

5.1 Introduction

5.1.1 Economically important freshwater fish species

Two indigenous cichlid species, namely the Mozambique tilapia Oreochromis mossambicus (Peters, 1852) and the redbreast tilapia, Coptodon (Tilapia) rendalli (Boulenger, 1896), are highly valued and extensively utilized target species of subsistence fishers in the Phongolo floodplain region of South Africa. In addition, O. mossambicus, was recently added to the Threatened or Protected Species list (TOPS) (NEMBA, 2013) mainly because of the threat posed to the genetic integrity of the species caused by potential for hybridization with the alien cichlid Oreochromis niloticus (van der Bank & Deacon, 2007). Another species of economic importance from this region is the tigerfish, Hydrocynus vittatus, one of South Africa's most coveted angling fish species (Van Loggerenberg, 1983; Smit et al., 2009). However, the numbers of this species has declined over the past decades not only due to their limited distribution, but as result of pollution, weirs, and over exploitation (Van Loggerenberg, 1983; Steyn et al., 1996; Skelton, 2001; Smit et al., 2009). The economic importance of the latter species is further emphasized through the Tigerfish Bonanza fishing compitition which is held annually at Pongolapoort Dam. This competition attracts recreational anglers from across South Africa and earn millions of rands in revenue for the local community. Recreational angling has proved to be a valuable activity that generates considerable income to local and national economies (Cooke & Suski, 2005).

Due to the above-mentioned social, economic and environmental importance of these indigenous fish species, the aim of this section of the study was to assess the health status of these three species (*H. vittatus, O. mossambicus* and *C. rendalli*) in the protected part of the Phongolo Floodplain, the Ndumo Game Reserve. Nyamiti Pan is a perennial pan located in the eastern section of the Ndumo Game Reserve and when full, the outflow decants into the Phongolo River (Figure 1). The pan receives water from a relatively small catchment and the outflow is controlled by a small weir.

A review of literature has shown that the role of this protected area as refugia for aquatic biodiversity has never been established, nor has the health status of the species within this area been determined.

5.1.2 Population structure

In order to accurately determine the population structure, extensive sampling has to be done to ensure that the sample represents the entire fish population. In other words, the sampling methods should enable the researcher to catch representative specimens of each size class of that population. The value of the knowledge generated through studying the population structure of fish has been discussed in Chapter 1, therefore the following section of this chapter will focus on the value of age determination and age structures of populations.

5.1.2.1 Age determination

Over the past decades scientists have used a variety of methods to study annuli deposited in calcified structures of fish. These structures include scales, opercular covers, spines, fin rays, vertebrae and otoliths (Secor et al., 1992; Wischniowski & Bobko, 1998; Campana, 2001). Age determination of fish involves an assessment of the age of individual animals by enumerating the daily, seasonal, or annual variations in growth in their calcified structures (Paxton & King, 2009). Otoliths are the preferred method for ageing since these are usually the first calcified structures to be laid down during the early life stages and are also believed to reflect growth more accurately than other structures (Campana & Neilson, 1985). Otoliths are the most common and accurate structures used for age determination of various species, since they contain the best permanent pattern of fish growth, as they are internal and are not exposed to harsh external conditions (Wischniowski & Bobko, 1998). The application of otolith studies revolutionized our understanding of fish populations, their integrity and management. The chronological properties of otoliths are unparalleled in the animal world, allowing accurate estimates of age and growth at both daily and yearly levels (Campana & Thorrold, 2001). The determination of age aids in understanding the biology and ecology of any given species. The knowledge generated from otoliths yields valuable information on growth, recruitment, mortality rates and response to environmental factors such as change in water temperature and food availability (Campana & Thorrold, 2001; Wischniowski & Bobko, 1998; Paxton & King, 2009).

There are two methods for validating the age of fishes. These can be either direct or indirect validation (Wischniowski & Bobko, 1998). Direct validation involves rearing known-age larvae, but this can be difficult for species such as *H. vittatus*, as a study done by Van Loggerenberg (1983) showed that female *H. vittatus* do not become sexually mature in captivity. A second direct validation is by chemically marking the otolith with chemical compounds (Oxytretracyline hydrochloride) which is incorporated into the calcium matrix of the bony structures

(Wischniowski & Bobko, 1998). This serves as a time reference that can be investigated at a later stage. This technique involves externally tagging the fish with dart-tags and recapturing them after a minimum period of one year. When these fish are recaptured, the structures such as otoliths are removed and the number of annular rings formed after the chemically marked ring is counted and related to time (Wischniowski & Bobko, 1998). The latter method is considered to be the traditional validation method, but it requires a large sample size and the effectiveness is dependent on the recapture rate.

Indirect validation is based on the estimation of age by measuring the frequency of occurrence of various parameters, of which length-frequency is the most common (Wischniowski & Bobko, 1998). This method is accurate during rapid growth of especially juvenile fish and becomes less accurate at maturity and/or slow growth rates. In this study no validation of the age determination was carried out due to time constraints; however, the ageing section of this study determined process errors in the form of errors in precision and accuracy due to the lack of validation (Campana, 1999; Gerber *et al.*, 2009).

Otoliths play an important function in the balance of fish as well as hearing. Fish have three pairs of otoliths located on each side of the head opposite to each other (bilateral symmetry), posterior of the cranium (Secor *et al.*, 1992) (Figure 5.1 A). The sagittae, the largest pair, are situated in close proximity to the pars inferior and are medial and ventral to the asterisci, the third pair (Secor *et al.*, 1992) (Figure 5.1 B). The lapilli are situated anterior of the pars superior (Secor *et al.*, 1992) (Figure 5.1 B).

The deposition of the patterns in otoliths is a result of fast summer growth and slow winter growth, and they are directly equivalent to seasonal changes (Wischniowski & Bobko, 1998; Campana & Thorrold, 2001). The rings formed are mainly differentiated by the amount of material deposited during summer or winter. This forms wide translucent rings during summer followed by thinner opaque rings during winter. The combination of one translucent ring and one opaque ring is referred to as an annulus and represents one year's growth (Wischniowski & Bobko, 1998).

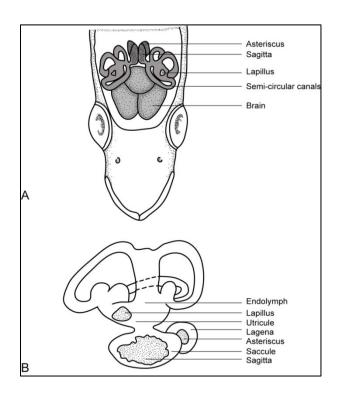


Figure 5.1: A) Dorsal view of the vestibular apparatus of a typical teleost where the dorsal part of the head is cut away, B) Anatomy of the vestibular apparatus (Adapted from Secor et al., 1992).

The discovery of daily growth increments (Panella, 1971) led to the determination of hatch dates and to relating these parameters to environmental variables. Daily growth increments result from the differential deposition of calcium carbonate (in the aragonite 2 configuration) and a protein (otolin) over a twenty-four hour period, resulting in a sequence of contrasting opaque and hyaline bands through the otolith (Paxton & King, 2009). Campana and Nielson (1985) suggested that the formation of daily growth increments is attributed to an endocrine-driven endogenous circadian rhythm that is entrained by photoperiod. The rate of increment deposition is species-specific (Jones, 1986) and there are many variables influencing the rate of deposition, such as temperature, feeding patterns and environmental factors (Brothers *et al.*, 1976; Paxton & King, 2009).

5.1.3 Fish health

The health of fish in this study was determined using a macroscopic assessment of health by applying the Fish Health Assessment Index (FHAI) (Adams *et al.*, 1993) and a microscopic histology-based health assessment (Zimmerli *et al.*, 2007). A combination of these two assessments was used to strengthen the determination of the health of the selected species.

The health of organisms reflects the biotic integrity of an ecological system (Adams *et al.*, 1993). Because of fishes' position in the aquatic food chain they are generally regarded as representative of the overall system (Adams *et al.*, 1993). Schmitt & Dethloff (2000) discovered that contaminants bio-accumulate in aquatic organisms. This implies that higher up in the food chain the contaminants will increase due to bio-magnification. In the Phongolo Floodplain concerns have been raised regarding the accumulation of DDT in fish species such as *H. vittatus* and *O. mossambicus* (Bouwman & Coetzee, 1990; McHugh *et al.*, 2011).

Fish are subject to a variety of stressors in their natural environment. These stressors can be either natural occurrences such as temperature fluctuation, predation, food availability, habitat availability and other episodic variables or it can be anthropogenic stressors such as pollution and water extraction (Adams *et al.*, 1993). When fish are challenged by these stressors, valuable physiological energy is diverted to deal with the stress, ultimately influencing growth and reproduction negatively (Barton & Schreck, 1987; Wedemeyer *et al.*, 1990; Adams *et al.*, 1993).

Adams *et al.* (1993) developed a quantitative fish health assessment index which alowed the comparison of fish from differrent systems. This index is a simple and inexpensive, rapid assessment of the health of fish populations in the wild (Adams *et al.*, 1993). Numerical values are assigned to particular alterations and the severity thereof. This index follows the necropsy approach set out by Goede and Barton (1990), which includes the following set of variables: (1) three blood parameters (haematocrit, leokocrit and plasma protein), (2) length, weight and condition factors, (3) fish with normal or abnormal eyes, gills, spleens, kidneys, pseudobranchs and livers (expressed in %), and (4) index values of damaged fins, skin, thymus, hindgut inflammation, fat deposits and the colour of the bile (Adams *et al.*, 1993). When zero abnormalities or alterations are observed, a score of 0 is assigned, a score of 10 is assigned to few alterations, a score of 20 is assigned if moderate alterations are noted and a score of 30 is assigned when severe alterations are noted. The analysis and evaluation of the data created by the variables mentioned involves a summary of the means for the variables in Group 4, a summary of the percentage of normal in Group 3, means and variances of the blood parameters in Group 1 and the length weight (condition factor) data in Group 2 (Adams *et al.*, 1993).

Adams et al. (1993) described the purpose of the fish health assessment index as the detection of changes in the health of fish populations early enough to take remedial action. Once a trend

is indicated by the health of individuals of a population, more detailed procedures should be applied to identify the problem (Adams *et al.*, 1993).

The histological assessment of selected organs can indicate the impacts of various environmental stressors such as pathogens, toxic compounds and even temperature fluctuations (Zimmerli et al., 2007). Because histology provides a rapid method of detecting the effects of irritants in various tissues and organs, Zimmerli et al. (2007) developed a classification system based on the work done by Bernet et al. (1999). This system allows for a semi-quantitative assessment of histological alterations in tissue structures and is based on pathological changes grouped into five different reaction patterns: (1) circulatory disturbances, (2) regressive changes, (3) progressive changes, (4) inflammation and (5) neoplasms. The next step in this assessment method was to calculate an index for each of the following organs, kidney, liver, gill, gonads. The indices are scored according to the severity of the histological alterations in order to convert morphological change into a quantitative value. These indices are then used to calculate the overall fish index, which can be compared to the classification table set out by Zimmerli et al. (2007).

5.2 Materials and methods

Data collected from a series of five surveys represented in Table 2.14 were used for the assessment of the various indices. Otoliths were collected during all the surveys for age determination. The fish collected in the September 2013 survey were used for the macroscopic health assessment (FHAI) and the microscopic histology based health assessment. The fish collected during the April 2014 survey were used to do the staging of the gonads in order to determine the sexual maturity based on gonadal development.

5.2.1 Ageing determination

Two different sets of otoliths were collected for the different species. Gerber *et al.* (2009) determined that the lapillus otoliths were the most accurate structures to age *H. vittatus*, and Weyl & Hecht (1998) used the sagittal otoliths of *O. mossambicus* and *C. rendalli*, thus the lapillus otoliths were collected from *H. vittatus* and sagittal otoliths from the two cichlid species.

Each fish was measured to the nearest 1 mm (SL), photographed and weighed and then dissected to remove the otoliths. Following removal, each otolith was carefully dried with tissue paper and transported to the lab. The left otoliths of each fish were baked on a hot plate at

150 °C (Figure 5.2 B) for three minutes on each side until they turned light brown (Figure 5.2 C). This enhances the opaque rings and makes the annular rings more visible under the light microscope (Wischniowski & Bobko, 1998; Weyl & Hecht, 1998). If the left otolith was damaged, the right otolith was used. Once the otoliths had cooled they were glued onto resin bases prepared beforehand using moulds, as described in Secor *et al.* (1992) (Figure 5.2 D). After all the otoliths had been mounted, a second layer of resin was poured over them (Figure 5.2 D). The moulds were then placed in a vacuum chamber and left to dry for 24 hours. After the resin had solidified, the otoliths were marked for sectioning with the otolith saw (Figure 5.2 E). A thin section was cut into the mounted otolith using a double-blade diamond-edged otolith saw. Cut sections were then mounted on microscope slides using DPX. This also enhances the clarity of the otoliths when viewed under a light microscope (Gerber et al., 2009a). A growth ring was considered to be valid when it consisted of an opaque and translucent zone that was continuous and could be followed around the entire structure of the otolith. If not, the growth ring was considered as a false ring (Gerber *et al.*, 2012).

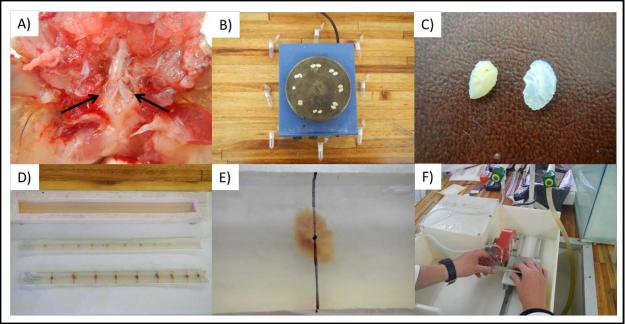


Figure 5.2: Otolith preparation and sectioning: A) removal of otoliths, B) baking otoliths, C) difference in colour of a baked otolith (left) compared to non-baked (right), D) embedding the otoliths in resin, E) marking of embedded otolith for the sawing process, F) sectioning the otolith with an otolith saw.

5.2.1.1 Data analysis

The annuli of each otolith were counted twice by two individual readers. The first readings were recorded and were repeated one week later. Readers worked independently and did not consult their previous count in order to reduce bias. Indices of bias and precision were calculated. The precision of all the replicate age estimates was assessed by using the Average Percentage Error (APE) (Beamish & Fournier, 1981) as well as the Coefficient of Variance (CV) (Campana, 2001). No process of elimination was followed when readings differed by more than one or two annuli. By convention a hatching date of 1 January was assigned to the fish species as described by Wischniowski & Bobko (1998).

A single relative age was assigned to each otolith and was derived from the mode of the four replicate readings. This implies that the most frequent reading was assigned to that otolith. If the mode could not be determined, the average of the four counts was taken. The data of the annual rings were fitted to the 3-parameter von Bertalanffy Growth Models (VBGM) in order to generate length-at-age curves. The VBGM was fitted to males and females separately and to both sexes combined.

5.2.2 Fish Health Assessment Index (FHAI)

Captured fish were transported to a field laboratory. Each specimen was weighed with a Berkley digital lip-grip scale to the nearest gram and the standard and total length measured to the nearest centimetre. The standard length and mass data were used calculate the condition factor (Carlander, 1969). Blood was drawn using 4 ml heparinized vacutainers, centrifuged at 3 000 r/min for 10 minutes, the plasma transferred to 2 ml cryo-tubes and stored in liquid nitrogen for the calculation of the total blood protein levels as part of the Adams *et al.* (1993) protocol. Fish were sacrificed by severing the spinal cord and a complete fish necropsy was done following the FHAI protocol (Adams *et al.* 1993). The health of fish populations from the Nyamiti Pan (this study) are expressed according to the FHAI formula used by Adams *et al.* (1993) (Table 5.5).

During the health assessment, parasites were counted and subsequently removed. Both the ecto- and endo-parasites were preserved in 70% ethanol for identification purposes.

5.2.2.1 Data analysis

The mean mass and standard length as well as the Condition Factor (CF), Hepato-somatic Index (HSI), Gonado-somatic Index (GSI), Spleeno-somatic Index (SSI) data were all tested for significance (p < 0.05) using one-way ANOVA (IBM SPSS ver. 22). If significant, the Tukey HSD test was used. Tukey's honest significant differences (HSD) test was applied where differences were significant and if not, the Dunnett T3 test was used.

5.2.3 Histology-based health assessment

Following the macroscopic health assessment, gill, liver and kidney samples were collected and fixed in 10% neutrally buffered formalin for 48 hours. Gonad samples were fixed in Bouin's solution for 24 hours. After fixation these samples were rinsed in distilled water and dehydrated in a series of increasing ethanol concentrations. The concentrations were changed every hour from 30% to 50% and finally to 70%. Tissue samples were prepared using the standard histological preparation techniques described by Humason (1962).

5.2.3.1 Histological analysis

Prepared slides from the different species were viewed at x10, x20 and x40 magnifications on a Nikon Eclipse 80i light microscope. The semi-quantitative histological assessment protocol proposed by Van Dyk *et al.* (2009) was used to quantify the histological alterations observed in the selected organs. The assessments of these histological alterations were done according to five reaction patterns, namely Circulatory Disturbances (CD), Regressive Changes (RC), Progressive Changes (PC), Tumours (T) and Inflammation (I). The results of this assessment were used to calculate the following indices; Gill index, Liver index, Kidney index, Testis index and/or Ovary index. The values of these indices were used to classify the severity of histological responses using the classification system set out by Van Dyk *et al.* (2009), which is based on the scoring system by Zimmerli *et al.* (2007).

Table 5.1: Histological criteria used to classify the severity of histological responses.

Classes	Description
Class 1 (Index < 10)	Normal tissue structure with slight histological alterations
Class 2 (Index 10-25)	Normal tissue structure with slight histological alterations
Class 3 (Index 26-35)	Pronounced alterations of organ tissue
Class 4 (Index > 35)	Severe alterations of organ tissue

5.2.3.2 Gonad development

The development stages to determine sexual maturity of testes and ovaries of the different species were determined by the histological assessment of the gonads using the criteria set out by Schmitt & Dethloff (2000) (Table 5.2).

Table 5.2: Histological criteria used in gonad staging of male and female Coptodon rendalli, Oreochromis mossambicus and Hydrocynus vittatus (Adapted from Schmitt & Dethloff, 2000).

Stage	Testis characteristics	Ovary characteristics
0	Undeveloped (immature): Little or no spermatogenic activity in germinal epithelium; immature states of spermatogenesis (largely spermatocytes); no spermatozoa observed.	Undeveloped: Pre-vitellogenic oocytes observed exclusively; oocyte diameter < 250 µm: cytoplasm stains basophilic with H&E.
1	Early spermatogenic (mature): Mostly thin germinal epithelium with scattered spermatogenic activity; spermatocytes to spermatids predominate; few spermatozoa observed.	Early development: > 90% of oocytes pre-vitellogenic, remaining oocytes early to mid-vitellogenic; oocytes slightly larger (up to 300 μm); late perinucleolus through cortical alveolar stages.
2	Mid-spermatogenic: Germinal epithelia of moderate thickness; moderate proliferation and maturation of spermatozoa and equal mix of spermatocytes, spermatids and spermatozoa present.	Mid-development: Majority of observed follicles early and midvitellogenic; oocytes larger 300–600 μm diameter, and containing peripheral yolk vesicles; globular and uniformly thick chorion (5–10 μm in black basses, 10–20 μm in common carp); cytoplasm basophilic, yolk globules eosinophilic.
3	Late spermatogenic: Thick germinal epithelium; diffuse regions of proliferation and maturation of spermatozoa; all stages of development represented, spermatozoa predominate.	Late development: Majority of developing follicles late vitellogenic; oocyte diameter 600–1000 μm, eosinophilic yolk globules distributed throughout the cytoplasm; chorion thickness 10–30 μm in black basses, 40–50 μm in common carp.
4	N/A	Late development/hydrated: Majority of developing follicles late vitellogenic; follicles much larger (> 1000 µm).
5	N/A	Post ovulatory: Spent follicles, remnants of the theca externa and granulosa present.

5.2.3.3 Data analysis

The data were tested for significance (p < 0.05.) using one-way ANOVA (IBM SPSS ver. 22). If significant, the Tukey HSD test was used. Tukey's honest significant differences (HSD) test was applied where differences were significant and if not the Dunnett T3 test was used.

5.3 Results

5.3.1 Age determination and population structure

Figure 5.3 shows the growth zone deviation between the different readings of the otoliths for each species. Of the 136 *H. vittatus* otoliths assessed, 31 did not deviate from one another, 52 deviated by one growth zone and four deviated by 2 growth zones. Of the 139 *O. mossambicus* otoliths assessed, 51 individuals did not deviate from one another, 40 deviated by one growth zone, 7 deviated by 2 growth zones, one by three growth zones and one by four growth zones. Of the 111 *C. rendalli* otoliths assessed, 41 did not deviate from one another, 57 deviated by one growth zone and 8 deviated by two growth zones.

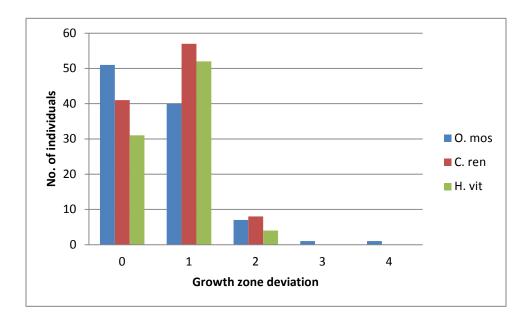


Figure 5.3: The deviation in age during the four counts for the different species used.

Precision of the replicate age estimation for the different species showed very little difference. The APE for *H. vittatus* was 10% and the coefficient of variance 13% (See Table 5.3). The APE for *C. rendalli* was 8% and the coefficient of variance 10.1% (See Table 5.3). The APE for *O. mossambicus* was 10.6% and the coefficient of variance 13.4% (See Table 5.3).

Table 5.3: Average percentage error (APE) and Coefficients of Variation (CV) of recorded age for different species (n = number of samples).

Species	n	APE	CV
H. vit	136	10.01%	13.06%
C. ren	111	7.99%	10.11%
O. mos	139	10.60%	13.39%

5.3.1.1 Hydrocynus vittatus

A total of 166 *H. vittatus* were caught during five surveys from 2012 to 2014 to determine the population structure of this species in Nyamiti Pan (Figure 5.8). The otoliths of 135 *H. vittatus* were successfully assessed for age determination. Figure 5.4 illustrates an example of a sectioned lapillus otolith from a four-year-old *H. vittatus* specimen.

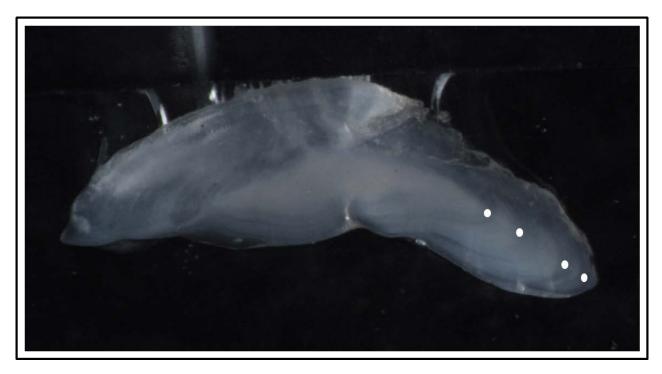


Figure 5.4: Micrograph of a sectioned lapillus otolith from a four-year-old *Hydrocynus vittatus* specimen.

The von Bertalanffy growth model indicates that females grow faster and larger than males. The largest *H. vittatus* female recorded during this study was 530 mm (SL) (Figure 5.6) compared to the largest male of 430 mm (SL) (Figure 5.7). Male and female *H. vittatus* otoliths were assigned a maximum relative age of six years (Figure 5.5).

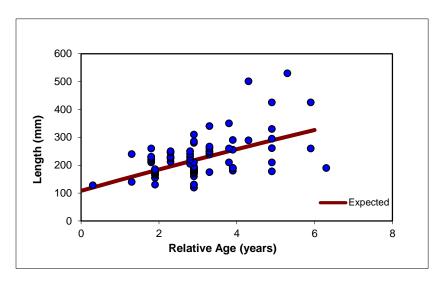


Figure 5.5: Size at relative age data of *Hydrocynus vittatus*, males and females combined, obtained from sectioned otoliths (n = 136). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

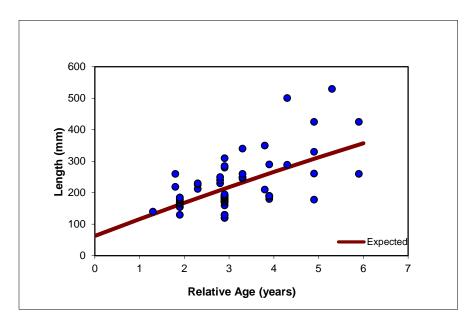


Figure 5.6: Size at relative age data of *Hydrocynus vittatus* females obtained from sectioned otoliths (n = 67). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

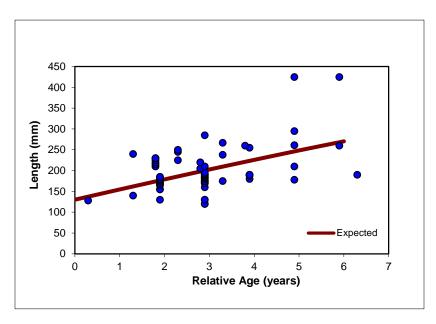


Figure 5.7: Size at relative age data of *Hydrocynus vittatus* males obtained from sectioned otoliths (n = 68). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

The majority of *H. vittatus* caught in Nyamiti Pan during 2012 to 2014 were within the 210–255 mm length class. These fish were estimated to be three years old. The second dominant class is 155–170 mm fish, estimated to be two years old (Figure 5.8).

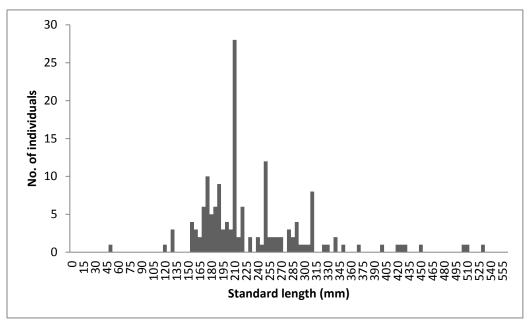


Figure 5.8: Population structure of all the *Hydrocynus vittatus* caught during 2012 to 2014 (n = 166).

5.3.1.2 Oreochromis mossambicus

A total of 383 *O. mossambicus* were caught during five surveys from 2012 to 2014 to determine the population structure of this species in Nyamiti Pan (Figure 5.13). The otoliths of 139 *O. mossambicus* were successfully assessed for age determination and comprised 61 females and 78 males. Figure 5.9 illustrates an example of a sectioned sagittal otolith from a 7-year-old *O. mossambicus* specimen.

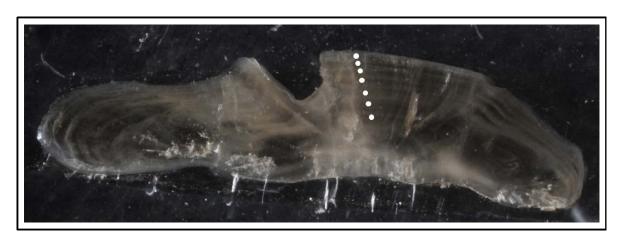


Figure 5.9: Micrograph of a sectioned sagittal otolith from a 7-year-old *Oreochromis mossambicus*.

The von Bertalanffy growth model indicates similar growth patterns for females and males, but males were larger than females. The biggest female recorded during this study was 290 mm (SL) (Figure 5.11) compared to the largest male of 390 mm (SL) (Figure 5.12). Male and female O. mossambicus otoliths were assigned a maximum relative age of 10 years (Figure 5.10).

The majority of *O. mossambicus* caught in Nyamiti Pan during 2012 to 2014 were within the 260–280 mm (SL) length classes (Figure 5.13). These fish were estimated to be four to six years old. The second dominant classes were 300–310 mm (SL) fish, estimated to be between 8 and 10 years old (Figure 5.13). Of the 383 *O. mossambicus* caught at Nyamiti Pan, 79% (n = 302) were adults.

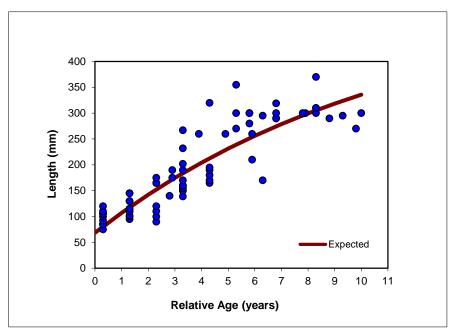


Figure 5.10: Size at relative age data of *Oreochromis mossambicus*, males and females combined, obtained from sectioned otoliths (n = 139). The solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

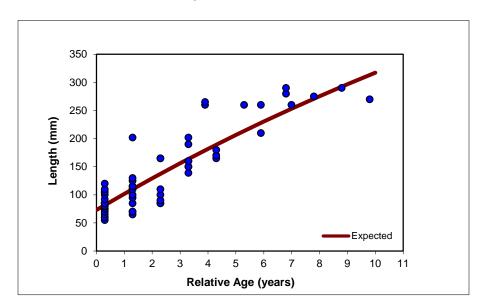


Figure 5.11: Size at relative age data of *Oreochromis mossambicus*, females obtained from sectioned otoliths (n = 61). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

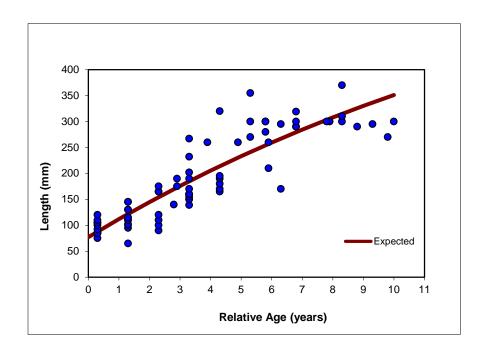


Figure 5.12: Size at relative age data of *Oreochromis mossambicus* males, obtained from sectioned otoliths (n = 78). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

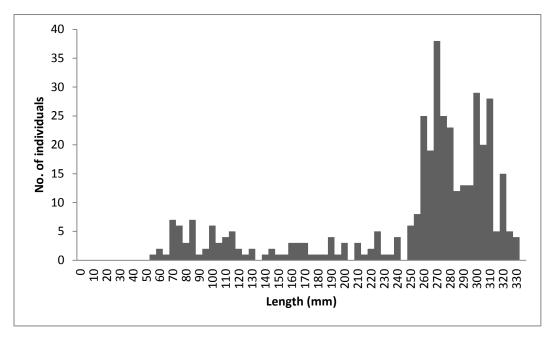


Figure 5.13: Population structure of all the *Oreochromis mossambicus* caught during 2012 to 2014 (n = 383).

5.3.1.3 Coptodon rendalli

A total of 345 *C. rendalli* were caught during five surveys from 2012 to 2014 to determine the population structure of this species in Nyamiti Pan (Figure 5.18). The otoliths of 139 *C. rendalli* were successfully assessed for age determination. Figure 5.14 illustrates an example of a sectioned sagittal otolith from a 9 year old *C. rendalli* specimen.

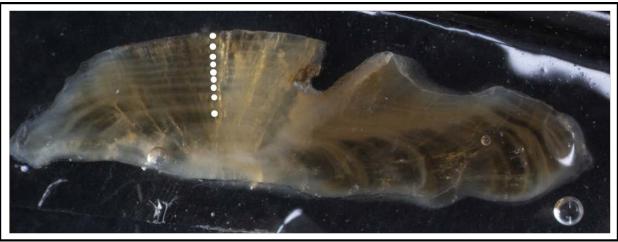


Figure 5.14: Micrograph of a sectioned sagittal otolith from a 9-year-old Coptodon rendalli.

The von Bertalanffy growth model indicates that males grow faster and larger than females. The biggest female recorded during this study was 290 mm (SL) (Figure 5.16) compared to the largest male of 315 mm (SL) (Figure 5.17). Female *C. rendalli* otoliths were assigned a maximum of 8 years compared to the male otoliths, which were assigned a maximum of 9 years.

Most of the *C. rendalli* caught in Nyamiti Pan during 2012 to 2014 were within the 250–260 mm (SL) length classes. These fish are estimated to be about five years old. The second dominant classes are 265–280 mm (SL) fish, estimated to be between 6 and 7 years old. Of the 345 *C. rendalli* caught at Nyamiti Pan, 91% of these individuals were adults (Figure 5.17).

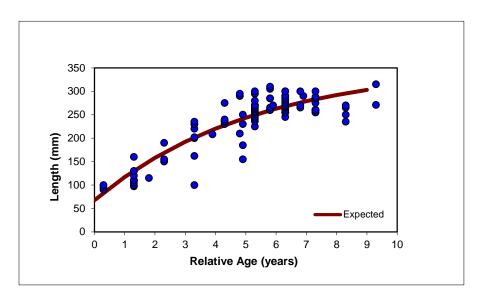


Figure 5.15: Size at relative age data of *Coptodon rendalli*, males and females combined, obtained from sectioned otoliths (n = 111). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

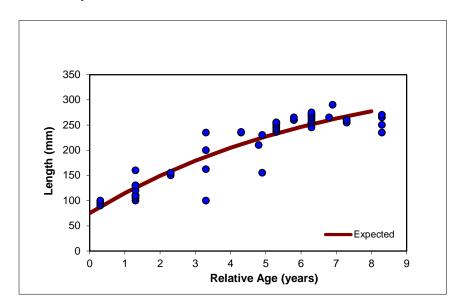


Figure 5.16: Size at relative age data of *Coptodon rendalli* females obtained from sectioned otoliths (n = 55). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

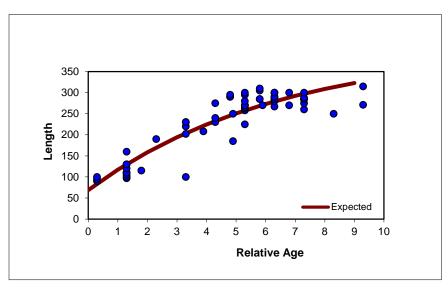


Figure 5.17: Size at relative age data of *Coptodon rendalli* males obtained from sectioned otoliths (n = 66). Solid line indicates the von Bertalanffy growth models fitted to the data. Parameters are provided in Table 5.4.

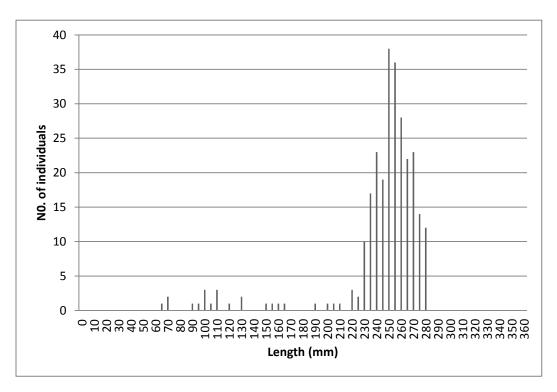


Figure 5.18: Population structure of all the Coptodon rendalli caught during 2012 to 2014.

Table 5.4: Parameters for the von Bertalanffy growth curves for each species used (n = number of samples; $L^{\infty} = L$ infinity; k = growth rate; $t_0 = function of the age of the fish).$

			L _∞		k		t _o	
Species	n	sex	mean	sd	mean	sd	mean	sd
H. vit	136	combined	1590.0	0.0002	0.027	0.004	-2.66	0.70
	67	f	1590.0	0.0008	0.036	0.005	-1.12	0.51
	68	m	1275.0	0.0005	0.022	0.004	-4.94	1.43
O. mos	139	combined	523.0	185.3	0.089	0.047	-1.58	0.34
	61	f	857.0	1360.3	0.037	0.072	-2.38	0.78
	78	m	716.7	454.5	0.056	0.047	-2.04	0.48
C. ren	111	combined	356.0	30.1	0.189	0.037	-1.12	0.20
	55	f	370.1	62.7	0.145	0.050	-1.58	0.37
	66	m	407.5	60.5	0.154	0.045	-1.20	0.25

5.3.2 Fish Health Assessment Index

During the April 2013 survey (High flow), data was collected in order to determine the general indices such as condition factor, hepato- spleno- and gonado-somatic indices. On return during the September 2013 survey (Low flow), an in-depth health assessment was carried out according to Adams *et al.* (1993) (Table 5.5).

5.3.2.1 April 2013:

The *H. vittatus* collected during the April 2013 survey had a condition factor of $K = 3.69 \pm 0.39$ (Table 5.5). The HSI value for *H. vittatus* was 0.64 ± 0.17 (Table 5.5). The GSI and SSI values were 0.30 ± 0.22 and 0.03 ± 0.02 , respectively (Table 5.5). *Oreochromis mossambicus* collected from Nyamiti Pan had a low CF ($K = 3.74 \pm 0.40$) compared to *C. rendalli* ($K = 4.45 \pm 0.59$) (Table 5.5). The HSI values of both species collected from Nyamiti Pan were low. The gonadosomatic index value for *O. mossambicus* was low. Spleno-somatic indices for most of the species caught during the April survey were high (Table 5.5).

5.3.2.2 September 2013:

The *O. mossambicus* collected from Nyamiti Pan were significantly larger (p < 0.05) than those collected during April 2013. However, the CF (1.94 \pm 0.19) of September 2013 was significantly lower (p < 0.05) than that of the April 2013 survey (Table 5.5). The *C. rendalli* specimens collected from Nyamiti Pan were slightly bigger than in the survey of April 2013 but their CF was also lower (3.85 \pm 0.25) than in the previous survey (Table 5.5). Hepato-somatic index values of

the *O. mossambicus* from Nyamiti Pan were slightly higher, but were not significantly different (p > 0.05) compared to the previous survey (Table 5.5). The *C. rendalli* from Nyamiti Pan, on the other hand, showed a significant difference (p < 0.05) in HSI compared to the April 2013 survey. *Oreochromis mossambicus* had an increase in the GSI value. *Coptodon rendalli* had a significant (p < 0.05) increase in GSI values compared to April 2013 (Table 5.5).

Health Assessment Index scores were calculated for both species and *O. mossambicus* from Nyamiti Pan had an average score of HAI score; 111 \pm 10 (Table 5.5). *Oreochromis mossambicus* from Nyamiti Pan were heavily infected with both external and internal parasites. The external parasite, *Lernaea cyprinacea*, had a prevalence of 100% and parasitemia ranged from 1–109, while the internal parasites (nematodes, *Contracaecum* sp.) had a prevalence of 52.4% and parasitemia ranged from 1–13. The *Coptodon rendalli* (HAI score; 39.5 \pm 10) caught in Nyamiti Pan were in a better condition than the *O. mossambicus* found in the pan, and very few parasites were found on this species (Table 5.5). The *H. vittatus* caught during September 2013 (low flow) were significantly smaller (p < 0.05) than those found during the April 2013 survey (high flow). The condition factor of *H. vittatus* dropped from K = 3.69 \pm 0.39 in April 2013 to K = 1.45 \pm 0.23 in September 2013 (Table 5.5). The HAI score of *H. vittatus* was 42.4 \pm 16.8 and ranged from 10 to 80 (Table 5.5). *Hydrocynus vittatus* were also infected with internal nematodes (*Contracaecum* sp.) in the liver, with a prevalence of 76%, ranging from few parasites to severely infected.

Table 5.5: Mean body mass (g), standard length (mm), Condition Factor (CF), Hepato-somatic Index (HSI), Gonado-somatic Index (GSI), Spleno-somatic Index (SSI) and Health Assessment Index (HAI) values for *Oreochromis mossambicus*, *Coptodon rendalli* and *Hydrocynus vittatus* from Nyamiti Pan during two surveys. Ranges are shown in parentheses

Survey	Species	n	Mass	Length	CF	HSI	GSI	SSI	HAI
Apr-2013	O. mos	15	468 ± 277	221 ± 50	3.74 ± 0.4	0.87 ± 0.14	0.10 ± 0.05	0.13 ± 0.03	-
			(60 - 880)	(115 - 292)					
	C. ren	13	744 ± 234	249 ± 0.6	4.45 ± 0.59	0.97 ± 0.22	0.15 ± 0.07	0.09 ± 0.03	-
			(36 - 960)	(97 - 288)					
	H.vit	11	688 ± 969	289 ± 118	3.69 ± 0.39	0.67 ± 0.17	0.30 ± 0.22	0.03 ± 0.02	-
-			(35 - 2860)	(128 - 530)					
Sep-2013	O. mos	21	837 ± 264	278 ± 43	1.94 ± 0.19	0.86 ± 0.14	0.79 ± 1.02	0.16 ± 0.05	110.5 ± 10.2
			(78 - 1080)	(140 - 319)					(90 - 130)
	C. ren	20	801 ± 257	269 ± 42	3.85 ± 0.25	1.45 ± 0.31	1.13 ± 1.20	0.13 ± 0.03	39.5 ± 10.0
			(53 - 1100)	(115 - 310)					(20 - 60)
	H.vit	19	195 ± 128	231 ± 35	1.45 ± 0.23	0.41 ± 0.07	0.15 ± 0.12	0.10 ± 0.15	42.4 ± 16.8
			(58 - 680)	(165 - 350)					(10 - 80)

5.3.3 Histology-based health assessment

A histology-based microscopic health assessment was done on 20 individuals of each species (*H. vittatus*, *O. mossambicus* and *C. rendalli*). Because *H. vittatus* is the only predator assessed in this chapter, it could not be compared directly or statistically to the cichlid species and therefore the results from this chapter will be compared to previous studies done on this species in the same system.

5.3.3.1 Hydrocynus vittatus

The average gill index was 1.5 and 100% of the fish were within Class 1 (Table 5.1). The liver index score was 1.4 on average and 100% of the individuals were within Class 1 (Table 5.1). The kidney index was 2.9 and 100% of the individuals fell in Class 1 (Table 5.1). The average fish index was 5.7 and the maximum score was 11. Thus 95% of *H. vittatus* were scored in Class 1 and 5% in Class 2 (Table 5.1). These indices are summarized in Table 5.6. There was no significant difference between the testis and ovary indices for this species (p < 0.05). Gill monogeneans were present in the secondary lamella (Figure 5.19 B)

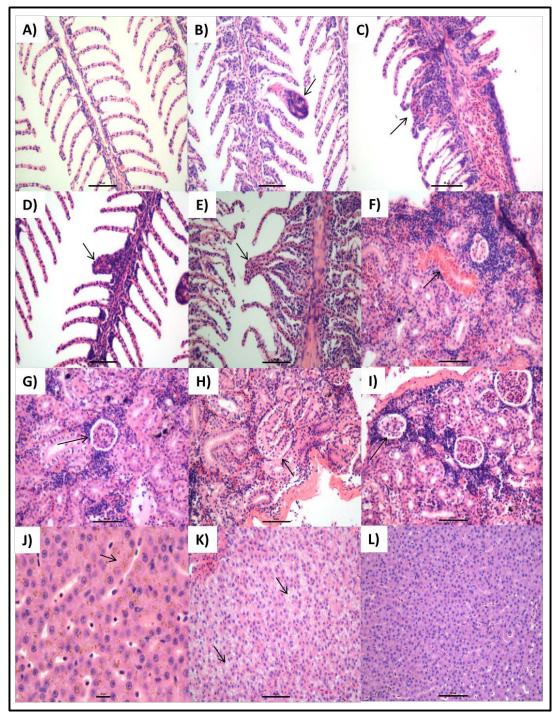


Figure 5.19: Micrographs of the gills, kidney and liver sections (5 µm) of *Hydrocynus vittatus* stained with H&E: A) Normal gill, B) gill monogenean between secondary lamella (arrow), C–D) hyperplasia of the gill epithelium (arrows), E) congestion of the gill epithelium (arrows), F) hyaline droplet degeneration in kidney tissue (arrow), G) increase in Bouwman's space in kidney tissue (arrow), H) vacuolation of renal tubials in kidney tissue (arrow), J) intercellular deposits in liver tissue (arrow), K) vacuolation of hepatocytes in liver tissue (arrows), L) normal liver.

5.3.3.2 Oreochromis mossambicus

The average gill index was 9.9 and 60% of the fish fell in Class 1, 35% in Class 2 and 5% in Class 3 (Table 5.1). The liver index was 6.4 on average, where 90 % of the individuals fell within Class 1 and 10% in Class 2 (Table 5.1). The kidney index was 2.7 and 100% of the individuals fell in Class 1 (Table 5.1). The average fish index was 19.3 and the maximum score was 34. Thus 5% of the individuals were scored in Class 1, 80% in Class 2 and 15% in Class 3 (Table 5.1). These indices are summarized in Table 5.6. There was no significant difference between the testis and ovary indices for this species (p < 0.05).

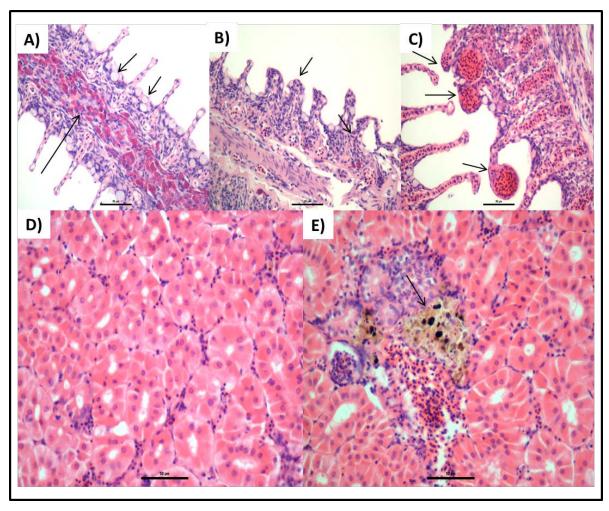


Figure 5.20: Micrographs of the gills and kidney sections (50 µm) from *Oreochromis mossambicus* stained with H&E: A) increase in mucus cells and infiltration of granulocytes (arrows), B) hyperplasia of the gill epithelium (arrows), C) telangiectasia and rupture of pillar cells (arrows), D) normal kidney tissue, E) increase in melano-macrophage centre in the kidney tissue (arrow).

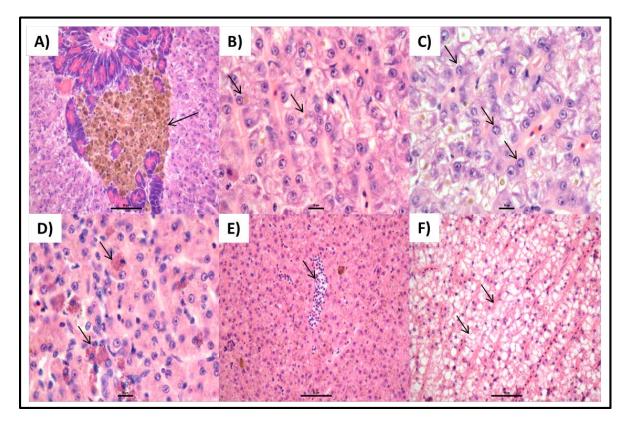


Figure 5.21: Micrographs of the liver sections (5 μm) from *Oreochromis mossambicus* stained with H&E: A) increase in melano-macrophage centre in the liver tissue (arrow), B) intercellular deposits in liver tissue (arrows), C) pycnosis of nuclei in liver tissue (arrows), D) infiltration of granulocytes in liver tissue (arrows), E) infiltration of lymphocytes in liver tissue (arrow), F) vacuolation of hepatocytes (arrows).

5.3.3.3 Coptodon rendalli

The average gill index was 5.1 and 95% of the fish fell in Class 1 and 5% in Class 2. The liver index was 4.5 on average and 100% of the individuals fell within Class 1. The kidney index was 1.4 and 100% of the individuals fell in Class 1. The average fish index was 11.8 and the maximum score was 18. Thus 20% of the individuals were scored in Class 1 and 80% in Class 2. These indices are summarized in Table 5.6. There was no significant difference between the testis and ovary indices for this species (p < 0.05).

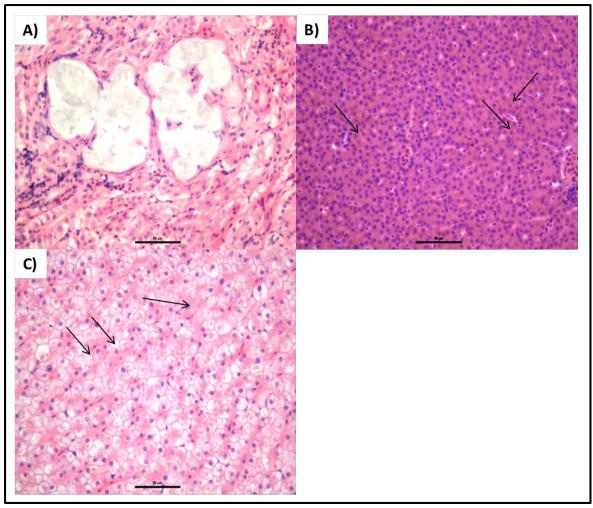


Figure 5.22: Micrographs of the kidney and liver sections (50 μ m) *Coptodon rendalli* stained with Hematoxinilyn and Eosine (H&E): A) nephrocalcinosis in kidney tissue, B) intercellular deposits in liver tissue (arrows), C) vacuolation of hepatocytes in liver tissue. Scale 50 μ m.

Table 5.6: Mean index values for *C. rendalli*, *O. mossambicus* and *H. vittatus*. Gill Index (GI), Liver Index (LI), Kidney Index (KI), Testis Index (TI), Ovary Index (OI) and Fish Index (FI). (Ranges are shown in parenthesis).

Species	GI	LI	KI	TI	OI	FI
C. rendalli	5.1 (2-11)	4.5 (0-9)	1.4 (0-5)	0	2.1 (0-3)	11.75 ±3.70 (4-18)
O. mossambicus	9.9 (4-25)	6.4 (0-12)	2.7 (0-6)	0.2 (0-3)	0.6 (0-3)	19.25 ± 6.80 (6-34)
H. vittatus	1.5 (0-5)	1.4 (0-4)	2.9 (0-6)	0	0	5.70 ± 3.50 (0-11)

5.3.4 Gonad development

5.3.4.1 Males

Of the 25 *H. vittatus* specimens used for staging analysis, 56% were males and 46% were females. For the males (standard length range: 175 mm–260 mm) 92% were classified as at stage 0 of development and 8% were at stage 1 of development. Thus, due to a lack of sample size of different size classes, age-length maturity curves could not be determined for *H. vittatus* males.

Of the 41 *O. mossambicus* specimens used for staging analysis, 78% were males and 22% were females. For the males (standard length range: 100 mm–310 mm) 50% were at stage 0 of development and 50% at stage 1 or >1. This means that 50% of the *O. mossambicus* males caught were sexually mature. Length-maturity logistic ogives for male *O. mossambicus* can be seen in Figure 5.23

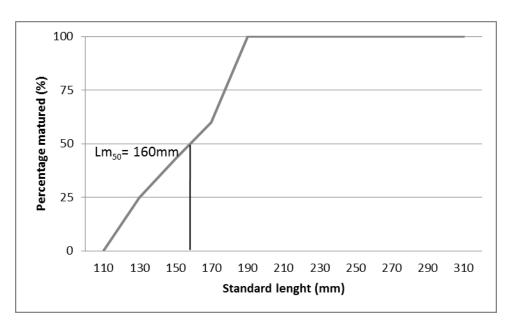


Figure 5.23: Logistic ogives depicting the length at which 50% maturity is reached for *Oreochromis mossambicus* males.

Sexual maturity for male *O. mossambicus* is reached at the relative size of 160 mm (SL) (Figure 5.23) and for females at the relative size of 250 mm (SL) (Figure 5.14). The results indicate that male *O. mossambicus* reach sexual maturity earlier than females.

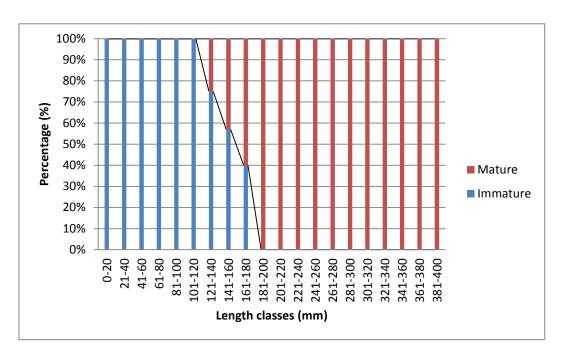


Figure 5.24: Bar graph showing the relationship in percentages of immature and mature male *Oreochromis mossambicus* in each length class.

Of the 71 *C. rendalli* sampled for staging analysis, 52% were males and 48% were females. For the males (standard length range: 190 mm–315 mm) 100% fell within stage 1 and higher. This indicates that all the males sampled were sexually mature. Due to a lack of sample size of different size classes, age-length maturity curves could not be determined for *C. rendalli* males.

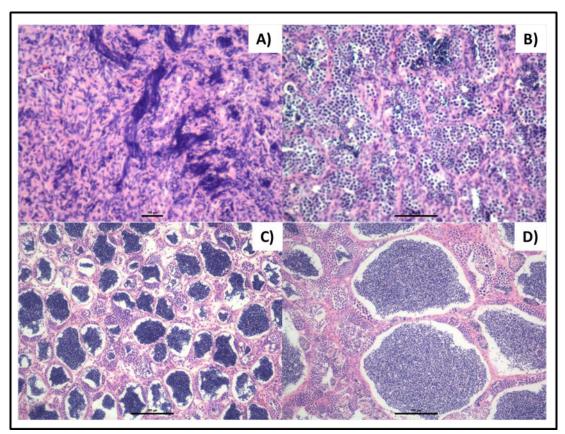


Figure 5.25: Micrographs of testis sections (5 μ m) stained with H&E: A) Stage 0, B) stage 1, C) stage 2, D) stage 3.

5.3.4.2 Females

For the total of 11 *H. vittatus* females (standard length range: 210 mm–350 mm), 100% fell within stage 0 of development. Due to a lack of sample size of different size classes, age-length maturity curves could not be determined for *H. vittatus* females.

For the total of 14 *O. mossambicus* females (standard length range: 110 mm–205 mm), 92% fell within stage 0 of development and 8% in stage 1 of development. Thus none the female *O. mossambicus* caught were sexually mature and no age-length maturity curves could be determined for this species due to a lack of sample size of different size classes.

For the total of 34 *C. rendalli* females (SL range: 150 mm–350 mm), 8% were in stage 0, 35% were in stage 1–3 of development and 47% were in the stage 5 of development. Due to a lack of sample size of different size classes, age-length maturity curves could not be determined for *C. rendalli females* because of the majority of adults.

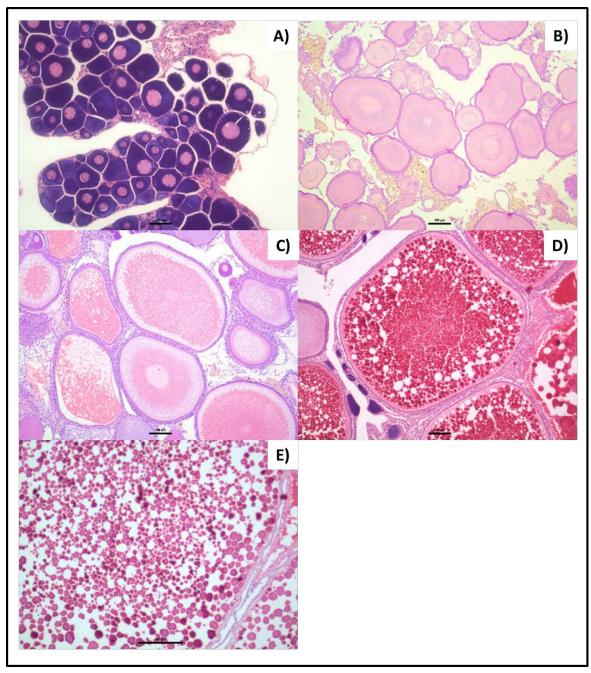


Figure 5.26: Micrographs of ovary sections (5 μ m) stained with H&E: A) stage 0, B) stage 1, C) stage 2, D) stage 3, E) stage 4.

5.4 Discussion

5.4.1 Population structure

Results from the age determination suggest that the growth rates of *H. vittatus* from Nyamiti Pan, which grew at an average rate of 33 mm per year, were slower when compared to H. vittatus from the Okavango Delta recorded by Gerber et al. (2009), which grew at an average rate of 160 mm per year. However, this might not reflect the true growth curve of this population due to the high number of small H. vittatus caught. Very few adult H. vittatus were caught and this might have skewed the data to show slower growth rates. Badenhuizen (1967) demonstrated that the catching ability of *H. vittatus* decreases when the stretched mesh sizes decrease. Efficiency of the seine net was limited due to the fact that H. vittatus jumped over the net as the water became shallower, despite the effort of lifting the nets higher. The presence of a large number of crocodiles limits the sampling efforts for H. vittatus, in that gillnets cannot be used. Males and females from Nyamiti Pan had similar growth; this was also the case for the H. vittatus from the Okavango Delta (Gerber et al., 2009). Although Gerber et al. (2009) showed that H. vittatus can live up to 20 years, the oldest H. vittatus caught during this study was six years old. Results from Gerber et al. (2009) and Soekoe (2010) showed a similar initial linear growth as the results from the present study but due to the lack of larger fish the growth model in the present study was skewed by the large numbers of smaller specimens.

The initial growth of *O. mossambicus* is rapid, with individuals reaching 100 mm within the first year. After the first year, fish grow at an average rate of 50 mm per year, till they reach a standard length of 300 mm. Fish that have a standard length of 300 mm are between 5 and 6 years old. *Oreochromis mossambicus* from Nyamiti Pan grew slower (32.3 mm per year) compared to other populations in Lake Chicamba (Zimbabwe) (± 50 mm per year) (Weyl & Hecht, 1998).

Male and female *O. mossambicus* have similar growth rates, but males tend to grow bigger than females and reach maturity at 160 mm (SL). The largest male was 390 mm compared to the largest female of 290 mm. The oldest male and female were both 10 years of age, but a higher numbers of old males was recorded than females.

Based on the population structures, the majority of *O. mossambicus* caught in Nyamiti Pan were between 6 and 10 years old, suggesting that these individuals were all sexually mature, and confirming that *O. mossambicus* does spawn in Nyamiti Pan. This species is known to breed in

still-standing water (Skelton, 2001; Russell *et al.*, 2012). The lack of small, younger *O. mossambicus* either suggest that they are preyed on by *H. vittatus* (which are abundant in this pan) or move into the river after being spawned. There seems to be a gap in the population structure of fish ranging from 140 mm to 240 mm (three to five years of age). This might be due to the drought of 2008, where there was no input recorded above the Pongolapoort Dam (Chapter 3; Figure 3.3). If this drought had a significant effect on the water level of Nyamiti Pan, it might have caused a loss in habitat for *O. mossambicus* to breed during the summer months of 2008.

The initial rapid growth rate of *O. mossambicus* was also observed in *C. rendalli*. Individuals reached 100 mm within the first year. The initial rapid growth of both these cichlid species was also observed by Weyl & Hecht (1998) in Lake Chicamba (Mozambique). After the first year, *C. rendalli* grow at an average length of ± 30 mm per year until they reach the age of five. Male *C. rendalli* grow faster and larger than the females. The largest male *C. rendalli* was 315 mm (SL) compared to the largest female of 290 mm (SL). The oldest male was 9 years old and the oldest female was 8 years old.

Population structures indicate that Nyamiti Pan is mostly populated by large adult C. rendalli ranging between 6 and 10 years old. There were very few small individuals caught (Figure 5. 17); however, larvae were spotted in nesting sites in Nyamiti Pan confirming that this species does breed in the pan. There was a high abundance of fish within the 250 mm size class (5 years), suggesting that during the drought of 2008 the C. rendalli population were not impacted as much as the O. mossambicus population. Due to the high parasite infestation (see below) on O. mossambicus, these species are already under pressure. The decrease in water level has resulted in more stress (Bain et al., 1988, Arthington et al., 1999), which has affected the population structure. This also explains why the numbers of C. rendalli have increased significantly since 1994 (Merron et al., 1993b, 1994a-e) (Chapter 3). However, the lack of smaller, younger C. rendalli is a concern. The smaller C. rendalli fall prey to the large number of H. vittatus in the pan. Very few C. rendalli were found in the Phongolo River (Chapter 3). This might be attributed to unsuitable habitat, but if this is the case for larger part of the river it might have a significant effect on the C. rendalli populations in the Phongolo River. Birds such as the African fish eagle (Haliaeetus vocifer) also prey on fish and possibly have an effect on the fish populations in Nyamiti Pan (Bouwman et al., 1990).

When comparing the sexual maturity of *O. mossambicus* males collected from Nyamiti Pan, they were smaller (160 mm SL) than the populations from a similar environment, Lake Chicamba in Zimbabwe (Weyl & Hecht, 1998), where maturity was reached at a total length of 251 mm. This is attributed to the fact that the histological assessment of gonads is more accurate in that spermatozoa are identified more easily than with the methods used by Weyl & Hecht (1998).

5.4.2 Fish Health Assessment Index (FHAI)

During the April 2013 survey, which is the high flow period for this region, the condition factors of all the fish were greater compared to the low flow period in September 2013. *Oreochromis mossambicus* from NP were heavily infected (100%) with *Lernaea cyprinacaea*, which potentially contributed to the low condition factor ($K = 1.94 \pm 0.19$) when compared to the same species caught in a similar environment ($K = 4.09 \pm 0.79$) with no *L. cyprinacaea* infection (Dalu *et al.*, 2013). Barely any of the *C. rendalli* or *H. vittatus* were infected with *L. cyprinacaea* and very few were infected with nematodes.

After the observation of the *L. cyprinacaea* on the fish from the pan and their condition from the previous survey, a complete assessment of the health status of the fish according to Adams *et al.* (1993) was done during the September 2013 survey. When these fish were dissected the heart cavities of the *O. mossambicus* were severely infected with nematode parasites and the exterior of the fish were heavily infected with *L. cyprinacea* (pers. obs.) (Figure 5.27). No *L. cyprinacaea* were observed on the *C. rendalli* and very few nematodes were removed from this species. *Hydrocynus vittatus* were also infected with nematodes, but no *L. cyprinacaea* were removed from this species.

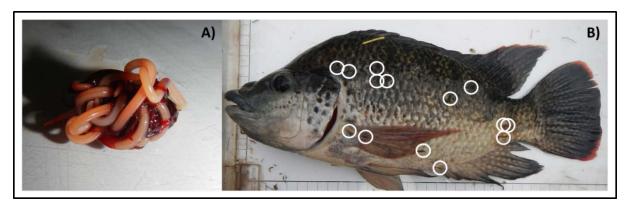


Figure 5.27: A) A photograph of a heart from an *Oreochromis mossambicus* infected with nematodes, B) infection of *Lernaea cyprinacaea* on *Oreochromis mossambicus* indicated by the white circles.

Based on the parasitemia, both these parasites seem to prefer the *O. mossambicus* as host. During the September 2013 survey (low flow) the *O. mossambicus* ($K = 1.94 \pm 0.19$) and the *C. rendalli* ($K = 3.85 \pm 0.25$) showed a lower condition factor than the *O. mossambicus* ($K = 3.74 \pm 0.40$) or *C. rendalli* ($K = 4.45 \pm 0.59$) collected during April 2013. The same trend was seen in the condition factor of *H. vittatus* ($K = 1.45 \pm 0.23$) in September 2013 compared to ($K = 3.69 \pm 0.39$) in April 2013. This decrease can possibly be attributed to fact that the pan is cut off from the river during the low flow season and thus formed a closed environment. The isolated pan becomes a breeding site for parasites, with an increase in parasite concentration. It is well documented that there is a big fluctuation in salinity levels in Nyamiti Pan over different seasons (Heeg & Breen, 1982) and that regular flooding plays an important role in the flushing of this system, but it is not known what role this fluctuation and flushing plays in the health of the fish in the pan.

The HSI of *H. vittatus* during the April 2013 survey (0.67 ± 0.17) was slightly higher when compared to the September (2013) survey (0.41 ± 0.07) . This falls within the ranges for *H. vittatus* from the Pongolapoort Dam (0.59 ± 0.81) (McHugh *et al.,* 2011). The HSI of both cichlids was within the normal range (1.30 ± 0.30) (van Dyk, 2007). The highest GSI values for *O. mossambicus* and *C. rendalli* were recorded during September at 0.79 ± 1.02 and 1.13 ± 1.20 , respectively. This may be because of the preparation for the breeding season in summer months from mid-October to mid-February (southern hemisphere) (Weyl & Hecht, 1998; Skelton, 2001).

The GSI values of *H. vittatus* did not indicate that this species was preparing to breed, but this may be due to the fact that the majority of the sample was small individuals. Based on the *H.*

vittatus from the Okavango Delta, this species only reaches sexual maturity at a total length of 451 mm for males and 522 mm for females (Gerber *et al.*, 2009). Due to the small sample size, the sexual maturity of H. vittatus from NP could not be determined; however, if this species is compared to that of the Okavango Delta, they would not have been sexually mature yet at the average standard length of 195 \pm 128 mm.

The GSI values for both cichlids increased from April to September, indicating that the gonads are developing during this period in order to breed during the summer months, and this was confirmed by the presence of large numbers of larvae in nesting sides in Nyamiti Pan (pers. obs.). Male *O. mossambicus* from NP reach sexual maturity at an average standard length of ± 160 mm (Figure 5.23). This is slightly earlier than *O. mossambicus* populations from Lake Chicamba (Zimbabwe), which reached sexual maturity at an average total length of 251 mm for females and 223 mm for males (Weyl & Hecht, 1998).

Cichlids collected from NP were larger and heavier than those found in the Phongolo River (Chapter 3). This confirms that NP is populated by adult cichlids and the fact that their GSI values increase towards the summer months confirms that they use the pan as a refuge for breeding purposes. No small cichlids were found in NP, which either means that they are preyed on by *Hydrocynus vittatus* (which are abundant in this pan) or move into the river after being spawned, or both. Although only a few adult *H. vittatus* were caught, the majority were young fish. This could be due to the lower capture rate of the larger *H. vittatus*.

Based on the HAI scores of the three targeted species O. mossambicus scored the highest (111 \pm 10) followed by H. vittatus (42 \pm 17) and C. rendalli (40 \pm 10), Oreochromis mossambicus is under severe stress due to the high prevalence of parasites, which seem to prefer them to the other two mentioned species.

5.4.3 Histology-based assessment

The gill index of *O. mossambicus* (9.85) was significantly higher when compared to *C. rendalli* (5.1) (p < 0.05). The liver index of *O. mossambicus* (6.4) was also a significantly higher than *C. rendalli* (4.5) (p < 0.05). There was no significant difference between the kidney indices of these two species (p > 0.05). There was also no significant difference between the gonadal indices when the males and females from the two species were compared (p > 0.05). However, the fish index score of *O. mossambicus* (19.25) was significantly higher than that of the *C. rendalli* (11.75) (p < 0.05) (Table 5.5)

The histological assessment of *C. rendalli* showed that this species has a good health status as most of them fell within Class 1, indicating that they have normal tissue structures. All the indices calculated for *C. rendalli* were normal. *Oreochromis mossambicus*, however, had the worst scores of the three species. Overall, *O. mossambicus* was scored in Class 2, indicating moderate histological alterations. These alterations included vacuolation of hepatocytes, which can be linked to exposure to pesticides (Velmurugan *et al.*, 2007). Hyperplasia and telangiectasia in the gill epithelium also occurred and were also linked to exposure to pesticides (Velmurugan *et al.*, 2007). The Phongolo Floodplain is known for the application of DDT to control malaria (Bouwman & Coetzee, 1990). DDT also has an effect on the reproduction of fish (Zaroogian *et al.*, 2001). Telangiectasia is also associated with the breakdown of pillar cells (Van Dyk *et al.*, 2009). Various circulatory disturbances were noted in the liver, which can be related to pathological conditions of blood and tissue fluid flow and regressive changes (McHugh *et al.*, 2011). All the indices fell within Class 1 with the exception of the gill index, which fell in Class 2.

The same histological alterations found in *O. mossambicus* were also observed in the organs of *H. vittatus* but not as severe as in *O. mossambicus*. These histological alterations can also be linked to the use of pesticides in the surrounding area and that their effect bio-magnifies as it moves higher in the food chain. The indices of *H. vittatus* from Nyamiti Pan indicate that their gills have more alterations compared to the *H. vittatus* from Pongolapoort Dam (McHugh *et al.*, 2011). However, the fish index suggests that *H. vittatus* from Nyamiti Pan are more healthy in that they have fewer alterations overall compared to the *H. vittatus* from Pongolapoort Dam (McHugh *et al.*, 2011).

5.5 Conclusion

Both indigenous cichlid species breed in standing water (Skelton, 2001) and since the pans outside of the Ndumo Game Reserve are heavily utilized by local communities, the pans in the protected area serve as very important refugia (Heeg *et al.*, 1980). From the results presented here it is evident that these two species do use Nyamiti Pan as a refuge area to breed in, and the community structures of the pan indicate that it is largely populated by cichlids between the ages of four and 10 years old. Very few young cichlids were found in Nyamiti Pan and this could be a cause of concern due to the pressure on the adult fish especially *O. mossambicus*. The majority of *H. vittatus* were small fish and this could be attributed to the fact that this species is

increasingly difficult to sample as they grow bigger. More sampling is needed to understand the biology and ecology of this species from Nyamiti Pan.

Results from the macro- and micro-health assessments indicate that the health of *O. mossambicus* is under major stress in Nyamiti Pan. High infestations of internal and external parasites are a major contribution to the deteriorating condition factor of this species. Another pressure these fish experience is predation from crocodiles and the piscivorous *H. vittatus*. *Coptodon rendalli* seem to be in a much better condition than *O. mossambicus* and do not suffer from the high parasite infestations that *O. mossambicus* do. What might be of concern, though, is the fact that very few small *C. rendalli* were caught in Nyamiti Pan and the Phongolo River during 2012–2014. This might mean that the recruitment of this species is under pressure. If this is the case in large stretches of the Phongolo River, it might be a cause for concern as *C. rendalli* forms an important part of the subsistence fishery in this region.

It is evident that all the species from Nyamiti Pan, especially the cichlids, are very dependent on the flow and its associated water level. The drought of one year (2008) had a significant effect on the population structures of both these species. It should therefore again be emphasized what an important role the flood regime plays on these economically important species. It is also well documented that there is a big fluctuation in salinity levels in NP over different seasons (Heeg & Breen, 1982) and that regular flooding plays an important role in the flushing of this system, but it is not known what role this fluctuation and flushing plays in the health of the fish in the pan. It is thus important that future studies should focus on whether the poor health of *O. mossambicus* in this important refuge area is related to irregular artificial flooding or the presence of the alien parasite *L. cyprinacaea*.