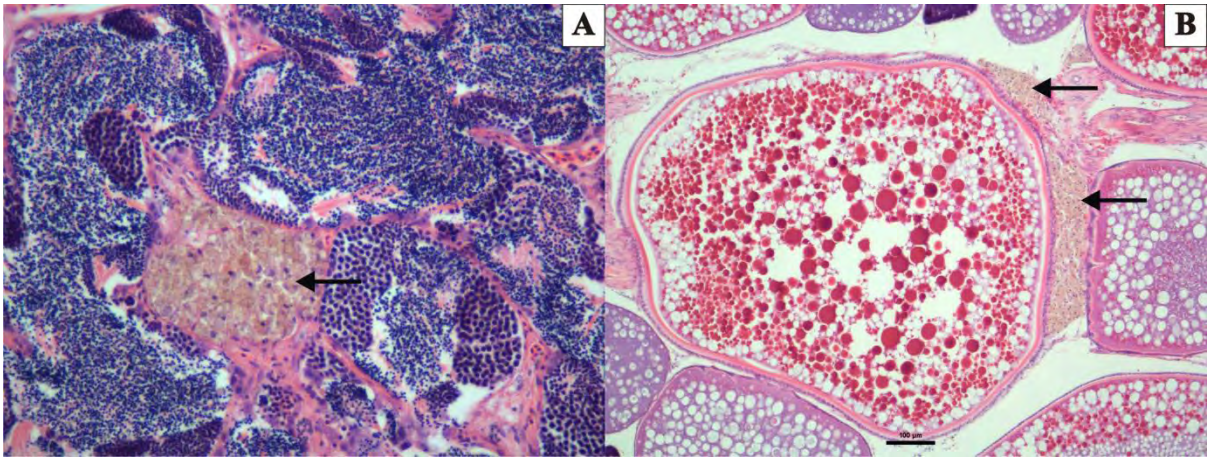


**Figure 3.9:** Micrographs of *Micropterus salmoides* testis (left) and ovary (right) sections (5 µm) stained with H&E. (A) increase in melano-macrophage centres (arrow); (B) increase in melano-macrophage centres (arrows).

## 3.4. Discussion

### 3.4.1. Water quality analyses

Morrison *et al.* (2001) reported that the Keiskamma River flowing into Sandile Dam was receiving significant levels of pollution in the form of ammonia, nitrate and phosphorus. Morrison *et al.* (2001) attributed these sources of pollution to the Keiskammahoek sewage treatment plant not working correctly and discharging nutrient-rich point source pollution into the Keiskamma River which may cause algae blooms in Sandile Dam, although the author did note that the nutrient levels were below South African water guideline levels. The South African water quality guidelines (DWAF 1996A) stipulate a threshold for ammonia levels of 0.007 mg/ℓ. All three of the impoundments were above this threshold limit. However, all the impoundments were below the previously recorded concentration of Morrison *et al.* (2001). The South Africa Water Quality guidelines for domestic supply set the threshold for nitrite at 6 mg/ℓ. Both the Binfield Park and Wriggleswade Dam results were below this threshold value. However, that of Sandile Dam was higher. Morrison *et al.* (2001) noted that none of the nitrate levels for the Keiskamma River flowing into Sandile Dam exceeded the threshold limits. However, the effluent being released by the Keiskammahoek sewage treatment plant was higher than the threshold limit. The pH of the three impoundments ranged between 8.96 and 9.51, which compared to the levels of Morrison *et al*. (2001) for the Keiskamma River (6.6 – 7.4), which is more neutral. However, the pH ranges are within the South African Water Quality Guideline range (DWAF 1996A, 1996B). The phosphate levels of the Binfield Park and Sandile Dams were similar, but slightly elevated in Wriggleswade Dam. According to the South African water quality guideline (1996A), 5µg/ℓ is the threshold limit for phosphates to prevent algal growth in water bodies. The phosphate levels of all three impoundments were higher than the South African water quality guidelines, as well as the levels previously recorded by Morrison *et al*. (2001).

According to Fatoki & Awofolu (2003), Cd, Hg and Zn were found in high levels in the water of the Tyume River and Sandile Dam. These authors also indicated that Cd, Hg and Zn were common components of environmental pollution. Fatoki & Awofolu (2003) attributed the high levels they found of these metals to be caused by rural and

urban runoff, both naturally from the geology of the area and from point source pollution from the Keiskammahoek Sewage Treatment Plant. The authors stated that the high levels would adversely affect the health of the aquatic ecosystem, as well the health of the rural community that depends on these water sources for their direct domestic use. The high levels of Cd and Zn reported in the water by Fatoki & Awofolu (2003) were not detected in any of the impoundments, and only Hg was detected in Wriggleswade Dam in similar concentrations to those reported by Fatoki and Awofolu (2003).

### 3.4.2. Sediment metal analysis

The concentration values of the metals in sediment where compared to international guideline concentration values of Australia – New Zealand (ANZECC 2000), Netherland (Friday 1998), Canada (Friday 1998) and other studies by Hamilton (2004) and Sheppard *et al.* (2005).

Sandile Dam sediment had the highest concentrations of Al, As, Co, Cu, Fe, Hg, Pb, Ti and Zn, while Binfield Park Dam sediment had the highest concentrations of Mn and Ni and Wriggleswade Dam had the highest concentration of U in the sediment. Cadmium (Cd) was not detected by Fatoki & Awofolu (2003) in the sediment of Sandile Dam, nor was it detected in any of the three impoundments in this study. However, Fatoki & Awofolu (2003) did detect trace amounts of Cd in the Tyume River. Mercury (Hg) was detected in trace amounts in Binfield Park, Sandile and Wriggleswade dam sediments. However, the levels were below the accepted guideline concentrations. In previous studies (Fatoki & Awofolu 2003), the authors did not detect any mercury. Zinc (Zn) was detected in all three impoundments, but was below the accepted concentration guideline values. However, the concentration values were higher than those of previous studies by Fatoki & Awofolu (2003) in the Tyume River. The concentration levels of Co, Mn and Ni in the sediment of Binfield Park and Sandile Dams were higher than the accepted guideline levels. Sandile Dam also had higher than guideline concentration levels for Cu in the sediment, while Wriggleswade Dam had higher than guideline levels for U.

### 3.4.3. Muscle tissue metal analysis

The statistical analyses showed that there were no significant differences between the three impoundments for the metals Al, As, Fe, Mn and Ti. However, Binfield Park Dam *M. salmoides* did have significantly higher Hg concentrations than the other two impoundments. According to Cizdziel *et al*. (2002), the safe total Hg concentrations for human consumption of *M. salmoides* is 0.16 µg/g wet weight of Hg in the muscle tissue. The mercury concentrations of the Binfield Park Dam *M. salmoides* far exceeded this safe guideline levels. The concentration of Zn in the muscle tissue of *M. salmoides* from Sandile Dam was also significantly higher than that in the other impoundments. Wriggleswade Dam fish had the highest levels of arsenic in their muscle tissue. Maher *et al.* (1999) showed that arsenic concentrations were the lowest in muscle tissue, compared to other organs such as gills and liver tissue. Maher *et al*. (1999) stated that the arsenic values in *Mugil cephalus* came from the consumption of sediment which, by microbial processes in the digestive system, allowed for the absorption of arsenic into the organism. This also accounted for the higher concentrations of arsenic in the liver tissue. However, there were low concentrations of arsenic in the sediment from Wriggleswade Dam. It is thus unlikely that the sediment is the source of the high arsenic concentrations in the muscle tissue. Although not significantly so, Sandile Dam had the highest concentrations levels of Al, Fe and Ti in the muscle tissue of *M. salmoides*. The sediment from Sandile Dam also had the highest concentration of Al, Fe and Ti when compared to the other two impoundments. The concentrations of manganese were higher in the muscle tissue samples collected from Wriggleswade Dam, and the values were similar to those found by Henry *et al*. (2004) in dab and flounder from the Somme bay, France. Henry *et al.* (2004) noted that, for metals such as manganese, the concentrations were higher in the liver tissue when compared to those in the muscle tissue, however, the liver detoxifies the body and is a natural occurence.

The concentrations of mercury in the muscle tissue of *M. salmoides* from Binfield Park Dam and the concentrations of the zinc for the muscle tissue of the Sandile Dam *M. salmoides* were significantly higher than of those in the other two impoundments. Mercury was not detected in the surface water but was present in the sediment samples, and was highest in the sediment of Sandile Dam. However, the concentration of mercury in the sediment was below the guideline

concentrations. The concentration level of zinc in the surface water was below detection limit but it was present in the sediment samples and highest in concentration at Sandile Dam. However, the concentration values were below the accepted guideline levels. The levels of Al were higher in the muscle tissue of *M. salmoides* collected from Sandile Dam than the concentrations of Al in the muscle tissue of *Labeo rosae* collected from Phalaborwa Barrage in the Olifants Rivers systems (Jooste *et al*. 2014). However, the concentrations of Al in muscle tissue of *M. salmoides* from Binfield Park and Wriggleswade Dams were lower than the concentrations reported by Jooste *et al*. (2014). The concentrations of Zn in muscle tissue of *M. salmoides* from Binfield Park, Sandile and Wriggleswade Dams were all higher in concentration than those in Flag Boshielo Dam, but lower than the concentrations reported from Phalaborwa Barrage (Jooste *et al.* 2014). Wriggleswade Dam had the highest concentration of As in the muscle tissue of *M. salmoides*. The concentrations were also greater than the levels reported by Jooste *et al.* (2014) from the Olifants River system. The Olifants River system is considered to be South Africa's most polluted river system (Heath *et al.* 2010, Jooste *et al*. 2014).

### 3.4.4. Biometric, necropsy and gross body indices

The samples of *M. salmoides* collected from Binfield Park had a significantly higher mass, fork length, CF and GCF. This may be a result of the potential food sources. The stomachs of the Binfield Park Dam samples were noted to contain *Lepomis macrochirus* (Rafinesque, 1819). *Lepomis macrochirus* was originally introduced into South Africa as a fodder fish for bass (de Moor & Bruton 1988), and are known to over-populate areas where they are stocked. In their native range in North America they have co-evolved with bass and are therefore generally less naive to the predatory threat of bass (de Moor & Bruton 1988). This experience with the North American bass species, and its presence in a system, may enhance the fitness of *L. macrochirus*, allowing it to better cope with the threat of bass over indigenous species in a system (de Moor & Bruton 1988). The stomach contents of the Sandile Dam bass included exclusively aquatic macro invertebrates such as crustaceans. The Wriggleswade Dam bass had mostly aquatic macroinvertebrates and *Gilchristella aestuaria* (Gilchrist, 1913). *Gilchristella aestuaria* are small estuarine fish

species that were released into Wriggleswade Dam as substitute fodder fish, primarily because of Wriggleswade Dam's importance as a competition bass angling venue.

There was no significant difference in the HSI of *M. salmoides* between the three impoundments, and they were similar to the HSI values reported by Brown & Murphy (2004), who found that the mean male HSI value was 0.87 and the mean female HSI value was 0.85 for *M. salmoides*. However, Brown & Murphy (2004) did note that there were no significant differences in the HSI values between male and female *M. salmoides,* nor were there any significant differences between the HSI values during different seasons (Brown & Murphy 2004). Sepúlveda *et al.* (2001) noted a marked increase in the HSI values of the male *M. salmoides* when exposed to increasing concentrations of effluent from a paper mill. However, the HSI value of the control fish were similar, as noted in this study at Wriggleswade Dam (HSI = 0.92). Huchzermeyer *et al*. (2013) noted that there was no significant difference between the HSI values for laboratory *M. salmoides* that had been surgically implanted with dummy tags and a control group that had not been implanted with tags. The HSI values for laboratory *M. salmoides* reported by Huchzermeyer *et al*. (2013) were also similar to the HSI values reported in this study. The lack of significant difference, and the close relatedness of the HSI values to those of other studies, indicates that the HSI values reported in this study are normal.

### 3.4.5. Histology analysis

The $I_G$ score revealed that there was no significant difference in the alterations to the gill sections of *M. salmoides* from Binfield Park, Sandile and Wriggleswade Dams. The gills come into direct contact with the water and therefore into direct contact with any pollutant in the water, and because of this they are sensitive indicators of environmental stress (van Dyk *et al.* 2009). The metal analysis of the surface water has already shown low concentrations of metals in the surface water. However, the $I_G$ score was highest for Sandile Dam. Previous research by Awofolu & Fatoki (2003) showed the presence of persistent organochlorines such as DDT, chlordane, hexachlorobenzene, heptachlor and endosulfan present in the surface water of the Tyume and Keiskamma River and Sandile Dam. However, the alterations observed

in the Sandile Dam fish were similar to the alterations observed in the gills of *M. salmoides* from Binfield Park and Wriggleswade Dams. The alterations observed in the gills of *M. salmoides* are more likely to be caused by the unidentified gill monogenean. Gill monogeneans are known to cause hyperplasia of the gill tissue and fusion of the secondary gill lamellae caused by the attachemt of hamuli to the gills and feeding on the gill mucus and gill epithelia (Kennedy 2007).

The Binfield Park Dam *M. salmoides* liver sections were significantly more affected than those from the other two impoundments. The Binfield Park Dam *M. salmoides* muscle samples also had significantly higher concentrations of Hg than the Sandile and Wriggleswade *M. salmoides* muscle samples. Raldúa *et al*. (2007) showed that mercury concentrations in the liver are higher than in the muscle tissue for feral *Barbus graellsii* collected from the Cinca River in North Eastern Spain. The histological alterations noted by Raldúa *et al*. (2007) were similar to the histological alterations observed in the liver of *M. salmoides* from this study, such as hepatocyte vacuolation, increase in melano-macrophage centres and intercellular deposits. Adams *et al*. (2010) noted pyknosis of the nuclei in the liver tissue in spotted sea trout *Cynoscion nebulosus*. The Binfield Park Dam *M. salmoides* kidney samples were also significantly more affected than the Sandile and Wriggleswade Dam *M. salmoides* kidney samples. Adams *et al.* (2010) noticed similar alterations in spotted sea trout *Cynoscion nebulosus* after mercury contamination, including increase in melano-macrophage centres and increase in the Bowman's space.

High levels of zinc (Zn) were also found in the muscle tissue of *M. salmoides* from Binfield Park, Sandile and Wriggleswade Dams. Van Dyk *et al*. (2007) exposed *Oreochromis mossambicus* to cadmium and zinc to determine the histological effect on the liver. Van Dyk *et al*. (2007) noted that that the effects would be limited by the exposure time. However, they did note that the main histological alterations were hyalinization, hepatocyte vacuolation, cellular swelling and congestion of blood vessels. In the present study, hepatocyte vacuolation was observed in the liver tissue in Binfield Park, Sandile and Wriggleswade Dams. However, the other alterations were not noted, which may more likely be caused by the exposure to cadmium, which was not detected in these systems. The alterations noted by as a result of zinc exposure.

The Binfield Park Dam $I_{FISH}$ score was significantly higher than that of the other two impoundments, the main cause of the significantly higher score was the significantly high $I_L$. The significantly higher $I_L$ may have been as a result of the high levels of Hg in the muscle tissue of the Binfield Park *M. salmoides*. According to Giari *et al.* (2008), the histology of the liver tissue is suitable and sensitive enough to be used to express the signs of injury from toxicant exposure. Although the exposure dose of Hg, was higher than what was found in the present study, a similar effect was observed in the liver tissue (Giari *et al.* (008). The $I_K$ for Binfield Park was also significantly higher than that for Sandile or Wriggleswade Dam, which also contributed to the higher $I_{FISH}$ score of Binfield Park Dam. According to Giari *et al.* (2008), the kidney tissue is the main target organ for mercury absorption. However, the kidney alterations in *Dicentrarchus labrax* reported by Giari *et al.* (2008) were more severe than those noted in this study.

### 3.5. Conclusion

The differences in the metal concentrations detected in this study, and those of Fatoki & Awofolu (2003), may be as a result of the different analytical techniques used. In the present study an inductively-coupled plasma mass spectrophotometer (ICP-MS) was used, whereas Fatoki & Awofolu (2003) used atomic absorption spectrometry (AAS). The ICP-MS is more sensitive and has lower detection limits. Wriggleswade Dam had the highest FHAI score, followed by Binfield Park Dam. Binfield Park Dam fish also had the highest $I_{Fish}$ score for histological alterations, compared to Sandile Dam and Wriggleswade Dam. The Hg concentrations were highest in the muscle tissue of *M. salmoides* from Binfield Park Dam and are possibly the cause of the increased liver and kidney alterations. Further research is required to determine the possible source of the high levels of Hg. Based on a hazard quotient and the weekly consumption of 150 grams of muscle tissue, as stipulated by Jooste *et al*. (2014), the high concentrations Hg in the muscle tissue from the Wriggleswade Dam *M. salmoides*, and the high concentrations of As and U from the Wriggleswade dam *M. salmoides*, would cause a human health risk, given continuous consumption of these fish. Further research is also required into the presence of persistent organo-pesticides in the surface water, fish muscle tissue and sediment of Binfield Park, Sandile and Wriggleswade Dams.

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# Chapter 4 : The health status of wild African longfin eel, *Anguilla mossambica*, with specific reference to the impact of the alien parasite, *Pseudodactylogyrus anguillae*.

## 4.1. Introduction

Anguillid eels are important commercial fisheries and aquaculture species in North America, Europe and Asia (Tesch 2003). In these regions eels are in a state of decline because of overfishing, habitat alteration, pollution and the introduction of alien parasites (Dekker *et al*. 2003, Haenen *et al*. 2010). As a result, European *Anguilla anguilla* (Linnaeus, 1758), Asian *Anguilla japonica* Temminck and Schlegel, 1846 and American *Anguilla rostrata* (Lesueur, 1817) have been the focus of much research.

Investigations into the health of European eels have primarily focused on parasites. According to Kennedy (2007), only the swim bladder nematode *Anguillicola crassus* Kuwahara, Niimi and Itagaki, and two monogenean species *Pseudodactylogyrus anguillae* (Yin and Sproston, 1948) and *Pseudodactylogyrus bini* (Kikuchi 1929) can be considered pathogens of eels. *Anguillicola crassus*, is a blood-feeding parasite found in the host's swim bladders that has spread from its natural host, *A. japonica*, to naive eel hosts on four continents (Lefebvre *et al*. 2012) and is thought to have contributed to the decline in *A. anguilla* populations in Europe (Wielgoss *et al*. 2008). *Pseudodactylogyrus anguillae* and *P. bini* are parasitic monogeneans originally described from the gills of *A. japonica*. After its original description, it was discovered to have invaded Europe, where it was first found in 1977 on the gills of *A. anguilla* from an eel farm in the western Soviet Union (Buchmann *et al*. 1987). *Pseudodactylogyrus anguillae* have subsequently been recorded from wild populations of *A. rostrata* in Canada (Cone & Marcogliese 1995), the United States of America (Hayward *et al*. 2001) and Africa (El Nagger *et al*. 1993; Christison & Baker 2007; Parker *et al*. 2011). *Pseudodactylogyrus anguillae* feed on mucus, gill epithelia and blood. Their large hamuli can cause bleeding and damage to the gill epithelium (Køie 1991). In fish farms mortality has been reported from *A. rostrata* in America and *A. anguilla* in Europe (Køie 1991; Kennedy 2007). While mortality has

not been reported for wild eels (Kennedy 2007), Køie (1991) noted that this parasite may cause some tissue damage, impair respiration and result in signs of stress in *A. anguilla*.

In contrast, very little research has been done on African eels, which are considered important components of freshwater ecosystems (Jubb 1964). Recent research on African eels include aspects of their biology and ecology (McEwan & Hecht 1984; Lin *et al*. 2012); descriptions of parasites (Moravec *et al*. 2000; Taraschewski *et al*. 2005; Moravec *et al*. 2013) and paratisitation rates and distributions (Parker *et al*. 2011; Weyl *et al*. 2013). No research has been done on the health or the effect of parasites on health status in any African eel population. Of the four eel species found in South Africa, the longfin eel *Anguilla mossambica* Peters, 1852 is the most abundant (Lin *et al*. 2012). Their distribution ranges from Madagascar to the east coast of Africa from Kenya to Cape Agulhas in South Africa (Jubb 1964). This species has recently entered the global trade and, according to Weyl *et al*. (2013), an assessment of its conservation status is urgently required. To contribute towards an understanding of the conservation status of this eel species, the aim of this chapter was to carry out a comprehensive fish health assessment on wild caught *A. mossambica*. The first objective was to identify any possible macroscopic abnormalities, the second was to assess the histology of selected target organs (gills, liver, kidney and gonads) and the third was to screen the eels for parasites and determine if the severity of the parasitism had an effect on the hosts.

### 4.2. Materials and methods

The study took place in Binfield Park Dam (S32° 40' 58.38"; E26° 54' 05.12"), Sandile Dam (S32° 42' 23.57"; E27° 06' 34.54") and Wriggleswade Dam (S32° 35.187; E27° 34.055) (see **Chapter 2, Section 2.1 and Figure 4.1**). The materials and methods used in this chapter are discussed in **Chapter 2**. For sampling (see **section 2.2)**, blood parameters, necropsy, and biometric indices (see **Chapter 2, Section 2.2.1**) were recorded. Fulton's condition factor (CF) was calculated following Haenen *et al*. (2010) for each eel as F = W (kg)/TL ($cm^3$). The gills, liver, kidney and gonads were prepared (see **Chapter 2, Section 2.2.2**) for histological analysis (see **Chapter 2, Section 2.2.2**). Statistical analyses were calculated for comparison (see **Chapter 2, Section 2.2.5**). *Anguilla mossambica* were collected from Binfield Park Dam (n = 12), Sandile Dam (n = 2) and Wriggleswade Dam (n = 5) in March and August 2012 by means of fyke nets (see **Chapter 2, Section 2.2**).

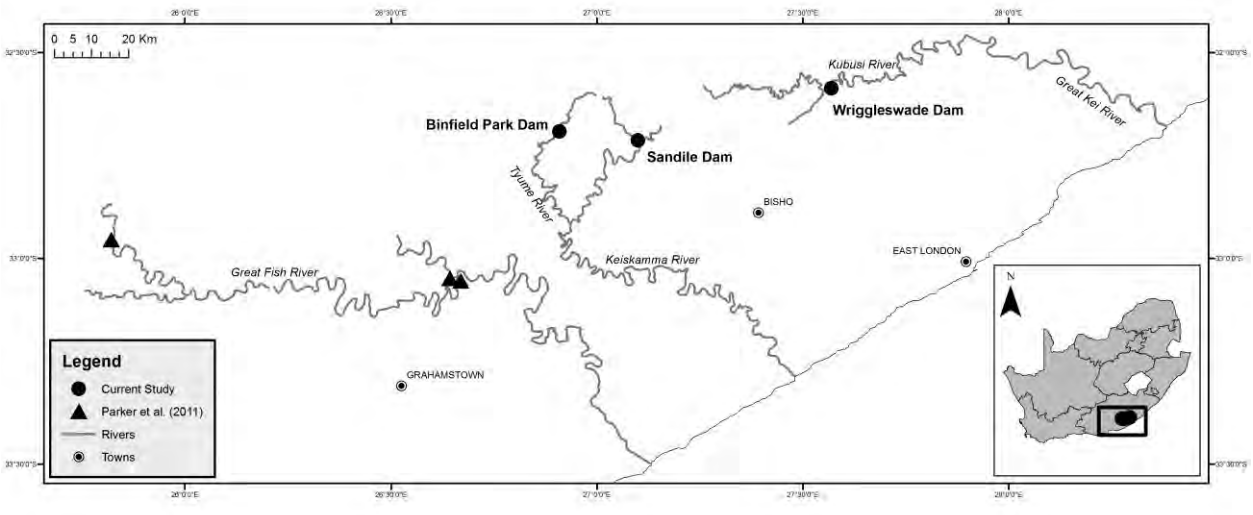


**Figure 4.1:** Map of the locations of Binfield Park Dam, Sandile Dam and Wriggleswade Dam, Eastern Cape, South Africa, as well as the locations of sites surveyed by Parker *et al*. (2011) to show previous known range of *Pseudodactylogyrus anguillae*.

## 4.3. Results

### 4.3.1. Necropsy and gross body indices

A total of 19 eel specimens were collected during all surveys. All fish appeared normal externally, and their mean body mass, total length, and health indices (CF, HSI and SSI), as well as the fish health assessment index (FHAI) are presented in **Table 4.1**. The CF for *A. mossambica* was 2.50 ± 0.05, the mean HSI was 0.62 ± 0.20 and the mean SSI was 0.05 ± 0.02. From the macroscopic examination of *A. mossambica* it was determined that 16% of the samples had frayed gills (**Figure 4.2A**) and 52% of the samples had discoloured livers (**Figure 4.2B**). One of the eels had bleeding gills. The leukocrit levels of all the samples were below 4%, which is considered normal (Heath *et al*. 2004).

**Table 4.1:** Mean body mass, total length, condition factor (CF), Hepatosomatic index (HSI), Splenosomatic index (SSI) and fish health assessment index (FHAI) from *Anguilla mossambica* sampled from impoundments in the Keiskamma and Kei River systems, Eastern Cape, South Africa

| Species | Mass (g) | Length (mm) | CF (kg $m^{-3}$) | HSI (%) | SSI (%) | FHAI |
|---|---|---|---|---|---|---|
| *Anguilla mossambica* (n = 19) | 844.2 ± 451.6 | 675.7 ± 124.6 | 2.50 ± 0.05 | 0.62 ± 0.20 | 0.05 ± 0.02 | 16.32 ± 8.95 |

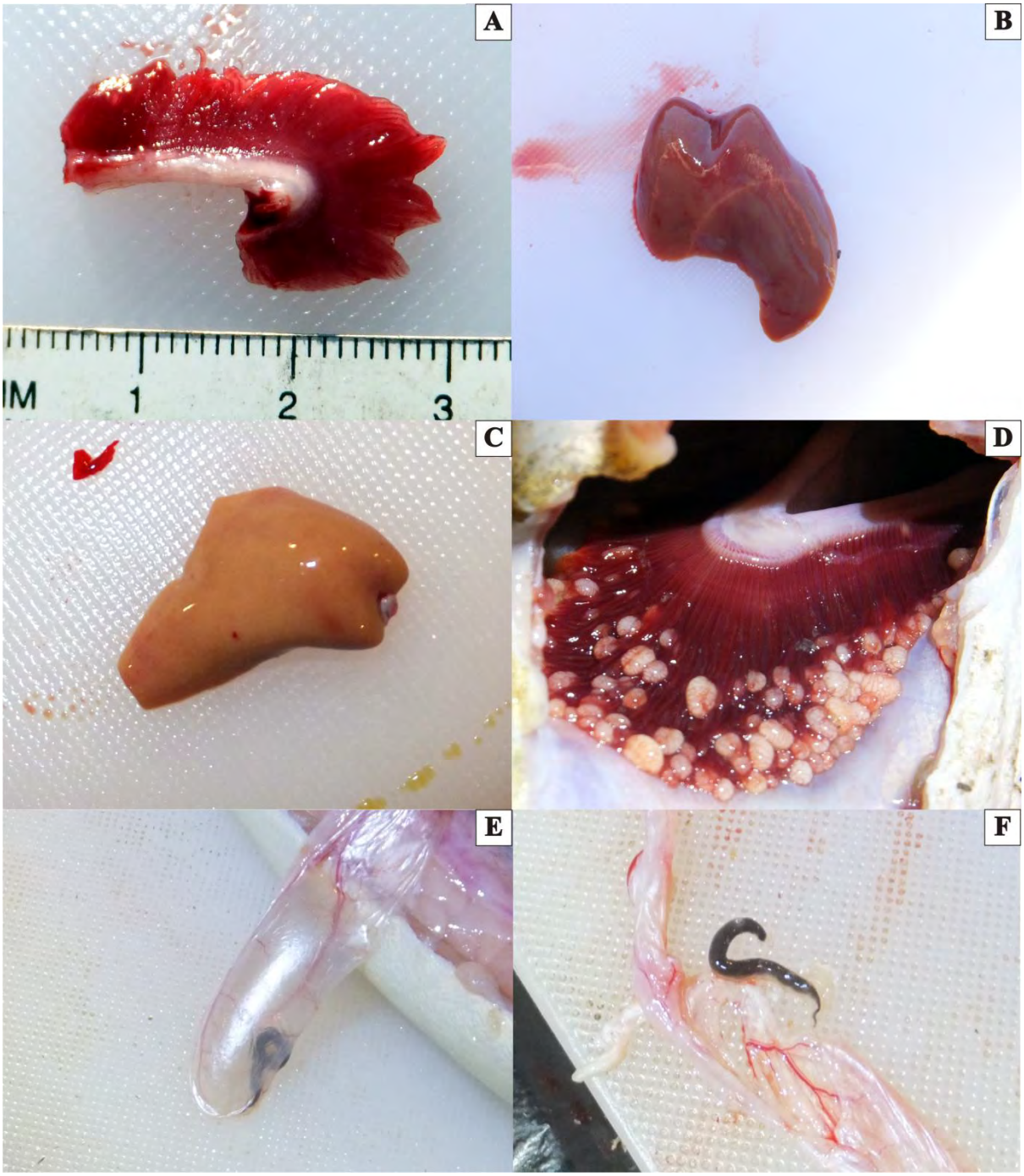


**Figure 4.2:** Macroscopic abnormalities and parasites identified in *Anguilla mossambica*; (A) frayed gills; (B) normal liver; (C) discoloured liver; (D) undescribed myxosporidian gill plasmodium; (E) *Anguillicola papernai* in the swimbladder; (F) swimbladder nematode *Anguillicola papernai.*

### 4.3.2. Parasitology

The prevalence and abundances of the different parasite species collected from *A. mossambica* are shown in **Table 4.3**. The parasitological assessment revealed the presence of three native nematodes; *Anguillicola papernai* Moravec & Taraschewski, 1988 (**Figure 4.2C**) in the swim-bladder, *Paraquimperia africana* Moravev, Boomker & Taraschewski 2000 in the gastrointestinal tract, and *Heliconema africanum* (Linstow, 1899) in the stomach. A monogenean was recorded from the gills of a large proportion of eels (84%) from all localities (**Figure 4.3A, 4.3B** & **4.4D**). Based on the morphological meusurements of this species it was identified as the alien *Pseudodactylogyrus anguillae* [see Table 4.2 for comparitive measurements with those by Christison & Baker (2007) and Iwashita *et al*. (2002)]. In addition, an undescribed myxosporeans species (**Figure 4.2D, 4.3C** & **4.3D**), possibly from the genus *Myxidium* Bütschli, 1882, was also observed in plasmodium on the gills.

This gill myxosporean was indentified, with the aid of the key to the Myxosporeans infecting freshwater fishes in Africa proposed by Reed (2003), as a member of the genus *Myxidium* Bütschli, 1882. The morphological characteristics of the genus *Myxidium* are the polar capsules discharging at both ends of the spores (**Figure 4.3D**). The spores have more or less pointed ends (**Figure 4.3D**). The spores have a fusiform shape with pointed ends (**Figure 4.3D**).

**Table 4.2:** Morphological measurements (in micrometres) of *Pseudodactylogyrus anguillae* from *Anguilla mossambica* from the Eastern Cape, South Africa.

| Measurement | *P. anguillae* Mean (Range) (n = 5) | *P. anguillae* Christison & Baker (2007) | *P. anguillae* Iwashita *et al.* (2002) |
|---|---|---|---|
| Hamulus | | | |
| Total length | 113.6 (104 – 124) | 113.5 (110 – 119) | 99 (94 – 105) |
| Length without foldable part | 95.6 (90 – 98) | 95.9 (92 – 100) | 87 (81 – 91) |
| Shaft length | 75.6 (74 -77) | 77.3 (73 – 81) | 73 (69 – 77) |
| Outer Root | 10.0 (9 – 11) | 9.9 (8 – 12) | 6 (5 – 9) |
| Length of foldable part of inner root | 35.6 (34 – 37) | 47.1 (42 – 51) | 35 (30 – 41) |
| Inner root | 68.6 (66 – 74) | 69.1 (65 – 74) | 51 (43 – 58) |
| Tip length | 31.6 (30 – 33) | 30.4 (27 – 33) | 29 (26 – 31) |
| Width of hamulus | 71.6 (70 – 73) | 72.9 (70 – 76) | 68 (64 – 72) |
| Hamulus ratio | 1.33 (1.28 – 1.36) | 1.30 (1.28 – 1.33) | 1.29 (1.25 – 1.32) |
| Dorsal bar | | | |
| Length | 54.0 (53 – 55) | 55.6 (51 – 60) | 50 (44 – 53) |
| Width | 10.6 (10 – 12) | 12.3 (9 – 15) | 9 ( 8 – 12) |
| Marginal hooklet | | | |
| Length | 16.3 (16 – 17) | 16.5 (16 – 17) | 16 (15 – 17) |

**Table 4.3:** Prevalence (P, %) and intensity (I) of parasites from *Anguilla mossambica* sampled during March and August 2012 from impoundments in the Keiskamma and Kei River systems, Eastern Cape, South Africa (n = 19). Comparative data from the Nahoon River (Taraschewski *et al*. 2005), Great Fish River (Parker *et al*. 2011) and Reunion Island (Sasal *et al*. 2008) are provided.

| Parasite | This Study | | Nahoon River | | Great Fish River | | Reunion | |
|---|---|---|---|---|---|---|---|---|
| | P | I | P | I | P | I | P | I |
| *Anguillicola papernai* | 32% | 1.16 ± 0.41 | 62% | 5.6 ± 4.2 | | | | |
| *Paraquimperia africana* | 58% | 3.36 ± 0.92 | 64% | 14.8 ± 12.2 | | | 13.3% | 1-2 |
| *Heliconema africanum* | 36% | 10.83 ± 2.13 | 92% | 59.6 ± 24.0 | | | | |
| *Pseudodactylogyrus anguillae* | 84% | 56.31 ± 10.64 | | | 73.2% | 63.8 ± 34.3 | 60% | 1-30 |
| *Myxidium* sp. | 21% | | | | | | | |

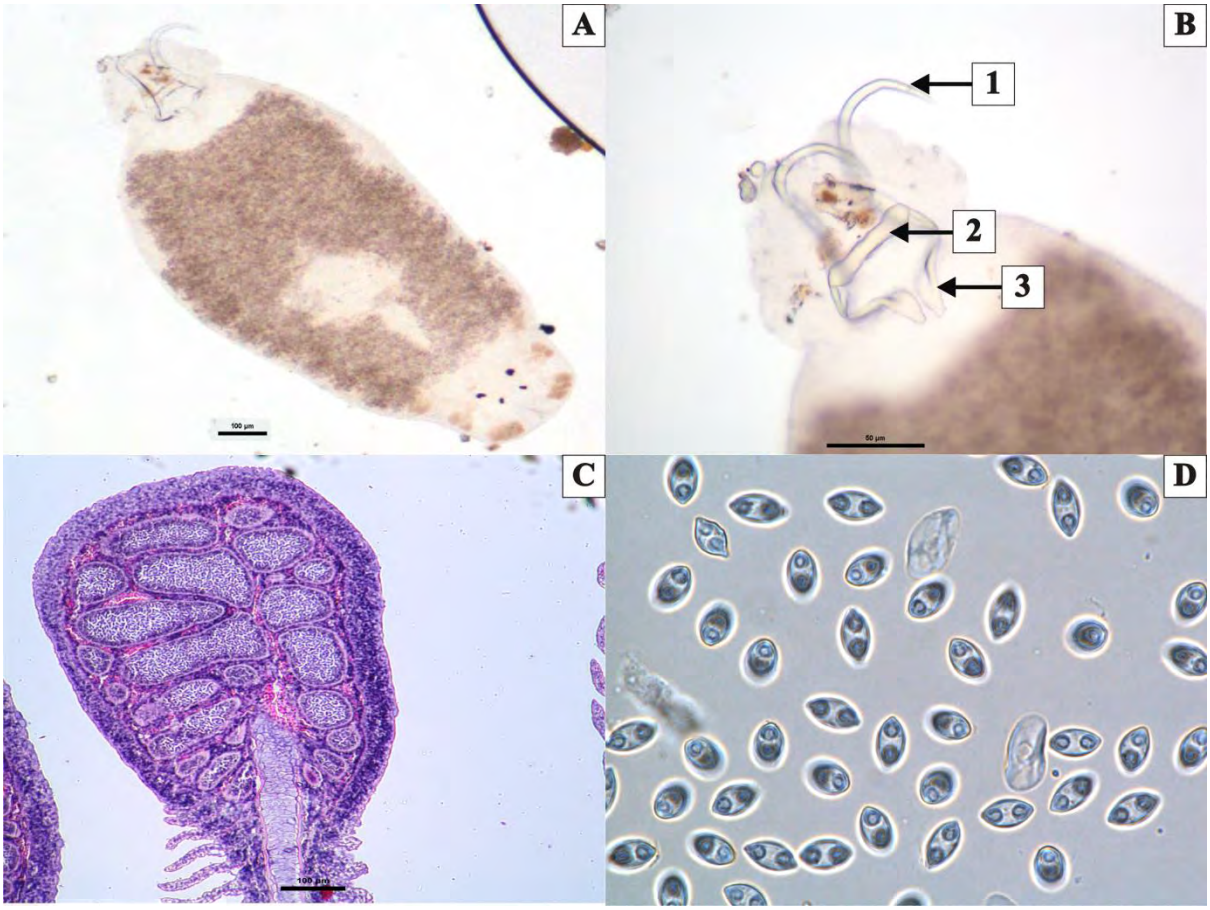


**Figure 4.3:** Micrographs of parasites collected off *Anguilla mossambica*. (A) *Pseudodactylogyrus anguillae*; (B) mouthparts of *Pseudodactylogyrus anguillae*, 1 – hamuli, 2 – dorsal bar, 3 – internal process; (C) unidentified myxosporean plasmodium and spores in the gill tissue stained with H&E (section 5 µm); (D) unidentified myxosporean spores.

### 4.3.3. Histological alterations

The percentage prevalence of histological alterations and the histological index are presented in **Tables 4.4** and **4.5,** respectively. The histological index indicated that the gills were the most severely affect tissue. Histological alterations of the gills included hyperplasia (**Figure 4.4B**) of the secondary gill epithelium, telangiectasia and rupture of pillar cells (**Figure 4.4C**) and increase in mucus cells (**Figure 4.4D**). The majority (74%) of the gill samples fell into Class 2, indicating a normal tissue structure with moderate histological alteration. The remaining 26% of the gill samples were in Class 1, which is normal tissue structure with slight histological alterations. The main liver histological alterations identified were hepatocyte vacuolation (**Figure 4.5B**), increase in melano-macrophage centres (MMCs) (**Figure 4.5C**), and intercellular deposits (**Figure 4.5D**). Most (95%) of the liver samples fell into Class 1 according to the histological index, while only 5% of the samples were in Class 2. According to the histological index (**Table 4.4**) the kidney tissue was second in ranking in terms of number and severity of alterations. The main kidney alterations observed were dilation of the renal tubule (**Figure 4.6A**), distortion of the glomerular tuft (**Figure 4.6B**), increase in the Bowman's space (**Figure 4.6C**), melano-macrophage centres (**Figure 4.6D**) and intercellular deposits. All (100%) of the kidney sample fell within Class 1.

No alterations were observed in the gonads, with both female and male gonads ranging in developmental stage from immature state to a developed state.

### 4.3.4. Statistical analyses

There were no significant differences between the individual samples for the CF, HSI and the SSI. There was also no significant difference ($p = 0.353; df1 = 1$) between the CF for *A. mossambica* infested with *P. anguillae* and un-infested eels. There was also no significant difference between the HSI ($p = 0.532; df1 = 1$) and the SSI ($p = 0.867$) for *P. anguillae*-infected and non-infected eels. There was also no significant difference for the CF ($p = 0.87; df1 = 1$), HSI ($p = 0.974$; $df1 = 1$), SSI ($p = 0.094; df1 = 1$) when uninfected eels were compared to eels infected with *Anguillicola papernai*.

**Table 4.4:** Prevalence (%) of histological alterations identified in *A. mossambica* from the Binfield Park Dam, Sandile Dam and Wriggleswade Dam for both March and August combined.

| Target Organ/ histological alteration | Percentage prevalence *Anguilla mossambica* n = 19 |
|---|---|
| **Gills** | |
| Hyperplasia | 68 |
| Increase in mucus cells | 79 |
| Rupture of Pillar cells | 53 |
| Telangiectasia | 47 |
| **Liver** | |
| Hepatocyte vacuolation | 42 |
| Increase in Melano macrophage centres | 58 |
| Intercellular deposits | 95 |
| **Kidney** | |
| Dilation of renal tubule | 74 |
| Distortion of glomerular tuft | 37 |
| Increase in Bowman's space | 58 |
| Increase in Melano macrophage centres | 47 |
| Intercellular deposits | 26 |

**Table 4.5:** Mean organ index and fish value index for *Anguilla mossambica*. $I_G$ = Gill Index, $I_L$ = Liver Index, $I_K$ = Kidney Index, $I_T$ = Testis Index, $I_O$ = Ovary Index and $I_{FISH}$ = Fish Index. Ranges are indicated in parentheses.

| Survey | $I_G$ | $I_L$ | $I_K$ | $I_T$ | $I_O$ | $I_{FISH}$ |
|---|---|---|---|---|---|---|
| *Anguilla mossambica* (n = 19) | 12.2 (0 – 21) | 2.7 (0 – 15) | 4.1 (1 – 7) | 0.0 | 0.0 | 18.9 (2 – 34) |

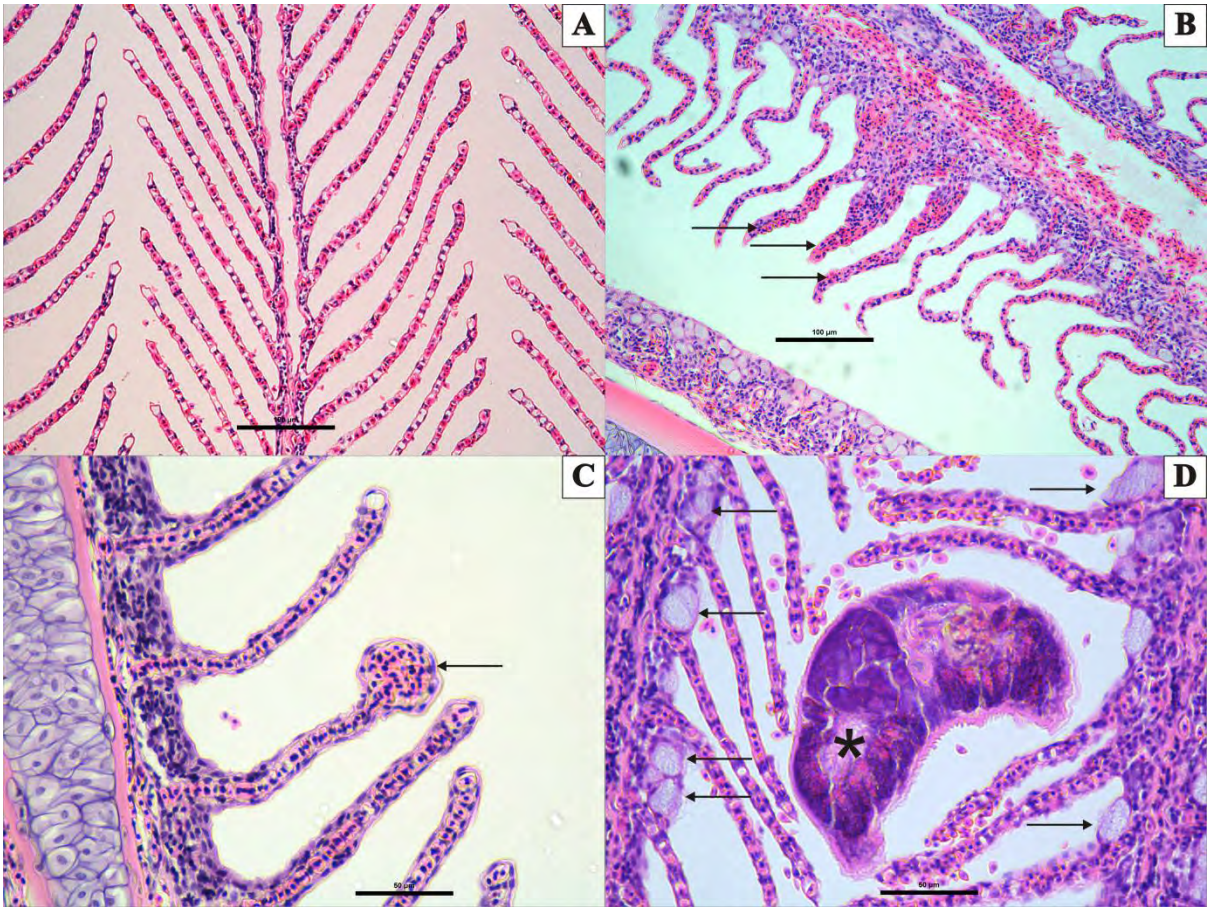


**Figure 4.4:** Micrographs of *Anguilla mossambica* gills sections (5 µm) stained with haematoxylin & eosin. (A) Normal *Anguilla mossambica* gills; (B) hyperplasia of the secondary gill lamella; (C) telangiectasia and rupture of pillar cells (D) increase in mucous cells (indicated by arrows) and *Pseudodactylogyrus anguillae* (indicated by asterisk).

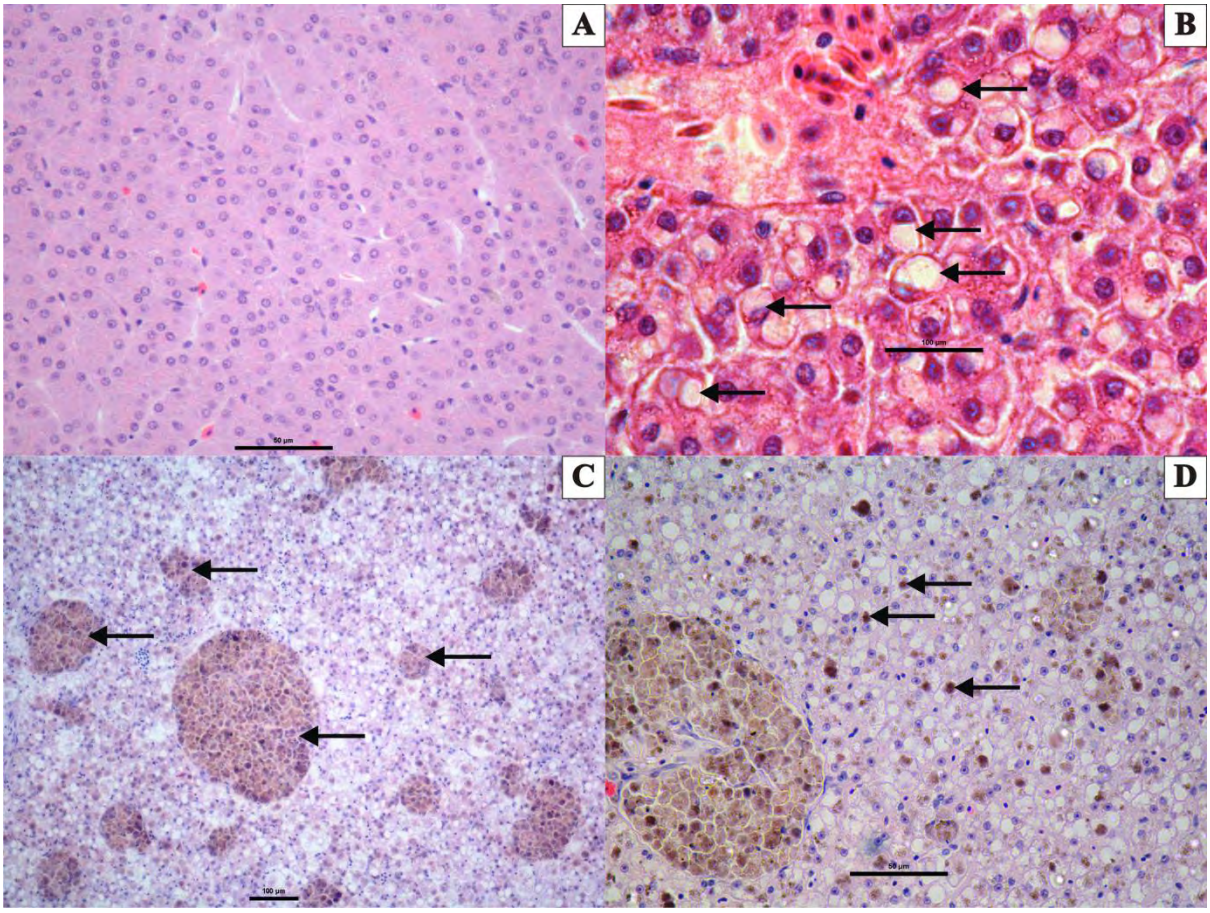


**Figure 4.5:** Micrographs of *Anguilla mossambica* liver sections (5 µm) stained with H&E. (A) Normal liver tissue; (B) hepatocyte vacuolation (arrows); (C) Increase in melano-macrophage centres (arrows); (D) intracellular deposits (arrows).

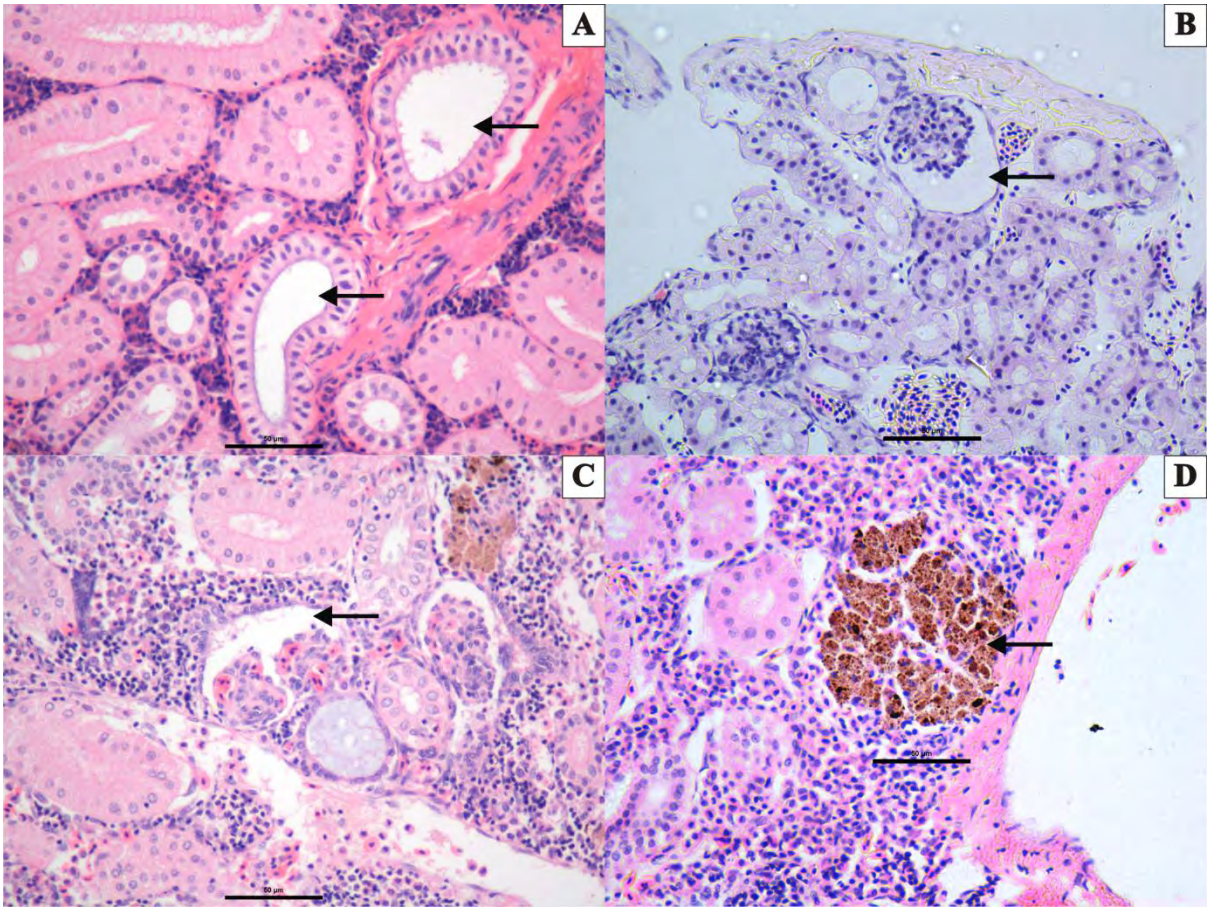


**Figure 4.6:** Micrographs of *Anguilla mossambica* kidney sections (5 µm) stained with H&E: (A) dilation of the renal tubule (arrows); (B) distortion of glomerular tuft (arrow); (C) increase in Bowman's space (arrow); (D) increase in melano-macrophage centres (arrow).

### 4.4. Discussion

Macroscopic necropsy indicated that there were no external macroscopic abnormalities in the fish. However, the HSI values were lower than the HSI values reported by Han *et al*. (2008) for farmed *A. japonica.* Whether this difference is species specific or whether the higher HSI values reported by Han *et al*. (2008) are because those were cultured eels kept under ideal conditions with an ample supply of food, is not known. However, Han *et al*. (2008) did note that there was a significant difference between the HSI values for eels infected with *A. crassus* and uninfected eels. In the present study there was no significant difference between the HSI values for eels infected with *P. anguillae* or *A. papernai* and uninfected eels.

Identification of *P. anguillae* was done by measuring the sclerotized structures of the monogenean according to the measurements of the hamulus, as described by Iwashita *et al*. (2002) (**Table 4.2**). Identification of *P. anguillae* was then confirmed by comparing the measurements with the measurements of Christison & Baker (2007), as well as of Iwashita *et al*. (2002). *Pseudodactylogyrus anguillae* had no significant effect on the condition factor of *A. mossambica*, supporting previous work (Kennedy 2007) that showed no effect on the condition factor of other *Anguilla* species. Haenen *et al*. (2010) showed that the swimbladder nematode *A. crassus* did have a significant influence on the condition factor of *Anguilla anguilla*, from the Rhine River. The indigenous swimbladder nematode *A. papernai* had no influence on the CF of *A. mossambica*.

Native parasite infestation rates (**Table 4.3**) were similar to those previously documented by Moravec *et al*. (2000), Taraschewski *et al*. (2005), Sasal *et al*. (2008) and Parker *et al*. (2011). The highest prevalence was by the alien gill monogenean *P. anguillae* with almost all (84%) individuals infested was similar to infestation rates reported by Parker *et al*. (2011) in the Great Fish River, South Africa. *Pseudodactylogyrus anguillae* was previously only reported in the wild from the Great Fish River system and this paper is the first report of this parasite in the Keiskamma and Kei River systems. In addition, the data strongly suggest that infestation by this parasite has severe impacts on the naive African eel host *A. mossambica*. Symptoms of infections included bleeding gills, hyperplasia of the

secondary gill epithelium, increase in mucus cells, telangiectasia and rupture of pillar cells (**Table 4.4**). Elsewhere, such impacts have previously only been documented from severely infected captive *A. anguilla* (Køie 1991; Kennedy 2007). Bleeding gills, for example, are most likely a result of damage to the gill epithelia by this parasites large hamuli (Køie 1991) and hyperplasia of gill tissue, fusion of the secondary lamellae and hyperanaemia have been linked to *P. anguillae* infestation in European eel (Kennedy 2007) and the observed increase in the mucus cells could be a response to *P. anguillae* feeding on mucus and gill epithelia (Buchmann *et al*. 1987). However, such effects have only been documented from aquaculture facilities where infestations have resulted in economic losses. Our findings are therefore the first demonstrable impact of *P. anguillae* on wild eel stocks. The finding that *P. anguillae* has a demonstrable impact on wild *A. anguillae* is significant, because well-defined impacts of invasive organisms are rare (Ricciardi *et al*. 2013).

In an attempt to characterise invasions, unified invasion frameworks (e.g. Blackburn *et al*. 2011) have been applied to a variety of taxa including fish (e.g. Jones *et al*. 2013), crustaceans (du Preez & Smit 2013) and plants (Weyl *et al*. 2014). Using the criteria proposed by Blackburn *et al*. (2011), we propose that *P. anguillae* can be considered fully invasive, with self-sustaining populations in multiple localities in discrete South African river systems. While the full extent and impacts of this invasion are currently not known, population level impacts of alien parasites might be severe, because the naïve host has not co-evolved with the parasite (Gozlan *et al*. 2008). This is partially supported by the evidence of impacts on wild *A. mossambica* that have previously only been reported from highly infested animals in stressful aquaculture conditions.

The histological alterations found in the gills were of the greatest concern, and our results indicated that it can be directly linked to the alien *P. anguillae*. The histological damage included an increase in mucous cells, hyperplasia of the gill epithelium, telangiectasia and rupture of the pillar cells. Such damage to the tissue of the gills may impair respiration and place additional stress on the eels.

The myxosporean on the gills of *A. mossambica* was identified using the key to the Myxosporeans infecting freshwater fishes in Africa (Reed 2003). The myxosporean was identified as a *Myxidium* species. *Myxidium* spores are usually fusiform, straight

or slightly crescent- shaped with pointed ends. There are two polar capsules that discharge at both ends, as well as their having a binucleate sporoplasm that lies between the two capsules (Reed 2003; Eiras *et al*. 2011). The most well-known species of *Myxidium* affecting various *Anguilla* species is *M. giardia*, which has been known to infect multiple organs, including the gills and the kidney of several eel species including *Anguilla anguilla*, *A. rostrata* and *A. japonica*. The infected elvers of these eels exhibited dropsy, ascites and swollen kidneys (Copland 1981; 1983; Ventura & Paperna 1984). Other known *Myxidium* species and their hosts are presented in **Table 4.6** (adapted from Eiras *et al*. 2011):

**Table 4.6:** The known *Myxidium* species infecting various different eels species from other countries, including the site of infections.

| *Myxidium* species | Author | Site of infection | *Anguilla* species | Locality |
|---|---|---|---|---|
| *M. acinum* | Hine, 1975 | Gills | *A. australis*<br>*A. dieffenbachi* | Australia |
| *M. anguillae* | Tshii, 1915 | Intestine | *A. japonica* | Japan |
| *M. durum* | Hine, 1980 | Gills | *A. australis* | Australia |
| *M. enchelyptergyii* | Hoshima, 1952 | Fins | *A. japonica* | Japan |
| *M. giardi* | Cépède, 1906 | Kidney | *A. vulgaris* | France |
| *M. illinoisense* | Meglirsch, 1937 | Kidney | *A. bostoniensis* | USA |
| *M. lentifome* syn. *M. fusiforme* | Fujita, 1927 | Kidney | *A. japonica* | Japan |
| *M. matsuii* | Fujita, 1929 | Skin | *A. japonica* | Japan |
| *M. mimdanoense* | Hine, 1980 | Gills | *A. bicolor pacifica* | Australia |
| *M. serum* | Hine, 1975 | Alimentary musculature | *A. dieffenbachii* | Australia |
| *M. uchiyamae* | Fujita, 1927 | Kidney | *A. japonica* | Japan |
| *M. zealandicum* | Hine, 1975 | Gills | *A. austalis*<br>*A. dieffenbachi* | Australia |

From the above information it is clear that there is a paucity of information regarding myxosporeans on South African eels. Further research is required on their morphology, genetics and distribution in order to identify this myxosporean correctly. Further research will also be required to determine the effects it has on its eel host.

The main histological alterations identified in the liver (see **Table 4.4**) were the hepatocyte vacuolation, and an increase in the melano macrophage centres (MMCs) were also of concern. Melano-marcophage centers are made up of different substances such as lipofuscin, melanin and ceriod. They can vary in number and size and often depend of the health of the species as well as its age and the species of fish (van Dyk *et al*. 2009). Hepatocyte vacuolation is often associated with a variety of stressors that can be ethier chronic and acute, it is difficult to associate these alternations with single causative agent without toxicity analysis of the tissue. The discolouration of the livers may have been as a result of the hepatocyte vacuolation observed in the histological assessment. Similar results were observed by van Dyk *et al*. (2012) in *Clarias gariepinus*, were it was noted that the macroscopically observed livers also had hepatocyte vacuolation as well as fatty deposits.

The kidney histology indicated that the Binfield Park Dam samples had more abnormalities in terms of number and severity of alterations identified. The alterations included increase in glomerular size, distortion of glomerular tuft and dilation of renal tubules. Similar alterations were noted by McHugh *et al*. (2013) in *Mugil cephalus* and *Myxus capensis* sampled from this locality (also see **Chapter 5**). Whereas these alterations were associated with nephrocalcinosis in *M. cephalus* and *M. capensis*, nephrocalcinosis was not detected in any of the Binfield Park Dam eel samples.

### 4.5. Conclusion

The data presented in this chapter demonstrate that *P. anguillae* has successfully invaded the Keiskamma and Kei River systems in the Eastern Cape. This increases the known range of this alien parasite to three river systems in South Africa. As a result of the impacts of this parasite on wild *A. mossambica*, it is important that the extent of the *P. anguillae* invasion and its potential population level impacts on *A. mossambica* in African river systems is assessed further.

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# Chapter 5 : A comprehensive fish health assessment of *Myxus capensis* (Valenciennes) and *Mugil cephalus* L. from Binfield Dam, South Africa.

## 5.1. Introduction

Fish of the family Mugilidae are tolerant of a wide range of salinities and are introduced into inland waters to enhance fisheries (Ellender *et al*. 2012). The freshwater mullet *Myxus capensis* (Valenciennes 1836) is endemic to southern Africa (Whitfield 1998), but is more common in Cape rivers than are other mullet species. It is a large fish species, reaching nearly 450 mm fork length. *Myxus capensis* move into freshwater as juveniles and are known to travel up freshwater rivers as far as 100 km inland (Bok 1979; Skelton 2001). The flathead mullet *Mugil cephalus* Linnaeus, 1758 is a bigger species of mullet, attaining 600 mm in fork length (Skelton 2001). *Mugil cephalus* are common throughout South African coastal rivers (Skelton 2001), as well occurring worldwide in coastal and estuarine waters of the tropical, subtropical and temperate zones of all seas (Whitfield *et al* 2012). The juveniles enter estuaries and may travel up rivers, where they remain for one or two years before returning to the sea (Skelton 2001; Whitfield *et al.* 2012). All members of the Mugilidae family can only spawn at sea (Whitfield *et al.* 2012).

There is a growing interest in the use of biological communities to assess the status of aquatic resources (Whitfield & Elliott 2002). As well as a growing demand for the use of fish species to detect environmental and ecological effects within estuaries (Whitfield & Elliott 2002). However, in order to do this, a suitable representative sentinel fish species is required, as well as a reference data set for this species, in order to determine what are normal environmental effects and what are man-induced effects. Mullet species such as *Mugil cephalus* have been chosen as sentinel species in Europe to determine the effect of organic pollutants in the estuarine and marine environments (Ferreira *et al*. 2004; Whitfield *et al*. 2012). The mullet species *M. cephalus* is a very widespread species and is capable of surviving in a range of habitats. It has several important characteristics that make it a useful indicator of aquatic pollution, such as its ability to tolerate a wide variety of salinities, and its wide

distribution that includes a variety of habitats (Whitfield *et al.* 2012). Previous studies that used *M. cephalus* as a bio indicator species include that by Frodello *et al.* (2001), who suggested that melano-macrophage centres in the liver of *M. cephalus* could be used as an indication of environmental pollution. In Australia the concentrations of selenium, cadmium, copper and zinc were recorded in the muscle tissue of *M. cephalus* from the southern basin of Lake Macquarie (Kirby *et al*. 2001). Yilmaz (2003) also investigated the concentrations of heavy metals (Fe, Cu, Ni, Cr, Pb and Zn) in the muscle tissue of *M. cephalus* from Iskenderun Bay, Turkey. Yilmaz (2001) found that metals present in the bay were taken up by *M. cephalus* through their diet. Ferreira *et al.* (2004) investigated the organochlorine pollutants in the Douro River estuary, Portugal, using the sentinel fish species *M. cephalus* and noted that the accumulation of organochlorines was much higher in the muscle tissue of the mullet than in any other sampled species. Ferreira *et al.* (2004) stated that *M. cephalus* should be used as the sentinel species for the monitoring of the presence of organic contaminants in southern European estuaries. Ferreira *et al.* (2005) also showed that oxidative stress in *M. cephalus* from the Douro River estuary was caused by pollutants. The use of *M. cephalus* as a sentinel species for aquatic pollution was suggested by Maruya *et al.* (2005). Tsangaris *et al*. (2011) also studied the effect of oxidative stress in *M. cephalus* from Saronikos Gulf, Greece, and stated that *M. cephalus* would be a useful species in assessing the impacts of pollution in the marine environment.

In September 2000, 75000 wild-caught young-of-the-year (20–40 mm total length) *Myxus capensis* and *Mugil cephalus* from the Keiskamma River estuary were stocked into Binfield Park Dam on the Tyume River (Ellender *et al*. 2012). The Tyume River forms a headwater tributary of the Keiskamma River system. This relocation was done because members of the Mugilidae family had been chosen as sentinel species because of their extreme tolerance to salinity (*Myxus capensis* 0 – 84; *Mugil cephalus* 0 – 49) (Bok 1979; Ferreira *et al*. 2004; Ellender *et al*. 2012).

The aim of this section of the study was to assess the health status of *M. capensis* and *M.l cephalus* using a macroscopic and a histology based fish health assessment protocol. The first objective was to compare any possible macroscopic abnormalities between the two different species. The second objective was to assess semi-quantitatively the histology of the selected target organs (gills, liver, kidney, testis

and ovaries), and to compare the results between the two species, since they share the same habitat and food preferences. The final objective was to categorise the results in terms of a classification system, indicating the severity of the histological responses identified.

### 5.2. Materials and methods

The study took place at Binfield Park Dam (see **Chapter 2, Section 2.1**). A detailed map of the impoundment has been provided (**Figure 5.1**). The materials and methods used in this chapter are discussed in **Chapter 2**. For sampling (see **section 2.2)**, blood parameters, necropsy, and biometric indices (see **Chapter 2, Section 2.2.1**) were recorded and the tissue sampling procedure was prepared (see **Chapter 2, Section 2.2.2**) for histological analysis (see **Chapter 2, Section 2.2.2**), and statistical analysis was performed for comparisons (see **Chapter 2, Section 2.2.5**). *M. capensis* was collected in 2011 (n = 15) and *M. cephalus* was sampled in 2012 (n = 20) by means of gill nets (see **Chapter 2, Section 2.2**). The techniques used will not be repeated here.

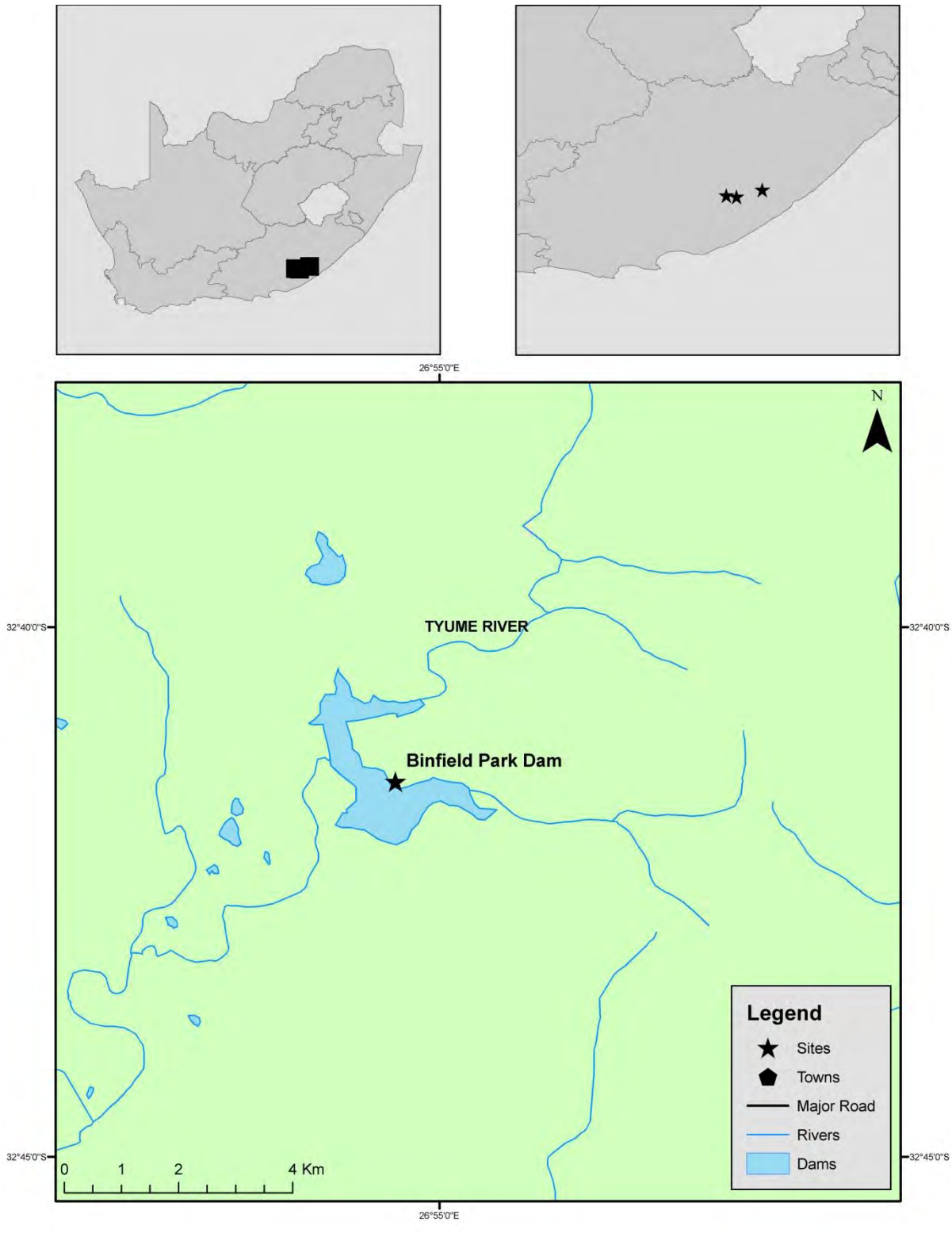


**Figure 5.1:** Location of Binfield Park Dam, near Bisho, Eastern Cape Province, South Africa.

## 5.3. Results

### 5.3.1. Necropsy, blood parameters and biometric indices

Macroscopic fish health assessment revealed that there were no external abnormalities on the *M. capensis* samples. The internal abnormalities noted in *M. capensis* were fatty and discoloured livers (20%) (**Figure 5.2A**), and some of the liver samples had cysts within the liver (5%), granular and enlarged spleens (20%) (**Figure 5.2B**) and swollen kidneys with visible white cysts (100%) (**Figure 5.2C**). The external examination of *M. cephalus* samples revealed slight (5%) skin damage (other than damage cause by the gill nets). The *M. cephalus* internal abnormalities noted were frayed gills (5%), discolouration and fatty livers (45%), granular spleens and cysts on the spleen (45%) (**Figure 5.2D**), and swollen kidneys with visible white cysts (100%).

The biometric indices are presented in **Table 5.1**. The masses of the collected samples of *M. cephalus* (2263 ± 428 g) indicated that they were, in general, slightly larger than the *M. capensis* (2233 ± 263 g). However, this difference was not significant ($p \geq 0.05$; $df1 = 2$). The same was true for the fork lengths, as there was no significant difference ($p \geq 0.05$; $df1 = 2$) between *M. capensis* (543.7 ± 17.1) and the *M. cephalus* (544.4 ± 37.5) samples. There was also no significant ($p \geq 0.05$; $df1 = 2$) difference between the condition factors (CF) for *M. capensis* (1.39 ± 0.12) and *M. cephalus* (1.40 ± 0.10). The gutted condition factor (GCF) was also not significantly ($p \geq 0.05$; $df1 = 2$) different between *M. capensis* (1.23 ± 0.09) and *M. cephalus* (1.24 ± 0.11). There was a significant difference ($p \leq 0.05$; $df1 = 2$) in the hepatosomatic indices (HSI), with the *M. capensis* (1.50 ± 0.32) liver samples being larger than the *M. cephalus* (1.30 ± 0.21) liver samples. There was no significant ($p \geq 0.05$; $df1 = 2$) difference between the splenosomatic indices (SSI) for *M. capensis* (0.14 ± 0.05) and *M. cephalus* (0.13 ± 0.06). There was no significant ($p \geq 0.05$; $df1 = 2$) difference in the gonadosomatic indices between the *M. capensis* females (2.91 ± 1.65) and the *M. cephalus* males (0.96 ± 2.24) or the *M. cephalus* females (2.73 ± 3.52). There also was no significant difference ($p \geq 0.05$; $df1 = 2$) between the GSI of *M. cephalus* males and females.

The mean total protein levels for *M. capensis* (58.56 ± 14.11 mg $dl^{-1}$) were not significantly ($p \geq 0.05$) lower than the total protein levels of *M. capensis* (64. 79 ± 19.56 mg $dl^{-1}$). Of the total protein levels of the *M. capensis* samples, 7% (19 – 29 mg $dl^{-1}$) fell below the normal range (30 – 69 mg $dl^{-1}$) of Adams *et al*. (1993), and 34% (>70 mg $dl^{-1}$) were above the normal range. Only 42% of the *M. cephalus* were above the normal range of Adams *et al*. (1993). The mean hematocrit levels for *M. capensis* (58.75 ± 14.11 %) were not significantly different ($p \geq 0.05; df1 = 2$) from the hematocrit levels of the *M. cephalus* (64.79 ± 19.56 %) samples. The hematocrit revealed that 86% of the *M. capensis* were above the normal range (30 – 45 %) of Adams *et al*. (1993) and that 92% of the *M. cephalus* hematocrit samples were above the normal range. Although the fish health assessment index (FHAI) indicated that *M. capensis* (34.67 ± 22.95) were in a poorer health status than *M. cephalus* (29.50 ± 8.25), this difference was not significant ($p \geq 0.05; df1 = 2$).

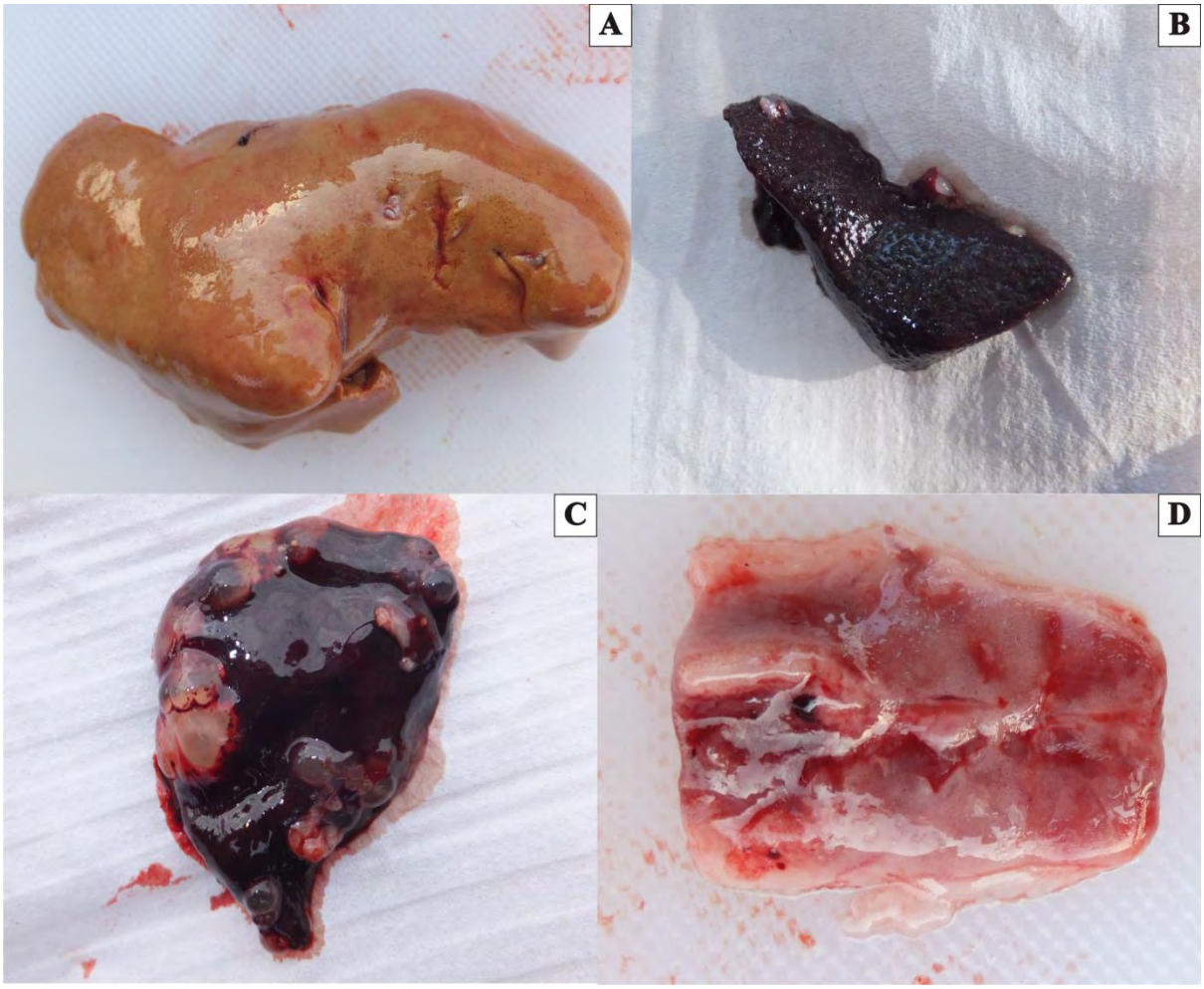


**Figure 5.2:** Internal abnormalities indentified in *Myxus capensis* and *Mugil cephalus* samples; (A) fatty and discoloured livers; (B) Granular and enlarged spleens; (C) spleens with cysts; (D) swollen kidneys with visible white cysts.

**Table 5.1:** Biometric indices for *Myxus capensis* and *Mugil cephalus*,: mean total body mass (g), fork length (mm), condition factor (CF), gutted condition factor (GCF), hepatosomatic index (HSI), spleenosomatic index (SSI), gonadosomatic index (GSI) and the fish health assessment index (FHAI).

| Survey | | *Myxus capensis* (n = 15) | *Mugil cephalus* (n = 20) |
|---|---|---|---|
| Mass | | 2233 ± 263 | 2263 ± 428 |
| Length | | 543.7 ± 17.1 | 544.4 ± 37.5 |
| CF | | 1.39 ± 0.12 | 1.40 ± 0.10 |
| GCF | | 1.23 ± 0.09 | 1.24 ± 0.11 |
| HSI | | 1.50 ± 0.32 | 1.30 ± 0.21 |
| SSI | | 0.14 ± 0.05 | 0.13 ± 0.06 |
| GSI | Male | 0 | 0.96 ± 2.24 |
| | Female | 2.91 ± 1.65 | 2.73 ± 3.52 |
| FHAI | | 34.67 ± 22.95 | 29.5 ± 2.25 |

### 5.3.2. Histological assessments

The percentage prevalence of the histological alterations that were indentified, as well as the histological index results, are presented in **Tables 5.2** and **5.3,** respectively. In terms of percentage prevalence, the gill samples of *M. cephalus* were more affected than those of *M. capensis* samples. The histological alterations noted in the gill samples included branching of the secondary lamellae (**Figure 5.3A**), congestion of the secondary lamellae (**Figure 5.3B**), hyperplasia (**Figure 5.3C**), rupture of pillar cells and telangiectasia (**Figure 5.3D**). The gill index ($I_G$) for *M. capensis* (4.80) was not significantly ($p \geq 0.05$; $df1 = 2$) lower than the $I_G$ of *M. cephalus*. Although the mean $I_G$ for *M. capensis* fell within Class 1, indicating normal tissue structure with slight histological alteration, 7% of the samples fell into Class 2, indicating normal tissue structure with moderate histological alterations. The mean $I_G$ for the *M. cephalus* samples fell within Class 1 and 15% of the *M. cephalus* $I_G$ fell within Class 2. The Liver samples were second in terms of histological alterations identified and percentage prevalence of the alterations observed. The main histological alterations observed in liver samples of *M. capensis* and *M. cephalus* were disarray of the cell structure (**Figure 5.4A**), hepatocyte vacuolation (**Figure 5.4B**), increase in the melano-macrophage centres (**Figure 5.4C**), intercellular deposits (**Figure 5.4C**) and pyknosis of the nuclei (**Figure 5.4D**). There was no significant difference ($p \geq 0.05$; $df1 = 2$) between *M. capensis* (20.73) and *M. cephalus* (22.40) in the liver index ($I_L$) classification, although the *M. cephalus* liver scores were slightly higher. The majority (80%) of *M. capensis* $I_L$ samples were in Class 2, however, 20% of the samples fell into Class 3, indicating that there are pronounced alterations in the *M. capensis* liver samples. 95% of the *M. cephalus* $I_G$ samples fell within Class 2 and 5% fell into Class 3. In terms of the histological alterations and percentage prevalence, the kidney samples were the most severely affected. The histological alterations identified in the kidney samples were dilation of the renal lumen (**Figure 5.5A**), eosinophillic granular cells (**Figure 5.5B**), an increase in the size of the glomerulus (**Figure 5.5C**), increase in melano-macrophage centres (**Figure 5.5D**) and nephrocalcinosis (**Figure 5.5E**). Although there was no significant ($p \geq 0.05$; $df1 = 2$) difference between the kidney index ($I_K$) scores, although the *M. capensis* (18.0) $I_K$ scores were slightly lower than the *M. cephalus* (20.75) kidney scores. For the *M. capensis* $I_K$ samples, 33% fell into Class 1, 27% were in Class 2 and 40% fell within Class 3. The $I_K$ for *M. cephalus* samples,

were in Class 2 (65%) and in Class 3 (35%). The histological alterations indentified in both the testes and the ovaries of the two mullet species were the lowest, and only an increase in the melano-marcophage centres (**Figure 5.6A** and **5.6D**) was identified. No male *M. capensis* samples were collected to compare with the males of *M. cephalus*. The testis index ($I_T$) for *M. cephalus* (5.00) was relatively low and all samples (100%) fell within Class 1. There was not a significant ($p \geq 0.05$; $df1 = 2$) difference between the ovary index ($I_O$) for the female *M. capensis* (4.33) and female *M. cephalus* (4.58) samples. The female *M. capensis* samples had 100% in Class 1 however, only 87% had observable histological alterations. The *M. cephalus* samples were all in Class 1 however, 92% of the samples had identifiable histological alterations.

### 5.3.4. Gonad development

The developmental stages of the testes and ovaries were determined according to the criteria given in the Biomonitoring of Environmental Status and Trends Program (BEST) (Schmitt & Dethloff 2000). The sex ratio of the *M. capensis* was 100% females collected. The female *M. capensis* comprised mostly Stage 2 (73%) of development, which is mid-development, the majority of the observed follicles were early and mid-vitellogenic, and the oocytes had diameters between 300 and 600µm (**Figure 5.6F**). The remaining 27% were in Stage 1 of development, indicating that they were in early development with the majority of the oocytes being pre-vitellogenic and having diameters up to 300µm (**Figure 5.6F**). The sex ratio for the *M. cephalus* samples was 40% males and 60% females, making it a 1:1.5 male to female ratio. The development stages of male *M. cephalus* samples were: Stage 1 (25%) early spermatogenic, with is a thin germinal epithelium with scattered spermatogenic activity (**Figure 5.6B**), Stage 2 (62%) mid-spermatogenic, epithelia are of moderate thickness and there is maturation of the spermatozoa (**Figure 5.6C**), and Stage 3 (13%) this is the late spermatogenic stage with a thick germinal epithelium, with diffuse regions of proliferation and maturation of spermatozoa.

### 5.3.4. Melano-macrophage centres

The large number of MMC's and intercellular deposits observed in the liver, kidney, testis and ovary samples, were stained with Perl's Prussian blue, Schmorl's and Masson-Fontana stains to identify the presence of hemosiderin, lipofuscin and melanin, respectively. The liver, kidney, testis and ovary samples all tested positive for hemosiderin (**Figure 5.7**), lipofuscin (**Figure 5.8**) and melanin (**Figure 5.9**). The intercellular deposits also tested positive for hemosiderin (**Figure 5.7A**), lipofuscin (**Figure 5.8A**) and melanin (**Figure 5.9A**). **Figures 5.7A** and **5.7D** show clearly how certain MMCs react to the special stains and how other MMCs adjacent to the stained MMC do not react.

**Table 5.2:** Percentage prevalence of histological alterations identified in *Myxus capensis* and *Mugil cephalus* from Binfield Park Dam.

| Target Organ/ histological alteration | Percentage prevalence | |
|---|---|---|
| | *Myxus capensis* n = 15 | *Mugil cephalus* n = 20 |
| **Gills** | | |
| Branching of secondary lamellae | 73 | 45 |
| Congestion of secondary lamellae | 87 | 95 |
| Hyperplasia | 20 | 30 |
| Rupture of pillar cells | 27 | 65 |
| Telangiectasia | 20 | 65 |
| **Liver** | | |
| Disarray of the cell structure | 40 | 75 |
| Hepatocyte vacuolation | 100 | 100 |
| Increase in melano-macrophage centres | 100 | 100 |
| Intercellular deposits | 40 | 5 |
| Pyknosis | 87 | 100 |
| **Kidney** | | |
| Dilation of the renal lumen | 47 | 85 |
| Eosinophilic granular cells | 40 | 35 |
| Glomerulus size increase | 67 | 70 |
| Increase in melano-macrophage centres | 67 | 95 |
| Nephrocalcinosis | 100 | 100 |
| **Testis** | **n = 0** | **n = 8** |
| Increase in melano-macrophage centres | | 100 |
| **Ovaries** | **n = 15** | **n = 12** |
| Increase in melano-macrophage centres | 87 | 92 |

**Table 5.3:** Mean organ index and fish value index for *Myxus capensis* and *Mugil cephalus*. $I_L$ = Liver Index, $I_K$ = Kidney Index, $I_G$ = Gill Index, $I_T$ = Testis Index, $I_O$ = Ovary Index and $I_{FISH}$ = Fish Index. Ranges are indicated in parentheses.

| Survey | $I_G$ | $I_L$ | $I_K$ | $I_T$ | $I_O$ | $I_{FISH}$ |
|---|---|---|---|---|---|---|
| *Myxus capensis* (n = 15) | 4.8 (0 – 12) | 20.7 (10 – 28) | 18.0 (5 – 30) | 0.0 (0) | 4.33 (0 – 5) | 47.9 (35 – 75) |
| *Mugil cephalus* (n = 20) | 6.8 (1 – 18) | 22.4 (20 – 26) | 20.7 (5 – 30) | 5.00 (5) | 4.58 (0 – 5) | 54.7 (36 – 74) |

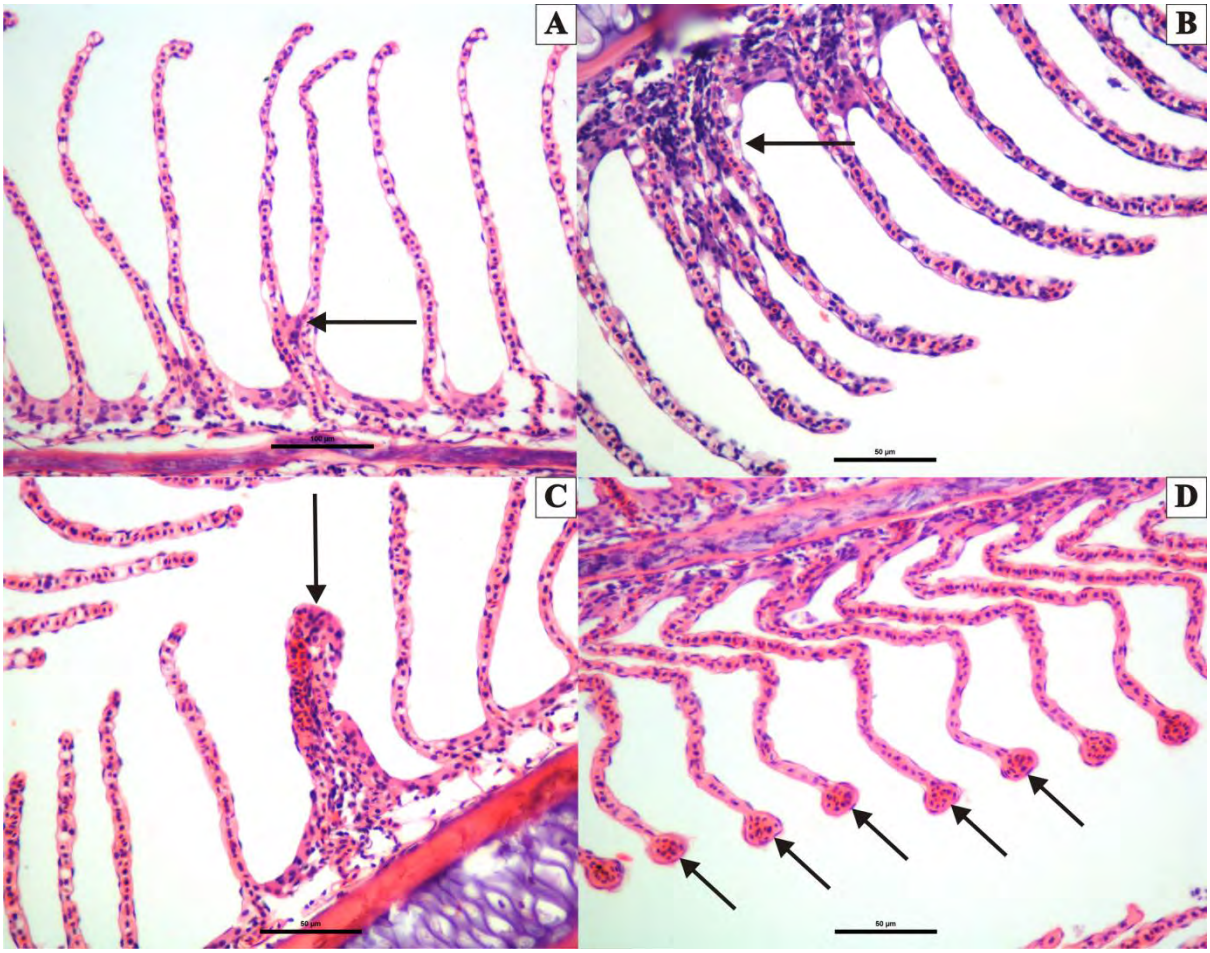


**Figure 5.3:** Micrographs of mullet gills sections stained with H&E. (A) Branching of the secondary gill lamellae (arrow); (B) congestion of the secondary gill lamellae (arrow); (C) hyperplasia (arrow); (D) telangiectasia and rupture of the pillar cells (arrows). Scale bars = 100 µm (A), 50 µm (B, C, D).

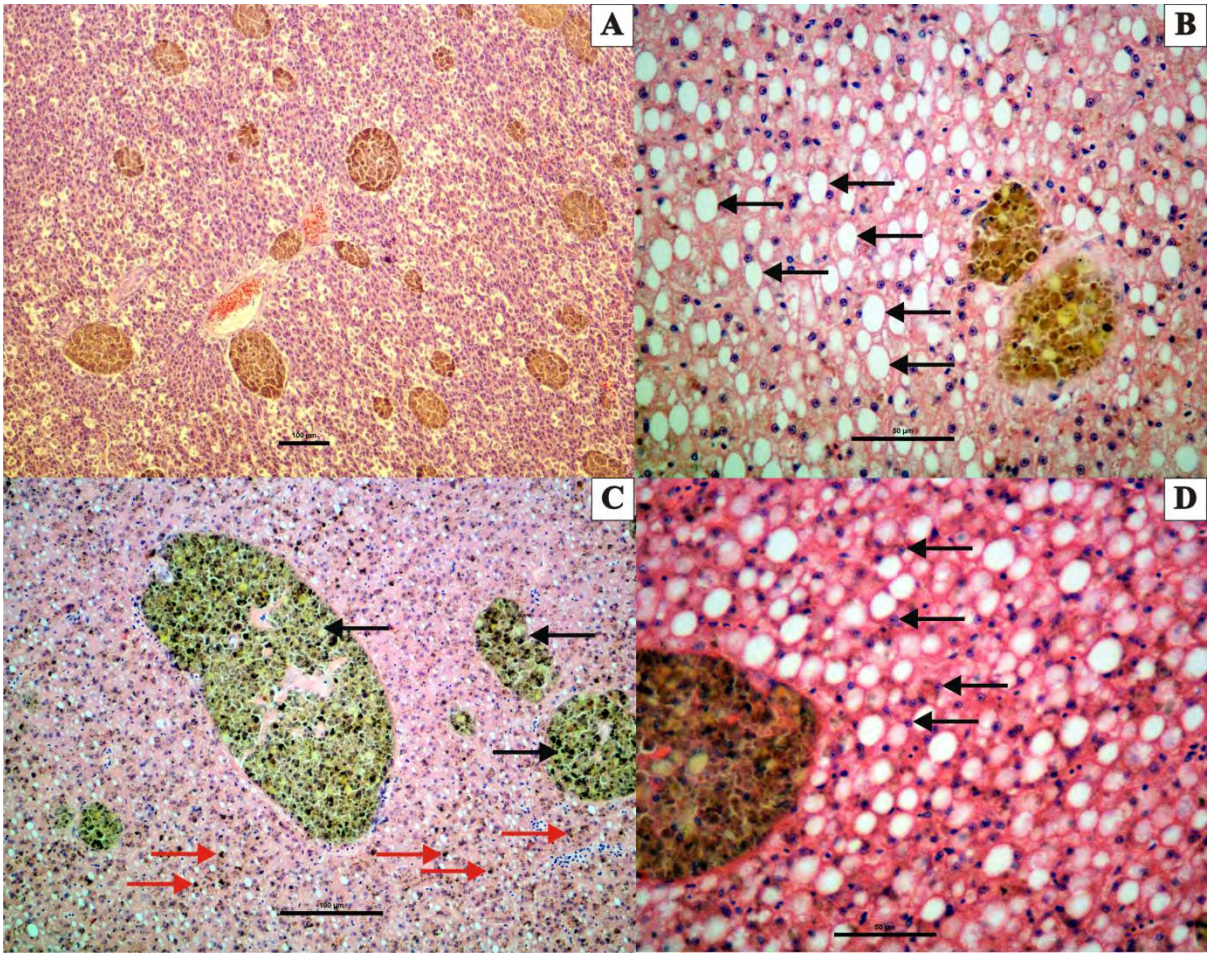


**Figure 5.4:** Micrographs of mullet liver stained with H&E. (A) Disarray of the liver tissue; (B) hepatocyte vacuolation (arrows); (C) an increase in the melano-macrophage centres (black arrows) and intercellular deposits (red arrows); (D) pyknosis of the nuclei (arrows). Scale bars = 100 µm (A, c), 50 µm (B, D).

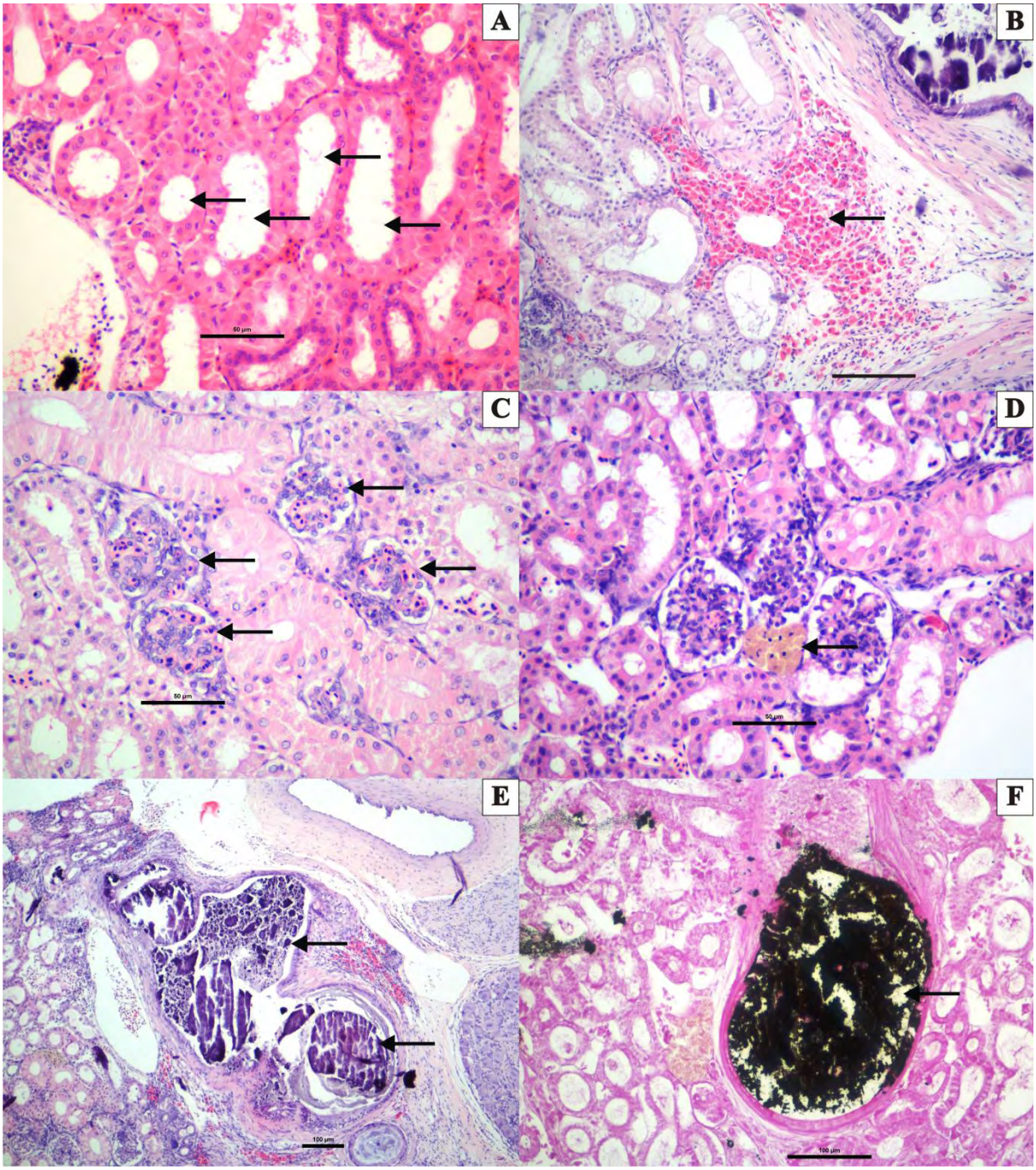


**Figure 5.5:** Micrographs of mullet kidney sections stained with H&E. (A) Dilation of the renal lumen (arrows); (B) eosinophillic granular cells (arrow); (C) increase in size and distortion of the glomerulus (arrows); (D) increase in melano-macrophage centres (arrow); (E) nephrocalcinosis (arrows); (F) nephrocalcinosis stained with the Von Kossa silver nitrate stain (arrow). Scale bars = 50 µm (A, B, C, D), 100 µm (E, F).

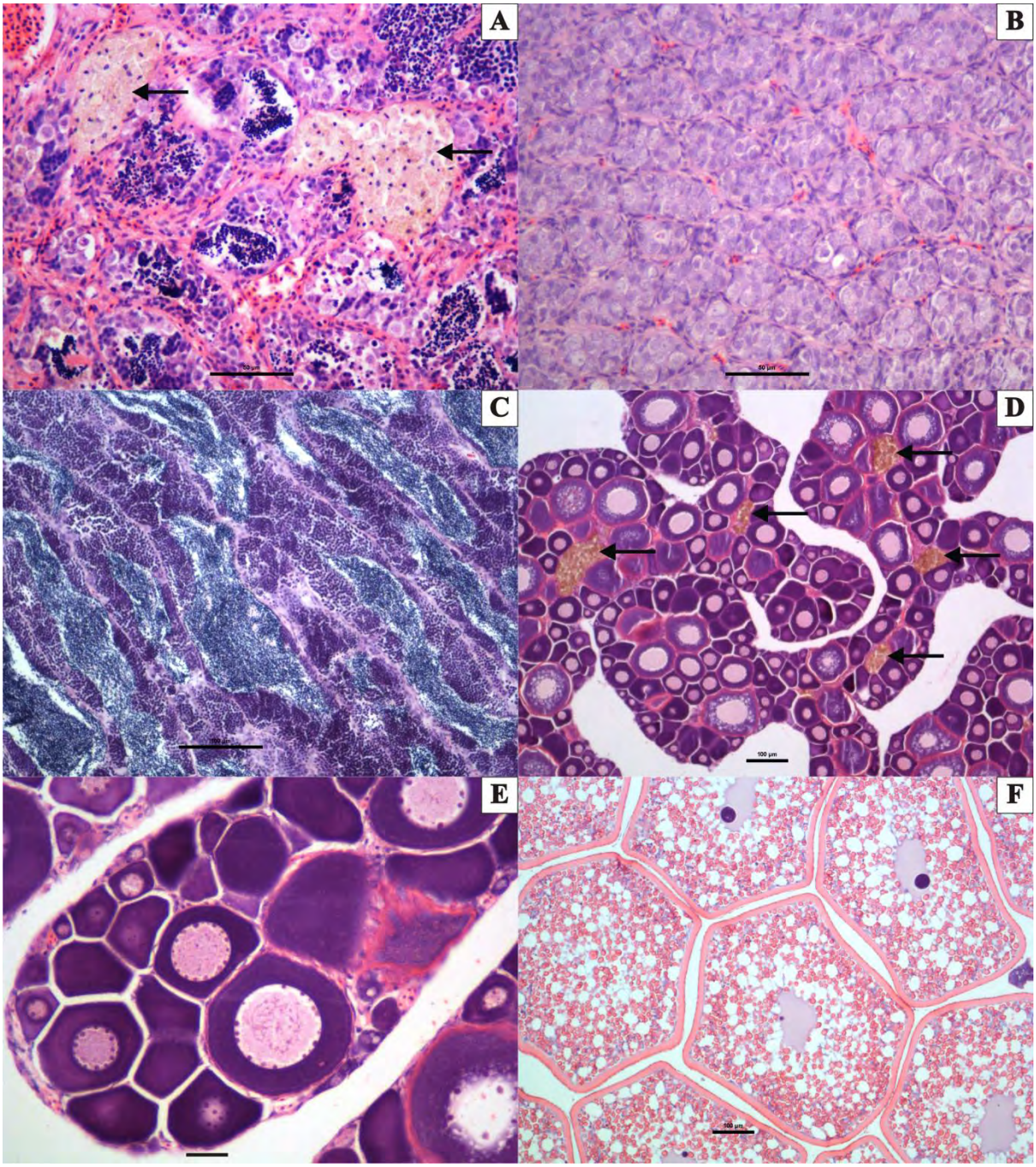


**Figure 5.6:** Micrographs of mullet gonad sections stained with H&E. (A) an increase in the melano-macrophage centres in the testis (arrows); (B) male testis at stage 1 of development; (C) male testis at stage 2 of development; (D) an increase in melano-macrophage centres in the ovaries (arrows); (E) female ovaries in stage 1 of development; (F) female ovaries in stage 2 of development. Scale bars = 50 µm (A, B, C, E, F), 100 µm (C, D).

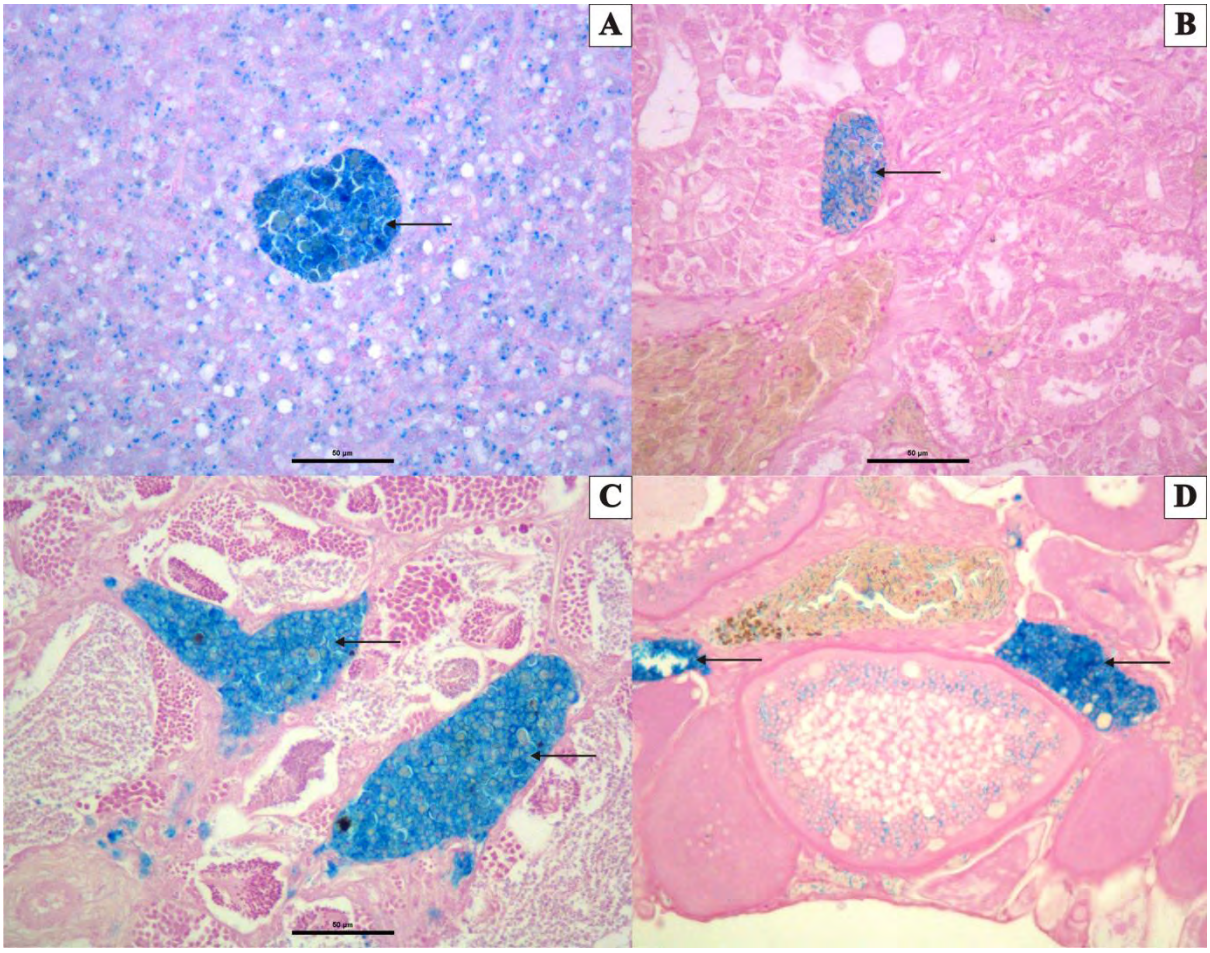


**Figure 5.7:** Micrographs of various mullet tissue sections stained with Perl's Prussian Blue. (A) Liver tissue with an increase in MMCs (arrow) as well as intracellular deposits; (B) kidney tissue with an increase in MMC's, stained (arrow) and unstained; (C) testis tissue with an increase in MMC's (arrows); (D) ovary tissue with an increase in MMC's, stained (arrow) and unstained. Scale bars = 50 µm (A, B, C, D).

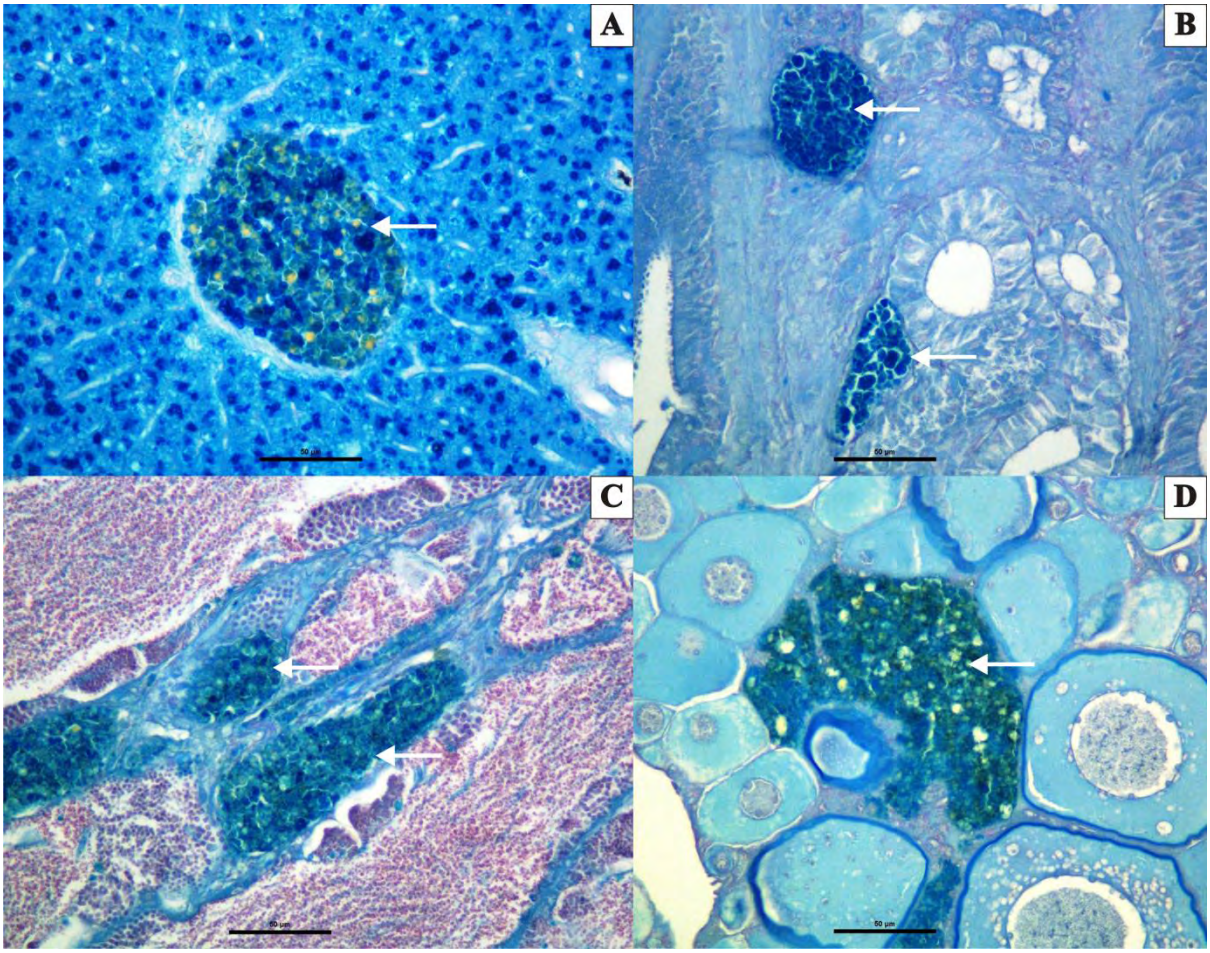


**Figure 5.8:** Micrographs of various mullet tissues sections stained with Schmorl's stain. (A) Liver tissue with an increase in MMCs (white arrows) as well as intracellular deposits; (B) kidney tissue with an increase in MMC's, (white arrows); (C) testes tissue with an increase in MMC's (white arrows); (D) ovary tissue with an increase in MMC's (white arrow). Scale bars = 50 µm (A, B, C, D).

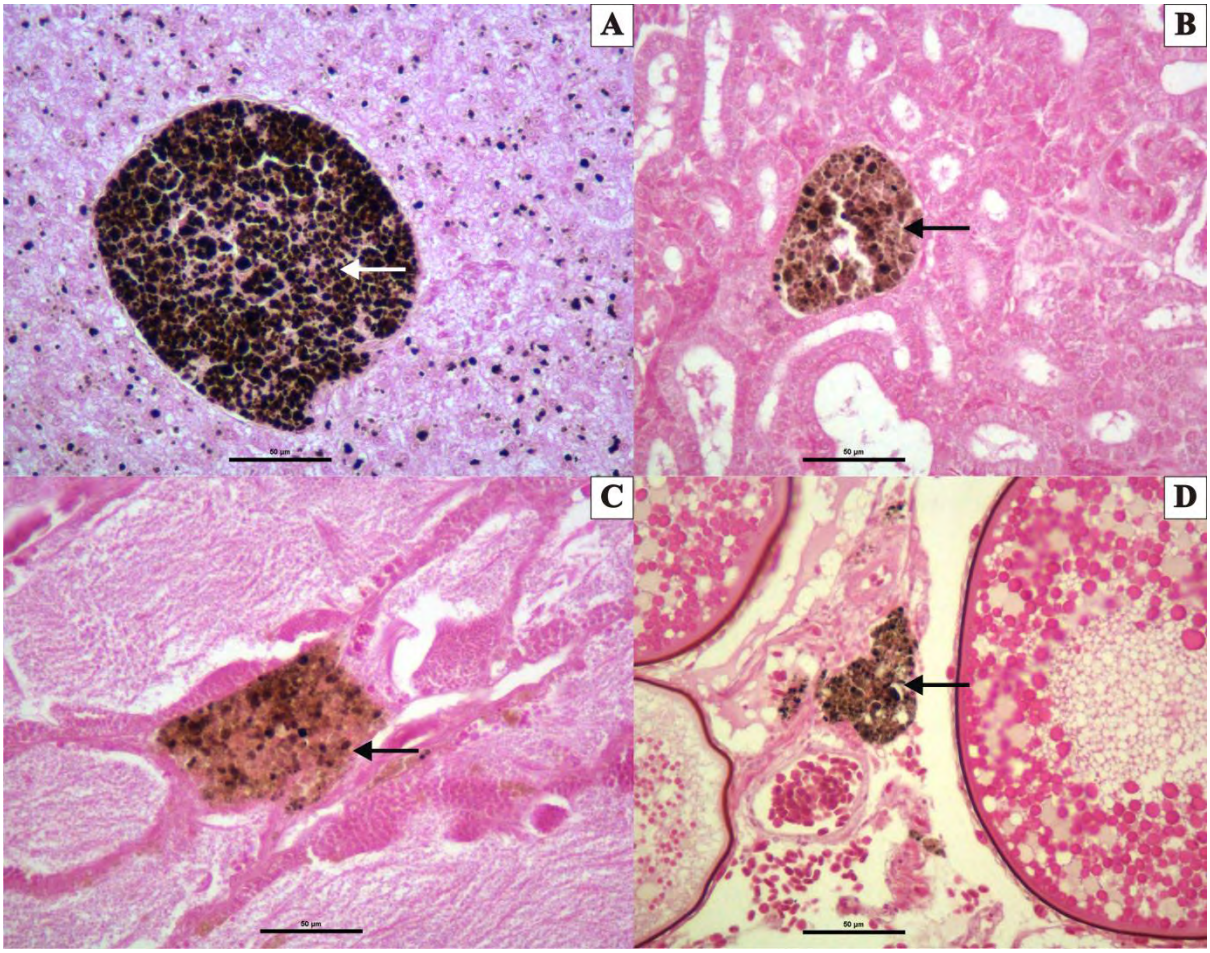


**Figure 5.9:** Micrographs of various mullet tissue sections stained with Masson-Fontana stain. (A) Liver tissue with an increase in MMCs (white arrow) as well as intracellular deposits; (B) kidney tissue with an increase in MMC's, (arrow); (C) testes tissue with an increase in MMC's (arrow); (D) ovary tissue with an increase in MMC's (arrow). Scale bars = 50 µm (A, B, C, D).

## 5.4. Discussion

The two mullet species are usually considered to be at the base of the food pyramid (Whitfield *et al*. 2012) and are therefore an important protein source for many predators. Predatory species are often used as indicator organisms because their position integrates the effects of environmental pollution acting on the system. However, because of their abundance as well as their importance as a food source, the health of these two mullet species is important. Fish can be used successfully as sentinel species of aquatic pollution. Biometric indices such as CF, HSI and SSI have previously been shown to be excellent tools in evaluating the effects of environmental pollution in aquatic organisms.

### 5.4.1. Necropsy, blood parameters and biometric indices

The macroscopic FHAI was higher for the *M. capensis* samples over the *M. cephalus* samples, although not significantly different. The main contributors to the higher *M. capensis* score were the abnormal total protein and hematocrit values. The high hematocrit percentages noted in both *M. capensis* and *M. cephalus* may have been linked to the abnormalities noted in the kidneys of the mullet samples. Nephrocalcinosis has been linked to an increase in the hematocrit values in humans (Markowitz *et al*. 2004). Chen *et al.* (2003) noted that Nile tilapia infected with nephrocalcinosis has significantly higher total protein levels than healthy Nile tilapia. However, nephrocalcinosis appeared to have no effect on the hematocrit levels (Chen *et al*. 2003). The other abnormalities noted in the necropsy of the two mullet species was fatty and discoloured livers which is an indication of steatosis, which is process of the retention of lipids within a cell and granular and enlarged spleens. The swollen kidneys with the visible white cysts were nephrocalcinosis.

There were no significant differences between *M. capensis* and *M. cephalus* for total mass and fork length, indicating that the two species were approximately the same size. Ellender *et al.* (2012), using sectioned otoliths, aged *M. capensis* from Binfield Park Dam to between 9 – 10 years and *M. cephalus* to between 9 – 11 years . As neither of these two species is capable of breeding in fresh water (Bok 1979), the

mullet species collected in this study will be of similar age to those of Ellender *et al*. (2012).

There was no significant difference between the CF for the *M. capensis* and *M. cephalus*. However, when compared to the CF of *M. cephalus* elsewhere, CF was generally higher. For example, the CF for the two mullet species collected in this study was higher than that for *M. cephalus* collected above (0.76) and below (0.96) a waste water treatment plant in Portugal (Pinto *et al*. 2010). The CF for the two mullet species collect in this study were also higher than the CF of *M. cephalus* collected by Ben Ameur *et al*. (2012) from the polluted Bizerte Lagoon in Tunisia (0.94) and the Mediterranean Sea (1.19). *Mugil cephalus* from Bizerte Lagoon showed levels of oxidative stress and as well as DNA damage, indicating severe environmental pollution (Ben Ameur *et al*. 2012).

Fish from polluted sites generally have an increased HSI value (Ben Ameur 2012). The HSI values for *M. capensis* and *M. cephalus* were both between 1 and 2% which is, according to Munshi and Dutta (1996), within the normal range. *Mugil cephalus* collected in a slightly less polluted upstream site had an HSI of 1.34% compared to those from the more polluted downstream site of 1.77% (Pinto *et al*. 2010). The HSI for *M. capensis* (1.50%) and *M. cephalus* (1.30%) from this study were closer to that of the less impacted upstream site of Pinto *et al.* (2010). The HSI values were lower than those of the *M. cephalus* HSI samples collected from the Bizerte Lagoon (2.90), and very similar to those of the Mediterranean Sea (1.50) samples (Ben Ameur 2012).

The GSI for the female *M. capensis* and *M. cephalus* samples showed that there were no significant differences, and both species matured to Stage 2 in the gonad development. Neither of these two species is capable of breeding in fresh water (Bok 1979), and they are capable of breeding only at sea or at the mouths of estuaries (Skelton 2001). Ferreira *et al*. (2004) found similar GSI values in *M. cephalus* to the GSI values presented in this study. Ferreira *et al*. (2004) noted that none of the *M. cephalus* samples were mature when compared using gonad histology. According to Skelton (2001), female *M. cephalus* remain in fresh water for up to seven years before returning to the marine environment to mature and spawn. However, according to Whitfield *et al*. (2012), male GSI values can peak at a value of 19 at the

height of the spawning season, while female GSI values are between 21 and 40. However, because *M. capensis* and *M. cephalus* can spawn at sea, the GSI values of these two mullet populations have no relevance, as they cannot return to the sea to spawn.

### 5.4.2. Histology assessments

The gill alterations that were observed in the two mullet species, such as branching and congestion of the secondary gill lamellae, hyperplasia, telangiectasia and rupture of the pillar cells, were similar to the gill alterations noted by van Dyk *et al*. (2009) in the gills of *Clarias gariepinus* from impoundments that receive effluent from sewage treatment plants as well as waste water from informal settlements. The histological responses noted in *M. capensis* and *M. cephalus* were mostly circulatory disturbances and regressive changes. Circulatory disturbances are pathological conditions that are related to the flow of blood. Telangiectasia is associated with the disintegration of the pillar cells (Takashima & Hibiya 1995) and is often caused by an irritant causing aneurisms in the secondary gill lamellae (Mallatt 1985).

Apart from the increase in the MMC's in the liver tissue, the vacuolation of the hepatocytes was more prevalent compared to any other type of alterations identified. Hepatocyte vacuolation was found in 100% of both *M. capensis* and *M. cephalus*. Hepatocyte vacuolation is characteristic of steatosis, which is the fatty degeneration of the liver observed in the macroscopic fish health assessment. Vacuolation of the hepatocytes is often associated with the inhibition of protein synthesis, energy depletion, disaggregation of microtubules and shifts in the use of substrates (Hinton & Laurén 1990).

The histological analysis of the kidney samples from the two mullet species revealed that both the species had nephrocalcinosis (McHugh *et al*. 2013). The presence of the nephrocalcinosis was confirmed using the Von Kossa silver nitrate stain, which stained indicated a black staining of calcium deposits (**Figure 5.5F**). Nephrocalcinosis is a non-infectious disease that is characterized by abnormal calcium deposition in the kidneys of humans and some fish species, including trout and salmon. Nephrocalcinosis maybe as a result of sulphamerazine toxicity (Smith *et al.* 1973), diet (Cowey *et al.* 1977) and/or exposure to environmental-free $CO_2$

(Smart *et al*. 1979). The other alternations noted in the kidney tissue, including dilation of the renal lumen, distortion and increase in size of the glomerular tufts, increase in MMCs and the presence of eoisinophillic granular cells are some histological alterations that are associated with nephrocalcinosis (Besse *et al*. 1968; Landolt 1975; Harrison & Richards 1979). The dilation of the renal tubule may be as a result of calcium deposit obstruction or as a result of inflammation.

The increase in melano-macrophage centres noted in the liver, kidney, testis and ovaries is a distinctive grouping of pigment-containing cells (Agius & Roberts 2003). According to Agius & Roberts (2003) is it the distinctive pigments that set them apart from aggregation of all other cells. Melano-macrophage centres are usually found close to vascular channels (as can be seen in **Figure 5.4A and 5.6B**, close to blood vessels), the MMCs are usually closely-packed to form large aggregates and are enlarged after phagocytosis of non uniform materials such as cell debris, melanin pigments, haemosiderin granules, lipofuscin residues, lipid droplets, basic protein aggregates and neutral mucoplysaccharides (Agius & Roberts 2003). The morphological appearance of the MMCs may vary between different species, or in the same species because of age, starvation, tissue breakdown, iron and haemoglobin metabolism, and pathological and inflammatory conditions (Agius & Roberts 2003). It is possible for macrophages of the melano-macrophage centres to carry different types of pigments, and frequently different types of pigments within the same cell (Agius & Roberts 2003).

According to the classification system of Zimmerli *et al*. (2007), the majority of gill, testis and ovary samples were in Class 1, with circulatory disturbances and regressive change being observed. The majority of the liver and kidney samples where in Class 2 and Class 3. The histological alterations noted were circulatory disturbances, regressive changes and progressive changes. The histological alterations identified as regressive changes are the functional loss or reduction of an organ because of processes which terminate its function (Bernet *et al*. 1999). The majority of the regressive changes indentified were structural changes, these changes in the in the tissue structure as well in shape and alignment of cells (Bernet *et al.* 1999). Plasma alterations and nuclear alterations were also identified in the liver samples. Plasma alterations are changes in cellular plasma caused by granular degeneration and vacuolation. Nuclear alterations are changes in the nuclear shape

and structure of chromatin (Bernet *et al*. 1999). Progressive changes were noted in the gills. Progressive change is the process of increased activity of cells or tissue (Bernet *et al*. 1999). The gill alteration was hyperplasia of the secondary gill epithelium, which is the enlargement of the tissue by a greater number of cells without a change in the volume of the cells (Bernet *et al*. 1999). In the kidney tissue inflammatory changes were noted. These are processes that are related to other reaction patterns. The alteration noted the kidney tissue was infiltration by eosinophilic granular cells, which penetrate the cell walls and infiltrate the surrounding tissue (Bernet *et al*. 1999)

The special staining techniques applied showed a positive stain for hemosiderin, lipofuscin and melanin. Hemosiderin (**Figure 5.7**) is a brown, granular, relatively insoluble pigment containing protein and iron component (Agius & Roberts 2003). According to Agius (1981), haemosiderin has been known to increase within the MMCs during prolonged starvation periods. A large accumulation of haemosiderin is often associated with the spleen and is known as haemosiderosis (Agius & Roberts 2003). This may have been the cause of the granular spleens noted in the macroscopic fish health assessment. Lipofuscin (**Figure 5.8**), according to Agius & Roberts (2003), results from the oxidative polymerization of polyunsaturated fatty acids. According to Pickford (1953) these pigments are as result of a fish species' lack of nutrition. An increase in the deposition of lipofuscin has been observed in multiple animal species, including humans during severe weight loss or wasting syndrome (Agius & Roberts 2003). According to Agius (1981), lipofuscin is often referred to as the 'wear and tear' pigment and is often associated with age and tissue destruction. It is the most widespread pigment in the MMCs of many fish species (Agius 1979). Melanin (**Figure 5.9**) was shown to be positive in the identified MMCs. Melanin is produced by melanocytes derived embryologically from the neutral crest and contained within spherical to oval melanosomes (Agius & Roberts 2003). According to Zuasti *et al.* (1989), melanins are complex polymers that can absorb and neutralize free radicals, cations and other potentially toxic agents that are caused by the degradation of phagocytosed cellular material. Agius & Agbede (1984) proposed the hypothesis that melanins play a role in neutralizing the free radicals realeased as a result of the catabolism of fatty acids derived from phagocytosis of cellular membranes at low temperatures. Another hypothesis by

Wolke *et al.* (1985) suggests that melanin may be important in the production of bacterialcidal compounds.

The main role of MMCs is to serve as metabolic dumps for the relocation of debris or damaged cells, including red blood cells (Agius & Roberts 2003). They play an important role in the response of fish to foreign materials, including infectious agents (Agius & Roberts 2003). These MMCs have been known to increase in size and number as fish grow older and tissues degenerate (Agius & Roberts 2003). Melano-macrophage centres have been used as indicators of environmental stress (Schwindt *et al.* 2006) and have a negative correlation with condition factor (Agius 1981).

The majority of the histological alterations observed in the *M. capensis* and *M. cephalus* have previously been linked to exposure to heavy metals (van Dyk *et al*. 2007), sewage from waste water treatment plants (van Dyk *et al*. 2009), and pesticides (McHugh *et al.* 2011). However, the histological alterations found here are not stressor-specific, and therefore comprehensive chemical analysis of the water, sediment and muscle tissue samples is recommended for future studies.

### 5.4.3. Parasitological assessments

No parasites were recorded on *M. capensis* or *M. cephalus* from Binfield Park Dam. The two mullet species were originally collected from the Keiskamma River estuary and, according to Whitfield (2012), wild *M. cephalus* are hosts to a wide variety of parasites. In South Africa, parasitic copepods have been described from the gills of *M. cephalus* (Oldewage & van As 1988; Baker *et al.* 2005), *Trypanorhynch plerocercoid* infestation in the muscle tissue (Schramm 1991) and haemoprotozoan infections in the blood of *M. cephalus* (Smit *et al*. 2002). According to Whitfield (2012), there is generally an increase in the number of parasite species with an increase in the age of the mullet hosts. The total absence of parasites on the two mullet species in Binfield Park Dam may have been either as a result of the lack of intermediate hosts or of their being trapped in a freshwater habitat which is not suitable for the parasites.

## 5.5. Conclusion

Although the external appearance of the *M. capensis* and *M. cephalus* would give the impression that these two fish species are in a healthy condition, closer inspection reveals this to be untrue. Although the exact cause of the alterations noted in this study cannot be determined without further research, one possible suggestion is that, although these two fish species are capable of surviving in freshwater, their health might be compromised in the long term. Another possible hypothesis is that the histological alterations observed may be age-related. According to Whitfield *et al.* (2012) and Ellender *et al*. (2012), the ages of the two mullet species from Binfield Park Dam are at the upper limits of the expected life spans of these species. The histological alterations observed in the two mullet species may be as a result of the advanced age of the two mullet species.

Although histological alterations are not stressor-specific, the histological alterations observed in *M. capensis* and *M. cephalus* from Binfield Park Dam did not provide the required reference data for further monitoring of aquatic environments using these two species. However the data provided in this study do provide important comparative data for future histological studies of these two species. This study achieved the aim of recording the histopathology of nephrocalcinosis in two mullet species and is the first report of this condition in these fish in South Africa. The research highlighted the need for regular monitoring of such fish stock populations and indicated that histopathology is an important tool in the early detection of nephrocalcinosis.

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# Chapter 6 : Health status of Labeo umbratus from Sandile Dam, Eastern Cape, with a first report of Lernaea barnimiana from this region.

## 6.1. Introduction

The moggel, *Labeo umbratus* (Smith, 1841), has shown potential as a fishery species in small impoundments and reservoirs in South Africa (Potts *et al*. 2006; Crafford *et al.* 2014). This is largely due to its occurring in large numbers in many freshwater impoundments (Gaigher 1984), as well as having a wide distribution (Skelton 2001). *Labeo umbratus* has been recognised as commercially important in Wuras Dam (Pieterse and Keulder 1982), Kalkfontein Reservoir (Merron and Tømasson 1984) and Bloemhof Dam (Potts *et al*. 2006) in the Free State Province; and Darlington Dam (formerly Lake Mentz), Dimbaza Reservoir, Sinqemeni Reservoir, Laing and Ndlambe Reservoirs in the Eastern Cape (Potts *et al.* 2006). As a result, *L. umbratus* has become the focal point of rural fishing projects in the Eastern Cape (Andrew *et al*. 2000).

Therefore, all the previous research into *L. umbratus* in the Eastern Cape has focused on its potential as an aquaculture species. However, previous research into the parasite diversity of *L. umbratus* from other localities has shown that they are hosts to various parasite species such as *Lernaea barnimiana*, *Lernaea* sp. (the latter only identified to genus and not to species level) and *Argulus japonicas* (van As & Basson 1984). At least one of these parasites, *A. japonicas*, is a known alien invasive parasite in South Africa (Fryer 1968, de Moor & Bruton 1988). More recent research (Dos Santos *et al*. 2013) reported the monogenean *Paradiplozoon vaalense* on the gills, and Crafford *et al*. (2012) found *Dactylogyrus larindae* on the skin of *L. umbratus* from the Vaal River system, South Africa. These recent discoveries of new parasite species indicate that there is a paucity of information regarding the parasite diversity of *L. umbratus* in general, and particularly in poorly-researched areas such as the Eastern Cape.

Research into freshwater parasites in the Eastern Cape Province has largely been limited to eel parasites such as *Anguillicola papernai* (Moravec *et al*. 2000; Taraschewski *et al*. 2005), *Heliconema africanum* (Moravec *et al*. 2013), *Paraquimperia africana* (Moravec *et al*. 2000) and the alien gill monogenean *Pseudodactylogyrus anguillae* (Christison & Baker 2007; Parker *et al.* 2011).

Although other parasites do occur within these Eastern Cape systems, the information on parasite diversity is limited, thus making their status as an indigenous, alien or translocated species difficult to determine. Among these species is *Lernaea barnimiana* Hartman 1865. *Lernaea barnimiana* is considered to be a pan African species indigenous to the African continent (Fryer 1968). Fryer (1968) recorded *L. barnimiana* in Africa from the Nile, Niger and Congo Rivers, the Awash River system, Ethiopia and eastern rivers (the latter referring to rivers north of the Limpopo River, South Africa, that flowed east and discharged into the Indian Ocean). In South Africa *L. barnimiana* has been recorded from Glen Alpine Dam, Boskop Dam, Wuras Dam, Smith's Drift, the Sabie River, as well as Chubu in the Eastern Cape (van As & Basson 1984). It has been recorded from both cyprinid and cichlid fish hosts from different water bodies in Africa (Fryer 1968; Thurston 1969). In South Africa, *L. barnimiana* has been recorded from the cichlid *Oreochromis mossambicus* and cyprinids including *Labeo capensis*, *L. rosae*, *L. rubromaculatus*, *L. umbratus*, as well as from *Labeobarbus aeneus* and *L. marequensis* (van As & Basson 1984).

The aim of this chapter was to determine health status of *L. umbratus* in Sandile Dam, Eastern Cape, and to determine the effects of *L. barnimiana* on *L. umbratus*. The objective was to determine the health status of *L. umbratus* using a macroscopic fish health assessment protocol and a histology-based fish health assessment index.

### 6.2. Materials and methods

The study took place at Sandile Dam (see **Chapter 2, Section 2.1**) (S32° 42' 23.57"; E27° 06' 34.54"), a 146ha impoundment, using subsistence anglers. A detailed map of the impoundment has been provided (**Figure 6.1**). The materials and methods used in this chapter are discussed in **Chapter 2**. For sampling, see **Section 2.2**. The blood parameters, necropsy, and biometric indices are discussed in **Chapter 2, Section 2.2.1**. The tissue samples were prepared (see **Chapter 2, Section 2.2.2**) for histological analysis (see **Chapter 2, Section 2.2.2**). Statistical analyses were perfomed for comparison, using methods described in **Chapter 2, Section 2.2.5**. *Labeo umbratus* were collected in July 2011 (n = 15) and March 2012 (n = 15) by means of gill nets (see **Chapter 2, Section 2.2**). The techniques used will not be repeated here.

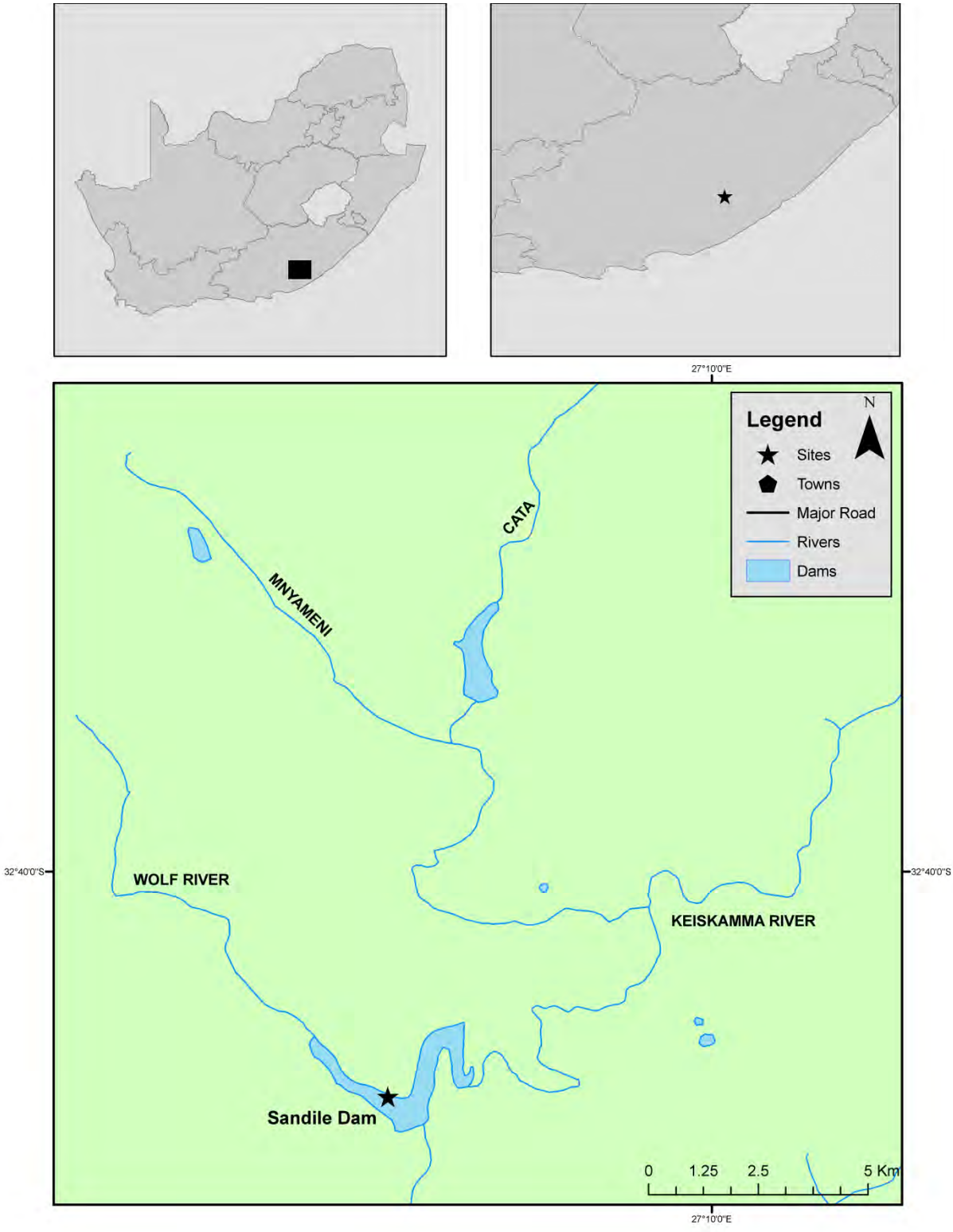


**Figure 6.1:** Map of Sandile Dam, Amatola region, Eastern Cape Province, South Africa.

## 6.3. Results

### 6.3.1. Necropsy, blood parameters and biometric indices

The biometric indices are presented in **Table 6.1**. There was no significant difference ($p \geq 0.05$; $df1 = 3$) between the masses of the July 2011 (344 ± 76 g) and March 2012 (356 ± 89 g) fish. Similarly, there was no significant difference ($p \geq 0.05$; $df1 = 3$) between the fork lengths of the July 2011 (296 ± 21 mm) and March 2012 (308 ± 22 mm) fish. However, the CF of the July 2011 sample (1.32 ± 0.16) was significantly ($p \leq 0.05$; $df1 = 3$) higher than that of the March 2012 (1.20 ± 0.10) sample. There was also a significant difference ($p \leq 0.05$; $df1 = 3$) between the GCF of the July 2011 (1.13 ± 0.13) and the March 2012 (1.01 ± 0.80) samples. For the somatic indices, the HSI of the July 2011 sample (1.35 ± 0.29) was significantly ($p \leq 0.05$; $df = 3$) higher than the HSI of the March 2012 (1.03 ± 0.29) sample. There was no significant ($p \geq 0.05$; $df1 = 3$) difference between the July 2011 (0.46 ± 0.03) and March 2012 (0.39 ± 0.03) samples for SSI. There was no significant ($p \geq 0.05$; $df1 = 3$) difference between the GSI values for the July 2011 males (0.62 ± 0.20) and the March 2012 males (0.80 ± 0.48). The GSI values for the July 2011 females (1.37 ± 0.46) and March 2012 (1.47 ± 0.45) females also showed no significant differences ($p \geq 0.05$; $df1 = 3$). The FHAI revealed that there was a significant ($p \leq 0.05$; $df1 = 3$) difference between the July 2011 (4.00 ± 7.38) and the March 2012 (16.67 ± 12.34) samples.

Macroscopic examination of *L. umbratus* during the July 2011 survey revealed that there were no external or internal abnormalities on any of the fish, though there were signs of previous external injuries or wounds that had healed. However, the external macroscopic examination of the March 2012 samples showed skin lesions (7%) and red, inflamed and frayed tail fins (7%). The internal examination showed pale gills (7%), and 60% of the fish had fatty and discoloured livers.

The mean total plasma protein for the July 2011 (52.02 ± 1.52 mg $dl^{-1}$) sample was significantly ($p \leq 0.05$; $df1 = 3$) higher than the mean total plasma protein levels of the March 2012 (46.84 ± 6.77 mg $dl^{-1}$) sample. The July 2011 and the March 2012 survey results all fell within the normal range (30–69 mg $dl^{-1}$) of Adams *et al*. (1993). There was no significant difference ($p \geq 0.05$; $df1 = 3$) between the mean hematocrit

for the July 2011 (41.96 ± 20.37%) and the March 2012 (33.47 ± 8.39%) samples. The July 2011 survey had 14% of its fish below (19–29%), the normal range (30–45%) of Adams *et al*. (1993). The March 2012 survey had 34% of its fish below the normal range, and 7% far below (< 18%) the normal range. For both the July 2011 and March 2012 surveys, the leukocrit was below 4% which, according to Adams *et al*. (1993), is within the normal range.

**Table 6.1:** Biometric indices for *Labeo umbratus*: mean total body mass (g), fork length (mm), condition factor (CF), gutted condition factor (GCF), hepatosomatic index (HSI), spleenosomatic index (SSI), gonadosomatic index (GSI) and fish health assessment index (FHAI). Sample size was 15 fish for each survey.

| *Labeo umbratus* | | July 2011 (n = 15) | March 2012 (n = 15) |
|---|---|---|---|
| Mass | | 344.0 ± 75.67 | 356.0 ± 89.18 |
| Length | | 295.8 ± 21.36 | 307.8 ± 22.50 |
| CF | | 1.32 ± 0.16 | 1.20 ± 0.10 |
| GCF | | 1.13 ± 0.13 | 1.01 ± 0.80 |
| HSI | | 1.35 ± 0.29 | 1.03 ± 0.29 |
| SSI | | 0.46 ± 0.03 | 0.39 ± 0.03 |
| GSI | Male | 0.62 ± 0.20 | 0.80 ± 0.48 |
| | Female | 1.37 ± 0.46 | 1.47 ± 0.45 |
| FHAI | | 4.00 ± 7.36 | 16.67 ± 12.34 |

### 6.3.2. Parasitological assessment

The prevalence and abundance of the different parasite species collected from *L. umbratus* during the July 2011 and March 2012 surveys are shown in Table 2. No external macro-parasites were observed on *L. umbratus* during the July 2011 survey. However, an unidentified gill monogenean (**Figure 6.4D**) was noted infecting the gills on 40% of the fish, and six percent of the kidney samples had a parasitic cyst (**Figure 6.4D**) encapsulated in the kidney tissue. In the March 2012 survey, 20% of the fish had *L. barnimiana* (**Figure 6.3A** & **B**) attached to their skin. Forty percent of the fish also had an unidentified gill monogenean. Parasitic cysts were also found in 26% of the kidney samples.

The presence of *L. barnimiana* had no effect on the biometric indices of *Labeo umbratus*. There was no significant difference between the condition factor for the samples infected with *L. barnimiana* and uninfected samples for the July 2011 ($p \geq 0.05$; $df1 = 3$) and the March 2012 ($p \geq 0.05$; $df1 = 3$) surveys. *Lernaea barnimiana* presence also had no effect on gutted condition factor, as there was also no significant difference between the July 2011 ($p \geq 0.05$; $df1 = 3$) and March 2012 ($p \geq 0.05$; $df1 = 3$) surveys. There was no effect on the blood parameters by the presence of *L. barnimiana*. There was no significant difference ($p \geq 0.05$; $df1 = 3$) in the total protein values between uninfected July 2011 samples and the March 2012 infected with *L. barnimiana*. However, the total protein levels were lower than the average for samples that had *L. barnimiana,* although they were not below the normal range. There was also no significant difference in the hematocrit values for the July 2011 ($p \geq 0.05$; $df1 = 3$) and March 2012 ($p \geq 0.05$; $df1 = 3$) values for samples infected with *L. barnimiana* and uninfected samples. Although two of the *L. barnimiana* infected samples fell within the normal range for hematocrit values, one of the samples was below the normal range. The presence of *L. barnimiana* also had no significant effect on the FHAI for the July 2011 ($p \geq 0.05$; $df1 = 3$) and March 2012 ($p \geq 0.05$; $df1 = 3$) values for infected and uninfected fish.

**Table 6.2:** Prevalence and abundance of parasite species collected from *Labeo umbratus* during the July 2011 and March 2012 surveys. Numbers of individual fish infected and intensity ranges are in parentheses.

| *Labeo umbratus* | July 2011 | | March 2012 | |
|---|---|---|---|---|
| | Prevalence | Intensity of Infection | Prevalence | Intensity of Infection |
| *Lernaea barnimiana* | - | - | 20 (3/15) | 1 (1) |
| Gill monogenean | 40 (6/15) | 1.16 (1–2) | 40 (6/15) | 2.16 (1–6) |
| Kidney cycts | 6 (1/15) | 1 (1) | 26 (4/15) | 1 (1) |

### 6.3.3. Histological assessment

The percentage prevalence of the histological alterations that were identified, as well as the histological index results, are presented in **Tables 6.3** and **6.4,** respectively.

The main histological alterations identified in the gills were fusion of secondary gill lamellae (**Figure 6.4A**), hyperplasia (**Figure 6.4B**), telangiectasia and rupture of pillar cells (**Figure 6.4C**). The alterations identified were more pronounced during the March 2012 survey. The majority of the alterations indentified were circulatory disturbances with regressive and progressive changes. The mean gill index ($I_G$) for the July 2011 survey was 2.53 ± 3.50, which was lower than that in March 2012 (4.40 ± 4.91). However, there was no significant difference ($p \geq 0.05$; $df1 = 3$) between the two surveys. For both the July 2011 and March 2012 surveys, 6.7% of the gill samples fell within Class 2, while the remainder (93.3%) fell within Class 1.

In terms of histological alterations in the liver samples, fish from both the July 2011 and March 2012 surveys had the same histological alterations. However, the percentage prevalence of the histological alterations was higher in fish from the July 2011 survey. The liver alterations identified were hepatocyte vacuolation (**Figure 6.5A**), an increase in melano-macrophage centres (MMC) (**Figure 6.5B**) and intra-

cellular deposits in the interstitial tissue (**Figure 6.5A**). All of the alterations identified in the liver samples from the two surveys were regressive changes. The mean liver index ($I_L$) was slightly higher in the July 2011 sample (5.06 ± 2.12) than in the March 2012 sample (4.26 ± 1.66). However, there was no significant ($p \geq 0.05$; $df1 = 3$) difference between the $I_L$ samples between the two sampling seasons. All of the July 2011 and the March 2012 liver samples fell within Class 1.

The main histological alterations indentified in the kidney samples were hyaline droplet degeneration (**Figure 6.6A**), an increase in MMCs (**Figure 6.6B**) and presence of intracellular deposits (**Figure 6.6C**). The histological alterations were all regressive changes. The mean kidney index ($I_k$) was higher in the March 2012 sample (3.73 ± 1.27) than in the July 2011 sample (2.80 ± 1.47). However, there was no significant ($p \geq 0.05$; $df1 = 3$) difference between the two surveys. All the kidney samples from both surveys fell within Class 1.

Histological assessment confirmed that the $I_{FISH}$ was higher for the March 2012 (12.40 ± 5.08) sample than for the July 2011 sample (10.40 ± 4.79). However, there was no significant difference ($p \geq 0.05$; $df1 = 3$) between the two surveys. The histological results were similar to the FHAI results, indicating that the samples collected during the March 2012 survey were in a poorer health status than the July 2011 fish samples. This can also be seen by the July 2011 survey fish condition factor being significantly higher than that in March 2012.

### 6.3.4 Gonad development

The developmental stages of the testes and ovaries were determined according to the criteria defined in the Biomonitoring of Environmental Status and Trends Program (BEST) (Schmitt & Dethloff 2000). The sex ratio of the July 2011 *L. umbratus* was 60% males and 40% females, and for the March 2012 samples the ratio was again 60% male and 40% females. This gave a male to female ratio of 1:0.6.

The developmental stages of the July 2011 males were 100% in Stage 1 (**Figure 6.7A**) and the females were also 100% in Stage 1 (**Figure 6.7D**), both indicating early development. The developmental stages of the March 2012 males were Stage

1: 67% (**Figure 6.7A**), Stage 2: 22% (**Figure 6.7B**) and Stage 3: 11% (**Figure 6.7C**), while the females were all (100%) in Stage 1 of development (**Figure 6.7D**).

**Table 6.3:** Percentage prevalence of histological alterations identified in *Labeo umbratus* from Sandile Dam in the July 2011 and March 2012 surveys.

| | Percentage prevalence | |
|---|---|---|
| Target Organ/ histological alteration | July 2011 n = 15 | March 2012 n = 15 |
| **Liver** | | |
| Hepatocyte vacuolation | 86 | 60 |
| Increase in melano-macrophage centres | 86 | 93 |
| Intercellular deposits | 46 | 13 |
| **Kidney** | | |
| Distortion of glomerular tuft | - | 6 |
| Hyaline Droplet degeneration | 6 | 73 |
| Increase in Melano macrophage centres | 93 | 86 |
| Intercellular deposits | 26 | 13 |
| **Gills** | | |
| Branching of secondary lamellae | - | 6 |
| Fusion of secondary lamellae | 13 | 33 |
| Hyperplasia | 27 | 46 |
| Rupture of Pillar cells | 13 | 20 |
| Telangiectasia | 6 | 20 |

Dashes denote that specific alteration was not detected

**Table 6.4:** Mean organ index and fish value index for *Labeo umbratus*. $I_L$ = Liver Index, $I_K$ = Kidney Index, $I_G$ = Gill Index, $I_T$ = Testis Index, $I_O$ = Ovary Index and $I_{FISH}$ = Fish Index. Ranges are indicated in parentheses.

| Survey | $I_L$ | $I_K$ | $I_G$ | $I_T$ | $I_O$ | $I_{FISH}$ |
|---|---|---|---|---|---|---|
| July 2011 (n = 15) | 5.06 (2–8) | 2.80 (2–6) | 2.53 (2–12) | 0.0 | 0.0 | 10.40 (4–20) |
| March 2012 (n = 15) | 4.26 (2–8) | 3.73 (2–6) | 4.40 (2–18) | 0.0 | 0.0 | 12.40 (6–26) |

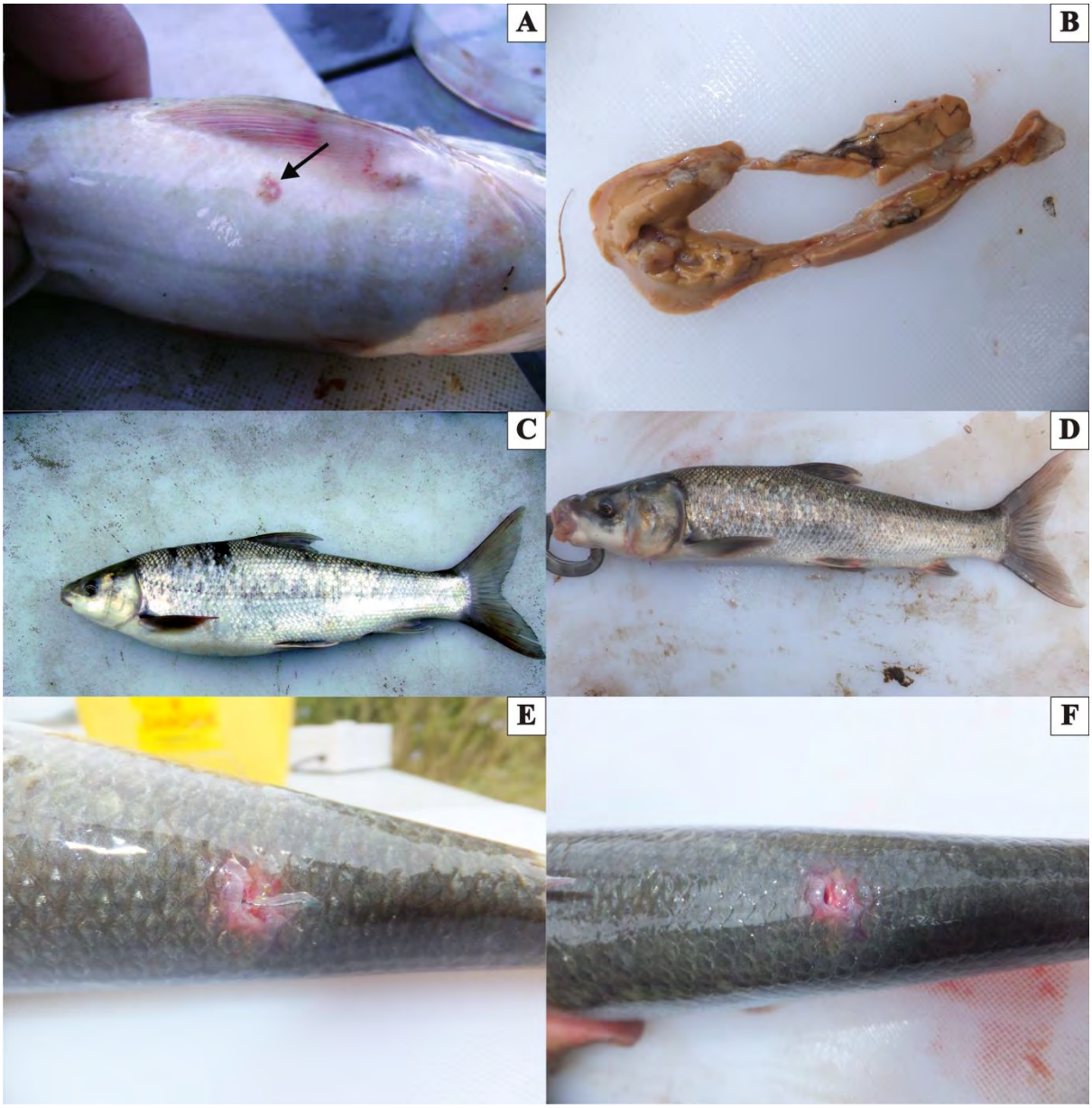


**Figure 6.2:** Macroscopic abnormalities identified in *Labeo umbratus*; (A) healed previous parasite attachment site on skin; (B) discoloured liver; (C) normal healthy condition factor; (D) unhealthy condition factor; (E) attachment site of *Lernaea barnimiana;* (F) damaged caused by attachment of *Lernaea barnimiana.*

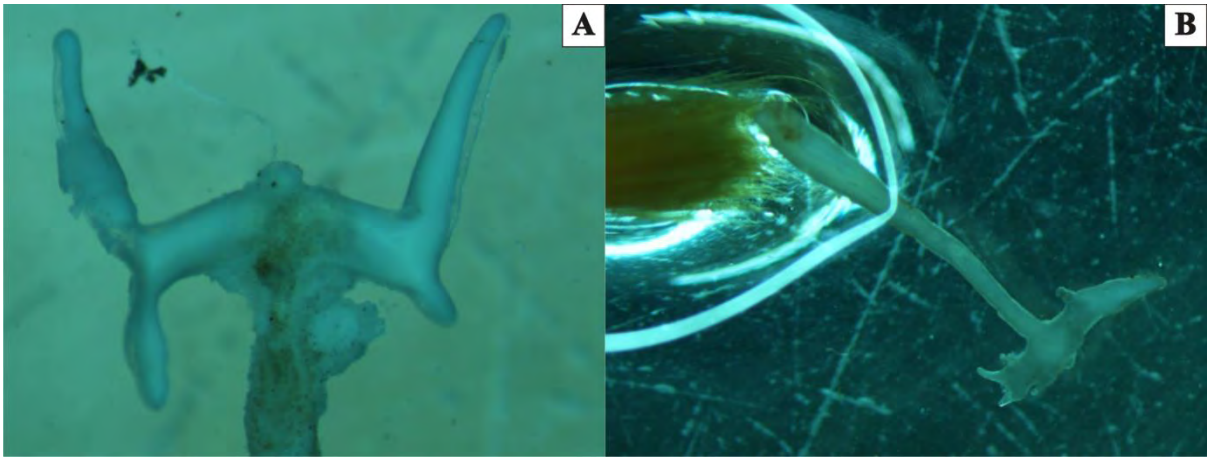


**Figure 6.3:** Micrographs and identification of *Lernaea barnimiana*, courtesy of Prof LL Van As. (A) Head region of *L. barnimiana*; (B) body region of *L. barnimiana*.

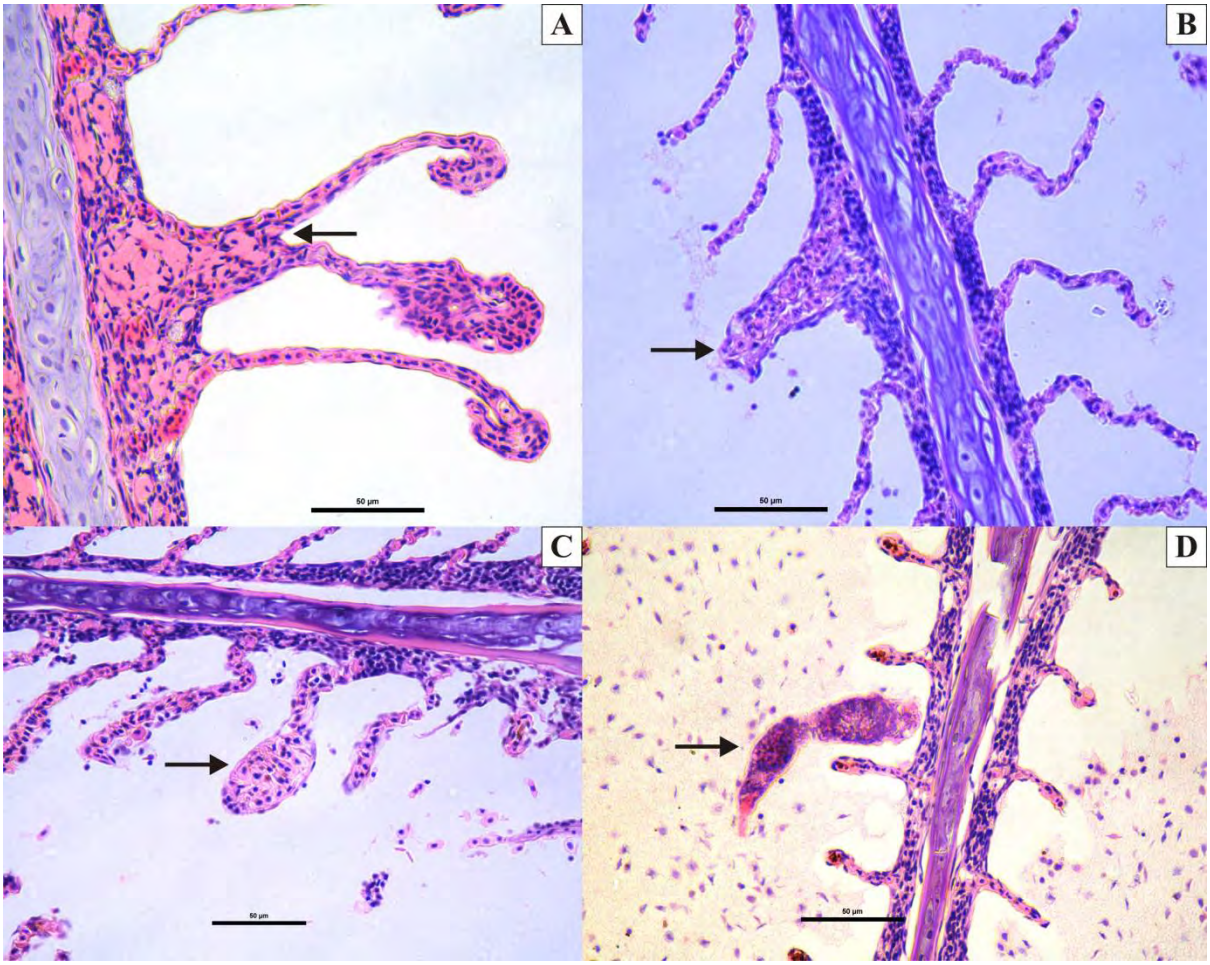


**Figure 6.4:** Micrographs of *Labeo umbratus* gill sections (5 µm) stained with H&E: (A) fusion of secondary gill lamellae (arrow); (B) hyperplasia of the gill epithelium (arrow); (C) telangiectasia and rupture of pillar cells (arrows); (D) parasite in between the secondary lamellae (arrows).

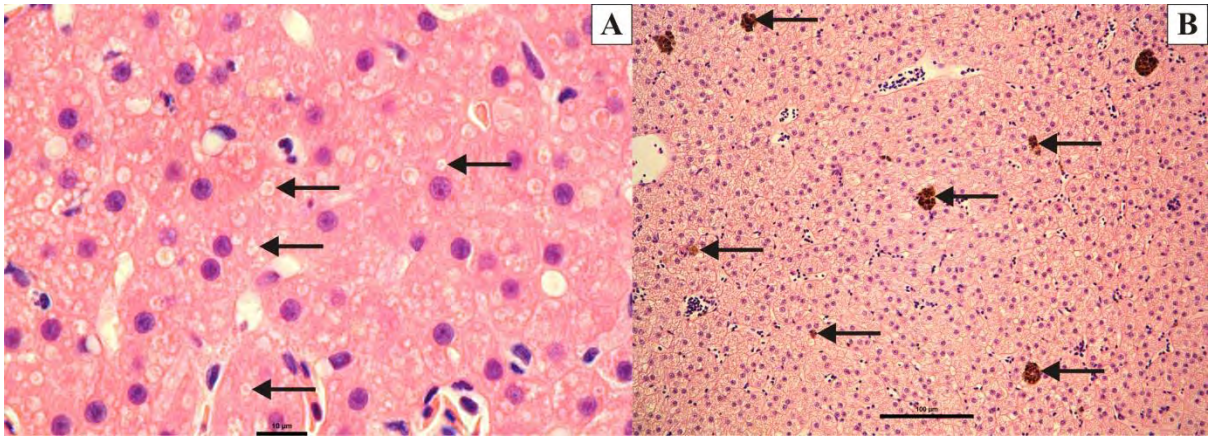


**Figure 6.5:** Micrographs of *Labeo umbratus* liver sections (5 µm) stained with H&E. (A) Vacuolation of the hepatocytes (arrows) with intercellular deposits; (B) increase in melano-macrophage centres (arrows).

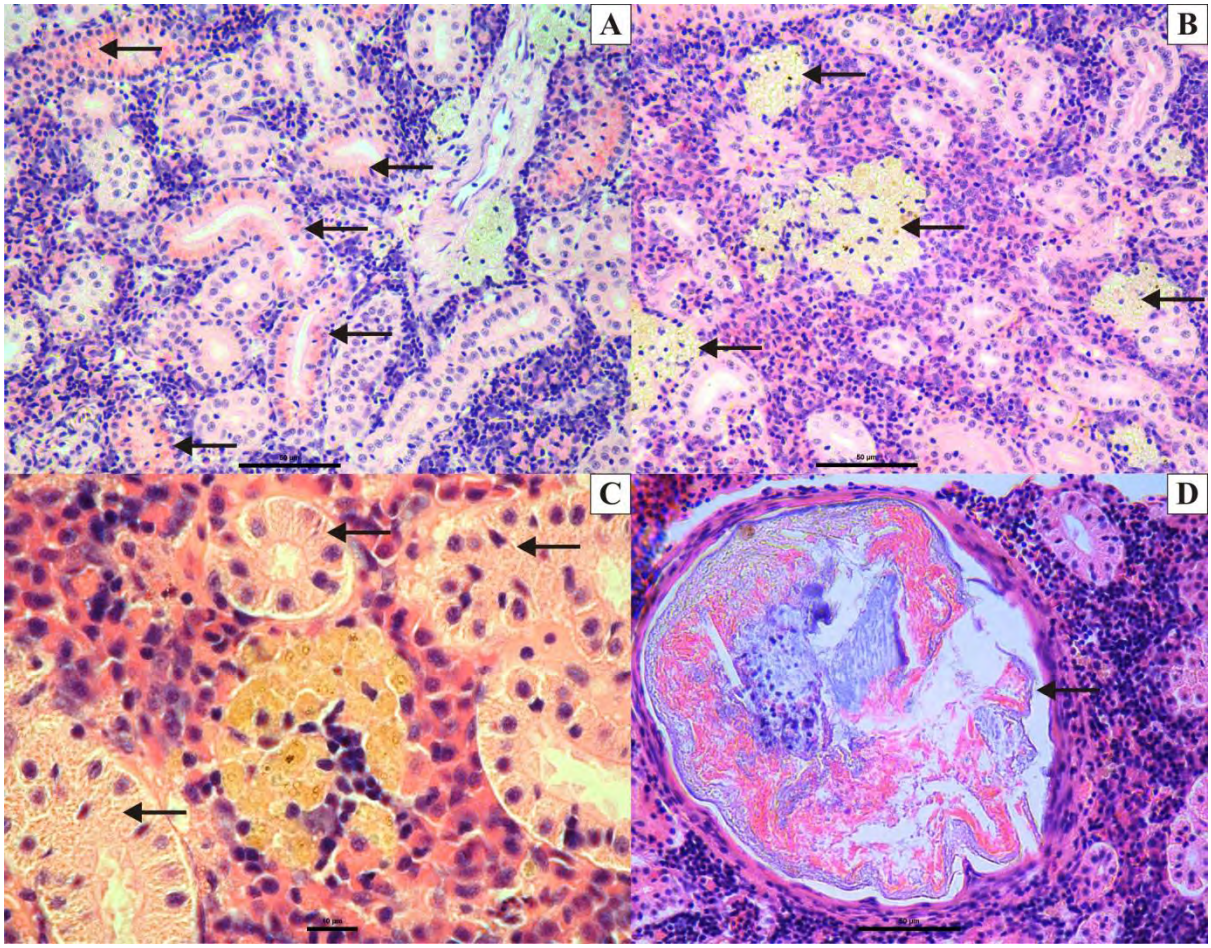


**Figure 6.6:** Micrographs of *Labeo umbratus* kidney sections (5 µm) stained with H&E: (A) Hyaline droplet degeneration (arrows); (B) an increase in melano-macrophage centres (arrows); (C) intercellular deposits in the renal tubule (arrows); (D) parasitic cyst (arrow).

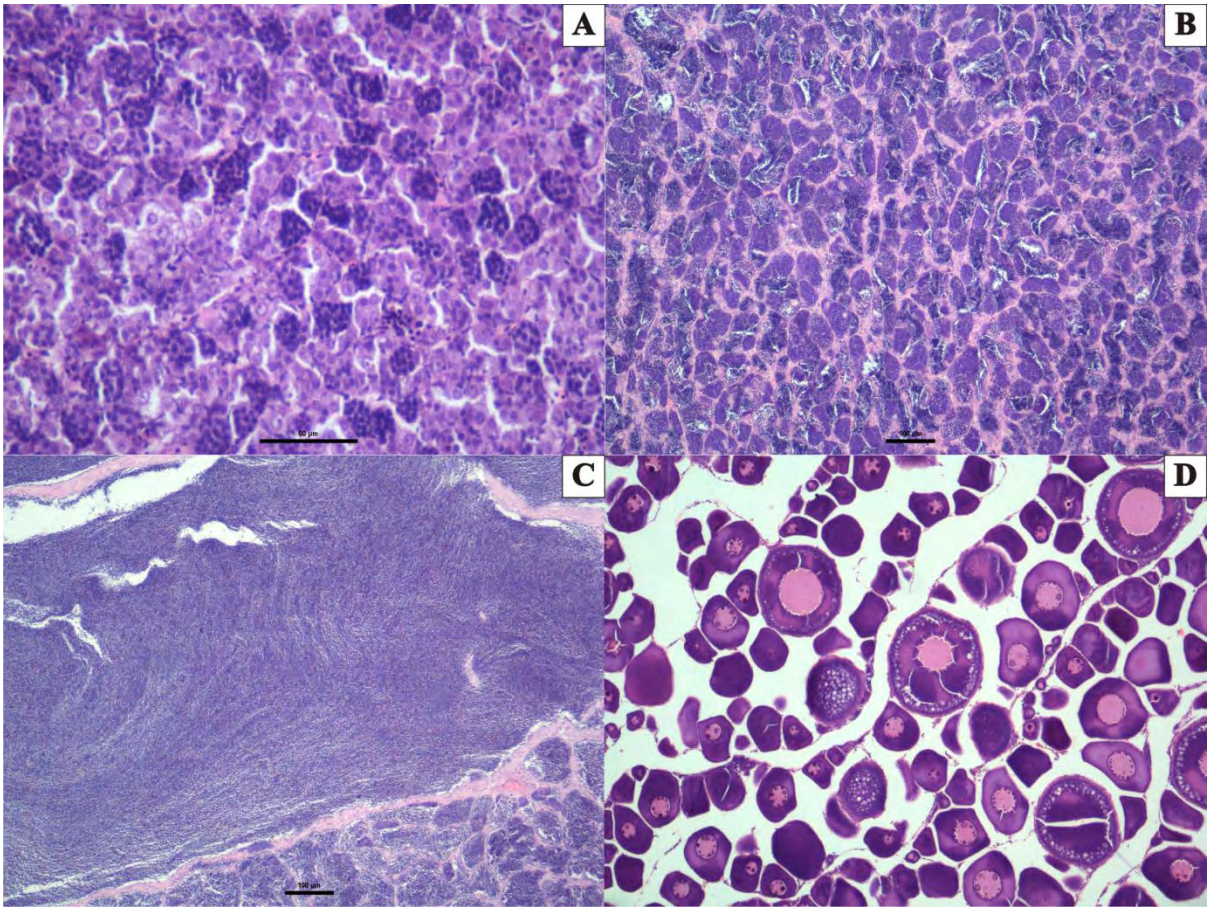


**Figure 6.7:** Micrographs of *Labeo umbratus* gonad sections (5 µm) stained with H&E: (A) mature stage 1 testis; (B) mature stage 2 testis; (C) mature stage 3 testis; (D) mature stage 1 ovaries.

### 6.4. Discussion

The macroscopic necropsy indicated that there were only healed wounds or injuries in the July 2011 sample, while the March 2012 sample had skin lesions, frayed fins and pale gills. The external abnormalities were consistent with those observed for parasite infections from anchor worm *Lernaea* infections (Shariff & Roberts 1989). The pale gills may have been as a result of an increase in the number of monogeneans feeding on the gills. The healed wounds on the samples from the July 2011 survey were possibly caused by *L. barnimiana* that were not able to over-winter and thus detached from the host. Shields & Tidd (1968) showed that *L. cyprinacea* had retarded anchor development, which hampered their ability to attach to the host, causing the parasite to drop off the host with prolonged exposure to cold water. This could also account for the presence of *L. barnimiana* during the March 2012 survey and not being present during the July 2011 survey. The mean total plasma protein for the July 2011 sample was significantly higher than the mean total plasma protein levels of the March 2012 sample. The blood plasma total protein levels are used as a nutritional indicator of the fish and coupled with the condition factor, which was significantly higher in July 2011 survey than in the March 2012 survey. This may indicate a lack of food supplies during the summer period. The hepatosomatic index was also significantly higher during the July 2011 survey than in the March 2012 survey. The HSI is a useful indicator of food intake and energy storage levels (Bulow *et al*. 1978), and indicates that there was a higher food availability during the July 2011 survey than during the March 2012 survey. The significant difference in the FHAI score also indicated that the July 2011 samples were healthier than those collected during the March 2012 survey, with the main contributors to the higher March 2012 FHAI score being poor biometric indices, as well as higher parasite loads and their associated effects.

*Lernaea barnimiana* is a pan-African species indigenous to the African continent (Fryer 1968). In South Africa *L. barnimiana* has been recorded from multiple northern localities, but only from one locality in the Eastern Cape (van As & Basson 1984). It has been recorded from both cyprinid and cichlid fish hosts from different water bodies in Africa (Fryer 1968; Thurston 1969). In South Africa, *L. barnimiana* has been recorded from the cichlid *Oreochromis mossambicus* from Glen Alpine Dam,

Limpopo Province (van As & Basson 1984). However, *O. mossambicus* has been translocated to Sandile Dam as a fodder fish for the resident population of *M. salmoides* (largemouth bass). Weyl *et al*. (2013) demonstrated the risk of introducing alien parasites with the movement of live fish between localities. There is only one previous documented case of *L. barnimiana* from the Eastern Cape (van As & Basson 1984), but no previous parasitological studies for this region, or on the presence of known translocated susceptible hosts. It is difficult to determine whether or not *L. barnimiana* is an indigenous or a translocated parasite. The limited effect of *L. barnimiana* on the host was not surprising, given the low intensity of infection with only one individual parasite infecting each fish. The only effect noted was the skin lesions, which could be potential sites for secondary infections or other pathogens.

Although not as significantly high as the FHAI score, the $I_{FISH}$ score for the March 2012 sample was higher than the July 2011 score. The main contributor to the July 2011 $I_{FISH}$ score was the liver index, (**Table 6.4**) which was higher than the March 2012 liver index score. However, the difference was not significant. The $I_{FISH}$ scores in both July 2011 (10.40) and March 2012 (12.40) were lower than the $I_{FISH}$ scores for *Clarias gariepinus* (22.0) from Pongolapoort Dam (McHugh *et al*. 2013). However, the $I_{FISH}$ scores in this study were higher than the $I_{FISH}$ score of *Hydrocynus vittatus* from two separate surveys also from the Pongolapoort Dam (March = 6.6 and July = 3.0) (McHugh *et al*. 2011). The liver and gill index were the main contributers to the high March 2012 $I_{FISH}$ value.

The main histological alterations noted where hepatocyte vacuolation, increase in melano-macrophage centres and intercellular deposits. The hepatocyte vacuolation accounts for the discolouration of the livers noted in the FHAI and, according to van Dyk *et al*. (2012), are characteristic of steatosis. Van Dyk *et al*. (2012) attributed the cause of hepatocyte vacuolation, increase in melano-macrophage centres and intercellular deposits to pollution of freshwater ecosystems. Awofolu & Fatoki (2003) recorded the persistent organochlorine pesticides Endosulfan and DDT from Sandile Dam. McHugh *et al*. (2011) also noted the liver alterations, hepatocyte vacuolation, increase in melano-macrophage centres and intercellular deposits as well as the kidney alteration hyaline droplet degeneration in *Hydrocynus vittatus* from a DDT-affected area impoundment. All the histological samples were with within Class 1, according to the classification scheme of van Dyk *et al*. (2009) adapted from

Zimmerli *et al*. (2007), which indicates normal tissue structure with slight histological alterations. According to this classification scheme and the parameters assessed, *L. umbratus* in Sandile Dam are in a healthy condition.

### 6.5. Conclusion

The aim of the chapter was to determine the health status of *L. umbratus,* to determine the effects of *L. barnimiana,* and to establish if *L. barnimiana* is a species alien, indigenous or translocated to this region. The results showed that *L. umbratus* in Sandile dam during the July 2011 survey were in a healthy state, according to the parameters assessed. However, their health status rapidly declined during the March 2012 survey, this being the summer period, with the main cause being a lack of nutrition, although the reason for the lack of food cannot be determined. Further research into this cause is required. The effect of *L. barnimiana* on *L. umbratus* was limited to the attachment site, causing skin lesions which seemed to heal during the winter period. The parasite does not appear able to over-winter while attached to the host. There seemed to be no other effects on the health status of *L. umbratus* due to *L. barnimiana* infestations. Determining whether or not *L. barnimiana* is indigenous to the Eastern Cape of South Africa was not possible, and further research into this will be required. This is mainly due to the paucity of information regarding freshwater parasites in the Eastern Cape and the introduction of an alien and translocated fish species to this region.

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# Chapter 7 : Health status of *Labeobarbus aeneus* and the potential threat of two alien fish parasites to cyprinids in the Eastern Cape of South Africa.

## 7.1. Introduction

Cyprinid fish make up approximately 30% of the total southern African freshwater fish fauna (Skelton 2001; Bloomer *et al.* 2007). Of the eight genera and 80 species of cyprinids found in Southern Africa the Eastern Cape Province has nine species of cyprinids of which three are alien invasive species (*Cyprinus carpio*, *Carassius auratus*, and *Tinca tinca*), one is a translocated species (*Labeobarbus aeneus*), one is near threatened (*Pseudobarbus afer*) and one is vulnerable (*Pseudobarbus asper*) (Skelton 2001). Impson *et al*. (2007) highlighted the main threats to indigenous and endemic cyprinids as being expanding agriculture and increasing levels of pollution, water abstraction as well as the introduction of large predatory fishes such as largemouth bass (*Micropterus salmoides*).

*Labeobarbus aeneus* (Burchell, 1822) has a natural distribution in the Orange-Vaal river system where it has become an important angling species (Brand *et al*. 2009). However, due to inter-basin water transfer schemes, and due to active stocking of this popular angling species (Skelton 2001; Gerber *et al*. 2012), *L. aeneus* has established extralimital populations in the Gouritz, Sundays, Great Fish and Kei Rivers (Skelton 2001, Weyl *et al.* 2009). However, it is also become important source of protein for many subsistence anglers (Ellender *et al.* 2010) and has shown good potential as a possible candidate for commercial fisheries (Richardson *et al.* 2009). This is because they occur in large abundances in impoundments (Tómasson *et al.* 1984; Weyl *et al.* 2009), however, inflowing rivers are required in order for them to breed successfully (Tómasson *et al.* 1984). Previous research on the movement and habitat preference of *L. aeneus* has been done by O'Brien *et al*. (2013). These authors showed that *L. aeneus* prefer shallow fast following water in the spring, summer and autumn months and shifted to deep slow flowing water in the winter months. O'Brien *et al*. (2013) also reported that in its native Vaal River system *L. aeneus* had a home range of up to 5.5 km. Using whole otoliths Gerber *et al*. (2012)

found that in its native Vaal River system male *L. aeneus* (maximum 19 years) live longer than females (maximum 16 years) and that males mature at three years of age and the females mature at five years. A major threat to *L. aeneus* in it native system has been the introduction of alien parasites such as the fish louse *Argulus japonicas* Thiele, 1900 and the Asian tapeworm *Bothriocephalus acheilognathi* (Yamaguti, 1934) (Impson *et al*. 2007; Stadtlander 2006). According to Avenant-Oldewage (2001) *A. japonicas* is known to cause localised damage because of the attachment process and in extreme cases retarded growth has been observed. However, despite the importance of *L. aeneus* as an angling species and its potential as a commercial species relatively little has been published on the impact of this species in areas that it has been tranlocated to.

Ecto-parasites that are kept in confined areas such as impoundments have the ability to multiply and disperse easily and rapidly, allowing them to reach high intensities (Rohde 1984, Thoney and Hargis 1991, Montero *et al.* 2004). *Lernaea cyprinacea* (Linnaeus, 1758) is such an opportunistic ectoparasite of many freshwater fish species as well as some amphibians (Bauer 1961; Paperna 1996; Piasecki *et al*. 2004). It is known to cause serious economic problems in crowded breeding ponds (Bulow *et al.* 1979). The female *L. cyprinacea* attaches to exposed body surfaces and causes acute haemorrhage and ulcers at the site of penetration, they have a negative effect on the hosts functioning and survival (Bauer 1961; Paperna 1996), fatality is as a result of blood loss and secondary infections (Putz & Bowen 1964). *Lernaea cyprinacea* has a cosmopolitan distribution and was first recorded in Africa on two tilapia species from Lake Victoria (Robinson & Avenant-Oldewage 1996). Fryer (1965) reported that the copepodid larvae from Lake Edward and made the assumption that *L. cyprinacea* invaded Africa via the Nile River. Robinson & Avenant-Oldewage (1996) stated that *L. cyprinacea* gained access to South African rivers via accidental sources for example the import trade of tropical fish from Europe and Asia. As a result *L. cyprinacea* has virtually spread throughout South Africa (van As & Basson 1984; Viljoen 1986).

Endo-parasites, such as the helminth *B. acheilognathi*, have also been introduced into South African waters by the introduction of other alien cyprinid fish species (Bertasso & Avenant-Oldewage 2005). The wide spread movement of the host species has enabled *B. acheilognathi* to infect cyprinid and non-cyprinid hosts

worldwide. Around the world they have been reported from 102 fish species in 14 families and 7 orders (Salgado-Maldonado & Pineda-López 2003). The natural hosts of *B. acheilognathi* are grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) and the silver carp (*Hypothalmichtys molitrix* Valenciennes, 1844) (Bertasso & Avenant-Oldewage 2005). The grass carp was introduced from Malaysia to Umgeni hatchery in KwaZulu-Natal in 1967, primarily for aquaculture and the control of aquatic vegetation (de Moor & Bruton 1988; Skelton 2001), and the silver carp was imported into Marble Hall, South Africa from Germany in 1975 for experimental fish farming in the Olifants River (de Moor & Bruton 1988; Skelton 2001). *Bothriocephalus acheilognathi* was first discovered in South Africa during routine monitoring of the common carp (*C. carpio*) from the Lowveld Fish Research Station in Marble Hall and was introduced in 1975 with the introduction of grass carp (*Ctenopharyngodon idella*) (de Moor & Bruton 1988; Retief *et al.* 2007). Since then it has been recorded from *Barbus argenteus*, *B. mattozi*, *B. motebensis, B. paludinosus*, *B. trimaculatus*, *Clarias gariepinus*, *C. carpio*, *L. aeneus*, *L. kimberleyensis*, *L. marequensis*, *Oreochromis mossambicus* and *Pseudocrenilabrus philander* (Retief *et al.* 2007). Severe infections of *B. acheilognathi* are known to cause high mortality rates in fish, however low infections will not cause any mortality (Retief *et al*. 2007). The damage caused by severe infections of *B. acheilognathi* are blocking of the intestine, inflammation of the intestine, perforation of the intestine wall as well as abnormal growth (Retief *et al.* 2007).

The aim of this chapter was to determine if *Labeobarbus aeneus* could be used as a sentinel species in the Eastern Cape of South African to determine the potential impacts of alien invasive parasites such *L. cyprinacea* and *B. acheilognathi* on the indigenous cyprinid species.

## 7.2. Materials and methods

The study took place at Wriggleswade Dam (see **Chapter 2, Section 2.1**) (S32° 35.187; E27° 34.055), a 1000ha impoundment used extensively by recreational bass and carp anglers (**Figure 7.1**).

The materials and methods used in this chapter were discussed in **Chapter 2**. For sampling (see **Section 2.2)**, blood parameters, necropsy, and biometric indices (see **Chapter 2, Section 2.2.1**) were recorded and the tissue sampling procedure was prepared (see **Chapter 2, Section 2.2.2**) for histological analysis (see **Chapter 2, Section 2.2.2**) and statistical analysis was calculated for comparison (see **Chapter 2, Section 2.2.5**). *Labeobarbus aeneus* were collected in July 2011 (n = 15) and March 2012 (n = 15) by means of gill nets (see **Chapter 2, Section 2.2**). Hence the techniques used will not be repeated here.

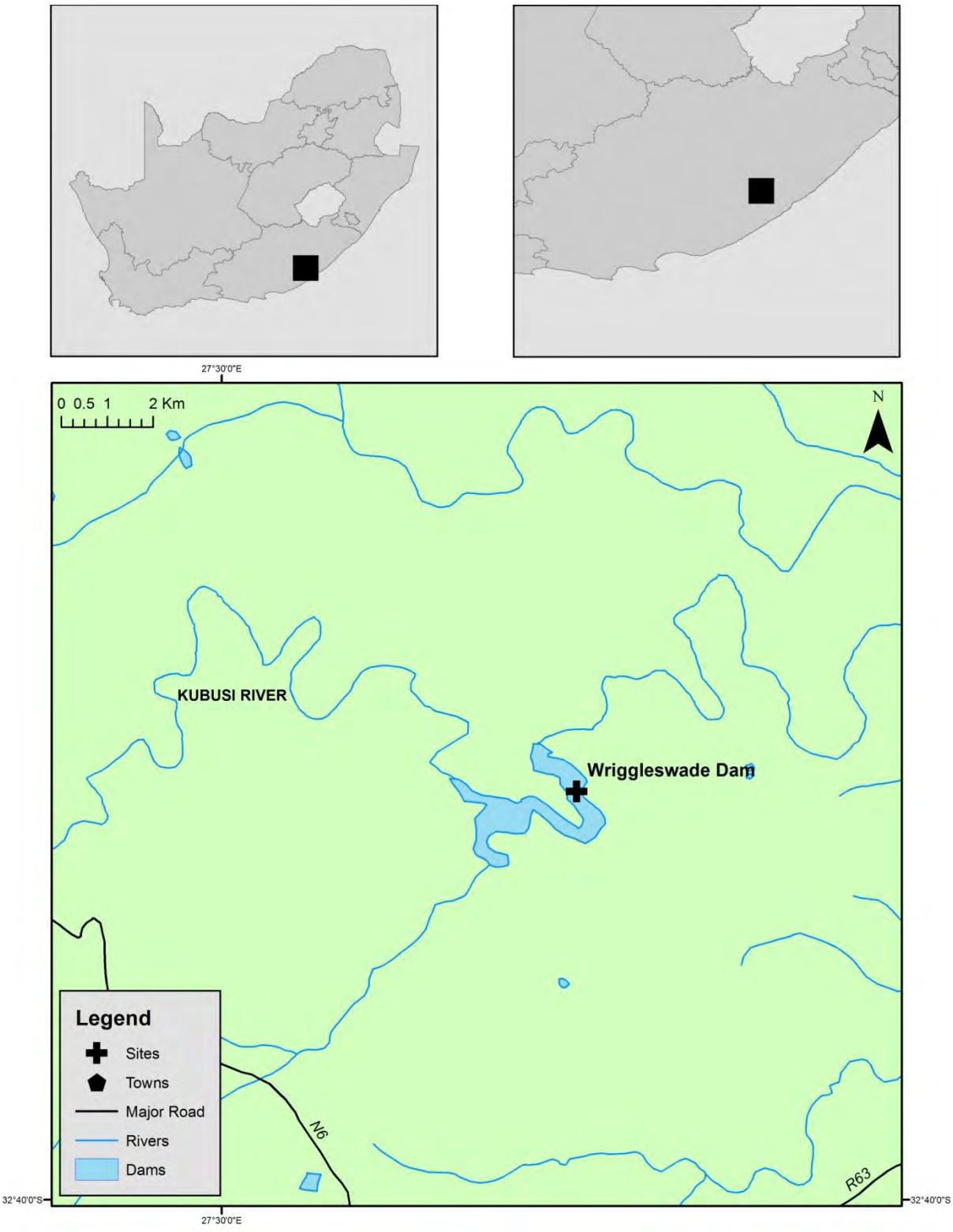


**Figure 7.1:** Map of Wriggleswade Dam (S32° 35.187; E27° 34.055), Amatola region, Eastern Cape Province, South Africa.

## 7.3. Results

### 7.3.1. Necropsy, blood parameters and biometric indices

The macroscopic external examination of the July 2011 *L. aeneus* revealed that 93% of the samples had severe skin lesions and red (**Figure 7.2A**), inflamed and damaged fins (**Figure 7.2C**). The opercula of *L. aeneus* had localised damage. The internal examination showed that 7% of the samples had discoloured gills and that 27% of the samples had fatty and discoloured livers. The macroscopic external examination of the March 2012 samples indicated all fish had severe skin and fin damage (**Figure 7.2A, 7.2B, 7.2C**), 20% of the sampled fish had pale gills and one of the fish had a missing left eye (**Figure 7.2D**).

The mean total plasma protein for the July 2011 (42.84 ± 5.96 mg $dl^{-1}$) was significantly ($p \leq 0.05; df1 = 3$) lower than the March 2012 (51.69 ± 8.31 mg $dl^{-1}$) survey. However, all the July 2011 total protein samples fell within the normal ranges (30 – 69 mg $dl^{-1}$) according to Adams *et al.* (1993) and only 7% of the samples in the March 2012 survey were above the normal range (> 70 mg $dl^{-1}$). There was no significant difference ($p \geq 0.05; df1 = 3$) between the July 2011 (40.62 ± 8.18 %) and the March 2012 (44.40 ± 6.86 %) hematocrit reading. The July 2011 hematocrit reading indicated that 7% of the samples hematocrit was below the normal range (30 – 45 %) (Adams *et al.* 1993), while 27% of the samples were above the normal range. The hematocrit reading for the March 2012 sample showed that 54% of *L. aeneus* samples were higher than the normal range of Adams *et al*. (1993). The leukocrit values for *L. aeneus* were all below 4% which is considered normal (Adams *et al.* 1993). There however, was no significant ($p \geq 0.05; df1 = 3$) difference between the July 2011 (61.33 ± 14.07) and March 2012 (59.33 ± 10.99) fish health assessment index (FHAI) scores.

The biometric indices are represented in **Table 7.1**. There was no significant difference ($p \geq 0.05; df1 = 3$) between the masses of the July 2011 (1734 ± 484 g) and the March 2012 (1796 ± 387 g) *L. aeneus* samples. There was no significant difference ($p \geq 0.05; df1 = 3$) between the July 2011 (1579 ± 540 g) and the March 2012 (1812 ± 463 g) females for the masses. There was also no significant difference ($p \geq 0.05$) between the masses of the male July 2011 (1579 ± 540 g) and

male March 2012 (1718 ± 300 g) samples. There was no significant difference ($p \geq 0.05$; $df1 = 3$) between the masses for the July 2011 (1579 ± 540 g) males and the March 2012 (1952 ± 526 g) females. There was also no significant difference ($p \geq 0.05$; $df1 = 3$) between the masses of the July 2011 (1812 ± 463 g) females and the March 2012 (1952 ± 526 g) females. Female fish were generally heavier than males. In July 2011, condition factor (CF = 1.28 ± 0.08) was significantly higher ($p \leq 0.05$; $df1 = 3$) than in March 2012 (1.15 ± 0.08). However, the gutted condition factor (GCF) was not significantly different ($p \geq 0.05$; $df1 = 3$) between July 2011 (1.10 ± 0.09) and March 2012 (1.02 ± 0.11). In July 2011, mean liver mass (1.16 ± 0.27) represented by the hepatosomatic index (HSI) was significantly ($p \leq 0.05$; $df1 = 3$) larger than in March 2012 (0.76 ± 0.09). The spleen index (SSI) for the July 2011 (0.10 ± 0.03) samples were also significantly ($p \leq 0.05$; $df1 = 3$) larger than for the March 2012 (0.07 ± 0.02) samples. The July 2011 male (1.06 ± 0.53) gonado-somatic index (GSI) was not significantly ($p \geq 0.05$; $df1 = 3$) larger than the March 2012 males (0.61 ± 0.15) GSI. The GSI for the July 2011 females (2.30 ± 0.31) were also not significantly ($p \geq 0.05$; $df1 = 3$) heavier than the GSI for March 2012 females (1.97 ± 0.81).

**Table 7.1:** Biometric indices for *Labeobarbus aeneus,* mean total body mass (g), fork length (mm), condition factor (CF), gutted condition factor (GCF), hepatosomatic index (HSI), spleenosomatic index (SSI), gonadosomatic index (GSI) and fish health assessment index (FHAI).

| Indice | | July 2011 (n = 15) | March 2012 (n = 15) |
|---|---|---|---|
| Mass | | 1734.93 ± 484.27 | 1796.00 ± 387.76 |
| Length | | 509.8 ± 43.52 | 535.6 ± 33.35 |
| CF | | 1.28 ± 0.08 | 1.15 ± 0.08 |
| GCF | | 1.10 ± 0.09 | 1.02 ± 0.11 |
| HSI | | 1.16 ± 0.27 | 0.76 ± 0.09 |
| SSI | | 0.10 ± 0.03 | 0.07 ± 0.02 |
| GSI | Male | 1.06 ± 0.53 | 2.30 ± 0.31 |
| | Female | 0.61 ± 0.15 | 1.97 ± 0.81 |
| FHAI | | 61.33 ± 14.07 | 59.33 ± 10.99 |

### 7.3.2. Histological assessments

The percentage prevalence of the histological alterations that were identified as well as the histological index results is presented in **Tables 7.2** and **7.3** respectively. In terms of histological alterations the gill samples were the worst affected. The main gill histological gill alterations were branching of the primary (**Figure 7.3A**) and secondary lamellae (**Figure 7.3B**), increase in mucous cells (**Figure 7.3C**) other alterations noted were hyperplasia (**Figure 7.3D**) of the secondary gill epithelium and telangiectasia with rupturing of the pillar cells (**Figure 7.3E**). Parasitic gill monogeneans (**Figure 7.3F**) were also noted in the gills of *L. aeneus* between the secondary lamellae. There was no significant difference ($p \geq 0.05$; $df1 = 3$) between the July 2011 (8.00 ± 5.07) and March 2012 (5.07 ± 3.37) Gill Index ($I_G$) score.

The main histological alterations that were identified in the *L. aeneus* liver samples were hepatocyte vacuolation (**Figure 7.4A**), increase in melano-macrophage centres (**Figure 7.4B**) and intercellular deposits (**Figure 7.4C** & **7.4D**). There was no significant difference ($p \geq 0.05$; $df1 = 3$) between the liver index ($I_L$) scores for the July 2011 (3.07 ± 1.98) and March 2012 (2.00 ± 1.69) sampling surveys.

The histological alterations that could be identified in the kidney tissue of *L. aeneus* were an increase in the Bowmans space (**Figure 7.5A**) and an increase in melano-macrophage centres (**Figure 7.5B**). There was no significant difference ($p \geq 0.05$; $df1 = 3$) between the July 2011 (1.60 ± 2.02) and the March 2012 (1.07 ± 1.48) survey for kidney index ($I_K$).

The only histological abnormalities that were identified in the *L. aeneus* testis and ovary samples, was an increase in melano-macrophage centres (**Figure 7.6A** & **7.6B**). There was no significant difference ($p \geq 0.05$; $df1 = 3$) for the July 2011 (2.00 ± 2.00) and the March 2012 (1.60 ± 1.06) testis index samples ($I_T$), nor was there a significant difference ($p = 0.864$) for the July 2011 (1.00 ± 1.05) and the March 2012 (1.60 ± 0.89) for the ovary index ($I_O$). The fish index ($I_{FISH}$) was significantly ($p \leq 0.05$; $df1 = 3$) higher in the July 2011 (13.60 ± 5.08) sampling period than during March 2012 (9.73 ± 4.59).

**Table 7.2:** Percentage prevalence of histological alterations identified in *Labeobarbus aeneus* from Wriggleswade Dam for the July 2011 and March 2012 surveys.

| | Percentage prevalence | |
|---|---|---|
| Target Organ/ histological alteration | July 2011 n = 15 | March 2012 n = 15 |
| **Gills** | | |
| Branching of primary lamellae | 7 | 7 |
| Branching of secondary lamellae | - | 7 |
| Hyperplasia | 7 | 14 |
| Increase in mucous cells | 100 | 93 |
| Rupture of pillar cells | 20 | 20 |
| Telangiectasia | 20 | 20 |
| **Liver** | | |
| Hepatocyte vacuolation | 73 | 20 |
| Increase in melano-macrophage centres | 7 | 20 |
| Intercellular deposits | - | 60 |
| **Kidney** | | |
| Increase in the Bowmans space | 47 | 7 |
| Increase in melano-macrophage centres | 20 | 40 |
| **Testis** | **n = 5** | **n = 10** |
| Increase in melano-macrophage centres | 60 | 60 |
| **Ovaries** | **n = 10** | **n = 5** |
| Increase in melano-macrophage centres | 50 | 80 |

Dashes denote that specific alteration was not detected

**Table 7.3:** Mean organ index and fish value index for *Labeobarbus aeneus*. $I_L$ = Liver Index, $I_K$ = Kidney Index, $I_G$ = Gill Index, $I_T$ = Testis Index, $I_O$ = Ovary Index and $I_{FISH}$ = Fish Index. Ranges are indicated in parentheses.

| Survey | $I_G$ | $I_L$ | $I_K$ | $I_T$ | $I_O$ | $I_{FISH}$ |
|---|---|---|---|---|---|---|
| July 2011 (n = 15) | 8.00 (4–20) | 3.07 (0–6) | 1.60 (0–4) | 2.00 (0–4) | 1.00 (0–2) | 13.60 (8–24) |
| March 2012 (n = 15) | 5.07 (2–12) | 2.00 (0–6) | 1.07 (0–4) | 1.60 (0–4) | 1.60 (0–2) | 9.73 (4–22) |

### 7.3.3. Gonad development

The developmental stages of the testes and ovaries were determined according to the criteria as defined in the Biomonitoring of Environmental Status and Trends Program (BEST) (Schmitt & Dethloff, 2000). The sex ratio of the *L .aeneus* was 33% males and 77% females for the July 2011 survey and 77% male and 33% females for the March survey.

The developmental stages of the July 2011 males were 100% in stage 3 (**Figure 7.7C**) and the females were also 100% in stage 3 (**Figure 7.7F**), both indicating late development. The developmental stages of the March 2012 males were stage 1 (30%) **(Figure 7.7A**), stage 2 (40%) (**Figure 7.7B**) and stage 3 (30%) (**Figure 7.7C**) while the females were all in stage 2 (100%) (**Figure 7.7D** & **7.7E**) of development.

### 7.3.4. Parasite assessment

The prevalence and the intensity of infection of the sampled species of parasites are represented in **Table 7.4**. The parasitological assessment revealed the presence of two alien invasive species and one species of unidentified gill monogenean (**Figure 7.3F**). The two alien parasite species were the ecotoparasite *L. cyprinacea* (Linnaeus, 1758) and the endo-parasite *B. acheilognathi* (Yamaguti, 1934).

**Table 7.4:** Prevalence (%) and intensity of parasites from *Labeobarbus aeneus* sampled during July 2011 (n = 15) and March 2012 (n = 15) from Wriggleswade Dam, Eastern Cape, South Africa.

| Parasite | July 2011 | | March 2012 | |
|---|---|---|---|---|
| | Prevalence | Intensity | Prevalence | Intensity |
| *Lernaea cyprinacea* | 100 | 20.5 (18 – 31) | 100 | 15.2 (8 – 21) |
| *Bothriocephalus acheilognathi* | 20 | 1.0 (1) | 20 | 1.0 (1) |
| Unidentified monogenean | 100 | 34.7 (8 – 56) | 100 | 39.5 (16 – 52) |

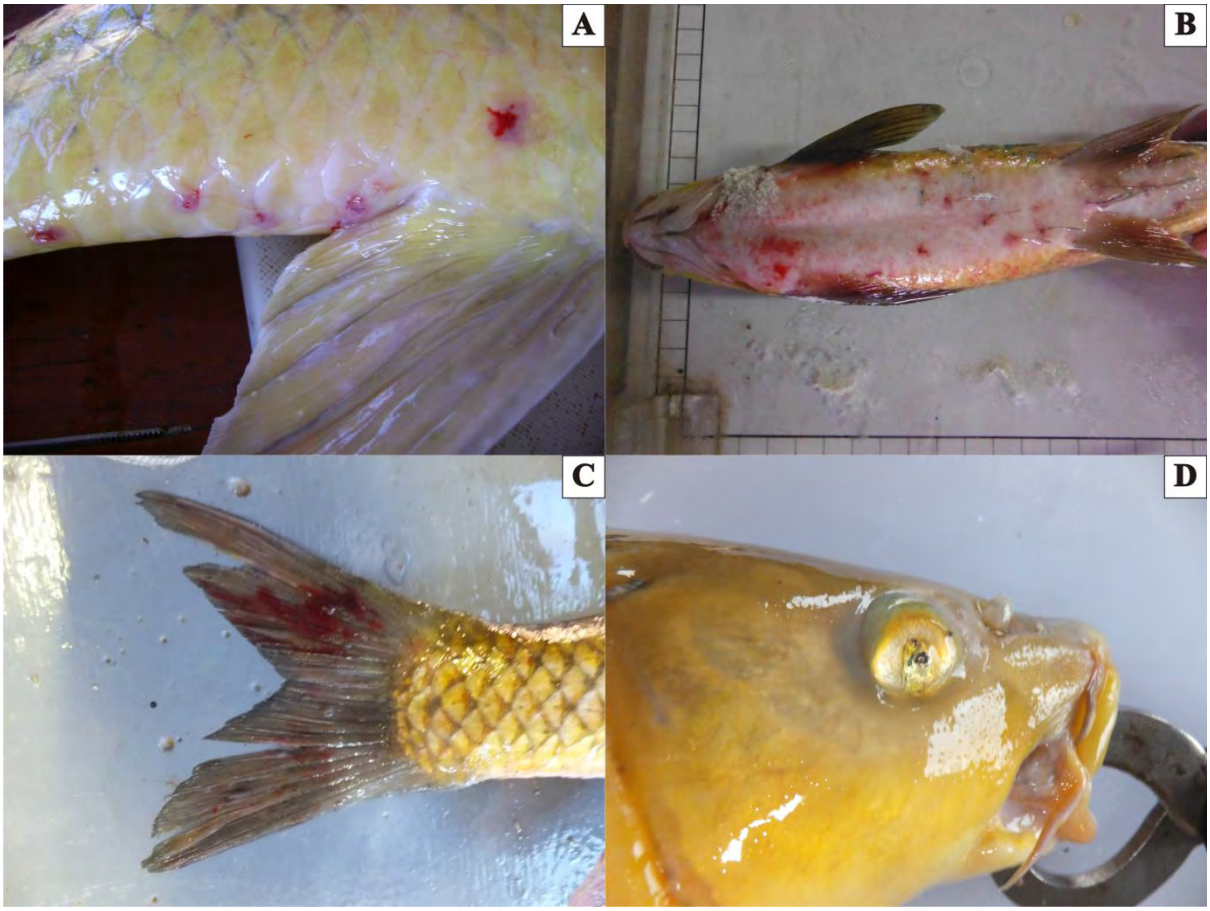


**Figure 7.2:** Macroscopic abnormalities identified in *Labeobarbus aeneus*; (A) damaged and bleeding skin; (B) red, inflamed and attachment sites of *Lernaea cyprinacea*; (C) bleeding a frayed tail fin; (D) missing eye.

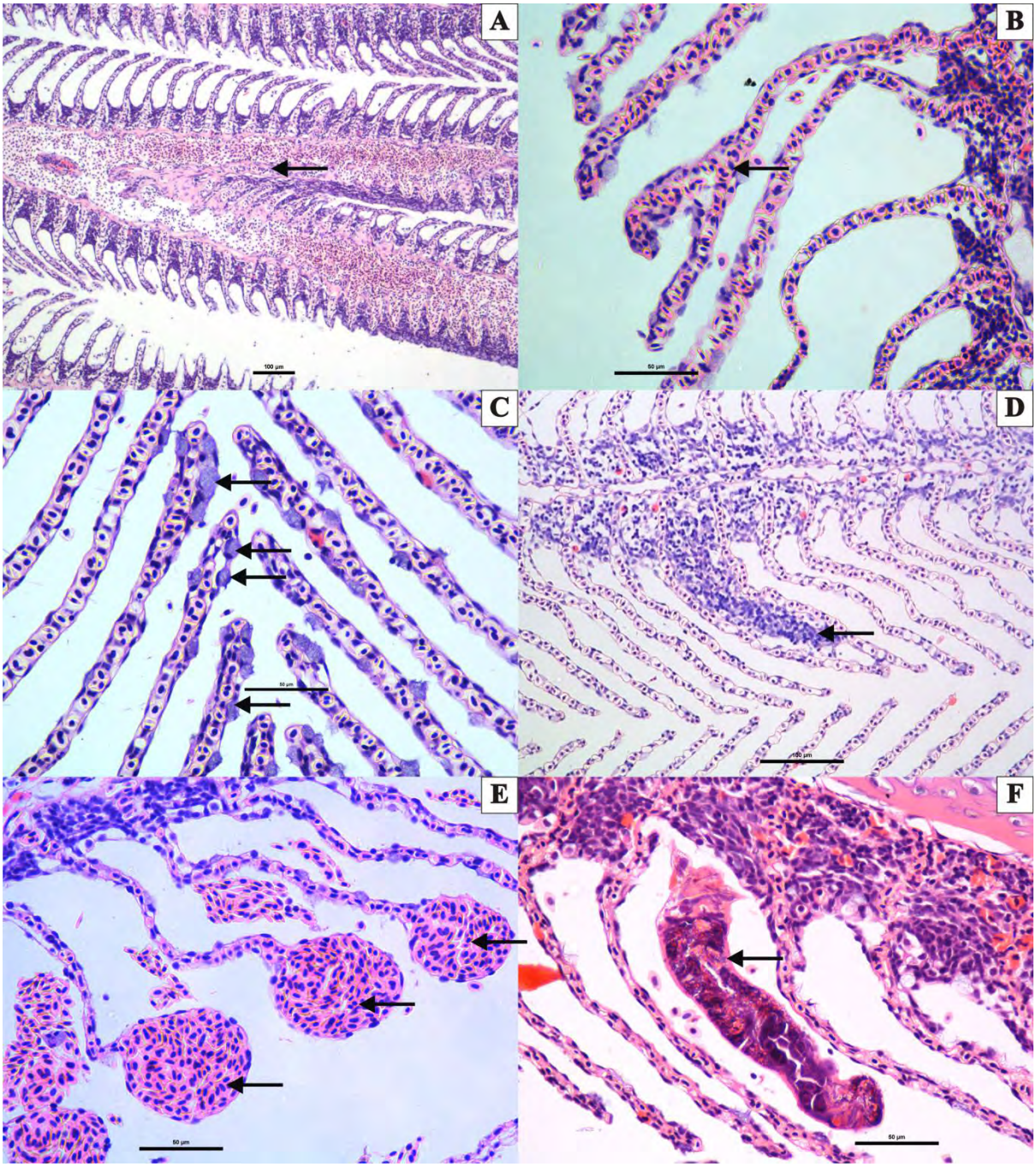


**Figure 7.3:** Micrographs of *Labeobarbus aeneus* gill sections (5 µm) stained with H&E: (A) Branching of the primary gill lamellae (arrow); (B) branching of the secondary gill lamellae (arrow); (C) Increase in the mucous cells (arrows); (D) Hyperplasia of the secondary gill lamellae (arrow); (E) Telangiectasia and rupture of the pillar cells (arrows); (F) parasitic gill monogenean species in between the secondary gill lamellae (arrow). Scale bars = 50 µm (A, C, E, F), 100 µm (B, D).

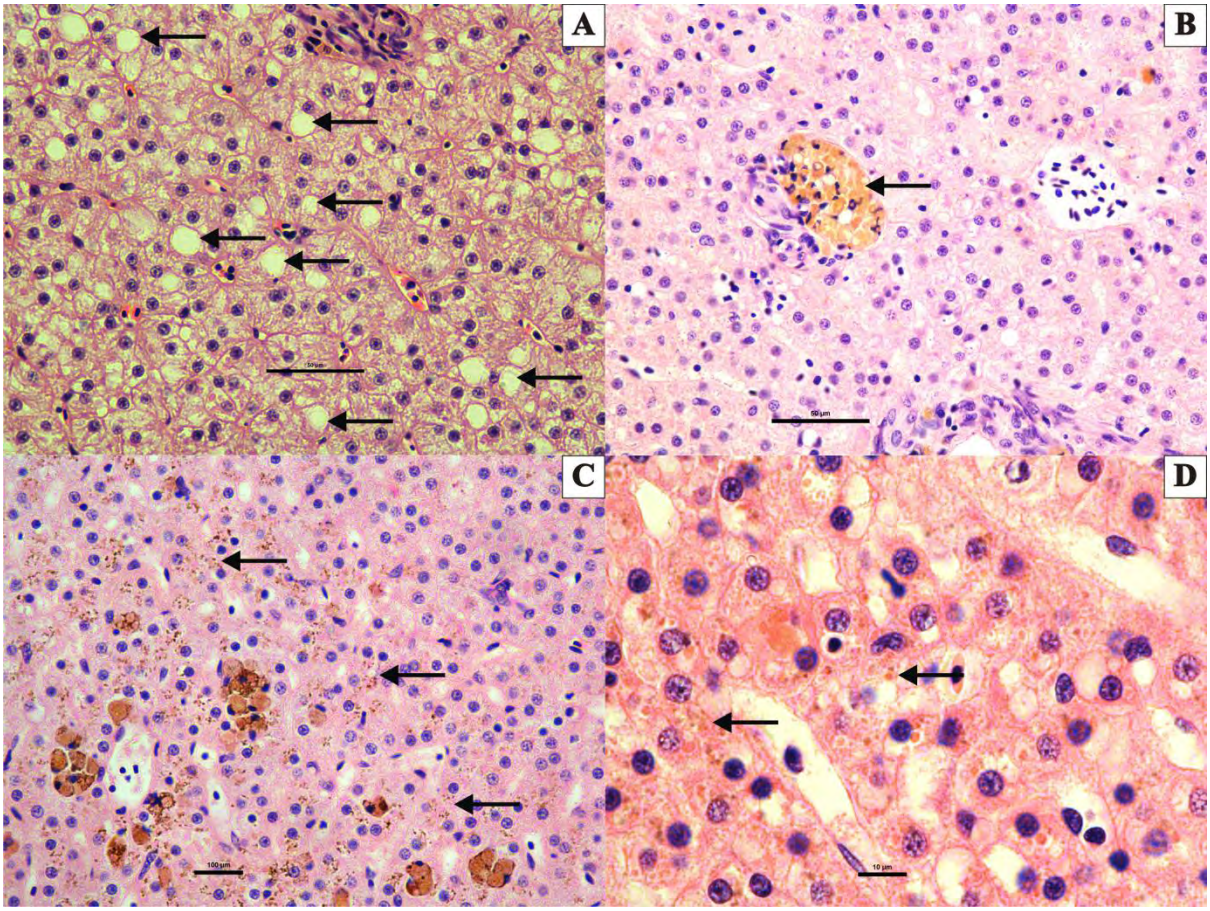


**Figure 7.4:** Micrographs of *Labeobarbus aeneus* liver sections (5 µm) stained with H&E: (A) hepatocyte vacuolation (arrows); (B) Increase in melano-macrophage centres (arrow); (C and D) intracellular deposits (arrows). Scale bars = 50 µm (A, B), 100 µm (C), 10 µm (D).

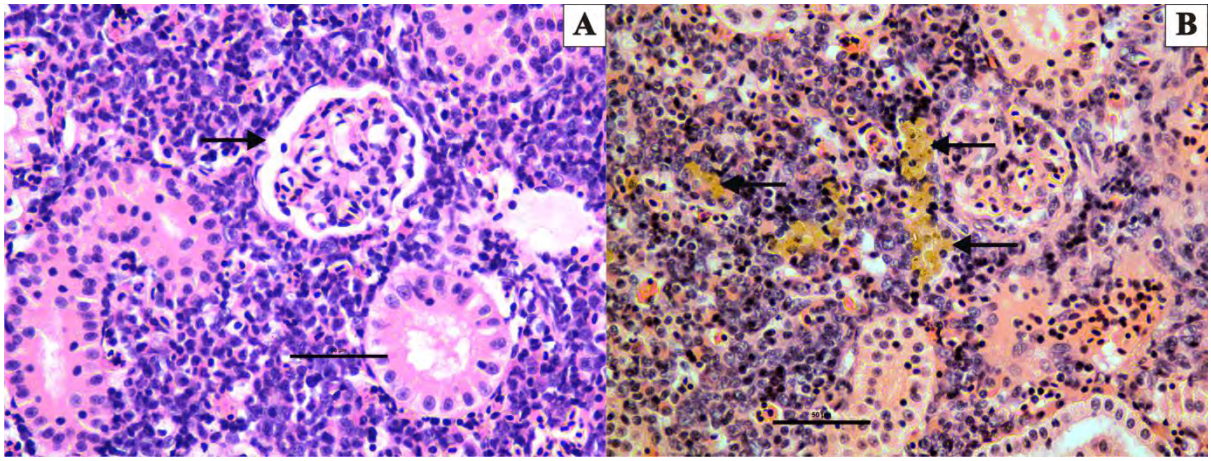


**Figure 7.5:** Micrographs of *Labeobarbus aeneus* kidney sections (5 µm) stained with H&E: (A) increase in Bowman's space (arrow); (B) Increase in melano-macrophage centres (arrows). Scale bars = 50 µm (A, B).

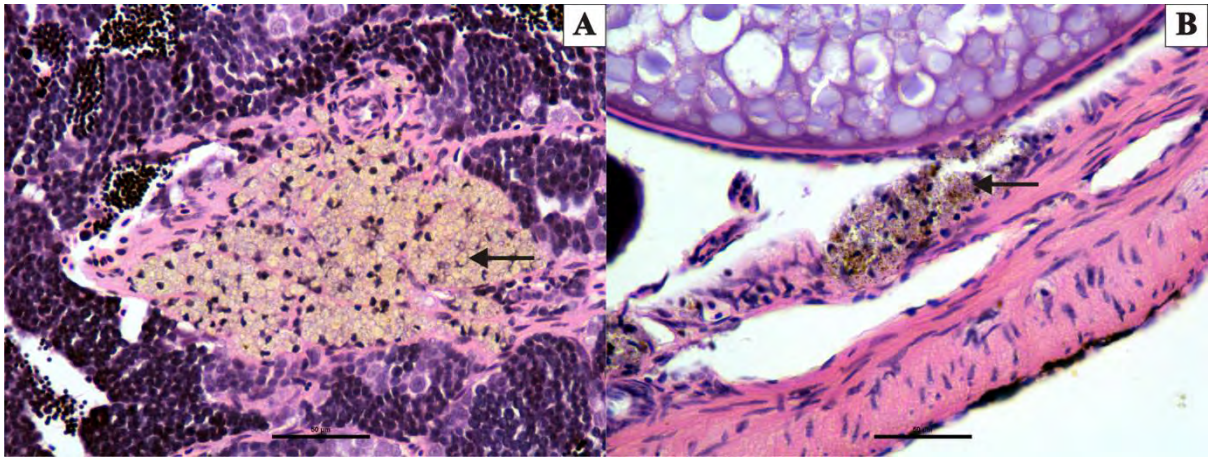


**Figure 7.6:** Micrographs of *Labeobarbus aeneus* testis and ovary sections (5 µm) stained with H&E: (A) increase in melano-macrophage centres in testis (arrow); (B) Increase in melano-macrophage centres in the ovaries (arrows). Scale bars = 50 µm (A, B).

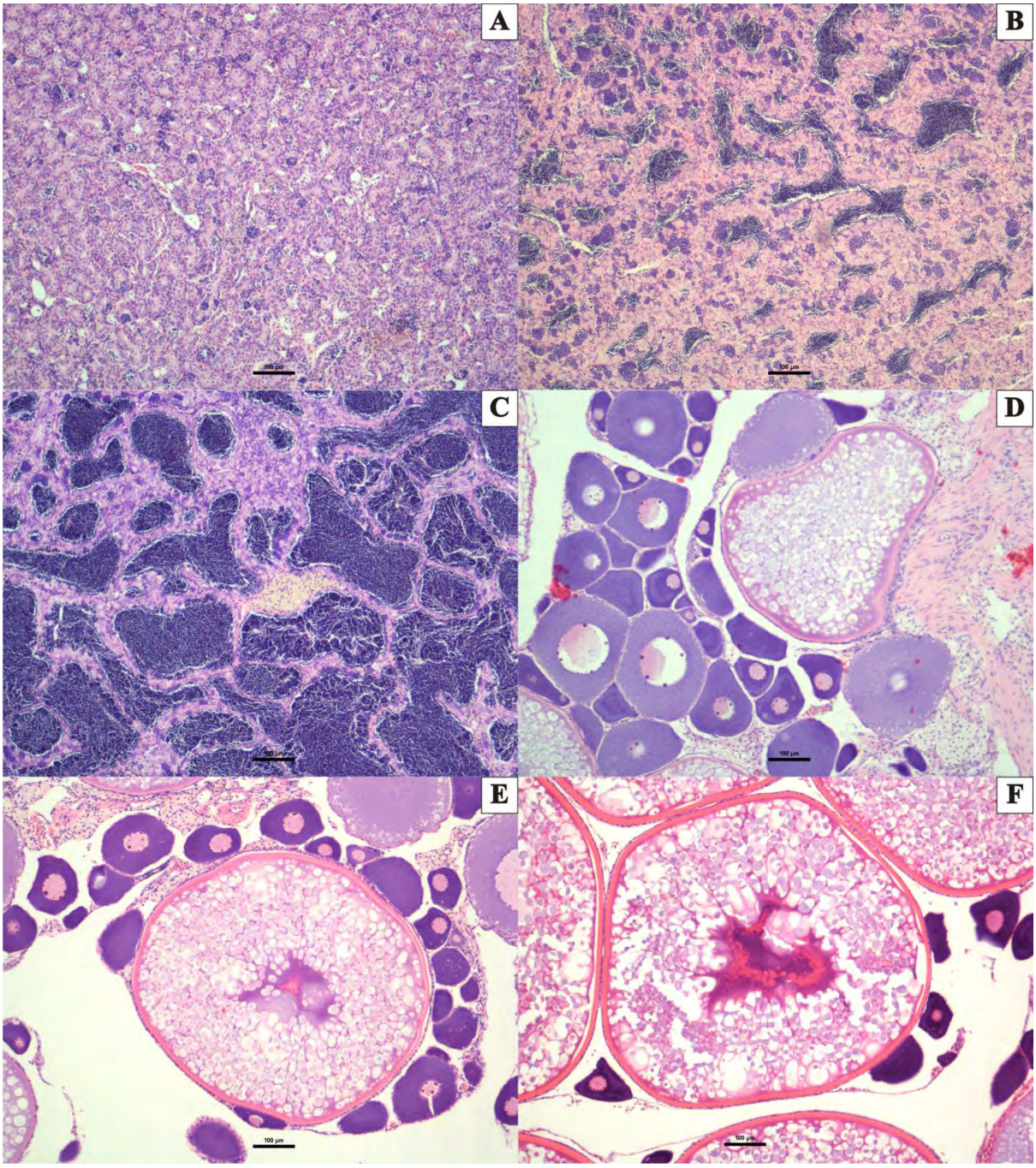


**Figure 7.7:** Micrographs of *Labeobarbus aeneus* gonad sections (5 µm) stained with H&E: (A) mature stage 1 testis; (B) mature stage 2 testis; (C) mature stage 3 testis; (D and E) mature stage 2 ovaries; (F) mature stage 3 ovaries. Scale bars = 100 µm (A, B, C, D, E, F).

### 7.4. Discussion

There was no significant difference between the FHAI scores for the two sampling periods, however, the score was considered to be high when compared to the FHAI values of Sara *et al.* (2014) for *C. gariepinus, O. mossambicus* and *C. carpio* collected from the Hout River, South Africa. The main contributors to the high FHAI scores where the skin lesions and damaged fins. The severe skin lesions and damage to the fins noted in the July 2011 and March 2012 sampling were as a result of the alien invasive ectoparasite *L. cyprinacea* attaching to the skin of *L. aeneus*. The localised damage observed on the opercula of *L. aeneus* was also a result of being attachment points for *L. cyprinacea.* Another contributing factor was the elevated to higher than normal hematocrit values. The increased hematocrit levels have been linked to stress in various fish species (Redding *et al.* 1987; Biron & Benfey 1994; Roche & Bogé 1996), the high infection rate of the alien parasite *L. cyprinacea* may have been the direct cause of the stress placed on *L. aeneus*.

The biometric indices indicated that the July 2011 samples had a better condition factor than the March 2012 samples however, there was no significant difference between the samples for the gutted condition factor and the GSI for the July 2011 sampling period was significantly higher than the March 2012 samples. Thus the increased CF for the July 2011 is as a result of *L. aeneus* preparing to spawn in the summer months. According to Skelton (2001), *L. aeneus* matures at 200 mm standard length in males and 240 mm standard length in females and breeds in spring to mid summer after the first rains of that season. The increased GSI noted in the July 2011 survey corresponds with the stage 3 gonad development stages. The annual cycles of gonad maturation is consistent with Weyl *et al.* (2009).

A large percentage of the *L. aeneus* samples had higher than normal hematocrit values, this is often as a result of spleen hyper-function. The samples that exhibited the lower than normal hematocrit values were as a result of the severe infection by *L. cyprinacea* causing blood loss at the sites of attachment. The spleen plays a vital role in filtering of blood, the spleen removes old erythrocytes and the high SSI values could be caused the severe infection by *L. cyprinacea*. *Lernaea cyprinacea* cause acute haemorrhage and ulcers at the site of penetration which results in blood loss

and secondary infections of leading to the death of the host (Putz & Bowen 1964). Although Tsotetsi *et al.* (2005) found no correlation between hematocrit percentages and intensity of infection *Lamproglena clariae* on the gills of *C. gariepinus.*

The significantly higher $I_{FISH}$ score of the July 2011 survey was largely as a result of the increased alterations. The increased $I_G$ was mainly due to alterations caused by the presence of gill monogenean parasites, the alterations observed were telangiectasia with rupturing of the pillar cells and increase in mucous cells other alterations noted were hyperplasia of the secondary gill epithelium and branching of the primary and secondary lamellae. Montero *et al.* (2004) noted similar effects with the gill monogenean *Zeuxapta seriolae* on the fish host *Seriola dumerili*. The fatty discolouration that was observed in the liver tissue during the macroscopic health assessment was an indication of liver steatosis. The liver steatosis was confirmed by the histological assessment revealing hepatocyte vacuolation. The hepatocyte vacuolation was more pronounced during the July 2011 survey which may account for the significantly higher HSI values during this sampling period. The intercellular deposits and increase in MMCs reported here are both circulatory disturbances, however, these are non-specific alterations and maybe normal histological features of liver tissue (van Dyk *et al*. 2012). Hepatocyte vacuolation has previously been linked with exposure to pesticides (McHugh *et al.* 2011), however there is no evidence that pesticides are currently being used in the surrounding areas that could affect the water and sediment of Wriggleswade Dam. There were no significant alterations identified in the kidney tissue of *L. aeneus*.

*Labeobarbus aeneus* collected during this study were all in a healthy state apart from the damage caused by the severe infections by the alien parasite *L. cyprinacea*. The medium sized cyprinid *L. aeneus* (Weyl *et al*. 2009) appear to be able to tolerate the high infection rate and wounds caused by the *L. cyprinacea*. However, according to Woo & Shariff (1990) *L. cyprinacea* cause high mortalities in young and small sized fish because of their relatively large size as well as their method of attachment and feeding. It is also possible for this parasite to transmit viruses and/or bacteria which result in secondary infections (Woo & Shariff 1990). Given that the majority of indigenous Eastern Cape cyprinids are smaller than 100 mm the potential threat of this alien parasite to the indigenous cyprinids is of high concern. Shariff & Roberts (1989) reported that in small fish the anchor of the parasite would penetrate into the

internal organs of the host, in these cases the smaller fish would die within a week of penetration. The attachment sites of *L. cyprinacea* to epidermis and dermis of *L. aeneus* skin resulted in open ulcers and severe acute inflammatory response. This inflammatory response has also been reported by Sharif & Roberts (1989). Shariff & Roberts (1989) conducted a histopathological study into the effects of *L. polymorpha* attachment to *Aristichthys nobilis*. These authors showed that the adult female embedded into the skin and the host's tissue at a slight angle, in order to slide in between the overlapping scales. This penetration causes extensive tissue disruption, necrosis and haemorrhaging along the entire path of entry, there is often an associated inflammatory response (Shariff & Roberts 1989).

The prevalence and intensity of *B. acheilognathi* was too low in *L. aeneus* to be of concern, as only severe infections of *B. acheilognathi* are known to cause high mortality in fish (Retief *et al*. 2007). However, *B. acheilognathi* has been reported from *L. aeneus* collected from the Glen Melville Reservoir located just north of Grahamstown in the Eastern Cape Province (Stadtlander *et al.* 2011), here 70% of the *L. aeneus* samples were infected with between 1 – 204 *B. acheilognathi* (Stadtlander *et al.* 2011). The CF *L. aeneus* values of the July 2011 survey were similar to those reported by Stadtlander *et al.* (2011) for the Great Fish River (1.28 ± 0.10), where there was no reported infection by *B. acheilognathi* however, Stadlander *et al.* (2011) did observe a drop in the CF values for the samples collected at Glen Melville Reservoir and reported that there was a significant drop in CF with an increase in the intensity of infection of *B. acheilognathi* which may be as a result of the parasite limiting the amount of available food for the host. Fish can become infected with *B. acheilognathi* by the consumption of the intermediate hosts such as freshwater cyclopoid or diaptomid copepods (Granath & Esch 1983) or by consuming an infected fish (Cole 2004). Stadtlander *et al.* (2011) determined that the stomach contents of the *L. aeneus* from Glen Melville Reservoir mainly contained terrestrial Hymenoptera and Coleoptera and aquatic components where mainly chironomid and trichopteran larvae. However, further research is required on the intermediate hosts of *B. acheilognathi*. Stadtlander *et al.* (2011) noted that no *B. acheilognathi* where found in the riverine fish and therefore, postulated that this could be as a result of the absence of the intermediate host(s), therefore *B. acheilognathi* could not complete its full lifecycle. The presence of the parasite in the

dam again highlights the potential of impoundments to act as reservoir for high infestation rates of parasites.

### 7.5. Conclusion

The quantitative fish health assessment used here was originally developed to reflect the health of the environment by reflecting the health of the organisms that reside in it. *Labeobarbus aeneus* from Wriggleswade Dam are, according to the parameters used to assess their health, in a healthy state. The only threat to the health of the cyprinids in the Eastern Cape of South Africa is the threat posed by invasive species such as alien fish species and alien parasite for example *L. cyprinacea* (Ellender & Weyl 2014). The original aim of this section of the study was to determine what the effect of alien parasites would be on the larger cyprinid to see if this species could be used as a sentinel species for comparative effects of the small indigenous cyprinid species. Both *L. cyprinacea* and *B. acheilognathi* have the ability to infect a wide range cyprinid and chiclid hosts and are a serious threat to native fish species in South Africa. According to Tweddle *et al.* (2009) endemic cyprinids in South Africa east and south flowing rivers make up the largest group of endangered fish species. The threat is that the smaller endemic cyprinids such as *Barbus trevelyani* and *B. amatolicus* become exposed to the harmful effects of *L. cyprinacea* and *B. acheilognathi* when *L. aeneus* moves up the Kubusi River to spawn bring these parasites into contact with the naive native cyprinids. Further research is thus required to determine whether these alien parasites have already infected the small indigenous cyprinids of the Kubusi River and if they have, what their effects are on these hosts. This is specifically relevant as this is one of the few localities where the endangered *Sandelia bainsii* are indigenous to.

### 7.6. References

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# Chapter 8 : General discussion and future recommendations

## 8.1. General discussion

Freshwater ecosystems are amongst the most threatened ecosystems on the planet (Dudgeon *et al*. 2006; Abell *et al.* 2007). Dudgeon *et al*. (2006) categorised the main threats as overexploitation, water pollution, flow modifications, destruction of habitat and invasion by alien species. In response to the growing need for the conservation of South Africa's freshwater ecosystems (Nel *et al*. 2011), South Africa attempts to address these threats through the National Freshwater Ecosystems Protected Areas (FEPA) strategy plan, which intends to protect 15% of South Africa's river lengths in order to provide protection to all of South Africa's fish species that are listed as critically endangered, endangered and vulnerable (Nel *et al.* 2011). To develop proactive strategies for protection and to prioritise conservation actions for these freshwater ecosystems, knowledge on the current health of these systems is essential.

The health status of an ecosystem can be illustrated by the health of the organisms that reside within that ecosystem (Adams *et al.* 1993). Fish can be used as biological indicators of ecosystem health (Adams *et al.* 1993, Bernet *et al.* 1999) because they are relatively long-lived and mobile (Karr *et al.* 1986), represent various trophic levels and their community structure is represented in ecosystem health (Barbour *et al*. 1999). Fish, particularly those at the top of the food chain, are excellent representatives for overall ecosystem health as they reflect the biotic and abiotic variables that are acting within the ecosystem (Adams *et al.* 1993). Due to South Africa's climate and rainfall patterns, it is described as a water-scarce country (Perret 2002). Because of this, many communities attempt to establish close to water sources in order to gain access to water for their domestic and recreational use (Heath *et al.* 2004), and large impoundments such as Lake Gariep may provide fish as a potential food source (Ellender *et al*. 2009). The pollution of South Africa's freshwater resources is becoming an ever-increasing issue, which threatens the communities surrounding the impoundment, the drinking water and the biodiversity within the impoundment (Heath *et al.* 2004). In South Africa the pollution of

freshwater resources can be linked to point source discharges such as waste water treatment plants and diffuse surface runoff from agricultural and industrial areas (Heath *et al.* 2004). The majority of South Africa's impoundments were built in order to act as reservoirs or for the supply water for drinking, domestic or agricultural uses and later, according to Ellender *et al.* (2014), were stocked with alien fish species for the enhancement of fisheries for either recreational or aquaculture purposes.

However, the introduction of alien fish species presented South Africa with additional threats to freshwater biodiversity. The introduction of largemouth bass *Micropterus salmoides* has had a negative impact on South Africa's aquatic biodiversity (Ellender & Weyl 2014, Ellender *et al.* 2014) by preying on and disrupting macro-invertebrate community structures (Weyl *et al*. 2010), as well as preying on indigenous fish species (Ellender & Weyl 2014). According to Gozlan *et al.* (2010) the threat is not the preying on native fish species but rather the reduction in native fish species numbers as well as the changes in community structure. The introduction of the common carp *Cyprinus carpio* has had a negative impact on freshwater ecosystems because it causes habitat destruction and because its feeding behaviour increases the turbidity of the water in inhabits (Koehn 2004). The introduction of *C. carpio* has also been responsible for the introduction of alien parasite species such as *Ichthyobodo necator, Chilodonella cyprinid, C. hexasticha, Apiosoma piscicola, Trichodina acuta, T. nigra,* and *Trichodinella epizootica* (Ellender & Weyl 2014). The introduction of grass carp *Ctenopharyngodon idella* was responsible for the introduction for the Asian tapeworm *Bothriocephalus acheilognathi* (Ellender & Weyl 2014).

These are just a few of the threats facing freshwater biodiversity in South Africa. However, research presented in this thesis will make a contribution to a better understanding the impacts of catchment inputs and parasitisation on the health of fishes in the Amatola region. The threats focused on in this thesis were water pollution and alien parasite species in the Keiskamma and Kubusi Rivers and will be discussed in this chapter.

### 8.1.1. Water pollution

In order to contribute to the understanding of the health of the fish in a freshwater ecosystem the levels of water pollution must be known. In **Chapter 3** I assessed the metal concentrations in the surface water and sediment from Binfield Park, Sandile and Wriggleswade Dams and used largemouth bass *M. salmoides* as a representative species for the determination of the metal concentrations in the muscle tissue to compare between the impoundments.

The results of the metal levels in the surface water indicated that Binfield Park Dam had the highest concentration of cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn), lead (Pb) and uranium (U), compared to Sandile and Wriggleswade Dams. However, the concentration levels of these heavy metals were lower than the South African water quality guideline levels for domestic use (DWAF 1996). Cadmium has previously been reported in the Keiskamma River by Fatoki & Awofolu (2003). However, the concentration levels reported in the current study were lower than previous levels. Mercury (Hg) was detected in the surface water of the Kubusi River and was similar to the levels reported by Fatoki & Awofolu (2003) from the Keiskamma River, indicating that the source of the mercury is higher up in the catchment. Cadmium, mercury and zinc are components of environmental pollution (Fatoki & Awofolu 2003). However, the concentration levels of cadmium, mercury and zinc were below the South African Water Quality Guideline levels for domestic use (DWAF 1996), indicating that the water is safe for domestic use.

The sediment of Binfield Park, Sandile and Wriggleswade Dam was also analysed for the presence of metal. The reason for this was because sediments are considered to be sinks for many contaminants and therefore pose the highest risk to the aquatic environment as sources of pollution (Bervoets *et al*. 1994, Wepener & Vermeulen 2005). The results of the metal levels in the sediment showed that Sandile Dam had the highest concentrations of aluminium (Al), arsenic (As), cobalt (Co), copper (Cu), Iron (Fe), mercury (Hg), lead (Pb) and titanium (Ti). However, the metal concentrations were below the guideline levels indicated by Pheiffer *et al*. (2014). However, the concentration values of cobalt (Co), copper (Cu), manganese (Mn) and nickel (Ni) in Sandile Dam exceed the international guideline levels (Pheiffer *et al.* 2014). Wriggleswade Dam had the highest concentration of uranium (U) in its sediment, which also exceeded the international guideline standards

(Pheiffer *et al.* 2014). Cadmium was not detected in the sediment in any of the three impoundments. However, previous studies by Fatoki & Awofolu (2003) did report trace concentrations of the metal. Mercury (Hg) was also not reported by Fatoki & Awofolu (2003) in the sediment from Binfield Park or Sandile Dams. However, it is now present, although below guideline levels. Zinc was only found in trace amounts in Binfield Park Dam by Fatoki & Awofulu (2003) and has now been shown to be present in all three impoundments, but below international guideline levels. The presence of these metals in the sediment shows that there is a historical input of these pollutants or that that the concentration values may differ between seasons.

The bio-availability of metals in the sediment is strongly dependent on the forms in which they occur (Baeyens *et al.* 2003). In order to assess for metal bioaccumulation in fish tissue between Binfield Park, Sandile and Wriggleswade Dams, largemouth bass *M. salmoides* was selected as a representative species because it occurs abundantly in all three of the impoundments. The level of mercury in the muscle tissue of the Binfield Park Dam *M. salmoides* was significantly higher than the mercury concentrations in the muscle tissue of *M. salmoides* from between Sandile and Wriggleswade Dams. Sandile Dam had significantly higher levels of zinc (Zn) in the muscle tissue compared to Binfield Park Dam and Wriggleswade Dam. In order to determine if the *M. salmoides* are safe for human consumption, human health studies would be required. However, given that the metals present in the sediment are also present in the muscle tissue of *M. salmoides*, the metals are bio-available and may pose a health risk to the ecosystem and humans.

The heavy metal concentrations in the surface water were below the South African water quality guideline levels, while heavy metals such as cobalt, copper, manganese, nickel and uranium in the sediment were above internationally accepted guideline levels. According to the US EPA (1995), heavy metal concentrations of cadmium, mercury and zinc found in the muscle tissue of fish must be routinely monitored. Because these heavy metals have recorded in this study and previously (Fatoki & Awofolu 2003), Binfield Park, Sandile and Wriggleswade Dams are polluted by heavy metals.

### 8.1.2. Alien parasites

Three alien parasites were sampled during this study. Two of the alien parasites had previously been reported from the Eastern Cape and the third is a new distribution record. The previously reported parasites were the gill monogenean *Pseudodactylogyrus anguillae* (Christison & Baker 2007; Parker *et al*. 2011; **Chapter 4**) and the Asian tapeworm *B. acheilognathi* (Stadtlander *et al.* 2011; **Chapter 7**). The third alien parasite was the anchor worm *Lernaea cyprinacea* (**Chapter 7**), infecting *Labeobarbus aeneus* from Wriggleswade Dam, which has not been previously reported in the Eastern Cape.

In **Chapter 4,** I assessed the effect that the alien gill monogenean *P. anguillae* was having on the native longfin eel *Anguilla mossambica*. The native host of *P. anguillae* is the Japanese eel *A. japonica* according to Christison & Baker (2007). According to Kennedy (2007), *P. anguillae* causes very little or no problems on wild eels, but can cause mortality and economic losses in an eel aquaculture system. In **Chapter 4,** I positively indentify *P. anguillae* on the gills of wild-caught *A. mossambica* from the Keiskamma and Kubusi Rivers. The damage caused to the gills of *A. mossambica* by *P. anguillae* was similar to the damage noted on the gills of the European eel *A. anguilla* (Kenndy 2007). This damage includes hyperplasia of the gill lamellae and fusion of the secondary gill lamellae (**Chapter 4**). This was also the first documentation of severe effects of *P. anguillae* on wild *A. mossambica*.

In **Chapter 5** the parasitological assessment of the *Myxus capensis* and *Mugil cephalus* revealed that these two fish species had no parasites. This was unusual, as members of the Mugilidae are host to a variety of parasites (Whitfield 2012). According to Whitfield (2012), there is generally an increase in the number of parasite species with an increase in the age of the mullet hosts. The total absence of parasites on the two mullet species was most likely because these fish had been in a freshwater impoundment for at least ten years (Ellender *et al*. 2012) which resulted in a lack of intermediate hosts. The two mullet species were collected from the Keiskamma estuary and released into Binfield Park Dam to enhance the fisheries potential (Ellender *et al*. 2012) and as a result the two mullet species may not have been suitable hosts to the indigenous freshwater parasites in this river stretch.

The parasitological assessment of *Labeo umbratus* in **Chapter 6** revealed the presence of the ecto-parasite *L. barnimiana*. *Lernaea barnimiana* is a pan African species indigenous to the African continent (Fryer 1968) and has previously been reported from Boskop Dam, North West Province (Viljoen 1986), and the Eastern Cape Province infecting *L. umbratus* from an area known as Chubu (van As & Basson 1984). In the July 2011 survey the parasite was not found. However, healed scars were noted on the fish from previous infections with *L. barnimiana*. In the March 2012 survey, samples were infected with *L. barnimiana*. However, the prevalence and intensity was very low. The low intensity of infection on *L. umbratus* did not cause any serious harm to the fish. The effect of *L. barnimiana* on *L. umbratus* was limited only to the attachment site, causing skin lesions.

Wriggleswade Dam has two species of well-documented alien parasite species, namely the Asian tapeworm *B. acheilognathi* and the anchor worm *L. cyprinacea*, both infecting the smallmouth yellowfish *L. aeneus* (**Chapter 7**). The prevalence and intensity of *B. acheilognathi* was very low in the *L. aeneus* population of Wriggleswade Dam. Low intensities of *B. acheilognathi* will have no or very little effect on the fish host (Brandt & Scoonbee 1980). However, severe infestations are known to cause fish mortalities (Brandt & Scoonbee 1980) by blocking the intestine, inflammation of the intestine, as well as perforation of the intestinal wall (Liao & Shih 1956; Hoffman 1980; Retief *et al.* 2007). *Bothriocephalus acheilognathi* has previously been reported in Glen Melville reservoir, Eastern Cape (Stadtlander *et al*. 2011). These authors noted that the parasite was present in the fish sampled in high prevalence's and intensity, but not present in the Great Fish River. *Bothriocephalus acheilognathi* has also been reported from the Vaal Dam, South Africa infecting the largemouth yellowfish *L. kimberleyensis* (Retief *et al*. 2007). Retief *et al.* (2007) reported that there was a 100% infection of *L. kimberleyensis* in the Vaal Dam and that there was no correlation between the intensity of infection and the size of the fish, indicating that intensity does not build up over the lifespan of the fish. According to Barkhuizen (1991), *B. acheilognathi* has been reported from rivers in Mpumalanga, Limpopo, Free State, KwaZulu-Natal and the Northern Cape Provinces. The alien anchor worm parasite *L. cyprinacea* was found infesting 100% of the *L. aeneus* samples from Wriggleswade Dam. In severe infections *L. cyprinacea* causes acute haemorrhage and ulcers at the site of penetration, which

have a negative effect on the host's functioning and survival (Bauer 1961; Paperna 1996) through blood loss and secondary infections (Putz & Bowen 1964). *Lernaea cyprinacea* has a cosmopolitan distribution in Africa (Viljoen 1986), but in South Africa it has only been previously reported from Hartbeespoort Dam in the North West Province (van As & Basson 1984; Viljoen 1986), the Lowveld Fisheries Station and the Limpopo River (van As & Basson 1984).

The findings of this thesis therefore demonstrate that alien parasites are present in the impoundments in the Amatola region. While the threat of these parasites to native fishes in the region has not been assessed, there is considerable risk of host-switching. Stadtlander *et al.* (2011) noted that the indigenous cyprinid fish species of Great Fish River are at risk because of *B. acheilognathi*'s ability to infect a wide variety of host fish species.

### 8.1.3. Fish health

A summary of the necropsy-based and histology-based fish health assessment scores for each fish species in each impoundment is represented in **Table 8.1**. In general the necropsy-based fish health assessment scores were higher than the scores represented by the histology-based fish health assessment index. The higher necropsy-based fish health assessment scores were often more as result of external mechanical damage caused by the presence parasites such as *L. barnimiana* species on *L. umbratus* in Sandile Dam (**Chapter 6**) and *L. cyprinacea* on *L. aeneus* and *M. salmoides* in Wriggleswade Dam (**Chapter 7**). The histology-based fish health assessment revealed that there were low scores for *M. salmoides* from Sandile and Wriggleswade Dams (**Chapter 3**), for *L. umbratus* from Sandile Dam (**Chapter 6**) and *L. aeneus* from Wriggleswade Dam (**Chapter 7**). The histology-based fish health assessment revealed high scores for *M. salmoides* in Binfield Park Dam, because of the alterations indentified in the liver tissue and the severe alterations observed in the liver and kidney tissues from the two mullet species (**Chapter 3**). The alterations identified in the gill tissue from *A. mossambica* from all three impoundments resulted from the damage caused by the alien parasite *P. anguillae*, resulting in the high histology fish health scores (**Chapter 4**).

**Table 8.1:** Summary of the necropsy-based and histology-based fish health assessment scores for each of the fish species used in this study from each of the impoundments.

| Impoundment | Species | FHAI | $I_{FISH}$ |
|---|---|---|---|
| Binfield Park Dam | *Micropterus salmoides* | 21.8 ± 20.4 | 19.1 ± 10.5 |
| Sandile Dam | *Micropterus salmoides* | 17.4 ± 18.8 | 11.8 ± 6.3 |
| Wriggleswade Dam | *Micropterus salmoides* | 26.0 ± 19.9 | 11.3 ± 5.3 |
| Binfield Park Dam | *Anguilla mossambica* | 15.0 ± 5.2 | 17.2 ± 9.1 |
| Sandile Dam | *Anguilla mossambica* | 15.0 ± 7.1 | 22.0 ± 4.24 |
| Wriggleswade Dam | *Anguilla mossambica* | 20.0 ± 15.8 | 21.8 ± 9.9 |
| Binfield Park Dam | *Myxus capensis* | 34.7 ± 22.9 | 47.9 ± 12.1 |
| Binfield Park Dam | *Mugil cephalus* | 29.5 ± 2.3 | 54.7 ± 9.7 |
| Sandile Dam | *Labeo umbratus* | 10.3 ± 11.9 | 11.4 ± 4.9 |
| Wriggleswade Dam | *Labeobarbus aeneus* | 59.0 ± 12.1 | 11.7 ± 5.1 |

The application of the necropsy-based fish health assessment allowed for the effects of the mechanical damage caused by the presence of the external parasites to be quantified. The advantage of the use of the necropsy-based fish health assessment is that it has now provided a base data set for the species used in this study for Binfield Park, Sandile and Wriggleswade Dams. The histology-based fish health assessment scores were in general higher for the fish species collected from Binfield Park Dam. The assessment of the metal concentrations between Binfield Park, Sandile and Wriggleswade Dams showed that Binfield Park Dam had the highest concentrations of metals in the surface water, as well as the highest concentrations of Hg in the muscle tissue of *M. salmoides*. These elevated metal concentrations may account for the increased histology-based fish health scores in *M. salmoides* in Binfield Park Dam. The histology-based fish health assessment also allowed for the

identification of the histological alterations to the gills caused by *P. anguillae* on wild-caught *A. mossambica.*

The continued use of necropsy-based and histology-based fish health assessment indexes will establish data-bases which would allow for the identification of trends in fish health over time, as well as allow for early detection of problems arising in the health of the fish, which in turn could allow for possible early remediation.

Some of the abnormities that were noted in this study were primarily as a result of the presence of alien parasite species, for example the alien gill monogenean *P. anguillae* infecting the gills of *A. mossambica* and the alien anchor worm *L. cyprinacea* attaching to the skin of *L. aeneus*. The use of both these assessment protocols allowed for the identification of the effects caused by these parasites to be observed and made quantifiable to show the effect on its host. The information produced in this study therefore contributes to addressing the knowledge gap on the impacts of introduced alien parasites in South Africa (Ellender & Weyl 2014).

## 8.2. Conclusion

This study, and previous research by Morrison *et al*. (2001), Awofolu & Fatoki (2003) and Fatoki & Awofolu (2003), indicated that there is water pollution in the Keiskamma, Tyume and Kubusi Rivers. Although the surface water heavy metal concentrations were below the South African water quality guideline levels, Cd, Hg, Zn and U are still present in the sediment, and high concentrations of Hg occur in the muscle tissue of *M. salmoides* from Binfield Park Dam. The reports of the non-functioning Keiskammahoek Waste Water Treatment Plant being a point source of pollution into the Keiskamma River are also a cause for concern because of the high levels of pollutants being discharged (Morrison *et al*. 2001). Therefore, there is a threat of water pollution in the Keiskamma and Kubusi Rivers.

The second threat identified in the Amatola Region was that of alien parasites. Parker *et al.* (2011) noted the presence of the alien gill monogenean *P. anguillae* in the Great Fish River, and the present study showed its further spread into the Keiskamma and the Great Kei Rivers. Stadtlander *et al*. (2011) also showed the

potential threat of *B. acheilognathi* in Glen Melville Reservoir, and this study showed that it is now also present in the Great Kei River, and also that the alien anchor worm *L. cyprinacea* is also present in this system. Therefore alien parasites do pose a potential threat to the freshwater fish biodiversity in these systems. Although *P. anguillae* is a specialist eel parasite (Christison & Baker 2007), it can only affect members of the Anguillidae family. However, the Keiskamma and Great Kei Rivers are home to three native eels species, namely *A. mossambica, A. bicolor bicolor* and *A. marmorata*. Therefore *P. anguillae* is a conservation threat to the indigenous eels of the Amatola region. The alien anchor worm *L. cyprinacea* is a potential threat to native fish species in the Amatola region, particularly to the small cyprinids such as *Barbus trevelyani* and *B. anoplus*. This largely due to its low host specificity and its ability to infect a wide variety of freshwater fish families such as the Amiidae, Catostomidae, Cyprinidae, Salmonidae, Sciaenidae and Umbridae (Hoffman 1976; Robinson & Avenanant-Oldewage 1996). Shariff & Roberts (1989) reported that, in small fish, the anchor of the parasite would penetrate into the internal organs of the host. In these cases, the smaller fish would die within a week of penetration. The Asian tapeworm *B. acheilognathi* has already been identified by Statlander *et al*. (2011) as a potential to threat to the indigenous fish species, because *B. acheilognathi* is able to infect a wide variety of fish species, particularly members of the Cyprinidae family. Native fish species generally show a low resistance to infection by alien parasite species (Sommerville 2009), and therefore native fish species are at risk from alien parasites.

Based on all the above information provided by this study, it is clear that the necropsy-based and histology-based fish health assessment protocols can successfully be implemented as tools to assess the effects of heavy metal pollution and alien fish parasites in freshwater fish from selected impoundments in the Amatola region, Eastern Cape Province, South Africa. Thus the original hypothesis of this thesis is accepted.

### 8.3. Recommendations and future research

Based on work done in this research, the following gaps have been identified. Further research in these areas would be beneficial, and help contribute to better understand the health of the fishes of this region. Potential studies should be established.

Due to the high levels of mercury indentified in the muscle tissue of *M. salmoides* from Binfield Park Dam in **Chapter 3**, a human health assessment and edibility study should be conducted in order to determine if the fish from Binfield Park Dam are safe for human consumption.

Awofolu & Fatoki (2003) identified the presence of persistent organic pesticides in the surface water and the sediment from the Keiskamma and Tyume Rivers. Further research is required to determine the source of these pesticides, as well as what effect they are having on the freshwater biodiversity in these rivers, particularly on the health of the indigenous fish species.

Gill monogeneans were observed on the gills of *M. salmoides* from Binfield Park, Sandile and Wriggleswade Dams and on the gills of *L. umbratus* from Sandile Dam, as well as on the gills of *L. aeneus* from Wriggleswade Dam. These gill monogeneans were not identified, and further research is required to identify whether these are known species, species new to science, or even alien species.

Stadtlander *et al*. (2011) noted that the Asian tapeworm *B. acheilognathi* may be a potential threat to the indigenous fish species of the Amatola region. However, further research is required on the small cyprinid fish species of the Amatola region such as *B. trevelyani* and *B. anoplus* to see what their natural parasite diversity is, as well as to determine if alien parasites have made them suitable hosts, and what effects these alien parasites are having on them.

In order for the Freshwater Ecosystem Protected Areas system to be successful, the threats to freshwater biodiversity must be known. The aim of the FEPA is to protect 15% of all river lengths in order to protect all their critically endangered, endangered

and vulnerable fish species. This study showed that, in the FEPA Amatola Region of the Eastern Cape, which is home to the critically endangered *B. trevelyani* and the endangered the Eastern Cape rocky *Sandelia bainsii*, numerous threats are already present. This study highlighted two of the threats as being heavy metal water pollution and alien parasites such as *P. anguillae, L. cyprinacea* and *B. acheilognathi*.

In order to conserve South Africa's freshwater fish biodiversity, country-wide surveys of indigenous fish species must be undertaken, so that their health and parasite diversity can be evaluated.

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