Chapter 2 provides a summarised literature review of relevant background information regarding specific aspects of this study. Section 2.2 explains in more detail how the study of normal histology fits into the larger framework of fish health research. The following section aims to familiarise the reader with the two freshwater indicator fish species, *Clarias gariepinus* and *Oreochromis mossambicus*, which were chosen for this project. Section 2.4 concludes this chapter with a summarised literature review on the five target organs which were selected for the qualitative and quantitative histological assessments. This will include the general morphology, function and histological structure as described for teleost species.
2.2 NORMAL HISTOLOGY AND FISH HEALTH

Various monitoring tools and assessment protocols have been developed to assist in evaluating fish health and subsequently monitoring aquatic pollution. These include the use of biological markers or biomarkers which are measurements at molecular, biochemical or cellular level in either wild populations of organisms from contaminated habitats, or in organisms experimentally exposed to pollutants. In other words, biomarkers indicate prior exposure to toxic chemicals, and the magnitude of the organism’s response to these contaminants (McCarthy and Shugart, 1990).

Biomarkers in indicator fish species are important if we are to maintain a viable fishery and a safe product for human consumption and health (Hinton and Laurén, 1990). Biomarkers measured in wild animals can directly contribute to detect, quantify, and understand the significance of exposure to chemicals in the environment. These measurements in fish species may also help assess the potential for human exposure to environmental pollutants and for predicting the associated human health risks (McCarthy and Shugart, 1990).

Biological and ecological responses to contaminant stressors may range from changes at molecular level, where genetic integrity and sub-cellular processes are evaluated, to population and community levels where dynamics and structure of entire food webs can be affected (Adams, 1990a; Shugart et al., 1992; Swee et al., 1996).

Some of the more commonly used approaches for assessing fish health are age and growth analysis and the condition factor (Le Cren, 1951; Bagenal and Tesch, 1978; Busacker et al., 1990), organo-somatic indices (Goede and Barton, 1990), and numerous measures of biochemical, physiological, and pathological condition (Neff, 1985; Adams, 1990a; Niimi, 1990).
According to Hinton and Laurén (1990), it is important to realise that physiological and biochemical alterations, if severe enough or protracted, will lead to structural alterations. Changes may be seen in the distribution of molecules on the cell surface, the organelle number, volume, shape or distribution, cell volume, morphology, cell distribution or number, and organ volume and relative weight.

Therefore, histological characteristics of specific organs express condition and represent time integrated endogenous and exogenous impacts on the organism stemming from alterations at lower levels of biological organisation (Chavin, 1973; Stebbing, 1985; Swee et al., 1996). In addition, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters. As an integrative parameter, histological changes provide better evaluation of an organism’s health than a single biochemical parameter (Segner and Braunbeck, 1988; Swee et al., 1996).

For field assessments, histopathology is often the easiest method of assessing both short and long-term toxic effects (Hinton and Laurén, 1990). If the level of a stressor is high enough, lethal changes may occur within an entire organ or specific regions within the organ. If tissues are properly fixed immediately after an animal dies, stressor-dependent pre-mortem cell death and necrosis can be differentiated from post-mortem changes (Trump et al., 1980). A histopathological examination should therefore be an essential part of fish health assessment.

However, histopathology in toxicity studies does require the ability to discriminate between toxicant-induced lesions and normal variations in structure (Hinton and Couch, 1984). It must also be noted that the functional capacity of organs is determined by both hormonal (Hoar and Randall, 1969) and environmental factors (Grant and Schoettger, 1972; Merle and Mayer, 1985) and changes in any of these factors can produce histological changes. Hinton and Laurén (1990) subsequently state that if the normal appearance of various organs and tissues of fish is known, alterations from the expected pattern may indicate stressor related injury. Errors in
histopathological assays can result if prior knowledge of the normal state for a given species is inadequate.

However, the paucity of carefully executed descriptive studies of the locale and morphology of individual cell types is a major drawback to field work (Hinton and Laurén, 1990). Hinton and Laurén (1990) state that one only has to compare micrographs of rainbow trout hepatocytes (Hampton et al., 1985; 1988) with those of Atlantic tomcod (Smith et al., 1979) to recognise the tremendous variation in normal morphology. Baseline studies of normal histological structure of indicator species are essential in ensuring accurate histopathological assessments and therefore plays a major role in biomarker and fish health related research.

As mentioned in Chapter 1, according to Hinton et al. (1992), a limited number of freshwater and marine finfish species has been subjected to detailed morphological analyses. It is stated that the normal morphological database at cell and tissue level needs expansion in promising indicator species. The following section will familiarise the reader with the two indicator species selected for this project.

2.3 TEST ORGANISMS

As mentioned in Chapter 1, two freshwater fish species endemic to southern Africa were selected for this project: *Clarias gariepinus* and *Oreochromis mossambicus*. These species were specifically chosen as both are of great interest as indicator species regarding aquatic health research at the University of Johannesburg. Also, for this project it was important that these species could be bred in captivity. The following sections provide a brief summary regarding background information on both these species in terms of natural distribution, habitat, reproduction, external anatomy, feeding habits and economic importance.
2.3.1 **Clarias gariepinus**

2.3.1.1 - Taxonomy and distribution

<table>
<thead>
<tr>
<th>Class:</th>
<th>Actinopterygii (ray-finned fishes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order:</td>
<td>Siluriformes (catfishes)</td>
</tr>
<tr>
<td>Family:</td>
<td>Clariidae (airbreathing catfishes / walking catfishes)</td>
</tr>
<tr>
<td>Species:</td>
<td><em>Clarias gariepinus</em> (Burchell, 1822)</td>
</tr>
<tr>
<td>Common name/s:</td>
<td>Sharptooth catfish / North African catfish (Afr. Skerptandbaber / Baber)</td>
</tr>
</tbody>
</table>

*Clarias gariepinus* occurs naturally from southern Kwazulu-Natal and the Orange River in South Africa (Figure 2.1), northwards through central, west and northern Africa to the Middle East and eastern Europe. *Clarias gariepinus* has also been translocated to certain rivers in the Western and Eastern Cape in South Africa (Skelton, 2001). This species is potamodromous, migrating within streams and rivers in which they occur (Teugels, 1986)

![Figure 2.1](image_url) Geographic distribution of *Clarias gariepinus* (grey areas) in southern Africa (adapted from Skelton, 2001) (translocated regions).

2.3.1.2 - Habitat and water quality range

This species inhabits a variety of freshwater environments including rivers and rapids, but favours flood plains, large slow rivers, lakes and dams. *Clarias gariepinus* is mostly a bottom dweller. However, they are also obligate air breathers, and do spend some time at the surface. They can survive in very poorly oxygenated waters
and are usually one of the last species present in relatively uninhabitable aquatic systems (Pienaar, 1968). They are also able to secrete mucous in extreme drought conditions to prevent them from drying out and can burrow in the muddy substrate of a drying body of water (Pienaar, 1968; Teugels, 1986; Skelton, 1993) to survive. *Clarias gariepinus* can adapt to extreme environmental conditions and can live in a pH range of 5.6 - 8. They are also able to live in very turbid waters and can tolerate temperatures of 8 - 35°C.

### 2.3.1.3 – Breeding and reproduction

This species participate in mass spawning. They are known to breed in summer after the rainy season. Vast numbers migrate to flooded grassy verges of rivers and lakes (Skelton, 1993). Their optimal temperature for growth is 28 – 30°C and they spawn at temperatures >8°C, usually >22°C. Eggs are laid on vegetation and hatch within 25-40 hours. The larvae are free swimming. This species is known to live eight years or more.

### 2.3.1.4 - General external anatomy

*Clarias gariepinus* (Figure 2.2) possess an elongated body with fairly long dorsal and anal fins. The dorsal fin has 61 – 80 soft rays and the anal fin has 45 – 65 soft rays. They have strong pectoral fins with spines that are serrated on the peripheral areas (Teugels, 1986). This species can attain sizes up to 1.7 m (total length) and can weigh up to 59 kg when fully mature. They possess nasal and maxillary barbels and relatively small eyes.

Their skin is smooth exhibiting a dark grey colour dorsally and a cream to white colour ventrally. Scales are absent. Adults possess a dark longitudinal line on either side of the head, however, this is absent in younger fish. The head is large, depressed, and heavily boned and is coarsely granulated in adults, while the head is smooth in the young. The mouth is quite large and sub-terminal (Skelton, 1993). This species is referred to as the ‘sharptooth catfish’ because of the presence of fine, pointed bands of teeth (Skelton, 1993).
2.3.1.5 - Feeding habits and behaviour

*Clarias gariepinus* is omnivores. They are not specific in their food requirements. They are known to feed on insects, plankton, snails, crabs, shrimp and other invertebrates and are also capable of eating dead animals, birds, reptiles, amphibians, small mammals, fish, eggs and plant matter such as fruit and seeds. They are poor swimmers and spend most of the time on the bottom of lakes and rivers (Pienaar, 1968). They are, however, able to move across land to other water sources during damp conditions (Skelton, 1993). This is achieved by simply extending their strong pectoral fins and spines allowing them to crawl through shallow pathways. Because they are mobile on land, they are able to sometimes prey on terrestrial organisms. This species may also hunt in packs on occasion by herding and trapping small fish. *Clarias gariepinus* has been known, during intra-specific aggressive interactions, to emit an electric organ discharge that is head-positive, lasting 5 – 260 ms (Pienaar, 1968; Teugels, 1986; Skelton, 1993).

2.3.1.6 - Economic importance

*C. gariepinus* is considered as one of the most commercially important freshwater fish species in Africa as a food source for humans. This species have also been imported in countries for aquaculture and game fishing (FIGIS, 2004).
2.3.2 *Oreochromis mossambicus*

2.3.2.1 - Taxonomy and distribution

| **Class:** | Actinopterygii (ray-finned fishes) |
| **Order:** | Perciformes (perch-like) |
| **Family:** | Cichlidae (Cichlids) |
| **Species:** | *Oreochromis mossambicus* (Peters, 1852) |
| **Common name:** | Mozambique tilapia (Afr. Bloukurper) |

Until the late 1970’s the tilapias were all classified into a single genus, *Tilapia*, however most taxonomists now classify them into three genera, *Tilapia*, *Sarotherodon* and *Oreochromis* according to their breeding behaviour. *Oreochromis mossambicus*, commonly known as the Mozambique tilapia occurs naturally along the eastern coast of Africa (Figure 2.3), in the lower Zambezi and its tributaries and eastward-flowing rivers and coastal lagoons southward to the Bushman’s river, near Port Elizabeth, South Africa (Bruton and Bolt, 1975).

![Figure 2.3](image)

**Figure 2.3** Geographic distribution of *Oreochromis mossambicus* (grey areas) in southern Africa (Skelton, 2001).

2.3.2.2 - Habitat and water quality range

This species is usually restricted to relatively shallow waters (Bruton and Bolt, 1975). Over portions of its native range, juveniles, however, appear better adapted to inhabit deeper waters compared to adults (Caulton and Hill, 1973). The movement of the species between shallow and deeper waters is mainly influenced by temperature changes (Bruton and Bolt, 1975), and its primary predator, *Clarias gariepinus* (Bowen and Allason, 1982). *Oreochromis mossambicus* can tolerate a broad range of...
salinities (Treweves, 1983). The species can not tolerate temperatures below 10°C which appear to be a limiting factor over its native range (Treweves, 1983). In Lake Sibaya (Africa), Bruton and Bolt (1975), reported seasonal movements to deeper waters during the cold season and to shallower waters in the warm season, with colder temperatures limiting the length of the breeding season. In tropical waters, *O. mossambicus* breed throughout the year (Neil, 1966; De Silva and Chandrasoma, 1980). Temperature tolerance ranges between 8 - 42°C (tropical areas) and 13 - 35°C.

### 2.3.2.3 – Breeding and reproduction

*Oreochromis mossambicus* is a maternal mouth brooder. Males construct nests in areas of sparse to moderately dense vegetation. Females produce anywhere from 50-1780 eggs, depending on the locality and the size of the specific female. Male specimens grow faster and become larger than female specimens (Bruton and Allason, 1974; Bruton and Bolt, 1975). Like most cichlids, optimal growth occurs near 30°C (Price *et al.*, 1985). However, growth rates vary depending on food availability and habitat quality (Bruton and Allason, 1974; Bowen, 1979; Arthington and Milton, 1986).

There are clear differences between the sexes in the tilapia species, particularly in terms of fin morphology and adult colouration. Maturation usually occurs between 150 and 160 mm in females and between 170 and 180 mm in males (Hodgkiss and Manson, 1978; Arthington and Milton, 1986). However, it has been reported that the average breeding size of *O. mossambicus* in food limited waters of Lake Sibaya, Africa, to be 100 mm in females and 120 mm in males, with the smallest breeding female 68 mm and the smallest breeding male 104 mm (Bruton and Allanson, 1974; Bowen, 1979).

*Oreochromis mossambicus* typically grows to about 380 mm (Treweves, 1983). The maximum age reported is 11 years. Various factors contribute to the suitability for culture of this species and include:
Resistance to poor water quality and disease
- Tolerance to a wide range of environmental conditions
- Good growth rate and easy to grow in intensive culture

2.3.2.4 - General external anatomy
The jaws (mandibles) of sexually mature males are enlarged, making their upper profile concave. Females and non-breeding males are silver in colour with two to five mid-lateral blotches (spots – usually black), and occasionally a few dorsal blotches. Breeding males have a distinct black colouration with a red colour on the periphery of their dorsal and caudal fins. In addition, males have simple genital papilla with a shallow distal notch. The pharyngeal teeth are very fine. The caudal fin is not densely scaled (Trewevas, 1983).

![External anatomy of Oreochromis mossambicus (adapted from Skelton, 1993)]
2.3.2.5 - Feeding habits and behaviour

In general, *O. mossambicus* is omnivorous and feeds on what is available, although they seem to show some preference for detritus and plant matter. Over their natural range, this species appears to be primarily detritivorous, with diatoms playing an important role in the nutrition of their diet (Bowen, 1979; Trewevas, 1983). There are reports of *O. mossambicus* feeding on algae, phytoplankton, zooplankton, vascular plant fragments, insects, crustaceans and small fish (Neil, 1966; Bruton and Bolt, 1975; Trewevas, 1983; Da Silva et al., 1984).

2.3.2.6 - Economic importance

*Oreochromis mossambicus* is economically important for the following reasons: (1) fisheries (highly commercial), (2) aquaculture (commercial), (3) game fishing (4) as a recreational aquarium fish (commercial) (5) and are also used extensively in biological, physiological and behavioural research (Skelton, 1993). It is not listed on the IUCN red list and because of its adaptability is generally regarded as a potential pest.
2.4 SELECTED TARGET ORGANS

According to the US EPA (2006), a target organ can be defined as a biological organ most adversely affected by exposure to a chemical substance. As mentioned in Chapter 1, for the purpose of this study, five target organs were selected for the histological assessment. These organs were specifically chosen as they are examined in histological assessments as part of fish health related studies within the department. The selected organs include the liver, gills, gonads, heart and kidney. In the following sections these organs will be discussed in terms of their general morphology as described for teleosts, and more detail on why these organs are regarded as target organs and subsequently form part of histological assessments in toxicity studies.

2.4.1 Liver

2.4.1.1 Liver as target organ

According to Hinton and Laurén (1990), fish liver microscopic structure is an integrator of physiological and biochemical function which, when altered, may produce biomarkers of prior exposure to toxicants. The liver has a key role in xenobiotic metabolism and excretion, digestion and storage, and the production of yolk protein. Thus, alterations in structure are expected under certain toxic conditions. These specific functions were summarised in more detail by Hinton et al. (1992):

(1) The liver of teleosts is the major site of the Cytochrome P450-mediated, mixed-function oxidase system (Stegeman et al., 1979). This system inactivates some xenobiotics, while activating others to their toxic forms.

(2) Nutrients derived from the gastro-intestinal absorption are stored in hepatocytes and released for further catabolism by other tissues (Walton and Cowey, 1982; Moon et al., 1985).
Bile synthesised by the hepatocytes (Schmidt and Weber, 1973; Boyer et al., 1976) aids in the digestion of fatty acids and carries conjugated metabolites of toxicants (Gingerich, 1982) into the intestine for excretion or entero-hepatic recirculation.

The yolk protein vitellogenin, destined for incorporation into the ovum, is synthesised entirely within the liver (Vaillant et al., 1988). Receptors in the liver must bind the hormone, estradiol, for initiation of the signal to begin synthesis of this reproductive component.

According to Bruslé and Gonzàlez (1996), many environmental parameters can alter liver structure and metabolism including pollutants (e.g. metals, pesticides, hydrocarbons, PCB), food (quantity and quality), biotoxins (algae and fungi), parasites, infectious germs (virus and bacteria) and physiochemical parameters (e.g. pH, oxygen, temperature etc.). Liver histology has been proven to be indicative of exposure to pollution (e.g. Meyers and Hendricks, 1985; Hinton and Laurén, 1990; Bernet et al., 2004). Pathological changes that are associated with fish liver according to Takashima and Hibiya (1995) include cloudy swelling, atrophy, necrosis, vacuolar degeneration, fatty degeneration, bile stagnation, hepatitis cirrhosis, congestion and tumours.

2.4.1.2 – Description of the liver in teleosts

Macroscopic structure
Macroscopically, fish liver is a large, dense organ and is ventrally located in the cranial region of the general cavity. The size, shape and volume of the organ are adapted to the space available between other visceral organs (Bruslé and Gonzàlez, 1996). It can consist of two to three lobes although no lobulation was recognised in Oncorhynchus mykiss (Robertson and Wexler, 1960), Liza spp. (Biagianti-Risbourg, 1991), Lutjanus bohar (Gonzàlez, 1992), Serranus cabrilla (Gonzàlez et al., 1993) or Lampetra spp. (Shin, 1977).
The colour of the liver usually varies from light brown to dark red (e.g. channel catfish) (Grizzle and Rogers, 1976). The gross variation in the colour of fresh liver may be related to differences in glycogen and fat content (Grizzle and Rogers, 1976) as well as its rich vascularization (Bruslé and González, 1996). The vascular organisation of the liver consists of two afferent blood vessels (hepatic artery and portal vein) and a single efferent vessel (hepatic vein) located at the hilum.

Microscopic structure
According to Takashima and Hibiya (1995), the lobular structure containing a small vein in the centre is present in the liver of higher vertebrates. In fish, however, these structures vary depending on the species and are generally obscure. The arrangement of the hepatocytes can rather be regarded as tubular (hepatic cords) (Hampton et al., 1988; Shore and Jones, 1989). In this arrangement, hepatocytes have their bases directed toward the sinusoids, mainly for absorption (Hampton et al., 1985; Schär et al., 1985; Robertson and Bradley, 1992) and their apices directed toward a biliary structure (Hinton et al., 1984a; Hampton et al., 1988; Hinton and Laurén, 1990) mainly for excretion. With this tubular structure, the hepatocytes are arranged as hepatic plates, usually two cells thick, but branching and anastomoses of hepatic cords can results in four or more cell layers per plate. These cords are not always clearly visible (Geyer, 1989). Takashima and Hibiya (1995) noted that the serous lining is extremely thin and can often not be recognised in light microscopy preparations.

The main cell type in the liver is the parenchymal hepatocytes, while endothelial cells, fat-storing cells, Kupffer cells, mesothelial cells and fibroblasts complement the basic liver architecture (Takashima and Hibiya, 1995). Grizzle and Rogers (1976) stated that the appearance of hepatocytes varies between individual specimens. The principle difference is the degree of vacuolation in routine preparations which results from the removal of fat and glycogen during slide preparation. Bruslé and González (1996) indicated that the hepatocytes are polygonal-shaped cells, often weakly basophilic (poor in organelles) compared to those of mammals. In addition, according
to Takashima and Hibiya (1995), the presence, internal arrangements, and peculiarities of the hepatocyte organelles are also known to vary among species, age, season, sex, spawning period, nutritional condition etc.

A typical hepatocyte contains a nucleus with variable amounts of dispersed and peripheral heterochromatin and a single prominent nucleolus. The strands of RNA within the nucleolus are tightly coiled in adult fish and its filamentous structure appears to be best visualised in early developmental stages. Nuclear contours are normally smooth and bear numerous nuclear pores. The size of hepatic cells in light microscopy preparations reflects their physiological functional state, and is markedly different in the hyper-functional and hypo-functional states. According to Takashima and Hibiya (1995), a good example of the variable appearance of the hepatic cell is hypertrophy of the cell body, nucleus and nucleoli, which occurs at the most active period of vitellogenesis, showed in *O. asou* and *P. altivelis* females, or, the shrinkage of these elements during prolonged starvation.

The hepatic artery and portal vein enter the liver. The latter, which carries venous blood from the stomach and intestine, gradually branches off and eventually divides into relatively wide blood capillaries known as sinusoids. The parenchymal cells are concentrically arranged around the sinusoids, which therefore may be considered the anatomical centre of the cord-like structures known as hepatic cords (Takashima and Hibiya, 1995). However, these authors state that some still recognise the bile canaliculi as the centre of the cords mainly out of functional considerations. The triads, constituted by a ramification of the portal vein, the hepatic artery and a biliary duct, are indistinct if not absent in almost all teleosts (Bruslé and Gonzàlez, 1996). According to Hinton and Laurén (1990), fenestrated endothelial cells line the sinusoids. Between endothelial cells and the hepatocytes is the peri-sinusoidal space of Disse containing the stellate, fat-storing cell of Ito.

Bile canaliculi within the laminae of the hepatocytes drain bile from hepatocytes to bile ducts to be transported to the gall bladder (Grizzle and Rogers, 1976). These bile
canaliculi are usually not visible with the light microscope. The walls of the bile ducts consist of a single layer of cuboidal to columnar epithelial cells over an underlying layer of connective tissue. The histological structure of the hepatic duct is similar but also includes a layer of smooth muscle (Takashima and Hibiya, 1995).

The main substances stored in fish liver are glycogen and to a lesser extent lipid. Glycogen particles may be found scattered in the cytoplasm or aggregated in large concentrations. Though lipid materials are not uncommon in fish liver, intensive accumulation is more often found in aqua-cultured fish, revealing nutritional inadequacy of artifactual feeds. Lipids can be found in small to medium-sized droplets interspersed with the organelles in the parenchymal cells, or nearly filling the cytoplasm of so-called fat-storing cells. Because these substances are poorly preserved or stained, the usual paraffin embedding and staining methods cause the appearance of many vacuolar structures in the hepatocytes. The presence of lipid or glycogen can be roughly inferred from the shape of the vacuoles. Lipid droplets tend to form round, single or coalescing droplets, whereas glycogen granules are often irregular shaped. To confirm the presence of either of these substances, electron microscopy or the use of specific stains is strongly recommended. Examples are PAS stain, as was used in this study, or frozen sections with Sudan Black or oil Red O stains (Takashima and Hibiya, 1995).

Takashima and Hibiya (1995) stated that the liver is present as a simple organ in the early life of all teleosts, but in many species including the channel catfish and carp among others, the pancreatic tissue invades the liver along the branches of the portal vein. The combined hepatic and pancreatic tissues are collectively called the hepatopancreas.

According to Bruslé and González (1996), the pancreatic exocrine develops around the portal vein during ontogenesis. It remains extra-hepatic or penetrates deeply into the liver parenchyma depending on the species. Thus the existence of the hepatopancreas makes the identification of the portal vein in these species relatively
Pancreatic tissue can be differentiated from hepatic tissue by its acinar arrangement and its characteristic stain with H&E. A thin septum of connective tissue separates the hepatocytes from the exocrine pancreatic cells.

Melano-macrophage centres (MMC) occur in the hepatic parenchyma of fish. Their size, number and content are highly variable depending on the species, age and health status (Agius, 1985). MMC’s are usually located in the vicinity of the hepatic arteries, portal veins or bile ducts. These structures concentrate heterogeneous materials such as lipofuscin, melanin, ceroid or hemosiderin. Such products may play a role in neutralising potentially toxic free radicals and cations produced during peroxidation of unsaturated lipids (Agius, 1985).

2.4.2 Gills

2.4.2.1 Gills as target organ

The gills perform a variety of critical physiological functions including gas exchange, ion-regulation, maintenance of acid-base balance, and excretion of nitrogenous wastes. Furthermore, they are continuously exposed to pollutants in the external medium (Hinton and Laurén, 1990).

The main function of the gills is gas exchange between the water and the blood (Grizzle and Rogers, 1976). As Yasutake and Wales (1983) state, the gill is a system for bringing the blood haemoglobin into close contact with the water, so that oxygen can be absorbed and carbon dioxide released. This function necessitates the exposure of a system of capillaries to water with sufficient surface area to ensure the required gas exchange. This exposure in turn, makes the tissue highly vulnerable to the external environment.

Mucous cells are scattered throughout the gill epithelium of the gill arch, filaments and lamellae. Mucous probably performs a variety of functions regarding the gills.
Interposition of a mucous coat between the environment and epithelium suggests that the primary function of mucous is insulative in nature (Olson, 1996).

Hinton et al. (1992) states that in constant contact with water, the gills are sensitive primary target organs for a variety of insults including low pH (McDonald, 1983), transition metals (Laurén and McDonald, 1985), heavy metals (Verbost et al., 1987), detergents (Abel and Skidmore, 1975) and polycationic agents, (Greenwald and Kirschner, 1976). Gill histological lesions are also associated with exposure to deltamethrin (Cengiz and Unlu, 2005) and oil sands process-affected water (Nero et al., 2005).

According to Takashima and Hibiya (1995), many pathological agents bring about epithelial oedema, vacuolation and necrosis in secondary lamellae and mucous cell death with excessive mucous secretions in primary lamellae. In general, oedematous change with inflammatory cell infiltration is interpreted as a defensive response to the agents. Seriously injured secondary lamellae have exfoliated epithelia, necrotic pillar cells and haemorrhage. Distortion of secondary lamellae is also frequent under various stimulations. Capillary lumens sometimes expand to induce blood congestion with a disintegrated pillar cell system. This is termed telangiectasia or aneurysm, sometimes with accompanying fibrinous exudates in the capillary wall. Hypertrophic cells and micro-projections of epithelial surface are the first signs of primary and secondary lamellae exposed to chemical or physical agents. Hyperplasia of mucous cells in primary lamellae, lamellar fusion, and epithelial hyperplasia of the secondary lamellae commonly occur as chronic responses to parasitic and bacterial infections or chemical irritants. Club-shaped lamellae are another example of chronic change in the gill.
2.4.2.2 – Description of the gills in teleosts

Macroscopic structure
Teleosts have five pairs of gill arches. In the front four pairs, the slender primary lamellae form two lines facing posteriorly, and these two lines are joined to each other at the base by a gill septum. The last pair of gill arches generally transforms into the pharyngeal bone and does not play a role in respiration (Takashima and Hibiya, 1995).

Microscopic structure
Numerous semicircular secondary lamellae are lined up along both sides of the primary lamella. These primary lamellae consist of cartilaginous support, a vascular system and multi-layered epithelium. Squamous epithelial cells with finger-like micro-ridges exist at the outermost layer of the primary lamellar epithelium. A double layer of epithelial cells constitutes the secondary lamellar epithelium. The outer layer is termed respiratory epithelial cells, which has sparse and small microvilli and marginal micro-ridges on the apical surface, whereas the inner layer supports epithelial cells situated on a basement membrane. Interstitial spaces are occasionally detectable between the two layers. The secondary lamellar epithelium is supported by many pillar cells, which are contractible and separate the capillary channels (Takashima and Hibiya, 1995).

In the branchial vascular system there are two principal pathways: arterio-arterial and arterio-venous systems. The arterio-arterial system is composed of afferent and efferent arteries in the primary lamellae branching off many afferent and efferent arterioles. Each afferent artery has an ampulla at a distance of one-third from the base, which functions as a branchial heart in the primary lamellae. The secondary lamellar capillaries with many anastomoses are interposed between the afferent and efferent companion vessels. The afferent and efferent vessels run parallel to the afferent and efferent primary lamellar arteries, whereas the central venous sinus occupies the whole length of the primary lamella along the cartilaginous support.
The central venous system shows variation among species in size and shape. The primary lamellar epithelium contains mucous cells and chloride cells. The mucous cells are principally located on the afferent and efferent edges of the primary lamellae. Chloride cells are seen at the basement of the secondary lamellae. These cells are acidophilic (easily stainable with acid dyes) and exists generally in marine fish and rarely in freshwater fish (Takashima and Hibiya, 1995).

### 2.4.3 Testis

#### 2.4.3.1 – Testis as target organ

Baseline reproductive biology of fish is increasingly being studied to support interpretation of tests with potential endocrine disrupting chemicals (EDCs) e.g the fathead minnow (*Pimephales promelas*) (Jensen *et al.*, 2001). These chemicals can have adverse effects on the reproduction and development of fish populations.

Findings have been published regarding EDCs and its subsequent effect on the gonadal histomorphology e.g. *Lepomis macrochirus* (Maxwell and Dutta, 2004), *Pimephales promelas* (Leino *et al.*, 2005) and *Rutilus rutilus* (Bjerregaard *et al.*, 2005). Intersex has also been identified in *C. gariepinus* exposed to estrogen polluted water in South Africa (Barnhoorn *et al.*, 2004). Apart from EDCs, exposure to certain metals has been shown to target the testes e.g. *Salvelinus fontinalis* (Sangalang and O’Halloran, 1972; Sangalang and Freeman, 1974), *Carassius auratus* (Tafanelli and Summerfelt, 1975), *Clarias batrachus* (Katti and Sathyanesan, 1985; Kirubagaran and Joy, 1992) and *O. mossambicus* (Pieterse, 2004). Examination of normal testis histology is therefore beneficial to better understand the effects of potential EDCs and metal exposures in fish.

Testicular necrosis and haemorrhage can be induced by pollutants in the water (Sangalang and Freeman, 1974). Adverse environmental conditions can increase the incidence of otherwise normal degenerative changes (Takashima and Hibiya, 1995). Water quality related degenerative conditions of the teleost gonad include abnormal

2.4.3.2 – Description of the testis in teleosts

**Macroscopic structure**

In most species, the testes are paired, elongated structures attached to swim bladder by mesenteries called mesorchia. The testes occur dorsally in the coelom and in most species extend almost the entire length of the coelom. In many teleosts, the walls of the gonads extend backward to form gonducts which fuse posteriorly before reaching the genital pore (Takashima and Hibiya, 1995).

**Microscopic structure**

Using a broad phylogenetic approach, Callard (1991), classified all vertebrate testes as either tubular (mammals, birds and reptiles) or lobular (amphibians and teleosts). In this classification, a tubule is an open-ended germinal compartment of the testes while a lobule is a blind-ended sac. Thus, most teleost testes would be of the lobular type despite differences in the patterns of spermatogonial distribution or the presence or absence of a lumen within the lobule. According to Takashima and Hibiya (1995), Grier (1993), agreed with Callard’s classification but also described a new type of tubular testes found in teleosts, the anastomosing tubular testis. It is characterised by a branching network of tubules that, like mammalian testicular tubules, loop at the periphery of the gonad (Grier, 1993).

Testicular lobules in teleosts are demarcated by a basement membrane and an overlying, sometimes incomplete, layer of boundary (myoid) cells (Takashima and Hibiya, 1995). The lobules contain spermatocytes, which are spherical units composed of germ cells and Sertoli cells. Spermatocytes are also delimited, partially or completely depending on the species, by a basement membrane. Germ cells within a spermatocyst are linked cytoplasmically by intercellular bridges and the differentiation is fairly synchronous. In most teleosts, spermatogonia as well as
spermatocytes at various stages of development can be seen throughout much of the length of the lobule, and mature spermatozoa are released into the lobular lumen. In many species, steroidogenic (interstitial Leydig) cells can be observed in the interstices between the lobules (Billard, 1986).

According to Takashima and Hibiya (1995), among chordates, the two general functions of Sertoli cells are to phagocytise degenerating germ cells and residual bodies, and to form the Sertoli cell barrier (Grier, 1993). Sertoli cells, germ cells and basement membranes constitute the germinal epithelium of vertebrate testes.

Various general patterns of testicular development can be observed in teleosts (Billard, 1986). In poeciliid fishes, spermatogenic activity is continuous throughout the year. In other teleosts, e.g. salmonids, distinct periods of spermatogenic activity can be seen in which a new cycle will not begin until the preceding cycle has been completed. Also, an intermediate condition can be found in the testes of carp, in which a quasi-continuous pattern of spermatogenic activity has been described (Takashima and Hibiya, 1995). A detailed description of testicular development of teleosts is available by Takashima and Hibiya (1995).

2.4.4  Ovary

2.4.4.1 – Ovary as target organ
According to Takashima and Hibiya (1995), abnormal as well as normal degenerative changes have been described in teleost gonads. For example, somatic or germ cell atresia and necrosis can be considered either a health-related pathological change or a normal degenerative condition accompanying seasonal changes in gonadal activity. Oocyte atresia is a normal histological condition observed after spawning (Billard and Takashima, 1983). However, adverse environmental conditions can increase the incidence of otherwise normal degenerative changes. Water quality related degenerative conditions of the teleost ovary include abnormal gross alterations of gonadal morphology and inhibition of oogenesis.
As mentioned in the previous section, findings have been published regarding endocrine disrupting chemicals and its subsequent effect on the gonadal histomorphology e.g. *Rutilus rutilus* (Bjerregaard *et al.*, 2005); *Lepomis macrochirus* (Maxwell and Dutta, 2004), *Pimephales promelas* (Leino *et al.*, 2005). Testicular oocytes have also been identified in *C. gariepinus* exposed to estrogen polluted water (Barnhoorn *et al.*, 2004).

### 2.4.4.2 – Description of the ovary in teleosts

**Macroscopic structure**

In most teleosts, the ovary consists of two lobes that join caudally with a short oviduct that exists between the anus and the urinary pore as is also described for the striped bass (Groman, 1982). The ovaries of teleosts are suspended from the abdominal wall by a mesentery and usually appear as a small cluster of minute orange-white spheres in immature females (Roberts, 2001). The mature ovaries can represent as much as 70% of the total body weight of a female specimen.

**Microscopic structure**

According to Takashima and Hibiya (1995), the teleost ovary is of the cystovarian type (Hoar, 1969), as it contains a lumen into which the eggs are released during ovulation. Ovigerous folds (ovarian lamellae) containing the developing follicles usually extends into the lumen. Before ovulation, the ovaries of ripe females are relatively large organs occupying much of the body cavity, and in many species the ovarian volume increases even further at ovulation due to hydration of the maturing oocytes.

From a morphological and functional point of view, the basic unit of the ovary is the ovarian follicle. The organisation of the follicle is similar in all teleosts. The centrally located oocyte is surrounded by an acellular envelope, the vitelline envelope, also known as the zona radiata or chorion. This envelope is in turn surrounded by the follicle cells. Follicle cells are organised into an inner monolayer of granulose cells.
and an outer layer of theca cells. A basement membrane separates these two cell layers (Takashima and Hibiya, 1995). Histologically, present evidence suggests that the granulose cell layer is largely composed of a homogeneous population of cells. However, this cell layer also contains the micropylar cell, which is involved in the formation of the micropyle – the opening in the vitelline envelope of ovulated eggs that provides access for the sperm to the egg surface during fertilisation.

The theca cell layer is a more heterogeneous layer composed of capillaries, fibroblasts and in many instances, special theca cells. Follicles at all stages of development can be found in the ovaries of teleosts with continuous or prolonged spawning seasons. Ovaries containing a heterogeneous population of follicles at different developmental stages are called asynchronous ovaries. Other developmental patterns include group-synchronous ovaries, in which clutches of follicles at different developmental stages exist in the same gonad, and synchronous ovaries, in which all follicles develop in unison (Takashima and Hibiya, 1995).

The ovaries of teleosts do not commit all oogonia to meiosis during early development (Tokarz, 1978). Resting and proliferating oogonia can be found in the interstices between larger ovarian follicles in the gonads of most adult female teleosts. Oocytes undergo remarkable nuclear and cytoplasmic changes during growth. In general, the growth stages include the chromatin-nucleolus stage, perinucleolar stage, cortical alveoli stage, vitellogenic stage, maturation stage and ovulation phase (Wallace and Selman, 1990).

The chromatin-nucleolus stage encompasses the various chromosomal stages from leptotene to the onset of pachytene during prophase of the first meiotic division. Leptotene oocytes can be difficult to distinguish from oogonia. However, under the light microscope the chromosomes may become evident as thin threads distributed throughout the nucleus. Paring of the homologous chromosomes occurs during the zygotene stage. The zygotene oocyte can be recognised with light microscopy by the conspicuous “bouquet” arrangement of the chromosomes on one side of the nucleus.
with a single nucleolus on the opposite side. Chromatin-nucleolus oocytes are partially surrounded by a layer of granulose cells and its associated basement membrane (Kanamori et al., 1985; Selman and Wallace, 1986).

The onset of the peri-nucleolar stage of oocyte growth is normally marked by the appearance and onset of migration of multiple nucleoli to the peripheral nucleoplasm. The large oocyte nucleus is now called a germinal vesicle. The diplotene stage of meiosis starts during the peri-nucleolar stage. During early diplotene, oocyte and follicular wall differentiations continue. The basic organisation of the ovarian follicle can be first observed during the peri-nucleolar stage, when the oocyte becomes enclosed within a continuous layer of follicle cells (Takashima and Hibiya, 1995).

The cortical alveoli stage is marked by the appearance of cortical alveoli in the cytoplasm. The term yolk vesicle is commonly used to describe an early cortical alveolus. Oil droplets may also form around the germinal vesicle during the cortical alveoli stage but the relative timing of this event is not uniform among species. Another significant event of the cortical alveoli stage is the appearance of well-defined microvilli and vitelline envelope. Uptake of hepatic vitellogenin and its deposition as yolk in the oocyte cytoplasm indicates the beginning of the vitellogenic stage. Most of the growth of the oocyte occurs during this stage. Yolk globules increase in mass and eventually occupy most of the cytoplasmic space in full grown oocytes (Takashima and Hibiya, 1995).

The architecture of the follicular wall increases in complexity (distinction of different layers) during vitellogenesis. In some species, such as salmonids, the germinal vesicle begins its migration towards the animal pole well before maturation during the latter stages of vitellogenesis, while in others, the germinal vesicle remains at the centre of the oocyte until the onset of final maturation (Takashima and Hibiya, 1995). The maturation stage is usually marked by the onset or resumption of germinal vesicle migration and is characterised by the resumption and completion of the first meiotic division. The lipid droplets coalesce into one or a few large droplets during
maturation in oocytes of some species. The germinal vesicle dissolves following its arrival at the animal pole, a process known as germinal vesicle breakdown. The mature oocyte is called an egg. Ovulation refers to the expulsion of the egg from its follicle. After ovulation, the ovary contains postovulatory follicles, unspawned eggs, full grown immature oocytes, oogonia and depending on the species, oocytes at various stages of development. In some species, the postovulatory follicles remain viable for considerable lengths of time, producing steroid hormones (Nagahama, et al., 1982; Smith and Haley, 1987).

According to Shanbhag and Saidapur (1996), it is a common feature of any vertebrate ovary that the oocyte is destined either to grow, mature, and ovulate and leave behind a corpus luteum (postovulatory follicle) or to undergo atresia (the degeneration and resorption of one or more ovarian follicles before a state of maturity have been reached) at some stage of its growth and development. While the corpus luteum is formed by the cells of the follicular envelope after expulsion of the mature ovum, the atretic follicles are formed by degeneration and resorption of oocyte and follicle cells in situ.

2.4.5 Heart

2.4.5.1 Heart as target organ

Although fish heart is not always regarded as a primary target organ for exposure to toxicants, several studies have shown histological lesion in cardiac tissue especially with regard to metal pollution (Exley, 1996; Borges et al., 2003). Nevertheless, little literature is available on fish heart histology and histopathology. One of the recognisable histopathological changes in the heart is muscular necrosis and inflammation of the cardiac muscle, endocardium or epicardium. Inflammation of the cardiac muscle is characterised by the presence of excessive accumulation of leucocytes and lymphocytes in the spaces among the muscle fibres. Collagen fibres in these spaces also show a tendency to proliferate (Takashima and Hibiya, 1995).
2.4.5.2 – Description of the heart in teleosts

Macroscopic structure

The heart of teleosts, as with other animals, can be described as a specialised muscular enlargement of the blood vessels and is responsible for pumping the blood throughout the circulatory system as stated by Groman (1982). In striped bass it is located within the pericardial sac which lies ventral to the buccal cavity and rostral to the liver (Groman, 1982) and is similarly described for the channel catfish (Grizzle and Rogers, 1976).

Microscopic structure

According to Takashima and Hibiya (1995), fish heart consists of an atrium and a ventricle. Blood flows into the sinus venosus and passes through the atrium, ventricle and bulbus arteriosus. The wall of all parts is composed of an internal membrane (endocardium), an intermediate layer (myocardium) and an external membrane (epicardium). The atrium wall is thin and contains few muscle fibres. The ventricle wall, on the other hand, is thick and rich in cardiac muscle. Two valves are recognised in the teleost heart: one between the atrium and ventricle, and the other between the ventricle and the bulbus arteriosus (Takashima and Hibiya, 1995).

Cardiac muscle fibres have striations and consist of one cell body containing only one nucleus. These fibres are joined to each other by special structures called intercalated discs. The cardiac muscles bifurcate and connect with other fibres forming a complex tri-dimensional mesh. The bulbus arteriosus wall is thick and composed mainly of connective tissue supplied with many elastic fibres (Takashima and Hibiya, 1995).
2.4.6 – Kidney

2.4.6.1 – Kidney as target organ
Groman (1982) stated that the kidney, specifically the trunk (posterior) kidney, is one of the more important excretory organs of teleost fish. The kidney, together with other organs maintains delicate osmotic balances between the fish and the environment. The lymphoid tissue of the head (anterior) kidney and inter-tubular tissue of the trunk kidney are haemopoietic in function. The kidneys are organs of obvious critical, though varied, function in fish species. One would assume that the renal tissues would be at major toxicological risk since they receive large volumes of blood flow from both the renal portal venous system and the renal arteries. In addition, urine produced collectively or individually through glomerular filtration, tubular reabsorption, or tubular secretion, serves as a major route of excretion for metabolites of various xenobiotics to which fish have been exposed to (Hinton et al., 1992).

In spite of these renal characteristics, no histopathological markers have been recognised that would provide a reliable indicator of the effects of toxicants in the environment (Hinton et al., 1992). However, Hinton et al. (1992) also state that the few histopathological studies regarding the kidneys probably contribute to the lack of biomarkers in this important organ. In recent years, more studies have incorporated the kidney in biomarker research and histological changes have been identified in the renal tissue after exposure to environmental pollutants e.g. *Salmo trutta* (Schmidt-Posthaus et al., 2001; Bernet, et al., 2004). According to Takashima and Hibiya (1995), pathological changes that are associated with the kidney include dilation or inflammation of glomerular blood capillaries and thickening of their walls, destruction and fibrosis of the glomerulus, capillary sclerosis, abnormal proliferation of epithelial cells within the Bowman’s capsule, cloudy swelling of epithelial cells of renal tubules, hyaline droplet degeneration of renal tubule epithelial cells, necrosis of epithelial cells and glycogen infiltration of tubular epithelium.
2.4.6.2 – Description of the kidney in teleosts

Macroscopic structure
The kidney is usually located in the retroperitoneal position up against the ventral aspect of the vertebral column. Beitch (1962) noted that the trunk (posterior) kidney of striped bass was located exterior to the dorsal wall of the peritoneal cavity and ventral to the axial skeleton (Groman, 1982). It is a light to dark brown or black organ normally extending the length of the body cavity. It is usually divided into an anterior or head kidney, and a posterior or excretory kidney (Roberts, 2001).

According to Takashima and Hibiya (1995), the external form of fish kidney varies according to species. Teleost kidney consists of head and body kidneys. In some fish species such as carp and goldfish, both kidneys regions are macroscopically discernible, but this distinction is difficult in other species, e.g. eel and rainbow trout. The head kidney consists of hematopoietic tissue, whereas many nephrons and interstitial lymphoid tissue constitute the posterior kidney. In the channel catfish, the kidney is fused bilaterally but is divided into completely separate anterior and posterior portions. The head kidney and trunk kidney of adults and juveniles are not connected by any kidney tissue or ducts (Grizzle and Rogers, 1976). In this species, the trunk kidney extends to the posterior end of the body becoming narrower posteriorly.

Microscopic structure
Histologically, the teleost kidney is composed of two parts: The renal parenchyma of which the functional unit is the nephron and the interstitial tissue which consists mainly of hematopoietic tissue (Takashima and Hibiya, 1995). The nephron of freshwater teleost kidney is divided into the following parts: (1) renal corpuscle (Malpighian body) consisting of the glomerulus and Bowman’s capsule (2) renal tubules divided into a neck segment, proximal convoluted tube (consisting of two segments P1 and P2), an intermediate segment and a distal convoluted tube (Takashima and Hibiya, 1995). Fish nephron is devoid of the thin segment of Henlé
found in the nephron of higher vertebrates. The nephron is followed by the collecting duct and the ureter. This portion is called the urinary bladder, but is not homologous to that of higher vertebrates. The glomerular capsule has an outer fibrous layer and an inner flattened epithelium. The capsular epithelium is continuous with the renal tubular epithelium. In the capsular epithelium, simple squamous cells with solitary cilia are delimited by marginal microvilli. The glomerulus is a lobulated tuft of capillaries which diverge, converge and wind to make the capillary loop. The glomerular capillaries reunite to leave the glomerulus as the efferent arteriole. Mesangial cells fill the space between the capillary loop. In the glomerular wall, there are three components: podocytes, basal lamina and endothelial cells (Takashima and Hibiya, 1995).

Renal tubules are thin and short in the neck segment and consist of a single layer of low epithelial cells with long cilia. The proximal convoluted segment is divided into two parts. In segment one the tubular epithelium is composed of cuboidal cells with cilia and densely arranged microvilli on the apical surface. These microvilli are recognised as a brush border under light microscopy. The nuclei of these epithelial cells are large and round or oval. They are situated in the central or basal region of the cells. The cytoplasm of these cells contains mitochondria and many secretory granules. In segment two, the renal tubules are composed of cuboidal epithelial cells. Cilia and microvilli are found in the tubular lumen. The diameters of the tubules and tubular lumen are about the same size or larger than those of segment one. In folding of the basal membrane is also noted in these cells (Takashima and Hibiya, 1995).

The hematopoietic tissue forms a support matrix for the nephrons of the posterior kidney but the anterior or head kidney is almost exclusively hematopoietic. The blast cells are situated within the stroma of the reticuloendothelial tissue similar to that of the bone marrow of the mammal (Roberts, 2001). Another cellular structure found throughout teleost hematopoietic tissue is the melano-macrophage centres (MMC) (Roberts, 1975b). Arterial blood is supplied to the kidney by renal arteries arising directly from the aorta or from segmental vessels (Roberts, 2001).
2.5 REFERENCES


