USE OF BIOINDICATORS AND BIOMARKERS TO ASSESS AQUATIC ENVIRONMENTAL CONTAMINATION IN SELECTED URBAN WETLANDS IN UGANDA

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DEDICATION

To my mother, Annet Nabirye Kakaire

Thank you for laying a strong academic foundation in me

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ABSTRACT

Pollution of aquatic resources in Uganda is on the increase and the trends are expected to increase with increase in population size and urbanisation. Assessment and mitigation of the environmental impacts on water quality and biodiversity have now become necessary. The aim of the study was to integrate invertebrate and fish as bioindicators and fish histopathology as a biomarker in the assessment of water quality deterioration in urban wetlands in Uganda. The integration harnesses the advantages and counteracts the shortcomings of each method and thus builds a more robust diagnostic tool that gives a better view of the impacts to the entire ecosystem. Four endpoints which included, physicochemical variables, benthic macroinvertebrate bioindicators, fish bioindicators and fish histopathology biomarkers were compared between varied effluent-impacted wetlands (Murchison Bay in Kampala, and Kirinya, Masese and Winday Bay in Jinja) and a non-impacted reference wetland (Lwanika in Mayuge).

Results from the effluent-impacted sites differed from the less impacted reference site. The two sampling locations at Murchison Bay (inshore and offshore) and one sampling location at Kirinya (inshore), that were highly impacted with urban effluent, showed elevated nutrient levels, low pH, dissolved oxygen and secchi depth readings. This corresponded with low invertebrate taxa and fish species diversity and richness; and severe histopathological responses in liver, gonads and gills of *O. niloticus*. Sensitive taxa such as ephemeroptera and trichoptera were completely absent while pollution tolerant taxa *Chironomus* sp, *Corbicula* and Oligochaeta were present. Also notable was the absence of many native *haplochromines and presence of m*ainly *Brycinus sadleri*, *Oreochromis niloticus* and *leucostictus.* The organs manifested high prevalence of severe inflammatory and regressive changes and higher organ indices that fell within the pathological category. These sites were consistently classified as highly polluted under the four endpoints. The reference site was classified as least polluted while Masese and Winday Bay were moderately polluted. Results suggested that the approach of using invertebrate and fish as bioindicators and the fish histopathology as a biomarker, in relation to water quality physicochemical variables was a useful tool in highlighting the spatial differences in environmental quality.

CHAPTER 1

General Introduction

1.1. Overview and rationale

The current drive for human economic growth and development continues to put pressure on aquatic resources across continents (Abdelgalil and Cohen 2007, Balsdon 2007, Chokor 2004, Agudelo et al. 2003, Bazaara 2003, Ravnborg 2003). Aquatic resources in the East African region today are being depleted and degraded as a result of rapid population growth, increased agricultural production, urbanisation, industrialisation, over fishing and poor waste management among others (Crona et al. 2009, Hecky et al. 2009, Oosterveer 2009, Odada et al. 2004, Bazaara 2003). Lake Victoria, a key component of the natural resource base in the region, is at the centre of rapid ecosystem change as a result of these stressors (Nyenje et al. 2010, Hecky et al. 2009, Madadi et al. 2007, Odada et al. 2004). At 68,800 square Kilometres, Lake Victoria services the three countries of Kenya (6%), Tanzania (49%) and Uganda (45%); all of which are heavily populated and pre-industrial with the majority of the economies depending on agriculture, processing and light manufacturing industries (Swallow et al. 2009, Geheb et al. 2008, Geheb and Crean 2003, Machiwa 2003). Fundamental to Lake Victoria are the wetlands in the lake's fringes, which serve as a backbone sustaining the livelihoods of rural and suburban economic communities (Crona et al. 2009, Swallow et al. 2009, Bakema and Iyango 2000).

However, wetlands on the Ugandan side are being extensively degraded as a result of various human activities among which are, habitat modification for agriculture, poor agricultural land use, urbanisation and infrastructure provision; discharge of industrial and municipal effluents, animal waste, runoff, storm water discharges and small scale economic production enterprises such as informal slaughter houses, poultry units, motor garages and car washing facilities (Munabi et al. 2009, Mugisha et al. 2007, Odada et al. 2004, Bakema and Iyango 2000, Balirwa 1998). These have resulted in depletion of resources, low biodiversity, species extinction, siltation and deterioration in the quality of both surface and groundwater (Mugisha et al. 2007, Odada et al. 2004, Kibwika 2000, Balirwa 1998). Efforts towards prevention and or reversal of these impacts demand efficient and reliable mechanisms of detection especially for systems contamination. Traditionally, methods of detection have been very broad and to a large extent pollutant specific, making pollution detection procedures tedious and costly for routine management practices in developing countries. In Lake Victoria for example systematic data collection and monitoring activities have been project based, and projects come and go; implying that data on water quality is both inconsistent and scattered. As a result, it has been difficult to rank the degree of pollution in the lake and its catchment as well as to establish an environmental policy, including management of water quality, which would abate environmental pollution (Machiwa 2003). This study tried to bridge the gap between extensive application, affordability and efficient outcomes of assessment methods.

Monitoring the state of the aquatic environment was traditionally almost entirely based on measurement of chemical and physical variables (chemical-based aquatic environmental monitoring), and only occasionally were biological variables incorporated (Lam 2009, Singh et al. 2005, Lam and Gray 2003, Davies 1996). However this approach has limitations that include: being inventory in nature, identifying a limited range of contaminants, and giving an understanding of the measured chemical concentration at a point in time. With the enormous and continuously increasing number of potentially toxic substances present in and released into the aquatic environment, coupled with the complex interaction between them, this approach alone is not only costly but is also not possible (Mann et al. 2009, Schiedek et al.

2007, Jin et al. 2004, Worrall et al. 2002). The bioindicator – biomarker approach, on which this study is based, has been shown to provide an assessment of the effects of environmental stressors on organism populations despite the diverse natural background variability (Lam 2009, Wepener et al. 2005, Vasseur and Cossu-Leguille 2003). Bioindicators and biomarkers can be effectively applied to poorly characterised or complex stressors such as mixed contaminants, making them ideal for field environmental quality assessments and assessment of sub-lethal stressor effects to provide early warning systems (Lam 2009, de la Torre et al. 2007, Linde-Arias et al. 2008, Napierska and Podolska 2005, Galloway et al. 2004).

Another limitation with chemical-based aquatic environmental monitoring is that it tends to overlook the information needed to assess biological effects of contaminants to organisms at different levels of biological organisation such as, molecular, cellular, tissue, individual, population community and ecosystems (Lam 2009, Lam and Gray 2003). With these limitations, the past two decades have seen development of a wide range of biomarkers for aquatic contaminant assessment. A summary of these is given in a number of reviews (Torres et al. 2008, Monserrat et al. 2007, Vasseur and Cossu-Leguille 2003, Van der Oost et al. 2003); and examples included molecular, biochemical, cytological, immunological and physiological biomarkers. A wide range of these biomarkers have been recommended for application in monitoring and assessment of toxic effects in biological systems but most of these have been limited to genotoxicity and immunotoxity biomarkers, which cannot be routinely assessed in developing countries due to lack of facilities and high analytical costs.

A number of studies have shown that there is increasing pollution of Lake Victoria and they recommend management to come up with policies of environmental protection (Banadda et al. 2009, Hecky et al. 2009, Matagi 2002, Mugidde 2001, Scheren et al. 2000). Most of the studies applied pollution inventory methods to estimate pollution sources. However, no study was encountered that developed an integrated pollution monitoring model that can be applied for pollution assessment locally. Thus there is need to establish a biological monitoring system (Nzomo 2005, Balirwa et al. 2003, Seehausen 1999, Kaufman 1992). The current conventional water quality physical-chemical tests are not only too expensive for regular water quality assessment but also need to be complemented as their test results only give snapshot conditions in time (GoU 2009, GoU 2006a, Kaufman 1992, Seehausen 1999, Balirwa et al. 2003). Biological monitoring systems are indispensable complements to the conventional physical and chemical tests as they integrate environmental effects on different taxonomic groups over longer time spans and thus have greater power as indicators of environmental change. Biological monitoring has also been widely applied and recommended because it is relatively cheap and easy to apply. In order to generate a biological monitoring tool for Lake Victoria, this study identified water quality assessment indicators using fish and invertebrate bioindicators and fish histopathology biomarkers, in five variably impacted wetlands along the Lake Victoria shoreline, four of which were urban and one rural.

Wetlands were selected for this study because they have a high biodiversity, perform several ecological functions and have extended activity contact regimes with humans (Kalisińska et al. 2004, Powers et al. 2002, Scholz and Xu 2002, Balirwa 1998). However, the multiple human activities make urban wetlands and their resident organisms highly susceptible to pollution stress resulting in deterioration of water quality, loss of biodiversity, absorption of toxins by resident organism some of which are lethal, and this may lead to reduced reproduction and productivity of the resident organisms (Kalisińska et al. 2004, Rees 2002, Abbot et al. 2000, Bakema and Iyango 2000). Furtrher, the lake’s shallow shoreline waters are the initial recipients of the watershed inputs and their ecological status defines the status of the whole lake. Assessing the ecological health status of the shoreline waters is therefore a good indicator of the environmental status of the lake.

The study undertook a comprehensive investigation of aquatic environmental contamination in the urban wetlands in Uganda in order to identify the bioindicator – biomarker indices of water quality assessment. Effects of pollution were assessed at three aspects of ecological level, and these included, water quality, species diversity and fish health (fish histopathology). The findings gave insight into the pollution status of the study sites. The interconnectedness of the pollution drivers, their effects and impacts, in relation to this study is illustrated in a conceptual framework in figure 1.1 below.

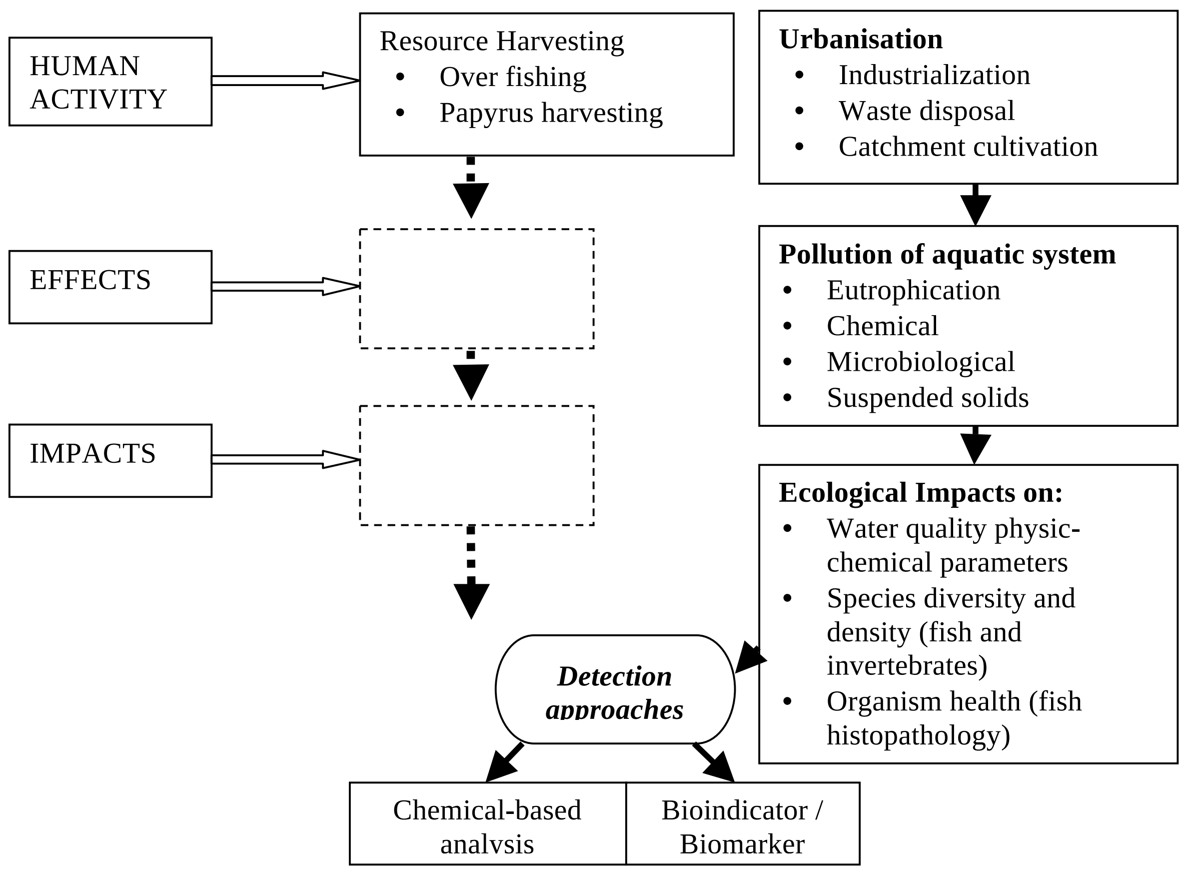


Figure 1.1 Human activity - pollution framework (*Would be contents of broken boxes are not central to this study*)

1.2. Pollution in the Lake Victoria Catchments

The Lake Victoria Basin (LVB) supports a complex arrangement of rural and urban population engaged in different socioeconomic activities and the basin also attracted development of large urban centres and industrial establishments; all of which are current and potential sources of pollutants. The Lake's catchment area of 258,700 square kilometers has a GDP of US$ 300-400 million and supports nearly one-third of the total population of East Africa. This state of affairs is a precursor and a proven accelerator of pollution advancement in the basin (Nyenje et al. 2010, Crona et al. 2009, Hecky et al. 2009, Odada et al. 2004, Machiwa 2003, Ogutu-Ohwayo 2000). The basin has alarmingly registered development of mega-cities characterised by large urban slums (Nyenje et al. 2010, Oosterveer 2009, Odada et al. 2004). Unlike other lake basins in Sub-Saharan Africa such as Malawi and Tanganyika, the LVB attracts a wider population because of its abundant resource base (Nyenje et al. 2010, Odada et al. 2004). The high population pressure has led to conflicting interest in the basin causing land use changes and degradation hence sediment and nutrient loading in the aquatic systems in the catchment (Odada et al. 2004, Machiwe 2003). In addition, there is increased pollution from industrial and municipal loading directly into the lake catchments or through the rivers feeding the lake.

Medium to large scale processing and manufacturing industries, factories, refineries and waste treatment plants discharge semi treated and sometimes untreated effluents that flow into the wetland ecosystems in the catchment. The major industrial polluters include; breweries, soft drink industries, textile factories, sugar industries, food processing industries, textile industries, dairy processing industries, metal factories, paints, oil and soap industries, fish processing industries, paper industries, tobacco processing industries and leather tanning industries (GoU 2006a, 2006b, Odada et al. 2004). There are also a number of cottage and semi-industrial activities such as battery manufacturers, garages, fuel stations, local gin distilleries, etc. which may affect the water quality in the vicinity of their location and the the lake as final recipient. The industries are poorly regulated and most lack pre-treatment facilities for wastewater (GoU 2006b). For example, the catchment of Masese and Kirinya study sites had dilapidated pre-treatment facilites and poor waste disposal methods. During data collection effluent were found disposed of on ground instead of in deep pits. Previously, the significance of these pollution sources was considered low due to the low economic activity. Presently, the magnitude of the industrial pollution is relatively unknown and with continued economic growth, water quality impacts from industrial activities will be an issue of high importance at the local level (GoU 2006b).

However, by far the most important problem concerning pollution of Lake Victoria and its catchments are related to urbanisation and eutrophication from both point and non-point sources. (Nyenje et al. 2010, Banadda et al. 2009, Crona et al. 2009, Hecky et al. 2009, Oosterveer 2009, Mugisha et al. 2007, Odada et al. 2004, Machiwa 2003, Lung'ayia et al. 2001, Scheren et al. 2000). There are several definitions of eutrophication but this study will use a definition according to Golterman and De Oude (1991) who defined eutrophication as a process by which lakes, rivers, and coastal waters become increasingly rich in plant biomass as a result of enhanced input of nutrients, mainly nitrogen and phosphorus. Most of the nutrients causing eutrophication originate from agriculture and urban areas. In developing countries like those in Sub-Saharan Africa, wastewaters from sewage and industries in urban areas, which are often discharged untreated in the environment, are increasingly becoming a major source of nutrients causing eutrophication of surface waters (Nyenje et al. 2010). In Lake Victoria and its catchment for example, although it has been reported that atmospheric deposition contributes the largest input of nutrients (Machiwa 2003, Scheren et al. 2000), comparative studies carried out later indicated stronger eutrophication effects in the nearshore compared to offshore waters (GoU 2010, Cózar et al. 2007, Kansiime et al. 2007). In addition, GoU (2010) reported that urban centres contribute the highest pollution loading into Lake Victoria shores followed by fishing villages and industries, with 72%, 15% and 13% respectively.

Other pollution issues in Lake Victoria and its catchments include chemical, microbiological and suspended solids (Odada et al. 2004). Large commercial agriculture employing chemical weed control and fertilization are sources of toxic chemicals and nutrients that end up in wetlands of the Lake Victoria systems (Munabi et al. 2009). This increased in pollution, as well as the demand for resource utilisation, together exerting environmental degradation to the basin's natural resources. The consequences have been deforestation and general land degradation, loss of water quality and clean air, unbalanced lake ecosystems that can no longer support full biological diversity and pollution (Nyenje et al. 2010, Hecky et al. 2009, Odada et al. 2004, Kibwika 2000).

1.3. Impacts of Pollution in the Lake Victoria Catchments

High levels of organic and inorganic pollutants from municipal and industrial sources can change aquatic communities by either altering the physicochemical environment of the ecosystem or by providing energy source to various trophic levels (Spieles and Mitsch 2003). This may compromise the quality and quantity of the resident aquatic organisms such as invertebrates and fish (Crona et al. 2009, Hecky et al. 2009, Jiang et al. 2007, Azrina et al. 2006, Bigot et al. 2006, Pyle et al. 2005, Paperno and Brodie 2004, Solis-Weiss et al. 2004, Van der Oost et al. 2003, Vasseur and Cossu-Leguille 2003). The main impact of pollution in Lake Victoria has been water quality deterioration due to eutrophication, coupled with toxicity to aquatic flora and fauna. Many causes of pollution, especially non-point sources such as storm water and fertilizers contain nutrients like nitrates and phosphates, heavy metals and pesticides could lead to eutrophication of the Lake and its catchments. There are also increased levels of nutrients and organic matter from urban and industrial point sources, resulting in eutrophication problems and oxygen deficiencies.

Eutrophication is reported to have several impacts on the aquatic system including compromising the ecological integrity of surface waters, extinction of fish species, abundance of toxic cyanobacterial blooms and reduced oxygen levels (Nyenje et al. 2010). In Lake Victoria, it led to the invasion and rapid proliferation of aquatic weeds, especially the water hyacinth. Emerging evidence suggests eutrophication as the main cause for elimination of cichlid species, a condition which has been predominantly attributed to Nile perch. It is apparent that eutrophication-induced loss of deep water oxygen started in the early 1960s and may have contributed significantly to the 1980s collapse of indigenous fish stocks starting with the elimination of suitable habitat for certain deep-water cichlids (Nyanje 2010, Verschuren et al. 2002, Hecky et al. 1994). When the sediment enters various bodies of water, fish respiration becomes impaired, plant productivity and water depth become reduced, and aquatic organisms and their environments become suffocated. On the other hand, pollution from organic material causes oxygen depletion in the water, and this may be harmful to fish at levels below 2ppm.

There are other impacts of point source pollution on water quality in the Lake Victoria catchment. For example, the urban sewerage (7%) and rural sanitation (49%) of Uganda is insufficient. Although the national water supply coverage in the year 2006 stood at 61% for rural areas and 70% for large urban areas, it is still among the lowest in the world (GoU 2006a). The main urban and industrial activities in Uganda are primarily concentrated in the Lake Victoria catchment for example, Kampala, Jinja, Entebbe and Masaka. There are poor sanitary facilities in all urban centres and these conditions result in increased risk of pathogenic contamination and epidemics. Increased incidence of water borne diseases such as cholera, dysentery and typhoid continue to be reported in several districts and is almost endemic (GoU 2006b). This calls for improved access to safe water and sanitation. Eutrophication and pathogenic contamination can make surface water inadequate for human and animal consumption and result in increased costs of production in water treatment.

In Uganda there are limited resources available for monitoring and assessment of water quality on a regular basis, and also for remediation (GoU 2006b). This makes it difficult to obtain a realistic picture of water quality and to map out protection zones at water sources and hot spots that need immediate attention. It also makes it hard to observe and predict future trends of the less polluted sites. In view of these limitations and constraints in chemical-based water quality assessment methods, and the availability of an increasing number of chemical parameters in recent years, a wide range of assessment techniques are now required. However this is not only impossible but is also not practical and sustainable due to the large expenses involved against the meagre government budgets allocated to the water sector. Thus the development and use of biomonitoring techniques as tools for assessment of water quality in natural water bodies has now become necessary. This thesis is the first in Uganda to integrate fish and invertebrate bioindicators and fish histopathology biomarkers as a biomonitoring tool in the assessment of the aquatic environment.

1.4. Monitoring of water resources in Uganda

The Government of Uganda (GoU) promotes an intergrated approach to manage water resources and provide water of adequate quantity and quality for all social and economic needs of the present and future generation with full participation of all stakeholders, inclusive of researchers. With regard to water quality and pollution in particular, the plan is to monitor water quality parameters for municipal wastewater, effluent and receiving body (GoU 1997, 1999). The variables monitored include temperature, pH, electrical conductivity, alkalinity, total suspended solids (TSS), biological and chemical oxygen demand, Total Phosphorus (TP), Phosphorus (P), nitrates, Ammonia, Total Nitrogen (TN), Faecal coliforms/ *Escherichia coli (E.coli)*. However these analytical services are irregular and if and when conducted are localised and at the centre, mainly in Kampala and Entebbe, and are expensive for wide application (GoU 2006b). Todate there are data collection and generation gaps, due to absence of a national water quality monitoring program and lack of standardized procedures or protocols. This has created a deficiency in obtaining meaningful data for both short and long term integrity of water quality. As it stands, a coherent picture of Uganda's water quality status is missing.

In addition, many water quality monitoring programs are not only independent of each other but are further not linked to the environmental values, as a result the relationship between the environmental variables and biological variables is neglected. It is apparent that the degradation of the quality of the water resource has prevailed, threatening public health, aquatic ecosystems integrity and the productive value of water. In this respect the GoU endorsed a national strategy for water quality management to ensure the recognition of water quality as a cross cutting issue and its management mainstreamed in all water, sanitation and environment management activities (GoU 2006b). The strategy targets the effective conservation and protection of water resources through effective water quality monitoring. It thus recognises that effective conservation and protection of water resources requires effective water quality monitoring. In this strategy, water quality criteria according to water quality indicators including physical, chemical and biological will be clarified and a wide range of tools employed, ranging from preventive to impact minimisation of pollution (GoU 2006b). Tools include among others, surveys, risk management, modelling, monitoring, regulatory instruments, and assessments.

In view of this, the present study addresses the deficiencies of the current water quality assessment strategy while taking into consideration some of the recommendations of the new one whereby a holistic approach to water quality monitoring was adopted. This involved linking environmental variables to water quality impacts at a biological level. The study set forth water quality assessment indicators encompassing physical, chemical and biological variables.

1.5. The bioindicator – biomarker approach of aquatic environmental assessment

Given that it is impossible to monitor all contaminants in the environments; indirect methods based on qualitative and quantitative observations of selected organisms have been developed through use of bioindicator / biomarker approach. This approach of assessing aquatic environmental contamination helps compensate for the deficiencies of chemical analysis. A biological indicator (bioindicator) is an organism whose presence or absence and or abundance provide information on environmental conditions or habitat quality (Van der Oost et al. 2003, Kaiser Jamil 2001, Johnson et al. 1993). Certain species have specific requirements for their survival and any effect on their requirement results in an ecological imbalance (Kaiser Jamil 2001). Different aquatic organisms therefore respond to environmental perturbations in different ways (Lenhardt et al. 2006, Islam and Tanaka 2004). The most extreme responses include death or migration to another habitat (Lenhardt et al. 2006, Islam and Tanaka 2004).

Fish and other aquatic vertebrates can indicate water and riparian quality. For example, freshwater macro-invertebrate species vary in sensitivity to organic pollution and thus their relative abundances have been used to make inferences about pollution loads. Macrobenthic species have been recommended for use to establish biological criteria to classify the aquatic ecosystem as being healthy or polluted (Azrina et al. 2006, Díarz-Pardo et al. 1998, Soto-Galera et al. 1998). Fish on the other hand are generally long-lived and integrate pollution effects of lower trophic levels over longer time periods and large spatial scales (Carr and Neary 2006). Also, fish provide a more publicly understandable indicator of environmental degradation. Fish life history information is available for many species, and because many are high order consumers, they often reflect the responses of the entire trophic structure to environmental stress. This study is the first to integrate invertebrate and fish bioindicators and fish histopathology biomarkers in environmental water quality assessment in Uganda. The study proposed a strategy for the application of fish and invertebrate bioindicators and fish histopathology biomarkers in water quality assessment of urban wetlands in Uganda.

A biomarker is a measurable change at molecular, biochemical, cellular or physiological level in individuals (Van der Oost et al. 2003, Kaiser Jamil 2001). Differences between individuals in their molecular, biochemical or physiological parameters reveal a present or past exposure to pollution (Van der Oost et al. 2003, Kaiser Jamil 2001). Biomarkers show that the individuals in which they are measured have been exposed to pollutants. Biomarkers represent biological evaluation that provides information on the health of individuals whereas bioindicator species account for the assessment of the environment. The integration of bioindicators and biomarkers allows for studying the effect of contaminants on different taxonomic groups by establishing a relationship between the environmental variables and the effects caused at biological level and the population. The integration also counteracts the shortcomings of each individual method and thus builds a more robust tool that gives a better view of the impacts to the entire ecosystem.

Many ecotoxicological field studies in the past applied an inventory-based chemical monitoring approach restricted to the identification of a limited range of contaminants in the biota (Avenant-Oldewage and Marx 2000, Kotze et al. 1999, Nussey et al. 1999, Lin and Dunson 1993, Du Preez and Steyn 1992, Miller et al. 1992). Apart from quantifying the selected chemical pollutant in biota, these studies did not address the biological significance of the contaminants. However due to their limitations highlighted in section 1.1 above, many researchers are now focussing on the use of bioindicators (Das and Chakrabarty 2007, Reiss and Kröncke 2005, Tagliapietra et al. 2005, Sekiranda et al. 2004, Fialkowski et al. 2003) and biomarkers (Giarratano et al. 2010, Cazenave et al. 2009, Hagger et al. 2009, Linde-Arias et al. 2008, de la Torre et al. 2007, Vlahogianni et al. 2007, Galloway 2006, Nigro et al. 2006, Quintaneiro et al. 2006, Valavanidis et al. 2006, Napierska and Podolska 2005, Bolognesi et al. 2004, Galloway et al. 2004, Handy et al. 2002, Lam and Gray 2003, Lionetto et al. 2003, Stentiford et al. 2003, Van der Oost et al. 2003, Hahn 2002, Regoli et al. 2002, Torres et al. 2002, Cajaraville et al. 2000, Wedderburn et al. 2000, Zelikoff et al. 2000, Teh et al. 1997).

Nonetheless, despite the rapid development of the bioindicator - biomarker approach in aquatic environmental quality assessments; most applications have been limited to genotoxicity and immunotoxity biomarkers, and mainly from the marine environment (Hagger et al. 2009, Vlahogianni et al. 2007, Galloway 2006, Nigro et al. 2006, Quintaneiro et al. 2006, Napierska and Podolska 2005, Bolognesi et al. 2004, Lionetto et al. 2003, Hahn 2002, Cajaraville et al. 2000, Wedderburn et al. 2000, Zelikoff et al. 2000), and to a lesser extent in freshwater river systems (Cazenave et al. 2009, Linde-Arias et al. 2008, de la Torre et al. 2007, Tegeda-Vera et al. 2007), with a few histopathological biomarkers in marine systems (Marigómez et al. 2006, Stentiford et al. 2003, Fournie et al. 2001, Teh et al. 1997). The most frequently used biomarkers include: energy metabolism, enzyme activities, ion regulation, RNA, DNA, amino acids and protein content. The challenge with the use of these biomarkers in developing countries is the lack of facilities and the expenses involved with the analysis that impedes their use in routine environmental monitoring programmes.

Although a few studies in Europe and North America (Stentiford et al. 2003, Fournie et al. 2001, Teh et al. 1997) have successfully used histopathology as a biomarker in environmental risk assessment, no related study in East Africa was encountered. In Uganda this is the first study to intergrate bioindicators and biomarkers in one assessment tool and to apply histopathology biomarkers in biomonitoring. Given the rapid deteriorating state of Lake Victoria and its catchments (Crona et al. 2009, Hecky et al. 2009, Oosterveer 2009, Odada et al. 2004, Bazaara 2003), it is invaluable to have in place efficient and reliable environmental pollution diagnostic/assessment procedures. In this study attempts have been made to characterise fish histopathological properties related to specific zones in the wetland ecosystems along the Lake Victoria basin; with the objective of developing histopathology biomarkers for assessing the aquatic environmental contamination in these wetlands, in integration with fish and macroinvertebrate bioindicators.

Microscopic examination of tissues has long been used in pathology laboratories for human and veterinary medicine both for disease diagnosis and toxicology investigations. While histopathology is not a biochemical / physiological technique, it is related to biochemical changes which are the result of lesions. In fish laboratory studies (Kinnberg et al. 2003, Thophon et al. 2003, Naigaga 2002) and field studies (Moore et al. 2003, Carletta et al. 2002, Simpson et al. 2000) have established links between exposure to different xenobiotics and development of toxicopathic lesions. These studies showed that 1) certain lesions in fish can be induced by environmental contaminants, 2) these lesions represent an ecologically relevant biological endpoint of exposure to pollution, and 3) histopathology may not be definitive but provides a useful insight into individual, population and overall ecosystem quality.

1.6. Aim and objectives of the study

The aim of the study was to integrate invertebrate and fish as bioindicators and fish histopathology as a biomarker in the assessment of water quality deterioration in urban wetlands in Uganda. To achieve this, a suite of endpoints were compared between varied effluent-impacted wetlands and a nonimpacted reference wetland. Endpoints for comparison included: (1) physicochemical parameters, (2) benthic macroinvertebrate bioindicators, (3) fish bioindicators and (4) histopathology biomarkers. Specific objectives included,

- to characterise the selected wetlands based on their physicochemical properties and identify possible underlying factors that influenced the physicochemical variables in the study sites,
- to investigate benthic macroinvertebrate and fish community structure in relation to the physicochemical variables so as to explore the use of macroinvertebrates and fish as biological indicators of water quality deterioration in selected Ugandan wetlands,
- and to investigate fish histopathology as a biomarker of water quality deterioration in selected Ugandan wetlands.

1.7. Thesis outline

This thesis explores the use of fish and invertebrate biological indicators (bioindicators) and fish histopathology biological markers (biomarkers) in water quality assessment of Lake

Victoria in Uganda. The thesis affirms the importance of this biological monitoring tool in water quality assessment given that there is increasing pollution of the lake in urban areas; and the fact that the conventional water quality physical chemical tests that are currently being used in Uganda are expensive for regular water quality assessment and need to be complemented as their test results only give snapshot conditions in time. The context for this study is that efforts towards prevention and/or reversal of pollution impacts demand efficient and reliable mechanisms of detection especially for systems contamination.

Chapter one articulates the study concept and rationale; highlights gaps in knowledge of the problems associated with pollution of urban aquatic resources in Uganda; underscores the limitations in the current water quality assessment methods in the country and the need to develop a biological monitoring system of water quality assessment for water bodies in Uganda. The chapter gives details on the pollution drivers in the catchments, important types of pollution and challenges of pollution management in the Lake Victoria catchments; and the need for management intervention. The different impacts of pollution on water quality and aquatic biodiversity were also addressed. The current Uganda national water monitoring strategy was assessed highlighting the strengths and weaknesses of the current aquatic environmental assessment methods, with respect to pollution. Key terminologies of biomonitoring, bioindicators, and biomarkers were defined; and the application and advantages of biological monitoring in aquatic environmental assessment was reviewed.

Chapter two describes the general methods used in data collection and laboratory analysis. Specific details of data processing and interpretation were given in the respective chapters. The chapter describes the five variably impacted wetland ecotones along the shoreline of Lake Victoria that were studied, four of which are urban, and one rural. Three of the urban wetlands directly received urban effluent through connected channels. The chapter also gives a detailed research design. Data for this study were collected 12 times, from August 2006 to July 2008. Sampling was conducted every two months making a total of 12 data points. For every study site (wetland), data were collected at two locations, nearshore and offshore locations. This helped to capture changes in temporal variability and to collect enough data points for statistical analysis. Samples included physicochemical variables, fish and invertebrates, and fish tissue for histopathology assessment. Physicochemical variables were sampled because they represent water quality deterioration, while fish and invertebrates were sampled because the two fauna are impacted differently by water quality deterioration. Fish histopathology was studied because fish cellular structure is sensitive to water quality deterioration and represents impacts occurring over time. Key statistics used in the study were also highlighted in this chapter.

Chapter three is an account of the physicochemical conditions of the study sites. Physicochemical conditions gave insight into the aquatic environmental quality of the different study sites and sampling locations. These conditions influenced invertebrate and fish species diversity, and fish histopathology examined in the later chapters. The following physicochemical parameters were sampled: total depth, secchi depth, temperature, oxygen, conductivity, pH, total nitrogen, total phosphorus, nitrite, nitrate, ammonia and chlorophyll a. These variables were sampled because they represent water quality deterioration. Carlson's Trophic State Index using total phosphorus, chlorophyll a and secchi depth was calculated and used to rank / classify the study sites according to their eutrophic status (Carlson 1977). Three multivariate statistical techniques that included cluster analysis (CA), principal component analysis (PCA) and factor analysis (FA) were used to evaluate spatial variation in water quality and to characterise the study sites according to their water quality status, subsequently defining their degree of contamination

Chapter four presents the aquatic environmental quality of the selected wetland ecosystems using invertebrates as bioindicators. The chapter included a review and highlight of the importance of invertebrates in water quality assessment, the response of benthic invertebrates to pollution, and the relationship between environmental physicochemical parameters and benthic invertebrate diversities. Species Diversity and Richness (SDR Version 2.65) was used to analyse and compare the invertebrate community structure in the different study sites (Seaby and Henderson 2000). The Margalef Index was used to estimate species richness. This index has been documented to have a very good discriminating ability to measure the number of species present for a given number of individuals. The Shannon diversity indices were computed and indices between sites compared using Randomization Test. The Shannon diversity is a very widely used index for comparing diversity between various habitats (Clarke and Warwick 2001). Canonical correspondence analysis (CCA) was performed to evaluate the relationship between invertebrate communities and environmental variables using the Environmental Community Analysis Package 2 (ECOM). CCA is a powerful tool for simplifying complex data sets and being a direct gradient analysis, it allows integrated analysis of both taxa and environmental data (Arimoro 2009, ter Braak and Smilauer 2002).

Chapter five characterises the aquatic environmental quality of the selected wetland ecosystems using fish as bioindicators. The chapter highlighted the importance of fish in water quality assessment, response of fish species to pollution and the relationship between environmental physicochemical conditions and fish species diversities. As in chapter four above, Species Diversity and Richness (SDR Version 2.65) was used to analyse and compare the fish community structure in the different study sites (Seaby and Henderson 2000). The Margalef Index was used to estimate species richness. The Shannon diversity indices were computed and indices between sites compared using Randomization Test. Canonical correspondence analysis (CCA) was performed to evaluate the relationship between fish communities and environmental variables using the Environmental Community Analysis Package 2 (ECOM).

Chapter six investigated fish histopathology in relation to urban effluent exposure in the study wetlands with a view to explore fish histopathology as a biomarker of water quality deterioration in selected Ugandan wetlands. Histological alterations in the liver, gills, gonads and spleen of *O. niloticus* from five variably impacted wetlands were assessed. The study sites were evaluated and compared in terms of the fish histopathology indices and lesion prevalence according to a modified protocol after Bernet et al. (1999).

Chapter seven synthesises the general findings of the thesis, and relates them to the contemporary understanding of environmental impacts on water quality and biodiversity. It also examines the potential for integration and application of fish and invertebrate bioindicators and fish histopathology biomarkers as a tool in water quality assessment of urban wetlands in Uganda.

In conclusion, this thesis provides a vital contribution to biomonitoring thereby providing baseline information useful in the development of a biomonitoring tool for shoreline water quality assessment in Lake Victoria. The current data on water quality characteristics in relations to invertebrate and fish bioindicators and fish histopathology biomarkers demonstrates that a biomonitoring tool can be developed and if successfully tested and applied, can be useful in water quality monitoring and assessment. Even more encouraging is that in some parts of the world, related tools are being successfully applied in water quality monitoring and assessment. For example, in India, Iraq, China and Japan, this has been successfully applied. South Africa has also successfully used the South African Scoring System (SASS) method. This research stands to motivate more efforts to protect and improve water resources for ecosystem health and human well-being in Uganda.

CHAPTER 2

General Methods

2.1. Study Sites:

Data for this study was collected in the bays of five wetland ecotones located in three districts under the same geo and agro ecological zones along the Ugandan shoreline of Lake Victoria. The wetland ecotones include Nakivubo, Kirinya, Masese, Winday and Lwanika. Nakivubo wetland is located in Kampala, Uganda's capital and business centre situated in Kampala District. Three wetlands of Kirinya, Masese and Winday, are located in Jinja municipality in Jinja district. Jinja is situated approximately 80km East of Kampala, and is one of the 13 municipalities and the second largest town in Uganda after Kampala. Lwanika wetland is located in Mayuge district. Mayuge is a rural district compared to Jinja and Kampala; located approximately 41 km and 122 km east of Jinja and Kampala respectively. For this reason Lwanika wetland was hypothesised to be less impacted by urban effluent and was thus used as a reference site during the study. Kirinya and Masese wetlands are located approximately 2 km apart along the Lake Victoria shoreline in Jinja municipality, both lying to the north east of the Napoleon gulf (Figure 2.1). Lying almost adjacent on the south west of the Napoleon gulf is Winday Bay (Figure 2.1). These wetlands are vegetated and shallow-water indentations along the coast of L. Victoria forming an important part of the lake's catchment. Accordingly they serve as intermediate natural resource between the uplands and the lake offering protection to the water resource and a source of livelihoods to the riparian communities.

Except for two wetlands of Winday Bay and Lwanika, the upland in Murchison Bay, Kirinya and Masese, consist of mainly urban centre developmental projects such as hotels, factories, industries, fuel depots, homesteads and sewage treatment ponds, untreated domestic and industrial effluent drainage channels and cultivated agricultural landscapes. These wetlands thus receive industrial and domestic effluent and storm-water, from point and non-point sources such as deficient sewage and industrial wastewater treatment plants, small-scale workshops, waste oil from parking lots and car repair garages before the effluent empties into Lake Victoria, and this waste matter may contain pollutants. The sewer system in Kampala city serves only a small fraction of the city population and only 10% of all sewage generated in Kampala gets treated. Guesthouses, slum dwellings and industries discharging untreated wastewater in Nakivubo channel, which flows through Nakivubo wetland into inner Murchison Bay contribute pollution load and lead to depleted oxygen levels in Lake Victoria. Riding a motorboat at the point where Nakivubo channel discharges into Murchison Bay churns up a trail of black sewage sludge. Nakivubo Channel carries approximately 75% of the nitrogen and 85% of the phosphorus nutrient load discharged daily into Murchison Bay (Nyenje et al. 2010, Mugisha et al. 2007). The high nitrogen and phosphorus levels are responsible for excessive eutrophication and algal blooms seen in the Bay.

Taking into consideration the wetland's pollutant retention properties (Mugisha et al. 2007, Bakema and Iyango 2000), the assumption is that the vegetative part of these wetlands would effectively trap sediments and remove nutrients and pollutants from surface water runoff before emptying into the lake. However, encroachment of the wetlands has reduced wetland vegetative coverage which would serve as natural filters for trapping and retaining many pollutants such as sediments, nutrients, heavy metals and pathogens before reaching water bodies (Machiwa 2003, Mdamo 2001). Nakivubo wetland and other major catchment wetlands, which when still lush and thick played the vital role of tertiary purification of effluent and stormwater discharging into the lake, have long been encroached and degraded by settlement and cultivation. Widespread lakeshore cultivation and soil erosion also contribute excessive sediment and nutrients into the lake (Machiwa 2003, Mdamo 2001). Stormwater flowing in Nakivubo Channel carries along tonnes of soil straight into the lake.

Nakivubo wetland is the largest of the twelve main wetland areas in Kampala city, covering approximately 5.29 km^2, with a total catchment extending over 40 km^2 (COWI and VKI 1998). Nakivubo forms a permanent swamp and is fed by the Nakivubo River and its tributaries the Katunga, Kitante, Lugogo and Nakulabye Rivers. The wetland runs from the central industrial district of Kampala, entering Lake Victoria at Murchison Bay. Much of the shallow upper part of the wetland has been reclaimed for settlement and industrial development, or is under cultivation. The Nakivubo wetland has become severely degraded over recent years, and is particularly threatened by the spread of industries and residential development (Emerton et al. 1998). The wetland receives secondary treated effluent from the Bugolobi sewerage treatment works and heavily polluted wastewater comprising runoff and effluent from guesthouses, slum dwellings and industries in Kampala city, through the Nakivubo channel, and discharges into Lake Victoria at Murchison Bay (Figure 2.1). The Nakivubo channel feeding this wetland with wastewater (Figure 2.1) is 12.3 km long with a catchment area of about 50km^2. The degree of pollution in this channel as a result of point source pollution and landuse-based nonpoint source pollution have a direct impact on the water quality and the aquatic life in the inner Murchison Bay (Banadda et al. 2009, Kayima et al. 2008, Kansiime et al. 2007). Sampling for this study was carried out at two locations in the inner Murchison Bay. The inshore location was closest to the shoreline at the Nakivubo channel discharge point, and the offshore location was approximately 60m from the inshore location into the bay.

Kirinya wetland is located along the Northern shoreline of Lake Victoria in Jinja municipality, lying to the north east of the Napoleon gulf (Figure 2.1). The Kirinya wetland ecotone has an area of 0.47 km^2 and is dominated by *Cyperus papyrus* and *Phragmites mauritanus* vegetation. This wetland has been reported to receive increasing discharges of domestic, municipal and industrial effluent, surface runoff and storm water from Jinja town (Oguttu et al. 2008, Kelderman et al. 2007). Although this wetland is not an official waste dumping site, open waste dumping of both biodegradable and non biodegradable materials were frequently observed during the study. Kirinya wetland offers unique services to Jinja municipality as it is a home to many institutions such as hotels, industries, factories, metallurgy and Jinja municipality's wastewater treatment oxidation ponds, the Kirinya National Water and Sewerage Corporation Oxidation ponds that receive effluent from the municipality before discharging into Lake Victoria as secondary treated effluent. During the study, sampling in this wetland was carried out at two locations in the Napoleon gulf. The inshore location was closest to the shoreline at the wastewater discharge point, and the offshore location was approximately 60m from the inshore location into the gulf.

Masese wetland is located along the Northern shoreline of Lake Victoria in Jinja municipality, lying to the north east of the Napoleon gulf, 2km from Kirinya wetland (Figure 2.1). This wetland is used for open dumping of waste material in Jinja municipality, both biodegradable and non biodegradable including chemical waste due to lack of a landfill in the municipality where such material could be disposed off. Masese wetland was gazetted for waste disposal for over three decades now, at a time when there were no inhabitants. But in the early 1990s people started inhabiting the wetland forming a slum as is the case today (Jinja District Profile 2003). Masese wetland receives urban effluent and storm water runoff through a drainage channel. This channel stretches for a distance of approximately 1.4km and carries industrial and domestic effluent from several residential villages in the outskirts of Jinja Municipality through the urban centres of Jinja and pours into the Masese wetland before emptying into Lake Victoria. During the study, sampling in this wetland was carried out at two locations in the Napoleon gulf. The inshore location was closest to the shoreline at the drainage channel discharge point, and the offshore location was approximately 60m from the inshore location into the gulf.

Lying almost adjacent on the south west of the Napoleon gulf is Winday Bay (Figure 2.1). The catchment of Winday Bay is less disturbed compared to Kirinya and Masese with an expansive Jinja prisons headquarters. The main activity in this catchment is crop cultivation, with a recent aquaculture establishment in the catchment prison land. During the study, sampling in this bay was carried out at two locations with the inshore location closest to the shoreline while the offshore location was approximately 60m from the inshore location.

Lwanika wetland is located along the lake shoreline in Mayuge district (Figure 2.1). Mayuge is a rural district located approximately 41km and 122km east of Jinja and Kampala respectively. Lwanika wetland is presumed to be a rural undisturbed wetland that does not have any inlet feeding into it. Sampling in this wetland was carried out at two sampling locations in the bay, with the inshore location close to the shoreline and the offshore location approximately 60m off the inshore.

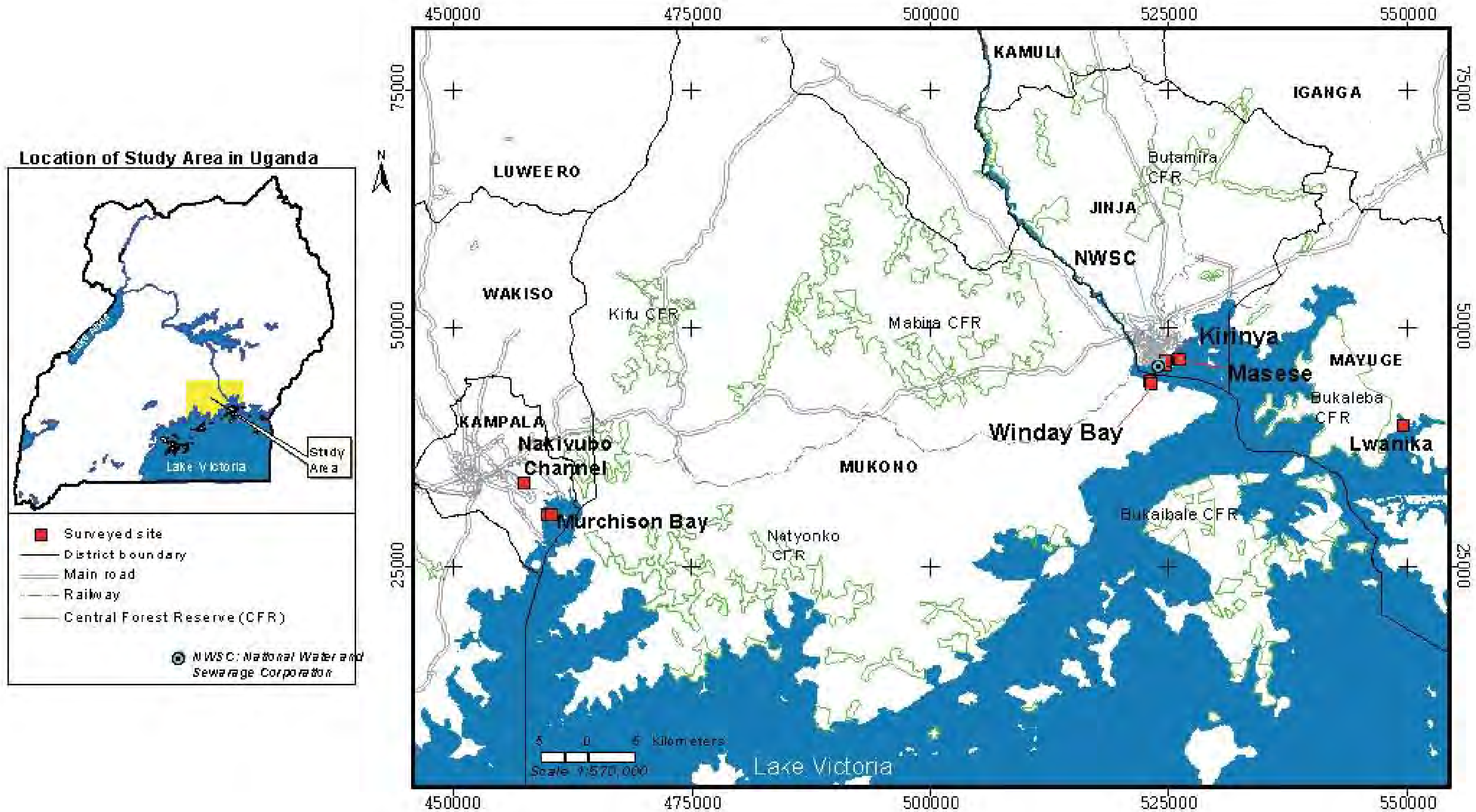


Figure 2.1 **A Map showing location of the study sites**

2.2. Research Design

The study involved collecting data every two months from August 2006 to June 2008 making a total of 12 field sampling trips. This entailed sampling for physical chemical parameters; invertebrates and fish for bioindicators; and fish for histopathology biomarkers.

2.3. Measurement of physicochemical variables

Physicochemical parameters were measured during sample collection at all sites and these included temperature, pH, dissolved oxygen, electrical conductivity, water transparency and dissolved nutrients (total dissolved phosphorus, total dissolved nitrogen, nitrite, nitrate, ammonia, and chlorophyll a). Temperature, pH, dissolved oxygen and electrical conductivity were measured in situ using portable meters. Water transparency at each site was measured using a 25 cm Secchi disk. Water samples were collected for measurement of total phosphorus (TP), total nitrogen (TN), nitrite (NO_2), nitrate (NO_3), ammonia, ($NH^+{}_4$) and chlorophyll a. Dissolved inorganic nutrients (NH_4^-, NO_2^-, NO_3) and chlorophyll-a (Chl-a) were determined spectrophotometrically following methods of APHA (1998). Ammonia was measured by the indophenol-blue method. Nitrate plus nitrite were determined using the cadmium method as described in APHA (1998). Total dissolved nitrogen and phosphorus concentrations were determined following oxidation of the filtrate of 20-ml subsamples in alkaline and acidic persulfate, respectively, and through subsequent analysis of nitrate and phosphate as in APHA (1998).

2.4. Sampling and analysis of macro-invertebrate bioindicators

Macro-invertebrates were sampled in all the locations during the study. Sediment samples were collected using a Ponar Grab with a jaw area of about 245 cm^2. Two hauls were taken from each sampling point, packed and preserved with 5% formalin in polyethene bags. Preserved samples from each bag were later washed using a 500µm mesh net washing bag and placed in labelled bottles containing 70% ethanol. In the laboratory, each sample was rinsed poured in a flat tray, individual organisms sorted and taxonomically identified as far as possible using a simple dissecting microscope and macro-invertebrate identification manuals. Members of each taxon were numerated and the counts expressed as individuals per square meter.

2.5. Sampling fish for bioindicators and histopathology analysis

Fish samples were collected for bioindicator assessment and histopathology analysis. Fish for bioindicator analysis was obtained by the use of gill nets set overnight. This involved determining the species diversity and abundance after taking into account all the fish in an overnight catch. All the fish caught per net in each site were identified to species level, counted and the corresponding numbers recorded. For histopathology only live fish were considered during the study. Because many fish could not stay alive during the overnight gill netting, fish for histopathology assessment were sampled separately. In many cases the local fishermen were hired to assist with the sampling during the day. Fish were kept alive by continuous water exchange of running running water from a mobile tap unit in the field. The water used in the mobile tap was collected from the study site. Dissections and removal of the gills, liver, spleen and gonads was carried out as fast as possible to ensure that as many fish as possible were dissected in the field. The dissected organs were preserved in bouin's solution and labelled pending laboratory analysis. Sample sizes varied between sites. The target was a minimum of 30 fish per site.

2.6. Laboratory analysis for histopathology

The samples for histopathology were processed according to standard histology procedures (Hopwood 2002, Anderson and Bancroft 2002) and stained with H and E (Wilson and Gamble 2002). Light microscopy was used to examine the tissues and histological alterations were qualitatively assessed. The observations were semi-quantitatively evaluated according to a protocol proposed by Bernet et al. (1999), which was slightly modified in nomenclature and with the inclusion of gonadal and splenic histopathology that were not included in the Bernet protocol. Pathological changes in each organ were classified into five reaction patterns and these included: Circulatory disturbances (C), regressive changes (R), progressive changes (P), inflammation (I) and neoplasms (N). Following the Bernet et al. (1999) protocol, the lesions under each reaction pattern were identified and scored as described in detail in chapter six.

2.7. Statistical Analysis

Descriptive statistics was used to derive the means, standard deviations and ranges for the different physical chemical variables. Kruskal-Wallis nonparametric ANOVA was used to compare the water quality variables between sampling locations. Multivariate statistical methods were applied to analyse water quality and bioindicator variables using ordination techniques to relate bioindicators to environmental variables. Cluster analysis

(CA), principal component analysis (PCA) and factor analysis (FA) were used to determine the spatial variation in water quality (chapter three). The composition and occurrence of macroinvertebrate taxa and fish communities were explored using relative abundance and community indices. Site differences in invertebrate and fish taxonomic composition were tested using CA and PCA where sites with similar taxa / community characteristics were grouped under one cluster. CA and PCA were used concurrently for validation purposes. The relationship between macroinvertebrate taxa and environmental variables, and fish communities and environmental variables were assessed using CCA (chapters four and five). The essence was to assess the relationship between the invertebrate taxa and fish species distribution, and the environmental variables. CA and PCA were performed using the Community Analysis Package 1.52 computer programme (Henderson and Seaby 2000a), while SPSS 16 was used for the FA. CCA was performed using the Environmental Community Analysis Package 2 (ECOM) by Henderson and Seaby (2000b).

The routine multimetric methods commonly used in bioindicator studies were not considered in this study. Although reported to be robust tools of bioassessment, multimetric approaches are less capable of distinguishing between impacted and reference sites as compared to multivariate assemblage methods. Unlike multimetric methods, which summarise assemblage structure in a single index developed from individual metrics, multivariate approaches consider the biotic conditions of a site while summarizing the relationships between taxon abundances and environmental variables (Kenney et al. 2006, Reynoldson et al. 1997).

CHAPTER 3

Physical and chemical water quality characteristics of selected wetlands in Uganda

3.1. Introduction

Many environmental factors affect ecological biodiversity and consequently the use of bioindicators and biomarkers, it is thus important that the physicochemical and ecological conditions of the ecosystem be characterized in such studies. In aquatic environments, different physicochemical factors may give rise to responses of biochemical and physiological parameters used as biomarkers (Vasseur and Cossu-Leguille 2003). Such factors include: climatic conditions, physicochemical characteristics of the environment, species genetic idiosyncrasy, relationships between individuals and or species, or interactions between pollutants (Van der Oost et al. 2003, Vasseur and Cossu-Leguille 2003). The use of bioindicators and biomarkers to evaluate environmental quality therefore necessitates that the ecosystem be characterized precisely in physicochemical as well as ecological terms. Given the eutrophication pollution challenge in Lake Victoria and its urban wetlands, physicochemical parameters including nutrient levels were assessed during the study.

Among the most important physicochemical variables that were considered in this study were pH, conductivity, temperature, water transparency, nutrient and chlorophyll-a levels. Temperature and water transparency are physical variables, pH and electrical conductivity determine the dissolved ionic strength of the water, while dissolved oxygen and nutrients are additional important chemical variables (Carr and Neary 2006).

Nutrients and chlorophyll-a levels give an indication of the trophic status of the water. The wetlands under study are urban and receive industrial and domestic effluent and storm-water, from point and non-point sources, discharges known to contain pollutants that influence physicochemical water quality variables (GoU 2010, Nyenje et al. 2010, Banadda et al. 2009, Munabi et al. 2009, Kayima et al. 2008, Oguttu et al. 2008, Cózar et al. 2007, GoU 2006a, Muwanga and Barifaijo 2006, Matagi 2002). In tropical lakes, the characteristics and dimensions of the shoreline wetlands have a strong influence on the quality of the inshore waters (Bracchini et al. 2009, Cózar et al. 2007).

It is therefore important to have reliable information on trends of water quality as a prerequisite for the prevention and control of the lake pollution and sustainability of an effective water management programme (GoU 2009, Carr and Neary 2006, GoU 2006a, Nzomo 2005, Balirwa et al. 2003, Seehausen 1999, Kaufman 1992). This necessity is even more pronounced in East Africa with its sanitation problems in densely populated areas like Kampala and Jinja combined with a rapid population growth due to the large influx of people seeking employment in cities (Nyenje et al. 2010, Oosterveer 2009, Odada et al. 2004, Matagi 2002, Scheren et al. 2000). The steady increase in population size imparts pressure on catchment resources for settlement, agriculture, and urban and industrial infrastructure development consequently exacerbating water pollution problems as more wastewater is discharged into the lake. The result is reduced quality of the aquatic environment.

Urban wetlands, in particular, play a major role in assimilation of pollutants as wetlands carry off municipal and industrial wastewater and surface runoff from agricultural land

(Nyenje et al. 2010, Banadda et al. 2009, Hecky et al. 2009, Kayima et al. 2008, Kulabako et al. 2007, 2008, Cózar et al. 2007, Kansiime et al. 2007, Kelderman et al. 2007, Mugisha et al. 2007, van Dam et al. 2007, Muwanga and Barifaijo 2006, Matagi 2002). Whereas the surface run-off is a seasonal phenomenon, the municipal and industrial wastewater discharge constitutes a constant polluting source and the two scenarios contribute to the concentration of pollutants in the aquatic ecosystem (Bu et al. 2010, GoU 2010, Razmkhah et al. 2010, Kazi et al. 2009, Alkarkhi et al. 2008, Li et al. 2007, Koukal et al. 2004, Singh et al. 2005). Surface waters are particularly vulnerable to pollution due to their easy accessibility for disposal of wastewaters (Bu et al. 2010, Carr and Neary 2006, Singh et al. 2005). A recent review on eutrophication and nutrient release in urban areas of Sub-Saharan Africa reported that wastewaters from sewage and industries in urban areas, are often discharged in the environment untreated and are becoming a major source of nutrients, causing eutrophication of surface water bodies which leads to the depletion of dissolved oxygen (Njenje et al. 2010). This problem is very relevant in Uganda (GoU 2010, Banadda et al. 2009, Kayima et al. 2008, Oguttu et al. 2008, GoU 2006a, Matagi 2002). Lake Victoria is noted in this review as the most prominent example highlighting the adverse effects of excess nutrients in freshwater resources.

Much of the lake's shoreline is covered by extensive wetlands, often dominated by dense papyrus stands that extend out over the lake waters and these wetlands play a role in the physical, chemical and biological conditions of the inshore waters (Cózar et al. 2007). The activities in the catchments reflect on the quality of the shoreline waters and consequently of the main lake. The water quality status of the shoreline waters is therefore an important indicator of the environmental status and trends of the whole lake. As water quality deteriorates, ecosystem services may be lost and organisms suffer. For example, the fluctuation of the physical and chemical characteristics of a lake impacts on the distribution and abundance of organisms such as macroinvertebrates and fish assemblage composition (Sheldon et al. 2002). As will be seen in the following chapters, deterioration in water quality negatively impacted on macroinvertebrates and fish assemblage composition and fish histopathology. There is a whole range of physical and chemical variables that could be measured but this chapter characterises the study sites according to eleven commonly recorded variables that are used to assess Lake Victoria's waters. The objective of this chapter was to characterise the selected wetlands based on their physicochemical variables and identify possible underlying factors that influenced the physicochemical variables in the study sites. The chapter set the stage for the proceeding chapters that relate the biological indicators and biomarkers to water quality. The hypothesis was that physicochemical variables were negatively impacted by effluent and this consequently impacted negatively on fish and invertebrates.

3.2. Literature Review

3.2.1. Factors that influence surface water quality:

Water of adequate quality is one of the key requisites in maintaining a healthy environment. Regardless of Lake Victoria being a significant freshwater resource in East Africa, the challenges of rapid population growth, increased urbanization and industrialization, uncontrolled environmental degradation and pollution are leading to the lake's accelerated degradation. The impact and extent of this degradation on water quality is complex and not fully understood. This defines the entry point of this study. As far as the lake's water quality is concerned, a number of reviewers and several authors who studied the Ugandan part of the Lake put emphasis on tracing the anthropogenic causes of pollution (Nyenje et al. 2010, Banadda et al. 2009, Oguttu et al. 2008, Kansiime et al. 2007, Muwanga and Barifaijo 2006, Odada et al. 2004, Matagi 2002). Related studies were conducted on the Kenyan and Tanzanian part of the Lake (Gakuma-Njugu and Hecky 2005, Nzomo 2005, Machiwa 2003). All studies agree that pollution loads from point and non-point diffuse sources of industrial, municipal, and agricultural wastewater are the most rampant in influencing the quality of the water.

Surface water quality in a region is largely determined by the diverse natural biochemical processes that are taking place in the aquatic system and the anthropogenic inputs from the catchments (Bu et al. 2010, Razmkhah et al. 2010, Kazi et al. 2009, Alkarkhi et al. 2008, Li et al. 2007, Panda et al. 2006, Koukal et al. 2004). Seasonal weathering and soil erosion processes are some of the natural processes causing variations in water quality. Many studies revealed that urbanisation and or catchment agriculture are not only a major anthropogenic origin of water quality deterioration but are also a constant source of pollution in aquatic systems (Nyenje et al. 2010, Banadda et al. 2009, Muwanga and Barifaijo 2006, Odada et al. 2004, Koukal et al. 2004, Matagi 2002). Other causes such as atmospheric pollution, municipal effluent discharges, use of agricultural chemicals, eroded soils and land use are embedded within these two broad sources, and are all driven by human activities in the catchment. Urbanisation is the most important factor responsible for surface water quality deterioration of many water bodies globally (Nyenje et al. 2010, Koukal et al. 2004).

In Uganda for example, most water pollution in Lake Victoria is reported to be domestic in origin, but the recent increased economic growth has resulted into increased industrialization, which led to setting up a number of medium to large-scale processing and manufacturing industries, factories, refineries including sugar, textiles, oil, and distilleries located mainly in the towns of Kampala and Jinja that are located close to Lake Victoria. This has increased industrial pollution (Banadda et al. 2009, Hecky et al. 2009, Mugisha et al. 2007, GoU 2006a, Matagi 2002). Most of these industries have no wastewater pre-treatment facilities thus they discharge directly into the water bodies (GoU 2006a, Matagi 2002). Discharge of raw sewage and untreated industrial effluent into Lake Victoria is a common occurrence in many major towns in Uganda where the lake spans such as, Entebbe, Masaka, Kampala and Jinja. This is mainly attributed to the lack of or existence of poor sewerage treatment facilities in these towns and has greatly contributed to poor quality of water in many surface water bodies around these towns (GoU 2006a). There is a lack of data for comparison of sites as studies that looked at the impact of pollution on water quality in the Ugandan part of Lake Victoria have been spatially and temporally limited (Munabi et al. 2009, Oguttu et al. 2008, Cózar et al. 2007, Kansiime et al. 2007, Sekiranda 2006). These authors suggest anthropogenic causes as the main underlying factors responsible for surface water quality deterioration.

For instance, Munabi et al. (2009) assessed the effect of the Kakira Sugar Works Limited sugarcane plantation on the water resources of Lake Victoria for a period of six months covering the wet and dry season. There were higher electrical conductivity values in the stream draining through the workers settlement (1412.3 μS/cm) than at a sampling site located in Lake Victoria which had values <300 μS/cm. Although the study reported nutrient concentrations within permissible limits, it was concluded that annual nutrient loads of nitrogen and phosphorus could increase the trophic level of Lake Victoria. Oguttu et al. (2008) observed that nutrient and heavy metal levels in effluent from many industries in Jinja town were above the allowed effluent limits in Uganda, thus contributing to eutrophication of the lake. Kansiime et al. (2007) also showed that Lake Victoria's Murchison Bay, due to the inflow of wastewater from Kampala (Uganda), had deteriorated in the past decades, as evidenced by a higher level of nutrients that was estimated to be 28mg/l NH_4^--N from the initial 5.6 mg/l NH_4^--N in 1999. Lastly, Sekiranda (2006) observed that the extent of eutrophication in the shoreline shallow bays along Lake Victoria was highest in the urban bays, followed by the semi-urban and least in the rural bays.

These studies concur with other studies conducted in surface water bodies in many urban centers elsewhere in Africa where increased sewage and domestic effluents have been reported to cause water quality deterioration (Kemka et al. 2006, Koukal et al. 2004, Nhapi and Tirivarombo 2004). Kemka et al. (2006) showed that Yaounde Municipality Lake in Cameroon is experiencing hypertrophic eutrophication as a result of the inflow of increasing quantities of domestic wastewater from Yaounde city. In Zimbabwe, eutrophication in Lake Chivero has been suggested to be a result of increased sewage effluent (Nhapi and Tirivarombo 2004). The pollution in Lake Chivero is a result of the eutrophying influence of the Marimba River which receives treated wastewater from the Crowborough Sewage Treatment Works in Harare and then discharges into Lake Chivero. In North Africa, the assessment of water quality and toxicity of polluted rivers Fez and Sebou in the region of Fez in Morocco suggested that sites located close to the most urbanized and industrialized areas were severely impaired, while remote sites downstream showed signs of physicochemical recovery (Koukal et al. 2004). Low dissolved oxygen (DO), high turbidity, organic matter and ammonia contents, high chromium and copper pollution and high acute and chronic toxicity were reported for the affected sites. The authors cautioned that use of water for drinking or for agriculture from the rivers or from some wells without treatment may expose the population to health risks.

Similar trends were observed in studies carried out on surface water bodies in other continents (Alkarkhi et al. 2008). Alkarkhi et al. (2008) assessed surface water quality in sites located adjacent to industrial areas operational industry that included electronics, textiles, basic and fabricated metal products, food processing and canning, processing of agricultural products, feed mills, chemical plants, rubber based industry, timber based wood products, paper products and printing works, and transport equipment. There was a large increase in conductivity and chemical oxygen demand which is characteristic of industrial pollution. From all these studies we note that wastewater discharges especially in densely populated urban areas are responsible for the deteriorating water quality as it directly impacts on many physicochemical variables such as reduced dissolved oxygen and pH levels, increased nutrient levels and higher conductivity levels.

The other anthropogenic activity that has been reported to cause seasonal surface water quality deterioration in Lake Victoria was agricultural activity in the catchments (Banadda et al. 2009, Munabi et al. 2009). In the Ugandan lake catchment, farming is done on marginal areas such as slopes and swamps. Unfortunately, these are fragile ecosystems prone to degradation. The indiscriminate drainage of swamps in city environments and growth in the use of inorganic chemicals in agriculture is well documented (Munabi et al. 2009, Matagi 2002). Large commercial agriculture employing chemical weed control and fertilization are sources of toxic and eutrophic chemicals that end up in wetlands of the Lake Victoria systems and drain into the lake (Munabi et al. 2009). At subsistence level, over 70% of the urban population earn below the poverty level, and a large number of city residents resort to urban agriculture to supplement their income (Banadda et al. 2009, Matagi 2002). In this respect Banadda et al. (2009) traced the main land-use-based pollution sources within Kampala City borders and around Murchison Bay and observed that wastewater discharge in Lake Victoria's Murchison bay is from diffuse sources including urban agriculture. In many other studies, water quality deterioration was a result of two factors, occurring at the same time, i.e., agricultural runoff alongside municipal and industrial effluent (Razmkhah et al. 2010, Kazi et al. 2009, Li et al. 2007, Shrestha and Kazama 2007, Wang et al. 2007).

3.2.2. Eutrophication in the shallow bays of Lake Victoria

Eutrophication due to nutrient enrichment of mainly nitrogen (N) and phosphorus (P) from a variety of anthropogenic sources, including agricultural, urban and industrial runoff and atmospheric deposition is reported as one of the long standing problems of Lake Victoria (Nyenje et al 2010, Hecky et al. 2009, Munabi et al. 2009, Kulabako et al. 2007, 2008, Oguttu et al. 2008, Cózar et al. 2007, Kansiime et al. 2007, GoU 2006a, Sekiranda 2006, Nzomo 2005, Odada et al. 2004, Machiwa 2003, Scheren et al. 2000). In the last 4 decades there have been reports of increased nutrient concentrations of N and P levels and all these studies agree that Lake Victoria's eutrophication is on the increase but reports on the main source of nutrients in the lake are conflicting. GoU (2006a) and Scheren et al. (2000) reported that atmospheric deposition contributes the largest input of nutrients together accounting for approximately 90% of phosphorus and 94% of nitrogen. Comparative reports by Nyenje (2010) and Hecky et al. (2009) and studies done by Cózar et al. (2007) between inshore and offshore lake waters suggest stronger eutrophication effects in the inshore areas of Lake Victoria, where nutrient and chlorophyll-a concentrations are markedly higher.

Nyenje et al. (2010), Hecky et al. (2009) and Cózar et al. (2007), agree with earlier studies (Machiwa et al. 2003, Muggide 1993, 2001, Hecky 1993, Hecky and Bugenyi 1992) which asserted that human settlement in the numerous urban and rural centers along the shoreline enhances eutrophication in the inshore regions, where nutrient loads from municipal and agricultural effluents are high. This account has since been supported by a number of other reports (GoU 2010, Munabi et al. 2009, Oguttu et al. 2008, Kansiime et al. 2007, Sekiranda 2006). Munabi et al. (2009) reported increased nutrient loads from domestic effluent as compared to subsistence agricultural farm land. Oguttu et al. (2008) also reported increased nutrient loading from industrial wastewater point sources in the Jinja (Uganda) catchment areas. A related report by Kansiime et al. (2007) also showed an increased loading of nutrients in Murchison Bay. The authors attributed the nutrient increase to the inflow of wastewater from Kampala (Uganda). On the other hand, large urban centres contribute 72% of the pollution loading into Lake Victoria shores compared to 13% by industries and 15% by fishing villages (GoU 2010). Lastly Sekiranda (2006), reported a variation in the extent of eutrophication in three shallow coastline bays along the Ugandan part of Lake Victoria, with the highest eutrophication in the urban Murchison Bay as compared to moderate eutrophication in a semi-urban Fielding Bay and least in Hannington bay, located in the rural areas.

The impacts of eutrophication in Lake Victoria are enormous. Eutrophication causes change in the physicochemical properties of water (Nyenje et al. 2010, Mugidde 2001). The long water residence time (140 years) and the long (3440 km) and highly indented shoreline that include numerous bays has been reported to exacerbate eutrophication effects in Lake Victoria (Nyenje et al. 2010, Cózar et al. 2007, Mugidde 2001). Nyenje et al. (2010) reckon that eutrophication-induced loss of deep water oxygen is one of the major problems in Lake Victoria and could also be responsible for the extinction of many cichlid species. Nutrients, such as phosphates, ammonia and nitrates, stimulate blooms of algae or aquatic vegetation with resultant oxygen depletion (Nyenje et al. 2010, Dodds 2007, Carr and Neary 2006, Mugidde 2001). Hecky et al. (1994) argue that high nutrient concentrations support elevated algal biomasses which on sedimentation and decomposition, contribute to increased oxygen demand which partly accounts for the pronounced hypolimnetic anoxia in Lake Victoria compared to the 1960s.

Other impacts of eutrophication on physicochemical properties of water are low pH, decreased water transparency and increased turbidity (Dodds 2007, Sekiranda 2006). Respiration and decomposition processes of the excessive algal blooms caused by organic nutrients reduces pH, as the organic matter produces CO_2, which dissolves in water as carbonic acid (Carr and Neary 2006, Carlson 1977). Thus eutrophication influences physicochemical properties through alteration of the ecosystem primary productivity (Nyenje et al. 2010, Dodds 2007, Carr and Neary 2006, Sekiranda 2006, Mugidde 2001, Carlson 1977). The potential for biomass growth of primary producers in an aquatic ecosystem can be assessed based on the system's trophic state. Trophic states are usually defined as oligotrophic (low productivity), mesotrophic (intermediate productivity), and eutrophic (high productivity). Ultraoligotrophic (TSI < 40) and hypereutrophic (TSI > 70) states represent opposite extremes in the trophic status classifications of aquatic environments (Carr and Neary 2006, Carlson 1977).

3.2.3. Assessment of water quality using multivariate statistical techniques

Environmental analytical chemistry generates multidimensional data that requires multivariate statistics to analyze and interpret relationships between variables (Razmkhah et al. 2010). Many studies have shown multivariate statistical techniques to be powerful tools to assess water quality and evaluate aquatic ecosystem health through data exploration and analysis. The application of multivariate methods such as cluster analysis (CA), principal component analysis (PCA), and factor analysis (FA) for analyzing aquatic environmental data to better understand causes for changes in water quality and the ecological status of the studied systems has increased in recent years (Bu et al. 2010, Razmkhah et al. 2010, Noori at al. 2010, Rodrigues at al. 2010, Kazi et al. 2009, Alkarkhi et al. 2008, Hussain et at. 2008, Shrestha and Kazama 2007, Panda et al. 2006, Koukal et al. 2004). The techniques have been used to characterize and evaluate surface and ground water quality and are useful in relating temporal and spatial variation to natural and anthropogenic factors (Razmkhah et al. 2010, Kazi et al. 2009, Alkarkhi et al. 2008, Hussain et at. 2008, Shrestha and Kazama 2007, Panda et al. 2006). Ultimately, multivariate statistical techniques have been applied in the identification of potential factors and or sources that influence water systems, and offer a valuable tool for reliable management of water sources.

Although the focus of many of the studies on freshwater systems has been on rivers (Bu et al. 2010, Razmkhah et al. 2010, Alkarkhi et al. 2008, Koukal et al. 2004), the few studies that employed multivariate methods on lake systems agree that the techniques are useful and encourage their incorporation into lake water quality management programmes (Kazi et al. 2009, Li et al. 2007). From these studies, it is evident that the multivariate techniques have been successfully applied and recommended in national water quality monitoring programmes and networks (Kazi et al. 2009, Li et al. 2007). However, no study on Lake Victoria was encountered that evaluated water quality variables using multivariate statistics. The usual technique of interpretation of water quality data from the lake is a univariate procedure, and this is the approach that most studies used (Munabi et al. 2009, Cózar et al. 2007, Kansiime et al. 2007). Razmkhah et al. (2010) on the other hand demonstrated that the univariate approach does not adequately characterize simultaneous similarities and differences between samples or variables. It was of great interest therefore to incorporate the usefulness of multivariate statistical techniques to analyse this study’s water quality data in order to characterise the study sites based on water quality variables.

Razmkhah et al. (2010) used principal component analysis (PCA) and hierarchical cluster analysis (CA) methods to investigate the water quality of Jajrood River (Iran) and to assess and discriminate the relative magnitude of anthropogenic and natural influences on the quality of river water. Exploratory analysis of the data was carried out by means of PCA and CA in an attempt to discriminate sources of variation in water quality. PCA allowed identification of a reduced number of mean 5 varifactors, pointing out 85% of both temporal and spatial changes. CA classified similar water quality stations and indicated one site (Out-Meygoon) as the most polluted one. Ahar, Baghgol, Rooteh, Befor Zaygan, Fasham, Roodak and Lashgarak were identified as affected by organic pollution. Similarly, surface water quality data for the Karoon River (in southwest Iran) were evaluated for spatial variations and the relationship between physical and chemical parameters (Noori at al. 2010). Principal component analysis was applied to determine important monitoring stations and water quality parameters. Altogether the authors concluded that the methods offered an effective solution to water quality management (Noori at al. 2010).

Kazi et al. (2009) used cluster analysis (CA) and principal component analysis (PCA) to evaluate and interpret water quality data sets and apportion pollution sources to get better information about water quality and to design a monitoring network. The chemical correlations were observed by PCA, and used to classify the samples by CA, based on the PCA scores. Three significant sampling locations were detected on the basis of similarity of their water quality. The major causes of water quality deterioration could be related to inflow of effluent from industrial, domestic, agricultural and saline seeps into the lake at the first site and also resulting from people living in boats and fishing at the other two sites (Kazi et al. 2009). In a related study to evaluate spatial variations and to interpret water quality data sets of two rivers (Juru and Jejawi) in Malaysia, factor analysis resulted in two factors explaining more than 82% of the total variance. The factors suggested that the possible variances in water quality may be due to either sources of anthropogenic origin or due to different biochemical processes that are taking place in the system (Alkarkhi et al. 2008).

The above studies and other related studies demonstrated the usefulness of multivariate statistical techniques for analysis and interpretation of multidimensional data sets in water quality assessment, identification of pollution sources and underlying latent factors and understanding temporal and spatial variations in water quality for effective water quality management in different aquatic systems. This study will apply these techniques to contribute to the much needed scientific knowledge on the spatial magnitude of water quality covering three districts in Uganda, along the shoreline of Lake Victoria. This chapter therefore used multivariate statistical techniques based on hierarchical clustering analysis, principal component analysis and factor analysis to evaluate and characterize the study sites water quality based on the 11 commonly assessed physico-chemical variables. Specifically this involved: extraction of information about the similarities or dissimilarities of the study sites based on the physico-chemical properties; identification of the possible latent factors that influence the physico-chemical variables in the study sites and lastly discussion of spatial variations in water quality and the likely pollution sources at the sites.

3.3. Specific Methods

3.3.1. Measurement of physicochemical variables

The principle that governed the choice of the physicochemical variables to measure in this study was to utilize the variables that are commonly monitored by the water resource management. These were pH, conductivity, temperature, dissolved oxygen, secchi disk depth, total phosphorus (TP), total nitrogen (TN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonia-nitrogen (NH_4-N), and chlorophyll a (Chl-a). These variables were monitored because they define the status and quality of the water and can cause mortality of aquatic organisms in concentrations beyond the normal ranges (Bracchini et al. 2009, Larson et al. 2007, Carr and Neary 2006, EPA 2003, Lessard and Hayes 2003, Zhang et al. 2003). Temperature affects the speed of chemical reactions, the rate of photosynthesis, the metabolic rate of aquatic organisms, as well as how pollutants, parasites, and other pathogens interact with aquatic residents. Temperature also influences the solubility of dissolved oxygen (DO) and other molecules in the water column such as ammonia (Carr and Neary 2006, Lessard and Hayes 2003). Oxygen influences inorganic chemical reactions and is required for aerobic metabolism (Carr and Neary 2006).

The pH of an aquatic ecosystem is important because it is closely linked to biological productivity. Secchi disk depth determines the water transparency, a measure of water quality that quantifies the depth of light penetration in a body of water. Water bodies that have high transparency values typically have good water quality (Bracchini et al. 2009, Larson et al. 2007, Carr and Neary 2006, Zhang et al. 2003). Specific conductivity measures how well the water conducts an electrical current, a property that is proportional to the concentration and strength of ions in solution and can also be used to detect pollution sources (Carr and Neary 2006). Nutrients were considered because they regulate the productivity and define the trophic status of aquatic ecosystems. Phosphorus and nitrogen are reported to be the primary drivers of eutrophication of aquatic ecosystems, where increased nutrient concentrations leads to increased primary productivity (Hecky et al. 2009, Carr and Neary 2006, Madadi et al. 2007, Zhao et al. 2001). These two elements together with Secchi disk depth were relevant in the calculation of Carlson's trophic index (Carlson 1977).

At every study site, physicochemical variables were measured at two locations. One location was at the shoreline (inshore site) and the other 60m off the shoreline (offshore site). At each location water quality variable were measured at different depths. Where the total depth was 0.5 m to 1.0 m samples were taken at 0 m (surface) and 0.5 m. At each depth, temperature, pH, dissolved oxygen and electrical conductivity were measured on site using portable meters. Water transparency was measured using a 25 cm diameter black and white Secchi disk. Water samples were collected using a Van Dorn water sampler, for measurement of total phosphorus (TP), total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+) and chlorophyll a (Chl-a).

The samples were analyzed for TN, TP, NH_4^+, NO_2^-, NO_3^-, and Chl-a using a spectrophotometer. Dissolved inorganic nutrients (NH_4^+, NO_2^-, NO_3^-) and Chl-a were determined spectrophotometrically following the methods of Stainton et al. (1977). Ammonia was measured by the indophenol-blue method. Nitrate plus nitrite were determined using the cadmium method as described in Stainton et al. (1977). The water sample was passed through a column packed with cadmium granules. Then the sample colour was developed with a combined sulfanilamide and 1-naphthyl-ethylenediamide dihydrochloride reagent and absorbance was read at 540 nm within 2 hours. Total dissolved nitrogen and phosphorus concentrations were determined following oxidation of the filtrate of 20-ml sub-samples in alkaline and acidic persulfate, respectively. The water quality physico-chemical variables, their units and tools of analysis are summarised in table 3.1.

Table 3.1 Water quality physicochemical variables, units and analytical methods used during the study

Parameter	**Abbreviation**	**Units**	**Analytical Tools**
Temperature	Temp	^oC	Portable meters
Dissolved Oxygen	DO	mg/l	Portable meters
Conductivity	Cond	µS/cm	Portable meters
pH	pH		Portable meters
Secchi Depth	SD	m	Secchi disk
Nitrite nitrogen	NO_2^-,	mg/l	Spectrophotometer
Nitrate nitrogen	NO_3^-	mg/l	Spectrophotometer
ammonia nitrogen	NH_4^+	mg/l	Spectrophotometer
Total Nitrogen	TN	mg/l	Spectrophotometer
Total phosphorus	TP	mg/l	Spectrophotometer
Chlorophyll-a	Chl-a	Mg/l	Spectrophotometer

3.3.2. Assessment of trophic status of the sampling sites

Secchi disk depth also called secchi disk transparency (depth to which you can easily see through the water), chlorophyll a (an indirect measure of phytoplankton abundance), and total phosphorus (an important nutrient and potential pollutant) are often used to define the degree of eutrophication or trophic status of a lake (Carlson 1977). The concept of trophic status is based on the fact that changes in nutrient levels (measured by total phosphorus) cause changes in algal biomass (measured by chlorophyll a) which in turn causes changes in lake clarity (measured by Secchi disk transparency). A trophic state index is a convenient way to quantify this relationship. This study applied the most popular index developed by Carlson (1977) to characterise and classify the sampling sites according to their trophic status. The trophic state index of Carlson (1977) is recommended as the simplest method of calculating and explaining trophic state concepts.

Carlson's index uses a log transformation of Secchi disk readings as a measure of algal biomass on a scale from 0 to 110. Each increase of ten units on the scale represents a doubling of algal biomass. Because chlorophyll a and total phosphorus are usually closely correlated to Secchi disk measurements, these variables can also be assigned trophic state index values. The Carlson trophic state index is useful for comparing lakes within a region and for assessing changes in trophic status over time. Thus it is often valuable to include an analysis of trophic state index in lake monitoring programs, more so for Lake Victoria which many studies have reported to be eutrophic. It is important to note that the Carlson's trophic state index was developed for use with lakes that have few rooted aquatic plants and little non-algal turbidity (Carlson 1977). Lake Victoria's turbidity has been attributed to high algal turbidity, rendering the application of Carlson's trophic state index appropriate. The Carlson's TSI for Secchi disk, Chlorophyll a and phosphorus was calculated according to the standard equations below.

$$TSI = 60 - 14.41 \ln \text{Secchi disk (meters)}$$

$$TSI = 9.81 \ln \text{Chlorophyll a (ug/L)} + 30.6$$

$$TSI = 14.42 \ln \text{Total phosphorus (ug/L)} + 4.15$$

Where TSI = Carlson trophic state index and ln = natural logarithm

3.3.3. Statistical Analysis

Descriptive statistics comprising the means, standard deviations and ranges for each parameter were derived. Data were not normally distributed hence the Kruskal-Wallis nonparametric ANOVA was used to compare the water quality variables between sampling locations. In order to evaluate spatial variation in water quality and to characterise the study sites according to their water quality status, subsequently defining their degree of contamination, the water quality datasets were subjected to three multivariate statistical techniques that included cluster analysis (CA), principal component analysis (PCA) and factor analysis (FA). CA and PCA were performed using the Community Analysis Package 1.52 computer programme (Henderson and Seaby 2000a), while SPSS 16 was used for the FA.

Cluster analysis is a group of multivariate techniques whose primary purpose is to assemble the objects based on the characteristics they possess. It groups objects (cases) into classes (clusters) on the basis of similarities within a class and dissimilarities between different classes. As a result, each object within the cluster exhibits high internal (within-cluster) homogeneity and high external (between-cluster) heterogeneity. The class characteristics are not known in advance but may be determined from the analysis. Hierarchical agglomerative clustering is the most common approach which provides intuitive similarity relationships between one sample and the entire dataset and is typically illustrated by a dendrogram (Bierman et al. 2011, Henderson and Seaby 2000a, 2007, Manly 2005).

The dendrogram provides a graphic summary of the clustering processes providing a picture of the groups and their proximity with a dramatic reduction in dimensionality of the original data. The Euclidean distance usually gives the similarity between two samples and a distance can be represented by the difference between analytical values from the samples (Bierman et al. 2011, Henderson and Seaby 2000a, 2007, Manly 2005). In this study, a hierarchical agglomerative CA by means of the Wards method using Euclidean distances as a measure of similarity was performed on transformed and normalised data sets. The data were transformed using log (x+1) and normalised by subtracting the mean from the values and divided by the standard deviation value (Henderson and Seaby 2000a). The Ward's method uses an analysis of variance approach to evaluate the distance between clusters in an attempt to minimise the sum of squares of any two clusters that can be formed at each step.

PCA of the normalised variables was performed to correlate the variables with the study sites and extract significant components. PCA is designed to transform the original variables into new uncorrelated variables (axes), called the principal components which are the linear combinations of the original variables. The new axes lie along the directions of maximum variance. PCA provides an objective way of finding indices of this type so that the variation in data can be accounted for as precisely as possible (Henderson and Seaby 2000a, 2007, Manly 2005, Sarbu and Pop 2005). Principal components (PC) provide information on the most meaningful parameters which describes a whole data set affording data reduction with minimum loss of original information.

FA analysis follows the same pattern as PCA but the main purpose of FA is to reduce the contribution of the less significant variable to further simplify the data structure coming from PCA. This purpose can be achieved by rotating the axis defined by PCA according to well established rules and constructing new variables also called varifactors (VFs). It is important to note that PC represents a linear combination of the observable water quality variables whereas VF can include unobservable variables, hypothetical, and latent variables (Bierman et al. 2011, Belkhiri et al. 2010, Manly 2005). The total number of varifactors generated from a typical factor analysis indicates the total number of possible sources of variation in the data. Varifactors are ranked in order of importance with the first having the highest eigenvector sum and thus representing the most important source of variation in the data (Belkhiri et al. 2010). Factor loadings on the factor loadings tables are interpreted as correlation coefficients between the variables and the factors (Belkhiri et al. 2010). They are calculated using eigenvalues greater than 1 (Alkarkhi et al. 2008, Shrestha and Kazama 2007, Panda et al. 2006). Thus in this study only factors with eigenvalues greater than one were considered. The study employed R-mode FA, the most common mode of FA where, the sampling sites were the grouping (dependent) variables, while all the measured variables constituted the independent variables as compared to the Q-mode factor analysis, also called inverse factor analysis where the parameters are the grouping variables (Shrestha and Kazama 2007, Panda et al. 2006, Manly 2005). The purpose was to compare the compositional patterns between sites and to identify the various latent factors that influence each of them.

3.4. Results

3.4.1. Water quality variables in the study sites

In all the study sites, the inshore sites were shallower than the offshore sites (Table 3.2). There was no significant difference between inshore locations ($P > 0.5$). Likewise there was no significant difference in total depth between offshore locations ($P > 0.5$). The range, mean and standard deviations of 12 water quality physicochemical variables in the 10 sampling locations are summarised in Table 3.2 and 3.3. Notable are the low temperatures, low secchi depth, low dissolved oxygen and higher conductivity values for the inshore and offshore sites in Murchison Bay and the Kirinya inshore site as compared to the other sites (Table 3.2). These three sites also registered higher total phosphorus and total nitrogen levels (Table 3.3). The reference site (Lwanika inshore and offshore) registered the lowest nutrient loadings (Table 3.3).

Table 3.2 Mean ± standard deviation, minimum and maximum total depth, secchi depth, temperature, dissolved oxygen and conductivity; and pH range in the study sites (n = 12).

Sampling Site	**Total Depth (m)**	**Secchi Depth (m)**	**Temperature (^{o}C)**	**Dissolved Oxygen (mg/l)**	**Conductivity (µS/cm)**	**pH**
Kirinya Inshore	1.0 ± 0.4 (0.35, 1.6)	0.5 ± 0.1 (0.35, 0.78)	26.8 ± 0.8 (25.6, 27.9)	3.1 ± 1.1 (1.26, 4.8)	301.9 ± 96.4 (159, 448)	6 – 8
Kirinya Offshore	2.5 ± 0.6 (2, 4)	0.9 ± 0.1 (0.75, 1.2)	27.6 ± 0.7 (26.3, 28.6)	6.9 ± 1.0 (5.23, 8.55)	134.3 ± 35.0 (82.7, 192.7)	6.85 – 9
Masese Inshore	1.2 ± 0.3 (0.72, 1.5)	0.9 ± 0.3 (0.45, 1.21)	27.3 ± 0.7 (26, 28.6)	6.0 ± 0.6 (4.52, 6.7)	136.4 ± 24.4 (81, 176.7)	6.7 – 9
Masese Offshore	2.4 ± 0.5 (1.6, 3.22)	1.1 ± 0.2 (0.64, 1.41)	27.3 ± 0.8 (26.1, 28.9)	7.2 ± 1.1 (5.44, 9.02)	120.4 ± 18.5 (75, 158.8)	6.82 – 8
Winday Inshore	1.2 ± 0.5 (0.43, 1.73)	0.8 ± 0.3 (0.3, 1.09)	27.1 ± 0.7 (25.5, 27.8)	7.4 ± 1.0 (4.6, 8.3)	117.8 ± 14.5 (79, 135)	7 – 9
Winday Offshore	3.9 ± 0.8 (2.25, 5)	1.0 ± 0.3 (0.54, 1.45)	27.2 ± 0.6 (25.9, 27.8)	7.6 ± 1.0 (4.9, 8.66)	110.7 ± 13.4 (76, 131)	7.25 – 9
Murchison Inshore	1.4 ± 0.5 (0.7, 2.7)	0.4 ± 0.1 (0.18, 0.49)	25.3 ± 0.6 (24.1, 26.1)	2.4 ± 1.4 (1.1, 5.36)	580.2±119.5 (383, 707)	4.96 – 7
Murchison Offshore	2.4 ± 0.8 (0.9, 3.1)	0.6 ± 0.2 (0.33, 0.81)	25.5 ± 0.7 (24.1, 26.2)	5.2 ± 1.8 (3.02, 7.9)	473.5±145.7 (227, 635)	5.41 – 7.2
Lwanika Inshore	1.4 ± 0.2 (1, 1.8)	0.8 ± 0.1 (0.5, 0.87)	27.5 ± 0.6 (26.5, 28.6)	6.9 ± 0.6 (5.7, 7.53)	133.4 ± 5.2 (127, 140)	7 – 7.85
Lwanika Offshore	2.2 ± 0.2 (1.95, 2.5)	0.9 ± 0.1 (0.7, 0.98)	28.4 ± 0.6 (26.9, 29)	6.7 ± 0.9 (5.55, 8)	128.0 ± 7.5 (119, 138)	6.95 – 8.0

Table 3.3 Mean ± standard deviation (minimum and maximum) of NO_2, NO_3, NH_4, TN, TP and Chlr-a in the study sites (n = 12).

Sampling Sites	**NO_2 (mg/l)**	**NO_3 (mg/l)**	**NH_4 (mg/l)**	**TN (mg/l)**	**TP (mg/l)**	**Chlr-a (mg/l)**
Kirinya	0.030±0.027	0.093±0.098	0.251±0.458	34.083±35.133	0.408±0.261	0.038±0.007
Inshore	(0.0, 0.066)	(0.024,0.385)	(0.0, 1.525)	(3.509, 5.224)	(0.049,0.766)	(0.022,0.051)
Kirinya	0.013±0.007	0.053±0.027	0.065±0.139	11.119±10.512	0.148±0.114	0.020±0.004
Offshore	(0.0, 0.019)	(0.014,0.114)	(0.0, 0.479)	(1.305, 4.068)	(0.020,0.379)	(0.013,0.0280)
Masese	0.012±0.009	0.058±0.024	0.020±0.021	32.049±22.651	0.171±0.129	0.037±0.007
Inshore	(0.0, 0.031)	(0.016,0.088)	(0.0, 0.055)	(8.02, 69.892)	(0.028,0.449)	(0.025, 0.051)
Masese	0.012±0.006	0.056±0.026	0.011±0.014	11.834±9.739	0.134±0.160	0.018±0.003
Offshore	(0.0, 0.018)	(0.014,0.090)	(0.0, 0.041)	(0.81, 33.144)	(0.026,0.606)	(0.011, 0.024)
Winday	0.009±0.007	0.051±0.023	0.007±0.009	12.327±12.315	0.109±0.085	0.021±0.006
Inshore	(0.0, 0.019)	(0.017,0.096)	(0.0, 0.030)	(0.913,30.837)	(0.038,0.311)	(0.011, 0.032)
Winday	0.008±0.007	0.033±0.017	0.003±0.005	11.307±20.164	0.082±0.079	0.016±0.005
Offshore	(0.0, 0.020)	(0.009,0.071)	(0.0, 0.018)	(0.720,71.223)	(0.016,0.316)	(0.004, 0.024)
Murchison	0.025±0.019	0.202±0.268	0.642±0.603	40.348±21.112	0.822±0.461	0.046±0.025
Inshore	(0.004,0.056)	(0.010,0.706)	(0.0, 1.867)	(6.467,67.531)	(0.269,1.614)	(0.013, 0.092)
Murchison	0.023±0.013	0.056±0.029	0.353±0.401	29.346±15.833	0.808±0.574	0.043±0.025
Offshore	(0.005,0.048)	(0.017,0.123)	(0.0, 1.520)	(2.464,60.457)	(0.080,1.538)	(0.010, 0.092)
Lwanika	0.007±0.001	0.039±0.011	0.003±0.001	5.409±1.691	0.063±0.013	0.019± 0.003
Inshore	(0.004,0.009)	(0.013,0.051)	(0.0, 0.005)	(2.205, 6.945)	(0.043,0.082)	(0.014, 0.022)
Lwanika	0.006±0.002	0.033±0.011	0.002±0.001	4.007±2.275	0.060±0.018	0.018±0.003
Offshore	(0.002,0.009)	(0.013,0.049)	(0.0, 0.040)	(0.765, 6.108)	(0.031,0.088)	(0.014,0.0220)

3.4.2. Spatial diversity and site grouping based on water quality characteristics

Hierarchical cluster analysis grouped the 10 sampling location into three clusters based on the similarity of water quality characteristics (Figure 3.1). The three clusters as displayed in the dendrogram (Figure 3.1) included the relatively less polluted cluster comprising the inshore and offshore locations of the reference site (cluster 1), a medium polluted cluster (cluster 2) and a highly polluted cluster (cluster 3). The medium polluted cluster 2 included Winday Bay’s inshore and offshore location, Masese inshore and offshore locations and Kirinya offshore sampling location. While the highly polluted cluster 3 included Murchison Bay’s inshore and offshore, and Kirinya inshore sampling locations (Figure 3.1).

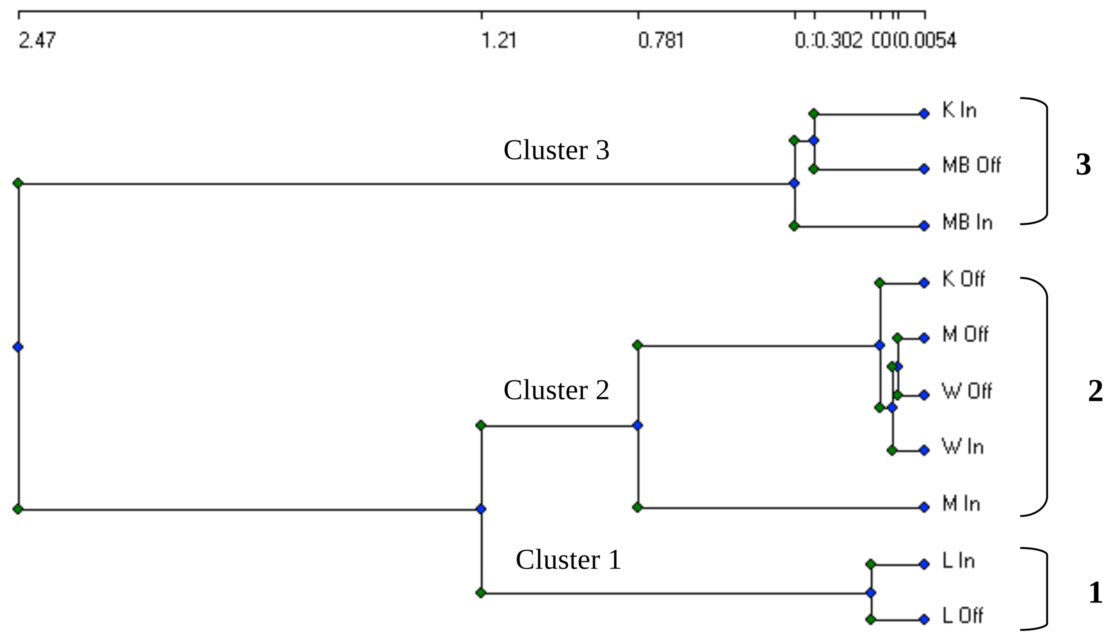


Figure 3.1 Hierarchical Cluster Analysis dendrogram showing three clusters of site composition, each cluster indicates sites with similar physico-chemistry. Homogeneity within clusters was based on Euclidean distance and the heterogeneity between clusters was based on Ward's method. Where sampling locations L In and L Off = Lwanika Inshore and Offshore, M In and M Off = Masese Inshore and Offshore, W In and W Off = Winday Bay Inshore and Offshore, K In and K Off = Kirinya Inshore and Offshore, MB In and MB Off = Murchison Bay Inshore and Offshore.

3.4.3. Relationship between sampling sites and physicochemical variables

The results of the PCA based on normalised data of physicochemical components are expressed in Table 3.4, and the sampling stations shown in Figure 3.2. Two components of PCA loaded eigenvalues greater than 1 with component one (X axis) registering 75.6% of the total variance and component two (Y axis) explained 18.7% of the total variance (Table 3.4). All together, the first two components explained 94.3 % of total variance and

10.4 of the 11 eigenvalues. The PCA plot brought out the three clusters observed in the hierarchical cluster analysis dendrogram above (Figure 3.2). Cluster 1, of the reference site, was highly correlated with Temperature, pH, oxygen and secchi depth and negatively correlated with conductivity and the nutrients on the main component of axis one. In contrast, cluster 3, the highly polluted cluster was highly correlated with conductivity and the nutrients (Figure 3.2). Cluster 2, the moderately polluted cluster lies in between cluster 1 and cluster 3.

Table 3.4 Eigenvalues, cumulative eigenvalues, percent of total variance and cumulative percent of total variance of correlation PCA for the physicochemical variables (n = 11) in the study sites

	Eigenvalues	**Cumulative Total**	**% of Total Variance**	**Cumulative % of Total Variance**
1	**8.31505**	**8.31505**	**75.5913**	**75.5913**
2	**2.05344**	**10.3685**	**18.6677**	**94.259**
3	0.426463	10.795	3.87694	98.1359
4	0.12473	10.9197	1.13391	99.2699
5	0.049905	10.9696	0.453683	99.7235
6	0.020498	10.9901	0.186342	99.9099
7	0.007245	10.9973	0.065864	99.9757
8	0.001612	10.9989	0.014652	99.9904
9	0.001056	11	0.009603	100
10	-2.22E-08	11	-2.01E-07	100
11	-1.30E-07	11	-1.18E-06	100

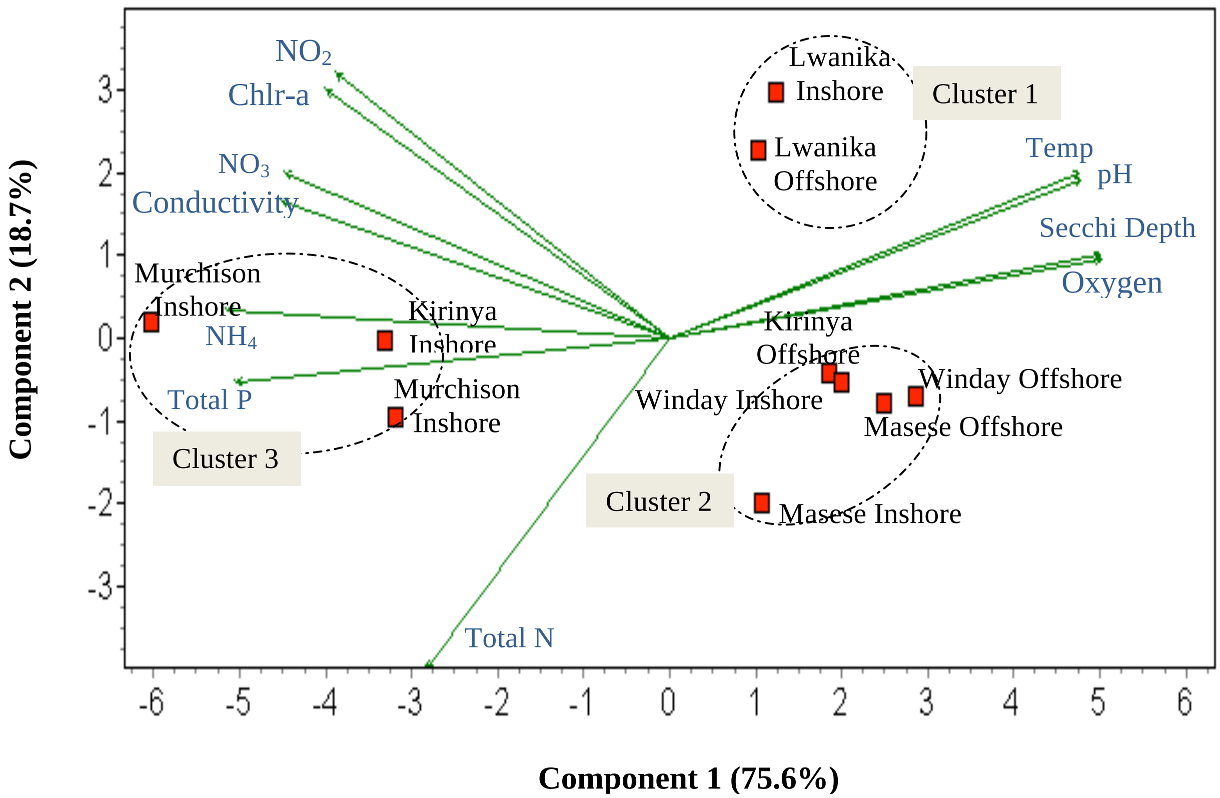


Figure 3.2 PCA plot correlating sampling sites scores of the 10 study locations with water quality quality vectors of the 11 physicochemical variables for plot component one (X axis) and plot component two (Y axis). Note the grouping of the three clusters.

3.4.4. Latent factors influencing water quality in the study sites:

Three factors were extracted explaining 71 % of the total variance in the water quality data set (Table 3.5). Eigenvalues >1 were taken as criterion for the extraction of the factors required for explaining the source of variances in the data set under Kaiser Normalization (Alkarkhi et al. 2008, Shrestha and Kazama 2007, Panda et al. 2006). The parameter loadings for the three identified factors, the factor eigenvalues, their percentage variance and cumulative percentage variance are given in Tables 3.4. The loading coincided with the correlation coefficients between water quality variables and the factors (Alkarkhi et al. 2008, Shrestha and Kazama 2007). Factor 1 accounted for 51 % of the total variance, and was positively correlated (loading > 0.50) with electrical conductivity, Nitrite, Ammonia, Total Nitrogen and Total Phosphorus (Table 3.5). Factor 2, explained 10% of the total variance and was positively loaded with temperature, dissolved oxygen and secchi depth. Unlike factor 1, factor 2 was negatively correlated with conductivity and nutrients. On the other hand, factor 3 explained 10% of the total variance and was positively loaded with Nitrate. Factor 3 correlated with different water quality variables in the same way as factor 1, with positive correlations of conductivity and nutrients just like factor 1 but with low loadings compared to factor 1. It is notable that factor 1 loading represented the changes and water quality status in the highly polluted cluster 3, while factor 2 loading explained the changes in the reference site cluster 1, and factor 3 expained cluster 2.

Table 3.5 R-mode Varimax rotated factor analysis of water quality variables (number of variables = 11, factors loadings > 0.50 in bold)

Variable	Factor 1	Factor 2	Factor 3
Temperature	-0.313	**0.664**	-0.145
Dissolved Oxygen	-0.359	**0.506**	-0.671
Conductivity	**0.535**	-0.585	0.426
pH	-0.549	0.254	-0.233
Secchi Depth	-0.151	**0.717**	-0.307
NO_2-N	**0.701**	0.098	0.448
NO_3-N	0.072	-0.147	**0.578**
NH_4-N	**0.575**	-0.442	0.154
Total Nitrogen	**0.502**	-0.264	0.111
Total Posphorous	**0.825**	-0.473	0.018
Chlorophyll a	0.476	-0.265	0.483
Eigenvalues	5.65	1.098	1.045
% Variance	51.368	9.982	9.501
Cummulative Variance	51.368	61.349	70.851

Extraction Method: Principal Axis Factoring.
Rotation Method: Varimax with Kaiser Normalization.
Rotation converged in 11 iterations.

3.4.5. Trophic condition (nutrient enrichment) in the study sites:

The Carlson's trophic indices for secchi disk depth, total phosphorus and chlorophyll a suggest that the study sites ranged between eutrophic and hypereutrophic (Table 3.6).

Table 3.6 Mean ± standard deviation (range) of Carlson's Trophic State Indices calculated on the basis of Secchi Disk, Total Phosphorus and Chlorophyll a, in the ten sampling sites. Standard TSI criteria: < 40 = Oligotrophic, 40 – 50 = Mesotrophic, 50 – 70 = Eutrophic, > 70 = Hypertrophic. N = 12.

Sampling Site	Secchi Disk (m)	TP (mg/l)	Chlorophyll	TSI Category	Classification
Kirinya Inshore	69.7 ± 3.7 63.6, 75.1	86.4 ± 13.8 60.3, 99.9	66.1 ± 2.0 61.0, 69.2	60 – 80	Eutrophic to hypereutrophic
Kirinya Offshore	61.0 ± 1.9 57.4, 64.2	72.0 ± 12.1 47.5, 89.8	59.7 ± 1.9 55.4, 63.4	60 – 80	Eutrophic to hypereutrophic
Masese Inshore	62.5 ± 50 57.3, 71.5	73.7 ± 12.9 52.0, 92.2	65.9 ± 1.8 62.2, 69.3	60 – 80	Eutrophic to hypereutrophic
Masese Offshore	59.7 ± 3.6 55.1, 66.4	69.0 ± 12.5 51.0, 97.0	58.8 ± 2.0 54.2, 61.7	50 – 70	Eutrophic
Winday Inshore	64.4 ± 6.4 58.8, 77.4	68.9 ± 8.94 56.6, 86.9	60.1 ± 3.0 54.2, 64.6	60 – 70	Eutrophic
Winday Offshore	60.7 ± 5.0 54.7, 68.9	63.6 ± 10.9 44.0, 87.2	57.3 ± 4.7 44.6, 61.6	50 – 70	Eutrophic
Murchison Inshore	75.3 ± 5.0 70.3, 84.7	98.7 ± 8.7 84.8, 110.7	66.7 ± 6.1 55.4, 74.9	60 – 100	Hypereutrophic
Murchison Offshore	67.5 ± 4.3 63.0, 76.0	95.5 ± 14.5 67.3, 110.0	65.8 ± 6.4 52.9, 74.9	60 – 100	Hypereutrophic
Lwanika Inshore	63.7 ± 2.4 62.0, 70.0	63.6 ± 3.2 58.4, 67.7	59.3 ± 1.7 56.4, 61.0	50 – 70	Eutrophic
Lwanika Offshore	62.3 ± 1.7 60.3, 65.1	62.7 ± 4.7 53.7, 68.7	58.9 ± 1.6 56.4, 61.0	50 – 70	Eutrophic

3.5. Discussion

Three study site groups / clusters of low pollution (two sampling locations), medium pollution (five sampling locations) and high pollution (three sampling locations) were observed based on their water quality physicochemical characteristics following different multivariate analyses. The groups were first observed using hierarchical clustering based on the similarity of their physicochemical characteristics and were confirmed by PCA and FA. The relatively less polluted cluster comprised of the two reference sampling locations, of Lwanika inshore and Lwanika offshore and correlated highly with temperature, dissolved oxygen and secchi transparency on the PCA plot. This cluster was confirmed following the varimax rotation factor analysis and was linked to the positive loading of temperature, dissolved oxygen and secchi depth in factor two. These sites registered a higher water transparency / secchi disk depth implying that there was more light penetration and potentially higher photosynthetic activity.

The high oxygen values and the high secchi depth leads to the supposition that the nutrients in the reference site could be mainly attributed to non-point sources of atmospheric deposition rather than point-source effluents given that there is no discharge inlet into the reference sites. This is in agreement with reports that atmospheric deposition constitutes the largest input of nutrients in Lake Victoria (GoU 2010, GoU 2006a, Scheren et al. 2000). The catchment in the reference site is dominated with subsistence agriculture and it is possible that this may not be contributing much organic matter into the lake. This agrees with many studies that reported that catchment agriculture contributed less to water quality deterioration than municipal and industrial effluent (Shrestha and Kazama 2007, Wang et al. 2007). Similar to the reference site findings, high oxygen levels were also observed by some authors in sites that were less polluted (Koukal et al. 2007, Wang et al. 2007). The temperatures in the reference site were also relatively stable and warmer with a minimum of 26.5 ^{o}C than the rest of the study sites whose minimum was less than this. This could be attributed to the fact that the site does not experience wastewater cooling effects since there is no wastewater inlet. This concurs with Sekiranda et al. (2006) who observed relatively warmer temperatures in bays that did not receive any effluent. This also supports Munro and Roberts' (1989), statement that shallow areas sheltered from water exchange, are warmer than the open waters in the tropics. However, the present study was limited to shallow coastline bays (coastal wetland areas) and cannot confirm how the temperatures compare with the open waters.

With regard to the highly polluted cluster three, the sampling locations under this group included Murchison Bay's inshore and offshore, and Kirinya inshore. This cluster correlated highly with conductivity, nitrite, ammonia, total nitrogen and total phosphorus on the PCA plot and these were confirmed by the positive loadings of these variables following the varimax rotation factor analysis under factor one. As a result factor one was linked to this cluster and the results agree with the descriptive statistics which reflected high levels of conductivity and nutrients. The high electrical conductivity values also reflect the higher total dissolved solids in these sites. This concurs with the statement that municipal, agricultural, and industrial discharges can contribute ions to receiving waters increasing the conductivity of the receiving waters (Carr and Neary 2006). These authors also reported that specific conductance can be used to detect pollution sources. Overall, these results reflect the high dissolved nutrients and organic pollution stemming from the different catchment activities, implying that factor one originates from the anthropogenic activities that are industrial, municipal and agricultural in nature. This is in agreement with the findings by many researchers that traced the pollution causal chain in these study sites (Banadda et al. 2009, Kayima et al. 2008, Oguttu et al. 2008, Kansiime et al. 2007, Muwanga and Barifaijo 2006, Matagi 2002).

For example Murchison Bay is the receipient of raw sewage, municipal and industrial wastewater discharged from Kampala via the Nakivubo channel, a channel that traverses highly populated Kampala slums, markets and industrial areas. These findings agree with those of earlier researchers who highlighted the different sources of pollution into this bay (Banadda et al. 2009, Kayima et al. 2008, Kansiime et al. 2007, Matagi 2002). Banadda et al. (2009) observed that wastewater discharge in Lake Victoria's Murchison Bay is from diffuse sources including urban agriculture. These authors traced the main landuse-based pollution sources within Kampala City borders and around the Bay. Kayima et al. (2008) and Kansiime et al. (2007) reported that there is a high degree of pollution in the Nakivubo channel, with nutrient levels above the Uganda national environmental management standards. In another instance a broader study highlighted the major sources of environmental degradation and pollution in Kampala city as solid waste, abattoir waste, sewage, sanitation, drainage, industrial pollution, traffic pollution, atmospheric pollution, urban agriculture, rapid urbanisation and water hyacinth populations (Matagi 2002).

These scenarios also apply to the Kirinya inshore sampling location. Like Murchison Bay the Napoleon gulf receives effluent discharges from various inlets, one of which occurs at Kirinya wetland. This wetland receives domestic, municipal and industrial effluent, surface runoff and storm water from Jinja town (Oguttu et al. 2008, Kelderman et al.

2007, Kansiime et al. 2007). The wetland catchment houses different institutions which include, hotels, industries, factories, metallurgy and Jinja municipality's wastewater treatment oxidation ponds, the Kirinya National Water and Sewerage Corporation Oxidation ponds that receive effluent from the municipality before discharging into Lake Victoria as secondary treated effluent. However, unlike in Murchison Bay where both inshore and offshore sampling locations were classified as highly polluted, the Kirinya offshore site is less polluted compared to the inshore site and was grouped under the moderately polluted cluster. This demonstrates that the pollutant retention capacity of Kirinya wetland catchment is still high. This is in agreement with previous studies that demonstrated that Kirinya wetland can retain nutrients from secondary treated wastewater through retention by sediments and uptake by plants or by sedimentation of nutrient-rich particulate matter (Kansiime et al. 2005, 2007, Kelderman et al. 2007, Mugisha et al. 2007, Van Dam et al. 2007). These findings concur with the fact that coastal wetlands serve as buffers to eutrophication and constitute an important role in maintaining lake ecosystems.

The difference could also be attributed to the high dilution effect off the shoreline, which is still high in Kirinya compared to the more polluted Murchison Bay (Nyenje et al. 2010, Dodds 2007, Carr and Neary 2006, Mugidde 2001). Thus, Murchison Bay as a whole is highly polluted and reflects the high levels of organic and inorganic pollutants from municipal, industrial and agricultural sources (Banadda et al. 2009, Kayima et al. 2008, Kansiime et al. 2007, Matagi 2002). It also shows that the Nakivubo wetland, serving as the bay's catchment is overwhelmed by the pollution and there is low pollutant retention capacity of the wetland catchment (Kansiime et al. 2007). The present study cautions that although Kirinya wetland is less impacted compared to Murchison Bay, if its effluent is not reduced, the water quality along this wetland and the whole of Napoleon gulf could deteriorate further.

Also to note in the highly polluted cluster is the poor factor loading of oxygen, secchi depth and temperature. This was supported by the descriptive statistics which indicated the depletion of oxygen in these locations (less than 3.5 mg/l for Murchison Bay inshore and Kirinya inshore, and 5.1 mg/l for Murchison Bay offshore). The low dissolved oxygen and low secchi depth could be attributed to the high input of organic matter which causes turbidity and consumes oxygen as it decays. This was reflected in the high nutrient levels and hypereutrophic state of these sites as a result of the municipal, industrial and agricultural effluents. Oxygen depletion is reported to be the most common result of such discharges, especially sewage (Nyenje et al. 2010, Dodds 2007, Carr and Neary 2006, Mugidde 2001). These authors suggest that oxygen depletion arises from insufficient dilution coupled with microbial growth on particulate and soluble organic content required for the decomposition of organic compounds. And that effluent derived organic nutrients, such as phosphates, ammonia and nitrate may stimulate excessive blooms of algae or attached weed with resultant oxygen depletion. The low temperatures could be attributed to the cooling effect of the wastewater discharges. It also concurs with Koukal et al. (2004) who reported low dissolved oxygen loadings (< 1 mg/l DO) in the highly polluted site that received huge amounts of untreated wastewater along the Fez River in Morocco and attributed it to the high organic matter content in the effluent. This also concurs with Wang et al. (2007) who reported low dissolved oxygen levels and low pH in rivers influenced by sewage and oxygen.

The medium polluted cluster 2 was the largest cluster with five sampling locations and included Winday Bay’s inshore and offshore location, Masese inshore and offshore locations and Kirinya offshore sampling location. The cluster could not be well explained by PCA but became confirmed following the varimax rotation factor analysis under factor three. It was observed that factor 3 had the same positive and negative variables as factor 1, but with low loadings of conductivity and nutrients thus explaining and confirming the moderate pollution in these sampling locations. As noted above, Kirinya inshore was classified under the highly polluted sites, while Kirinya offshore location was classified under moderately polluted. This difference was attributed to the dilution effect of the sewage effluent as it moves off the shoreline and is in agreement with studies by Cózar et al. (2007) who indicated a stronger eutrophication effect in the inshore areas of Lake Victoria compared to the offshore lake waters. Munro and Roberts (1989) reported that sewage discharges may reduce water quality, depending on the degree of dilution achieved, the degree of treatment of the original material, its composition and the response of the ecosystem.

Overall, the study showed that inshore sites receiving urban wastewater discharges were more polluted than the rest of the sites, emphasising the impact of urban discharges to water quality as compared to the sites that are less impacted by urban discharges. This is in line with the vast number of researchers who point to urbanisation as the most important underlying factor responsible for surface water quality deterioration in Lake Victoria (GoU 2010, Nyenje et al. 2010, Banadda et al. 2009, Hecky et al. 2009, Munabi et al. 2009, Kayima et al. 2008, Oguttu et al. 2008, Madadi et al. 2007, Muwanga and Barifaijo 2006, GoU 2006a, Gikuma-Njuru and Hecky 2005, Odada et al. 2004, Balirwa et al. 2003, Machiwa 2003, Matagi 2002, Scheren et al. 2000).

Results from the cluster analysis, principal component analysis and factor analysis complemented each other and led to the establishment of the three groups/clusters. This harmonisation in results agrees with previous studies on water quality that recommended the application of different multivariate statistical techniques when dealing with environmental variables datasets (Bu et al. 2010, Razmkhah et al. 2010, Noori at al. 2010, Rodrigues at al. 2010, Kazi et al. 2009, Alkarkhi et al. 2008, Hussain et at. 2008, Shrestha and Kazama 2007, Panda et al. 2006, Koukal et al. 2004).

These findings also show that all the study sites are eutrophic but become hypereutrophic in sites that receive municipal, industrial and agricultural effluents. This concurs with Sekiranda (2006), who studied three shallow bays along the Lake Victoria shoreline and reported the highest eutrophication in the urban bay as compared to moderate eutrophication in a semi-urban and least eutrophication in the rural bay. The findings are also in agreement with other studies carried out in other African lakes. For instance Kemka et al. (2006) showed that Yaounde Municipality Lake in Cameroon is experiencing hypertrophic eutrophication as a result of the inflow of increasing quantities of domestic wastewater from Yaounde city. In Zimbabwe, serious eutrophication in Lake Chivero was a result of increased sewage effluent (Nhapi and Tirivarombo 2004). The pollution in Lake Chivero is due to the eutrophying influence of the Marimba River which receives treated wastewater from the Crowborough Sewage Treatment Works in Harare and then discharges into Lake Chivero.

The fact that all the study sites are eutrophic, including sites that are not receiving any discharge, gives validity to reports that atmospheric deposition contributes the largest input of nutrients in Lake Victoria together accounting for approximately 90% of phosphorus and 94% of nitrogen (GoU 2010, GoU 2006a, Scheren et al. 2000). Comparative studies done by Cózar et al. (2007) between inshore and offshore lake waters indicated stronger eutrophication effects in the inshore areas of Lake Victoria, where nutrient and chlorophyll a concentrations are markedly higher. Findings by Cózar et al. (2007) agree with other earlier related studies (Hecky, 1993; Muggide, 1993). Like the present study, these studies were conducted at significant point discharge loading sources. For example, Cózar et al. (2007) studied Katonga Bay which is fed by a river and Murchison Bay which receives Kampala wastewater through the Nakivubo channel.

In conclusion, surface water quality is largely determined by the diverse natural biochemical processes that are taking place in the aquatic system and or the different anthropogenic inputs from the catchments. Water quality is highly influenced by the catchment activities with the sites receiving urban effluent registering poor water quality compared to those that are not. Correspondingly, where the dilution effect is high, inshore sites receiving wastewater discharges are more polluted than their respective offshore sites. This implies that if management could aim at regulating the discharge through cleaner production technologies and law enforcement, then the water quality deterioration could be reduced and even prevented. Also noted is that catchment agriculture and urbanisation through sewage and industrial effluent are the major catchment anthropogenic origins of water quality deterioration in the study sites and preservation of urban wetlands could conserve the much required buffering ability of wetlands. Lastly, Lake Victoria as a whole is eutrophic but the trophic state increases at the shoreline loading points with increasing municipal, sewage and agricultural discharges. The study recommends that the top priority be given to water quality monitoring and cleaner production technologies be adopted to improve water quality in urban water bodies.

CHAPTER 4

Evaluating benthic macroinvertebrates as biological indicators of water quality in selected wetlands in Uganda

4.1. Introduction

Wetland ecosystems along the Lake Victoria shoreline support a diversity of aquatic biota (Ogutu-Ohwayo and Balirwa 2004, Balirwa et al. 2003, Balirwa 1998). However, water quality in the study wetlands was highly influenced by the catchment activities (chapter three), with some wetlands receiving urban effluent being of poor water quality while other regions were less polluted. The development of biota in surface waters is governed by environmental conditions which determine the selection of species as well as the physiological performance of organisms consequently forming the basis on which the system operates (Darwall et al. 2005, Revenga and Kura 2003, Meybeck and Helmer 1996). Most organisms are sensitive to changes in their environment, whether natural, such as increased turbidity during floods, or unnatural, such as chemical contamination or decreased dissolved oxygen arising from sewage effluent (Friedrich et al. 1996).

The aquatic physicochemical water quality characteristics therefore affect the ability of species to persist in a given habitat (Crona et al. 2009, Hecky et al. 2009, Jiang et al. 2007, Azrina et al. 2006, Bigot et al. 2006, Pyle et al. 2005). As follows, the description of the biological quality of a water body based on the resident flora and fauna can give an indication of water quality and environmental conditions of the system (Friedrich et al. 1996). In addition to the quantitative physicochemical determinations, the description of the quality of the aquatic environment can also be achieved through semiquantitative and qualitative descriptions of community indices (Meybeck and Helmer 1996).

Use of organisms as indicators of aquatic environmental quality (biomonitoring) is one of the most valuable tools available for assessing the environmental quality (Azrina et al. 2006, Verlecar et al. 2006, Tagliapietral et al. 2005, Hilty and Merenlender 2000, Meybeck and Helmer 1996). Aquatic ecosystems are particularly responsive to environmental stress because the biotic communities tend to be dominated by motile short to long lived species with high reproductive rates, and pollutants tend to be well distributed throughout zones of active mixing (Ford 1989). As a result, resident organisms are being used in lakes, rivers, and streams, as sensitive indicators of water quality, a process known as biomonitoring, based on the premise that abundance and diversity of living organisms are the best indicators of environmental quality (Mandaville 2002). A commonly applied approach is to study the effect of pollution on species composition (Friedrich et al. 1996). Biological communities are sensitive to their chemical environment, and the degree of sensitivity varies among species and communities (Friedrich et al. 1996, Ford 1989). Ultimately, response to environmental stress may involve rapid changes in species composition of aquatic ecosystems that can translate into changes in various aspects of community structure such as species richness and diversity. The presence of families of highly tolerant organisms usually indicates poor water quality (Arimoro 2009, Pamplin et al. 2006, Aguiar et al. 2002, Griffith et al. 2001, Coimbra et al. 1996, Friedrich et al. 1996). Species richness and diversity therefore represent biodiversity measures of prime importance in community ecology which are often used as basic information for implementing habitat protection and conservation measures (Mora et al. 2008, Tokeshi and Arakaki 2007).

Through gradual anthropogenic change in the Lake Victoria watershed, environmental degradation has exerted its influence on water quality and the abundance and composition of communities of resident aquatic organisms (Hecky et al. 2009, Odada et al. 2009, Njiru et al. 2008a, Sekiranda 2006, Ogutu-Ohwayo and Balirwa 2004, Balirwa et al. 2003). Important to this study is pollution of the shoreline wetlands (Nyenje et al. 2010, Banadda et al. 2009, Munabi et al. 2009, Odada et al. 2009, 2004, Kayima et al. 2008, Njiru et al. 2008a, Oguttu et al. 2008, Cózar et al. 2007). Despite the increasing indication that water quality degradation has negatively influenced the invertebrate and fish populations in Lake Victoria (Njiru et al. 2008a, Ogutu-Ohwayo and Balirwa 2004), the systematic assessment and use of aquatic organisms in assessing the lacustrine water quality has not been well studied. Within specific bio-geographical regions, aquatic macroinvertebrate assemblages respond in predictable ways to changes in environmental variables because many of them have limited migration patterns and are particularly well suited for assessing site-specific effects (Azrina et al. 2006, Aisemberg et al. 2005, Tagliapietral et al. 2005, Verlecar et al. 2006, Hilty and Merenlender 2000, Butcher et al. 2003, Friedrich et al. 1996).

Benthic macroinvertebrates are of particular interest to this study and the focus of this chapter. They are common inhabitants of lakes where they are important in moving energy through food webs (Liu and Wang 2008, Madenjian et al. 2002, Ruitton et al.

2000). Many of these species are benthic for at least part of their life cycle; the prefix "macro" refers to species that are collected by mesh sizes of 200-500μm (Rosenberg and Resh 1993). Due to their association with contaminated sediments, benthic invertebrates tend to be useful bioindicator organisms of pollution in water quality monitoring programmes (Tagliapietral et al. 2005, Böhmer et al. 2004, Butcher et al. 2003, Jea et al. 2003). Benthic macroinvertebrates are ideal in biomonitoring because they are ubiquitous and they are affected by perturbations in many habitats. They are relatively sedentary and long-lived, which allows determination of the spatial extent of a perturbation. They are species-rich and differentially sensitive to many biotic and abiotic factors in their environment. They are relatively easy to collect and identify using available taxonomic keys (Mandaville 2002). As a result, their community structure has been used in biomonitoring of mainly rivers and streams, and as indicators of the health of an aquatic system because they can integrate changes that reflect the characteristics of both the sediment and the water column (Ngupula and Kayanda 2010, Böhmer et al. 2004, Butcher et al. 2003).

Water quality monitoring in Uganda, however, and in the Lake Victoria Basin in general, has been based on measurement of physicochemical parameters which is not only expensive and irregular (Njiru et al. 2008a, Machiwa 2003) but also fails to integrate temporal changes as it measures variables at a particular point in time. Further more some chemicals have high detections limits (Meybeck and Helmer 1996). On the other hand, organisms studied *in situ* can show the combined effects of all impacts on the water body, and can be used to compare changes in water quality from site to site, or over a time

(Friedrich et al. 1996). With the increasing pollution of urban aquatic systems in Uganda, coupled with meagre resource investment into environmental monitoring, there is need for regular environmental water quality assessment, especially of the urban aquatic systems that are vulnerable to pollution.

However, these efforts demand efficient and reliable mechanisms of detection especially of aquatic systems contamination. To this, the past two decades have seen a number of researchers recommend a biological monitoring system that is based on elements of flora and fauna as part of the process to reverse the negative impacts on water quality (Nzomo 2005, Balirwa et al. 2003, Seehausen 1999, Kaufman 1992). It is necessary to understand how selected organisms respond to environmental changes. This study is the first to assess the potential of using invertebrate and fish as biological indicators and fish histopathology biomarkers as an integrative and cost-effective biomonitoring tool in urban wetland environmental monitoring in Uganda. This chapter investigated the benthic macroinvertebrate community structure in relation to the water quality physicochemical variables, with a view to exploring benthic macroinvertebrates as biological indicators of water quality deterioration in wetlands in Uganda. Changes in invertebrate community structure in relation to environmental water quality were assessed using multivariate techniques such as Cluster Analysis (CA), Principal Component Analysis (PCA), and Canonical Correspondence Analysis (CCA), and richness, diversity and dominance indices. Findings from the present study will add to the fundamental knowledge and expertise necessary to support biomonitoring in the region.

4.2. Literature Review

4.2.1. Importance of benthic macroinvertebrates in water quality assessment and their spatial diversity in the study wetlands

Macro invertebrate assemblages have been used as bioindicators of environmental integrity of streams, rivers and lakes (Kobingi et al. 2009, Collins et al. 2008, Miltner et al. 2004, Butcher et al. 2003, Klemm et al. 2003, Moss et al. 2003). Within this framework, the study of benthic macroinvertebrates has gained interest for the environmental assessment of rivers and streams in urban and suburban catchments (Collins et al. 2008, Miltner et al. 2004). Despite the growing number of benthic macroinvertebrate community studies being conducted in East African water bodies (Ngupula and Kayanda 2010, Efitre et al. 2001, Muli and Mavuti 2001, Balirwa 1998, Okedi 1990), the concept of using benthic macroinvertebrates in water quality assessment and monitoring, especially in urban wetlands in Uganda has not been fully explored. Two biomonitoring studies were conducted in riverine and stream flow environments (Kobingi et al. 2009, Raburu et al. 2009). Only one study in Uganda evaluated benthic macroinvertebrate communities along an urban-rural gradient of landuse using GIS techniques and community composition (Sekiranda et al. 2004). There is a need for more systematic data assessment in the region in order to broaden our understanding of macroinvertebrates, their spatial dynamics in relation to catchment impacts and the relationship between the different taxa and water quality so as to establish methods to predict the effects of water quality on these organisms.

On a global scale, the use of macroinvertebrates to monitor water quality based on clear guidelines has been implemented in many countries. For instance, in the Australian River Assessment System (Parsons et al. 2010), in protocols for measuring biodiversity in Canada (Rosenberg et al. 2005), for the River Invertebrate Prediction and Classification System (RIVPACS) in the UK (Clarke et al. 2003), as part of protocols for sampling macroinvertebrates in wadeable streams in New Zealand (Stark et al. 2001), in the European Union Water Framework Directive (2000), for the US rapid bioassessment protocols for use in streams and wadeable rivers (Barbour et al. 1999), and in the South African Scoring System, a river health programme in South Africa (Dallas 2007). A number of researchers of riverine systems have also tried to modify the biotic indices (Karr et al. 1986) to meet their own particular needs, primarily to accommodate different habitats and taxa in the different case scenarios (Arimoro 2009, Kobingi et al. 2009, Masese et al., 2009a, b, Raburu et al. 2009, Arimoro et al. 2007, Bozzetti and Schulz 2004). These studies are often surveys conducted as intensive programmes to measure and observe the quality of the aquatic environment for a specific purpose. These surveys are important as they help to establish regional trends in biodiversity, consequently laying the foundation for surveillance and monitoring programmes. In Uganda, there is a need for baseline information on the potential for use of faunal indicators in water quality assessment.

Global trends in the past decade show that studies on the role of macroinvertebrates in aquatic environmental assessment considered the riverine (Arimoro 2009, Azrina et al. 2006, Böhmer et al. 2004, Butcher et al. 2003, Fenoglio et al. 2002, Griffith et al. 2001);

stream (Arimoro et al. 2007, Lounaci et al. 2000); and lacustrine environments (Walters et al. 2009, Batzer et al. 2004, Doherty et al. 2000). However, most of the studies encountered in the East African region have been limited to riverine and stream ecosystems with minimal information on lake near-shore and coastal wetland environments (Kobingi et al. 2009, Masese et al. 2009a, b, Raburu et al. 2009). These riverine studies demonstrated the potential of biomonitoring in water quality assessment in the region. They also serve as motivation for further research on the distribution, abundance, spatial and temporal characteristics of the local biota and their relationship with increasing environmental challenges. For example, in the upper reaches of the Lake Victoria basin, a number of studies showed the usefulness of macroinvertebrates in assessing the biological integrity of rivers and streams (Masese et al., 2009a, b, Raburu et al. 2009). Macroinvertebrates have specifically been popular in the assessment of pollution to evaluate the overall water resource quality, food habits of benthic fishes and ecological function and effects of anthropogenic disturbances (Stephens and Farris 2004, Poulton et al. 2003).

Studies that investigated the use of macroinvertebrates to make inferences about the level of pollution in lakes have been conflicting, with the majority of the studies in wetlands and near-shore areas of lakes showing promise of using macroinvertebrates for pollution monitoring, and development of biological criteria for lakes and wetland protection (Parsons et al. 2010, Schartau et al. 2008, Batzer et al. 2004, Cooper et al. 2006, Lewis et al. 2001, Doherty et al. 2000). However other studies inferred that macroinvertebrate indicators may not be reliable for assessing and reporting the biological condition of lakes (FDEP 2007) and ponds (Batzer et al. 2004). For example, FDEP (2007) observed a correlation between macroinvertebrate diversity and water clarity (Secchi depth and color) and nutrient concentrations (TKN and TP), but could not find a significant correlation between human disturbance in the near-shore area around the lake and nutrient concentration and water clarity. The authors argued that the high correlation with nutrients and water clarity could have been a result of anthropogenic and natural sources.

This goes to support the fact that the use of macroinvertebrates as indicators of aquatic environmental health requires measurement of end points based on differences observed between sites experiencing different levels of pollution (Solimini et al. 2006, Lewis et al. 2001, Norris 1995, Barbour et al. 1992). It also implies that the use of macroinvertebrates as indicators of aquatic environmental health would require establishment of the relevant environmental factors of the studied ecosystem because application of bioindicators based on the extrapolation of data from other studies may be inappropriate. Uganda being equatorial, extrapolation of data from non-equatorial regions would be inappropriate as pollution and its impacts may be different in different ecoregions. Taking these points into consideration, data collection at focused localised sites is needed to obtain the knowledge and expertise upon which biomonitoring programmes can be based in Uganda. In this regard, the present study was carried out in five Lake Victoria coastal wetlands, three of which are subjected to urban effluent while two were not. Although no biomonitoring studies have been conducted for these sites except for Murchison Bay (Sekiranda et al. 2004), earlier studies on macroinvertebrates composition in the ecoregion give useful background information to the study (Mbahinzireki 1994, 1993, Mwebaza-Ndawula 1994, Witte et al. 1992, Okedi 1990, Mothersill et al. 1980).

4.2.1. Relationship between environmental physicochemical variables and benthic invertebrate communities

Different anthropogenic activities in the catchment variably influence the distribution and abundance of benthic macroinvertebrate communites in the aquatic environment, with urban impacts reducing diversity and causing proliferation of the pollution tolerant taxa like Oligochaeta and chironomids (Table 4.1).

Table 4.1 Effects of environmental physicochemical variables on benthic macroinvertebrate abundance and diversity in different aquatic systems

Aquatic system studied	**Associated anthropogenic factors**	**Aquatic Environmental Variables**	**Impact on benthic macroinvertebrate taxa**	**References (Country of study)**
Lakes	Sulphur and Nitrogen emissions	Low pH due to lake acidity	Increase in Chironomidae and Oligochaeta, decrease in Hyallelidae	Parsons et al. 2010 (Canada)
Seasonal ponds	Relatively pristine forests wetlands	pH, conductivity, alkalinity, total organic C, water colour, total N, NO_3^--N and NH_4^+-N	Macroinvertebrates were unresponsive to environmental variables	Batzer et al. 2004 (USA, Minnesota)

Table 4.1 continued: Effects of environmental physicochemical variables and benthic macroinvertebrates abundance and diversity in different aquatic systems

Aquatic system studied	Associated anthropogenic factors	Aquatic Environmental Variables	Impact on benthic macroinvertebrate taxa	References (Country of study)
Lake/ river mouth, wetland	Land use and cover	Temperature, dissolved oxygen, chlorophyll *a*, total dissolved solids, turbidity, pH and conductivity	Water quality and macroinvertebrate abundance and diversity correlated with surrounding land use and cover	Cooper et al. 2006 (USA – Michigan)
River	Rubber effluent discharges	High conductivity, BOD, phosphates and nitrates	• No sensitive taxa in effluent impacted site • Predominance of oligochaetes and chironomids	Arimoro 2009
	Urbanisation	• Dissolved oxygen • specific conductance and pH	The water quality variables did not predict macroinvertebrate diversity	Walters, et al. 2009 (USA – Georgia)
	Organic pollution and habitat disturbance	• High conductivity • Variable pH and dissolved oxygen values • High N and P,	• Low diversity • Dominated by chironomids (93.3% individuals/site) • Oligochaeta and Simuliidae frequently occurred	Aguiar et al. 2002 (Portugal)
	Farming activities and urban developments	• Physico-chemistry was insignificant • Land use impacts significant	Presence of pollution sensitive fauna in undisturbed stations	Thorpe and Lloyd 1999 (St. Lucia)
	Sewage effluent	• Low oxygen concentration • High pH, sulphate and nitrate	Presence of taxa tolerant to those physicochemical conditions	Coimbra et al. 1996. (Portugal)
	No effluent	• High oxygen concentration	High taxa diversity especially in the reference site	

Table 4.1 continued: Effects of environmental physicochemical variables and benthic macroinvertebrates abundance and diversity in different aquatic systems

Aquatic system studied	Associated anthropogenic factors	Aquatic Environmental Variables	Impact on benthic macroinvertebrate taxa	References (Country of study)
Reservoir	Eutrophication and organic pollution	• Depth • Dissolved oxygen • Substrate	• High densiy of Oligochaetes and Chironomidae • Low density of Ephemeroptera	Pamplin et al. 2006 (Brazil)
Lake (Shallow bays)	Land use patterns i.e urban, semi-urban and rural	High conductivity and Low dissolved oxygen in urban bay	• Low diversity • Presence of worms and Chironomus sp	Sekiranda et al. 2004 (Uganda)
Stream	Organic and industrial wastes	High nitrite and phosphate	Associated with: Molluscs (*Pisidium* and *Planorbis*) Diptera *Pentaneurella* Odonata *Onychogomphus*	Girgin et al. 2003 (Turkey)
Forest Stream	High levels of deforestation	High values of : • TP, TN, pH, • conductivity • temperature	Reduced macroinvertebrate taxa	Couceiro et al. 2007 (Brazil)
	Reference site	Higher oxygen, depth and velocity	Increased taxa richness	
Mountain Streams	Riparian disturbance from livestock grazing	• Temperature • Dissolved & total organic carbon • Mean bank height • Mean water surface gradient	High diversity of: Chironomidae • *Radotanypus,* • *Paracladopelma* • *Phaenopsectr* • *Parakiefferella* Mayfly *Tricorythodes*	Griffith et al. 2001 (Colorado)
	Toxicological effects associated with mining		Low diversity of all taxa.	

In some cases however, aquatic environmental variables may not influence macroinvertebrate communities as was observed in the relatively pristine forest wetlands in Minnesota (Batzer et al. 2007), in the Etowah River basin in northern Georgia (Walters et al. 2009), and in the Florida lakes (FDEP 2007). In Uganda, water quality degradation negatively influenced the invertebrate and fish populations in Lake Victoria (Ogutu-Ohwayo and Balirwa 2004, Balirwa et al. 2003). For example, the increasing hypolimnetic anoxia in the lake has resulted in changes in the macroinvertebrate community (Balirwa et al. 2003). Some authors (GoU 2009, GoU 2006a, Balirwa et al. 2003, Kaufman 1992, Seehausen 1999) recommended the establishment of a biomonitoring system. The authors argued that biological monitoring systems are indispensable complements to the conventional physical and chemical tests as they average environmental effects over long time spans and thus have greater environmental change indicator abilities. Biological monitoring has been widely applied and recommended because it is relatively inexpensive and easy to apply (Parsons et al. 2010, Dallas 2007, Rosenberg et al. 2005, Clarke et al. 2003, Stark et al. 2001, Barbour et al. 1999, Friedrich et al. 1996).

However, despite data suggesting that water quality degradation has negatively influenced the invertebrate and fish populations in Lake Victoria (Ogutu-Ohwayo and Balirwa 2004, Sekiranda et al. 2004), the use of these organisms in water quality biomonitoring has not been explored for this region. Much of the knowledge available on benthic macroinvertebrates in the Lake Victoria environment is related to their biology, emergence, colonization, biomass, standing crop and spatial patterns in relation to habitat uses and preference (Ngupula and Kayanda 2010, Muli and Mavuti 2001, Efitre et al. 2001, Okedi 1990, Balirwa 1998). Because of the inadequate studies documenting the composition, distribution and response of macroinvertebrate assemblages to water and habitat degradation, it is not possible to develop a sound biomonitoring programme as a basis for developing management strategies for the Lake.

This chapter studies the role of benthic macroinvertebrates as biological indicators of water quality and discusses their potential for use in the environmental quality assessment of effluent impacted wetlands in Uganda. It was hypothesised that invertebrate taxa are useful biological indicators of water quality deterioration in wetlands in Uganda. To test this hypothesis, the invertebrate taxa community structure in relation to the water quality physicochemical variables was investigated. The objective was to explore changes in invertebrate community structure in relation to deterioration in environmental water quality in the studied wetlands. The composition and occurrence of macroinvertebrate taxa was explored using relative abundance and community indices. Site differences in taxonomic composition were tested using CA and PCA where sites with similar taxa characteristics were grouped together under one cluster. The relationship between macroinvertebrate taxa and environmental variables was assessed using CCA.

4.3. Specific Methods

4.3.1. Field sampling

The study involved collecting macroinvertebrate data every 2 months, over 12 sampling trips. Sampling was carried out between 2:00 and 4:00 pm and involved collecting sediment samples using a Ponar Grab with a jaw area of about 245 cm^2. Sediment samples were obtained at two locations, with one location at the shoreline (inshore site) and the other 60 m off the shoreline (offshore site). Two hauls of sediment were taken from each sampling point. The hauls were pooled and preserved with 5% buffered formalin in polythene bags. Preserved samples from each bag were later washed using a 500 µm mesh net washing bag and placed in labelled bottles containing 70% ethanol. In the laboratory, each sample was rinsed and poured into a flat tray, all organisms sorted and taxonomically identified up to genus level using a dissecting microscope and macro-invertebrate identification manuals (Kellogg 1994). Members of each taxon were counted and the counts expressed as individuals per square meter. For each sampling location physicochemical parameters were measured (chapter three).

4.3.2. Statistical analysis

The study sites were characterized according to their level of environmental degradation based on the invertebrate community structure by comparing the effluent-impacted sites against the reference site. Comparison was based on a number of end points which included 1) Total numbers of taxa per site, 2). Total number of individuals per square metre per site, 3) Relative abundances of taxa, 4) Margalef richness index (D), 5)

Shannon diversity index (H), 6) Simpson’s dominance D, 7) Site classification using Cluster Analysis (CA) and Principal Component Analysis (PCA) 8) Relationship between invertebrate taxa diversity and the physicochemical variables based on Canonical Correspondence Analysis (CCA).

Biological indices, which include diversity, richness and similarity indices, have been developed to simplify data on species richness and faunal density, enabling numerical comparisons between sites (Mora et al. 2008, Tokeshi and Arakaki 2007, Clarke and Warwick 2001). Diversity indices, such as the Shannon Index combine species richness, measured as the total number of taxa and evenness, measured as the number of individuals of each taxon (Clarke and Warwick 2001). The Margalef Index discriminates the number of species present for a given number of individuals (Mora et al. 2008, Tokeshi and Arakaki 2007). The Simpson’s species dominance index captures the variance of the species abundance distribution (Maqurran 2004). CA groups objects into clusters on the basis of their similarities within a class and dissimilarities between different classes (Bierman et al. 2011, Henderson and Seaby 2007, 2000a, Manly 2005). The PCA accounted for the variation in the observed clusters based on the macroinvertebrate taxa (Henderson and Seaby 2000a, 2007, Manly 2005, Sarbu and Pop 2005), while CCA allowed for the integration analysis of both taxa and environmental data (Arimoro 2009, ter Braak and Smilauer 2002).

Multivariate statistical techniques, CA and PCA were used to test for spatial difference in the macroinvertebrate taxonomic composition and to characterise the study sites according to their macroinvertebrate community structure. CA and PCA tests were performed using the Community Analysis Package 1.52 computer programme (Henderson and Seaby 2000a). Hierarchical agglomerative CA by means of the Wards method using Euclidean distances as a measure of similarity was performed on log-transformed and normalised data sets (Henderson and Seaby 2000a). Details on the advantages of CA and PCA are given in the previous chapter. Taxa relative abundances were calculated according to the proportion of each taxa found at each site during the study period. The Species Diversity and Richness (SDR Version 2.65) programme was used to calculate and compare the macroinvertebrate community indices (Shannon and Margalef) for the study sites (Seaby and Henderson 2000). Comparison of indices for significant differences between sites was done using the randomisation test of Solow (1993). Canonical correspondence analysis (CCA) was performed to evaluate the relationship between macroinvertebrate communities and environmental variables using the Environmental Community Analysis Package 2 (ECOM) by Henderson and Seaby (2000b).

4.4. Results

4.4.1. Composition and occurrence of invertebrate taxa in the study sites

Twenty-nine (29) benthic macroinvertebrate taxa representing 20 families in 13 orders were recorded. Total invertebrate relative abundance and the abundance of the major taxa did not vary significantly across months / seasons but site differences were observed. The highest taxa number of 22 and 18 before and after exclusion of rare occurrences respectively was registered in Winday Bay. This was followed by Lwanika (reference site) and Masese with 21 taxa each before exclusion of the rare occurrences, and 14 and 15 taxa after exclusion of rare occurrences respectively. The lowest taxa numbers before and after exclusion of rare occurrences were recorded in Kirinya and Murchison Bay respectively (Table 4.2). Offshore locations registered significantly higher taxa numbers than the inshore locations, and the percentage of individuals m^{-2} were significantly higher in the offshore sites than the inshore sites, with offshore sites contributing above 50% of the total individuals (Tables 4.2 and 4.3).

Table 4.2 Number of invertebrate taxa (genera) and individuals/m^2 observed during the study before and after excluding the rare occurrence species (n = 12).

	Site	Number of taxa			Individuals/m^2		
		Total	Inshore	Offshore	Total	Inshore	Offshore
Including rare occurrences	Kirinya	14	11	12	18523	6176	12347
	Masese	21	18	19	23466	4847	18619
	Winday	22	20	19	25107	10207	14900
	Murchison	13	11	13	28955	7604	21351
	Lwanika	21	18	20	15184	5404	9794
Excluding rare occurrences	Kirinya	10	3	8	17064	4977	12087
	Masese	15	3	9	19311	2068	17243
	Winday	18	10	14	23441	8751	14690
	Murchison	11	2	7	23584	2864	20720
	Lwanika	14	7	12	12986	3976	9010

For the inshore and offshore sampling locations, the number of taxa was highest in Winday Bay inshore and Lwanika offshore locations with 20 taxa each followed by Winday Bay offshore and Masese offshore with 19 each and Masese and Lwanika inshore locations with 18 each (Table 4.2). Also of interest in table 4.2 is that, despite Murchison Bay having the lowest number of taxa, the bay registered a significantly high number of individuals per square meter. Lastly, the taxa reduced significantly after the exclusion of the rare occurrences but there was no significant difference in individuals/m^2 before and after exclusion of the rare occurrences.

Seven taxa were found at all sampling locations before exclusion of the rare occurrences and these included, *Chironomus* sp, *Clinotanypus*, *Tanypus*, *Bellamya*, *Melanoides*, *Corbicula* and Oligochaetes (Table 4.3). Diptera contributed the highest number of taxa and individual numerical abundances, with *Chironomus* sp and *Tanypus* taxa belonging to the Chironomidae family being the most abundant; followed by Molluscs, especially *Corbicula*, *Bellamya*, and M*elanoides* (Table 4.3). *Chironomus* species contributed over 50% of the individuals/m^2 in Kirinya inshore and Masese offshore sampling locations (Table 4.3). Murchison Bay supported a taxa invertebrate assemblage with low diversity mainly comprising stenotopic and eutrophic populations of pollution-tolerant groups that included Oligochaeta, *Chironomus* species and *Corbicula* (Tables 4.3 and 4.4). Pollution-sensitive groups of Emphemeroptera and Trichoptera were absent from Murchison Bay, Kirinya and Masese, but two taxa in each of these groups were recorded in Lwanika (reference site) and Winday Bay (Table 4.3). On exclusion of the rare occurrences, *Corbicula* was the most predominant in four out of the eight sampling locations, i.e. Kirinya and Masese offshore and Winday Bay’s inshore and offshore sampling locations (Table 4.4). In Murchison Bay, Oligochaeta were the most predominant in both the inshore and offshore locations (Table 4.4).

Table 4.3 The relative percentage abundances of benthic macroinvertebrates at the sampling stations in the study wetlands in Uganda. Sampling locations L In and L Off = Lwanika Inshore and Offshore (Reference sites), M In and M Off = Masese Inshore and Offshore, W In and W Off = Winday Bay Inshore and Offshore, K In and K Off = Kirinya Inshore and Offshore, MB In and MB Off = Murchison Bay Inshore and Offshore (n = 12).

Order	**Family**	**Taxon (Genus)**	**K-In**	**K-Off**	**M-In**	**M-Off**	**W-In**	**W-Off**	**MB-In**	**MB-Off**	**L-In**	**L-Off**
Diptera	Chironomidae	*Ablabesmyia*	0.91	0.00	1.73	1.16	2.29	1.38	0.00	0.00	0.52	0.14
		Chironomus sp	73.82	34.29	27.69	58.70	42.75	12.36	35.47	18.71	33.51	17.58
		Clinotanypus	0.00	1.91	0.29	3.08	0.41	2.33	3.06	0.98	3.12	1.57
		Procladius	0.00	0.00	0.29	0.30	0.00	0.09	0.00	0.00	15.58	35.59
		Tanypus	5.21	30.84	14.98	1.58	2.74	4.70	6.18	9.19	5.71	3.00
		Tanytarsus	0.00	0.00	17.19	1.05	1.37	0.66	0.00	0.00	0.00	0.00
	Chaoboridae	*Chaoborus*	0.23	1.65	0.29	0.45	0.00	6.48	0.00	0.00	20.00	7.58
	Ceratopogonidae	*Palpomyia*	0.89	0.68	0.58	0.08	8.23	2.72	0.00	0.00	0.52	0.57
Odonata	Libellulidae	*Dythemis*	0.00	0.00	0.00	0.37	0.82	0.47	0.00	0.00	0.00	0.00
		Libellula	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00
		Perithemis	0.00	0.11	0.85	0.23	0.00	0.00	0.00	0.00	0.26	0.00
		Sympetrum	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ephemeroptera	Caenidae	*Caenis*	0.00	0.00	0.00	0.00	0.82	0.09	0.00	0.00	0.00	1.14
	Polymitarcyidae	*Povilla*	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.78	3.14
Trichoptera	Leptoceridae	*Leptocella*	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.52	1.00
	Dipseudopsidae	*Phylocentropus*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.04	1.72
Hemiptera	Notonectidae	*Notonecta*	0.00	0.00	1.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Podocopida	Cyprididae	*Ostracods*	0.45	0.00	0.00	2.03	0.00	0.00	0.00	0.00	0.00	0.57
Architaenioglossa	Viviparidae	*Bellamya*	3.82	6.28	2.52	7.06	8.76	24.99	5.26	7.43	1.82	5.43

Table 4.3 continues:

The relative percentage abundances of benthic macroinvertebrates at the sampling stations in the study wetlands in Uganda. Sampling locations L In and L Off = Lwanika Inshore and Offshore (Reference sites), M In and M Off = Masese Inshore and Offshore, W In and W Off = Winday Bay Inshore and Offshore, K In and K Off = Kirinya Inshore and Offshore, MB In and MB Off = Murchison Bay Inshore and Offshore (n = 12).

Order	**Family**	**Taxon (Genus)**	**K-In**	**K-Off**	**M-In**	**M-Off**	**W-In**	**W-Off**	**MB-In**	**MB-Off**	**L-In**	**L-Off**
Basommatophora	Planorbidae	Biomphalaria	0.00	0.23	1.40	2.09	2.06	3.66	0.55	0.07	1.82	0.29
		Bulinus	1.13	0.11	0.00	0.00	0.41	0.00	0.00	0.78	0.00	1.00
Sorbeoconcha	Birthyniidae	Gabbia	0.00	0.00	5.05	0.68	0.69	3.01	0.54	1.57	0.00	3.72
	Thiaridae	Melanoides	2.04	11.07	0.58	3.16	5.86	11.81	13.45	10.53	3.12	5.72
Pulmonata	Euconulidae	Plegma	0.00	0.00	0.00	0.00	0.27	2.50	0.00	0.00	0.00	0.00
Veneroida	Sphaeriidae	Byssanodonta	0.00	0.00	4.77	1.95	0.41	1.22	0.00	0.52	0.00	0.00
		Sphaerium	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.22	0.00	0.00
	Corbiculidae	Corbicula	9.28	7.39	19.50	10.24	18.16	17.05	32.19	31.12	6.23	7.51
Arhynchobdellida	Hirudinidae	Hirudinea	0.00	0.00	0.00	0.00	0.69	0.09	0.74	0.39	1.30	0.14
Haplotaxida	Naididae	Oligochaetes	2.22	5.43	0.87	5.64	2.98	4.38	2.20	18.50	4.16	2.57

Table 4.4 Ranks of invertebrate taxa (genera) in the study locations based on variance of abundance distributions calculated using Simpson's species dominance index for the commonly occurring species (n =12).

	Kirinya		**Masese**		**Winday Bay**		**Murchison Bay**		**Lwanika**	
	Inshore	**Offshore**	**Inshore**	**Offshore**	**Inshore**	**Offshore**	**Inshore**	**Offshore**	**Inshore**	**Offshore**
n	4	8	2	9	10	14	2	7	7	12
1	Ablabesmyia	Corbicula	Tanypus	Corbicula	Corbicula	Corbicula	Oligochaetes	Oligochaetes	Tanypus	Chironomus sp
2	Bellamya	Tanypus	Chironomus sp	Bellamya	Tanypus	Bellamya	Chironomus sp	Corbicula	Chironomus sp	Chaoborus
3	Melanoides	Clinotanypus		Chironomus sp	Caenis	Melanoides		Chironomus sp	Phylocentropus	Oligochaetes
4	Chironomus sp	Melanoides		Clinotanypus	Gabbia	Chironomus sp		Bellamya	Oligochaetes	Corbicula
5		Oligochaetes		Byssanodonta	Hirudinea	Oligochaetes		Gabbia	Corbicula	Povilla
6		Bellamya		Melanoides	Oligochaetes	Biomphalaria		Tanypus	Clinotanypus	Procladius
7		Chironomus sp		Biomphalaria	Melanoides	Clinotanypus		Melanoides	Chaoborus	Tanypus
8		Palpomyia		Gabbia	Ablabesmyia	Byssanodonta				Leptocella
9				Oligochaetes	Bellamya	Chaoborus				Phylocentropus
10					Chironomus sp	Gabbia				Caenis
11						Plegma				Bellamya
12						Tanypus				Melanoides
13						Palpomyia				
14						Ablabesmyia				

4.4.2. Spatial diversity and site grouping based on invertebrate taxa characteristics

Hierarchical cluster analysis grouped the 10 sampling locations into three clusters based on the similarity of invertebrate taxa characteristics (Figure 4.1). The three clusters as displayed in the dendrogram (Figure 4.1) were similar to the clusters obtained based on water quality physicochemical variables (chapter three Figure 3.1), with the less polluted cluster comprising the inshore and offshore locations of the reference site (cluster 1). The medium polluted cluster 2 included the inshore and offshore sampling locations of Winday Bay and Masese (Figure 4.1). In this case, Kirinya offshore was grouped in the highly polluted cluster together with Murchison bay inshore and offshore, and not in the medium polluted cluster as was the case in the classification based on water quality variables (Figures 4.1 and 3.1).

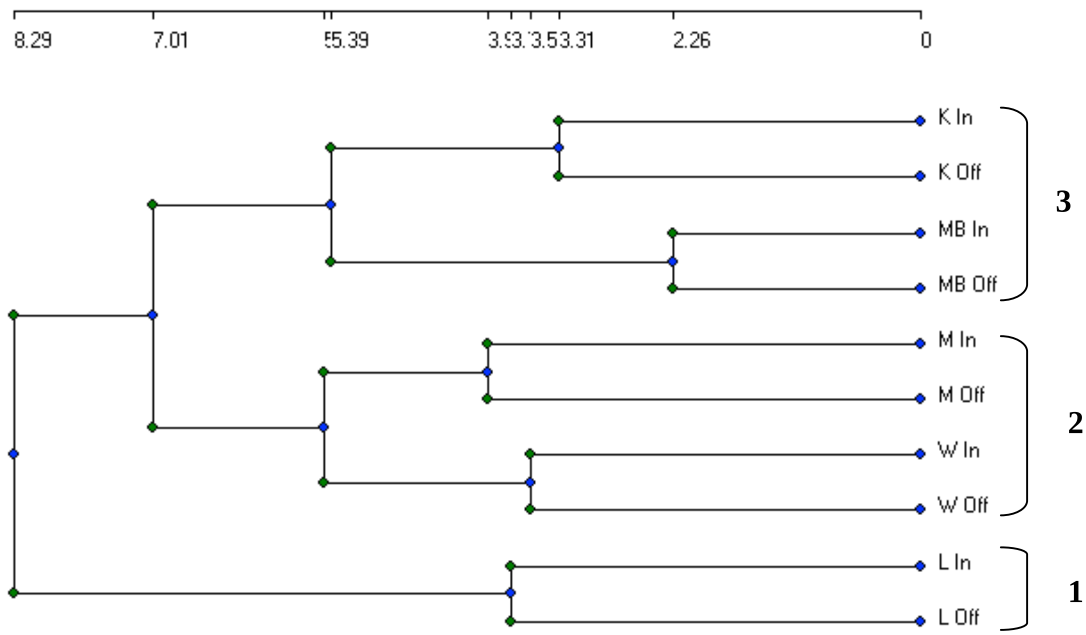


Figure 4.1 Hierarchical Cluster Analysis dendrogram, each of 3 clusters indicates sites with similar invertebrate taxa. Homogeneity within clusters was based on Euclidean distance and the heterogeneity between clusters was estimated according to Ward's method. Sampling locations L In and L Off = Lwanika Inshore and Offshore, M In and M Off = Masese Inshore and Offshore, W In and W Off = Winday Bay Inshore and Offshore, K In and K Off = Kirinya Inshore and Offshore, MB In and MB Off = Murchison Bay Inshore and Offshore.

The PCA plot results (Figure 4.2) complemented those of the CA, as it produced a three-dimensional distribution of sites similar to the grouping formed by the hierarchical cluster analysis dendrogram in figure 4.1. Cluster 1, comprising the reference site, was highly associated with the two Ephemeroptera taxa *Caenis* and *Povilla*, the two Trichoptera taxa, *Leptocell*a and *Phylocentropus*, and two diptera taxa, *Procladius* and *Chaoborus*

(Figure 4.2). The highly polluted cluster 3 was highly associated with stenotopic and eutrophic populations of pollution tolerant groups that included three Chironomidae taxa, such as *Chironomus* sp, *Clinotanypus* and *Tanypus*; Molluscs such as *Corbicula*, *Melanoides*, *Sphaerium*, *Bellamya* and *Bulinus*; and Oligochaeta (Figure 4.2). The moderately polluted cluster 2, was mainly associated with molluscs and odonates.

Applying the Shannon diversity and Margalef richness community indices to assess the macroinvertebrate assemblage at the sampling locations revealed a decline in biotic integrity in the inshore locations at the effluent impacted sites. The indices varied significantly among the sampling locations ($p < 0.05$). The indices were highest in the offshore sampling locations than the inshore sampling locations (Figure 4.3). At both locations, Winday Bay and the reference site (Lwanika) registered the highest indices while high effluent-impacted sampling locations of Murchison Bay and Kirinya registered the lowest indices (Figure 4.3).

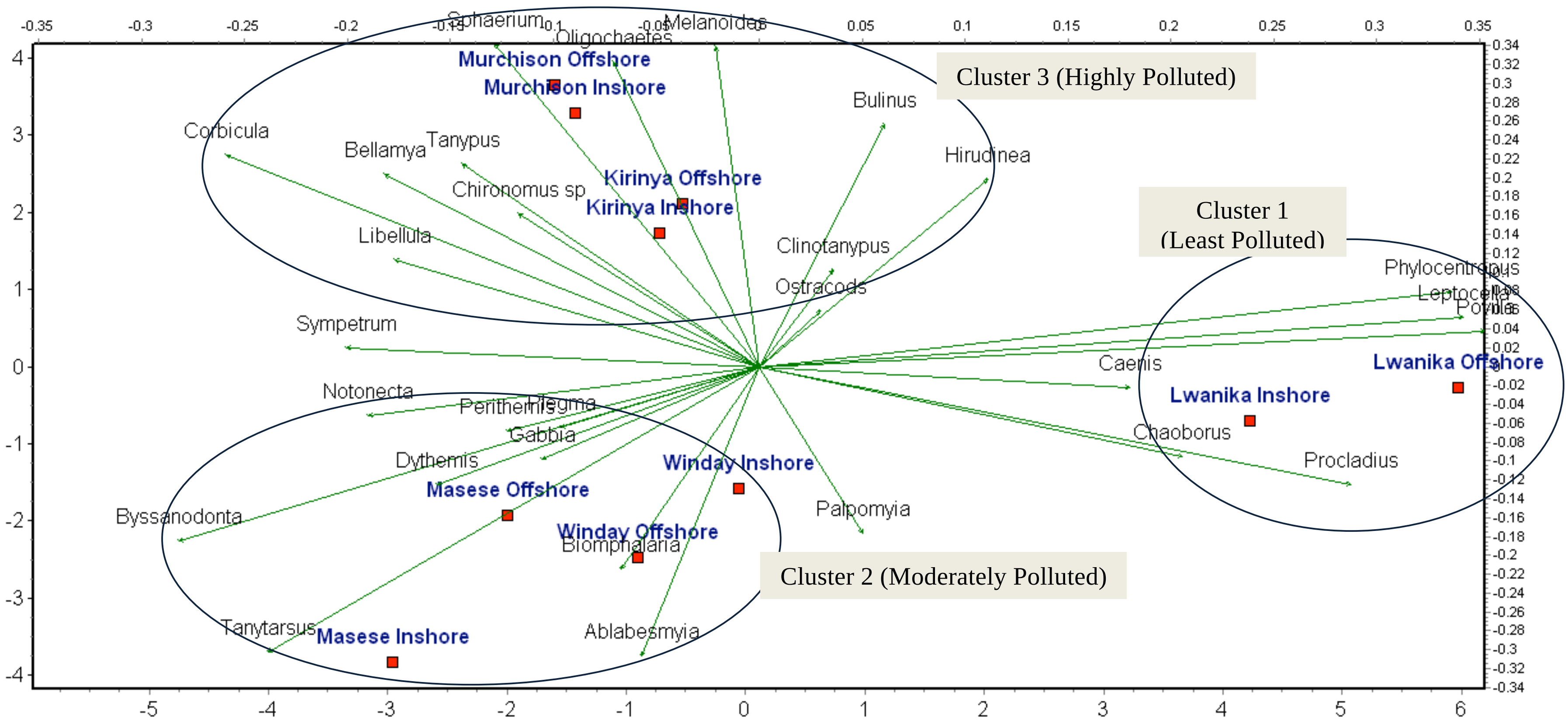


Figure 4.2 PCA ordination biplot of the ten sampling locations based on the benthic macroinvertebrate variables for plot component one (X axis) and plot component two (Y axis). Note the 3 groups (clusters) of the highly polluted Murchison Bay and Kirinya, the medium polluted Masese and Winday Bay and the less polluted reference site (Lwanika)

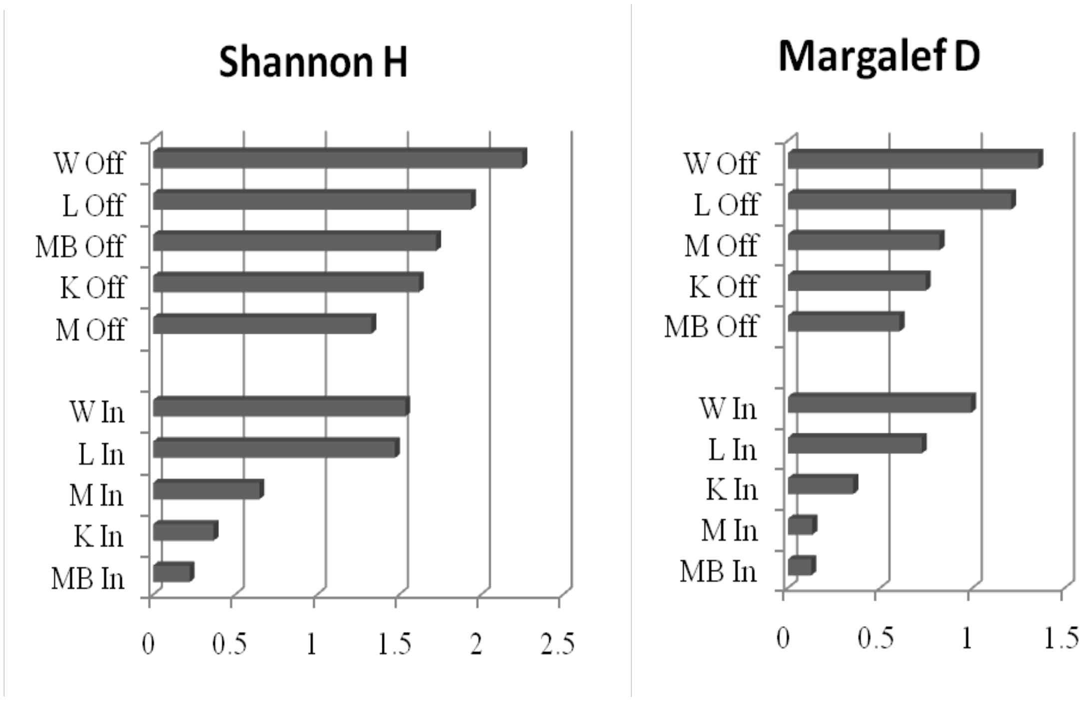


Figure 4.3 Shannon diversity H and Margalef richness D indices for macroinvertebrate taxa in the study sampling locations. Where: L Off = Lwanika Offshore (reference site), W Off = Winday Bay Offshore, M Off = Masese Offshore, MB Off = Murchison Bay Offshore, K Off = Kirinya Offshore, L In = Lwanika Inshore (reference site), W In = Winday Bay Inshore, M In = Masese Inshore, MB In = Murchison Bay Inshore and KI Kirinya Inshore.

4.4.3. Relationship between invertebrate taxa and environmental variables

The Canonical Correspondence Analysis (CCA) ordination showed a good relationship between invertebrate taxa distribution and the measured environmental variables in the different study sites as reflected by the high species-environmental correlation coefficient associated with each axis (Table 4.5). The strongest explanatory factors were oxygen, temperature, total depth, total phosphorus and conductivity. Nitrate-N, ammonium-N and pH, were excluded from the CCA plot due to a high multicollinearity with Nitrate-N, Total-N and oxygen respectively. The pollution-sensitive taxa of Ephemeroptera (*Caenis* and *Povilla*) and Trichoptera (*Leptocella* and *Phylocentropus*) were more abundant in the reference site's offshore location and were mainly associated with the high temperatures in these sites (Figure 4.4). Murchison Bay's inshore and offshore (denoted by MBI and MBO), associated with high total phosphorus and high conductivity favoured the pollution-resistant Oligochaetes (Figure 4.4). Lwanika and Winday Bay locations were associated with high oxygen and higher total depth and the majority of the taxa were collected under these conditions (Figure 4.4). The first three axes accounted for 52.8% of the total taxa variance (Table 4.5) explained in the observed patterns in the CCA plot (Figure 4.6).

Table 4.5 Summary of CCA axis length showing axis eigenvalues, correlation between taxa and the environmental gradients, and variance of taxa, following canonical correspondence analysis of invertebrate taxa abundance data in five Uganda wetlands.

	CCA Axis 1	CCA Axis 2	CCA Axis 3
Canonical eigenvalue	1.563	0.2071	0.127
Cumulative percentage variance	42.99	48.69	52.18
Species environmental correlation	0.9561	0.8903	0.7469
Number of species (response variable)	19		
Number of environmental variables	12		
Total variance in species data	3.635		

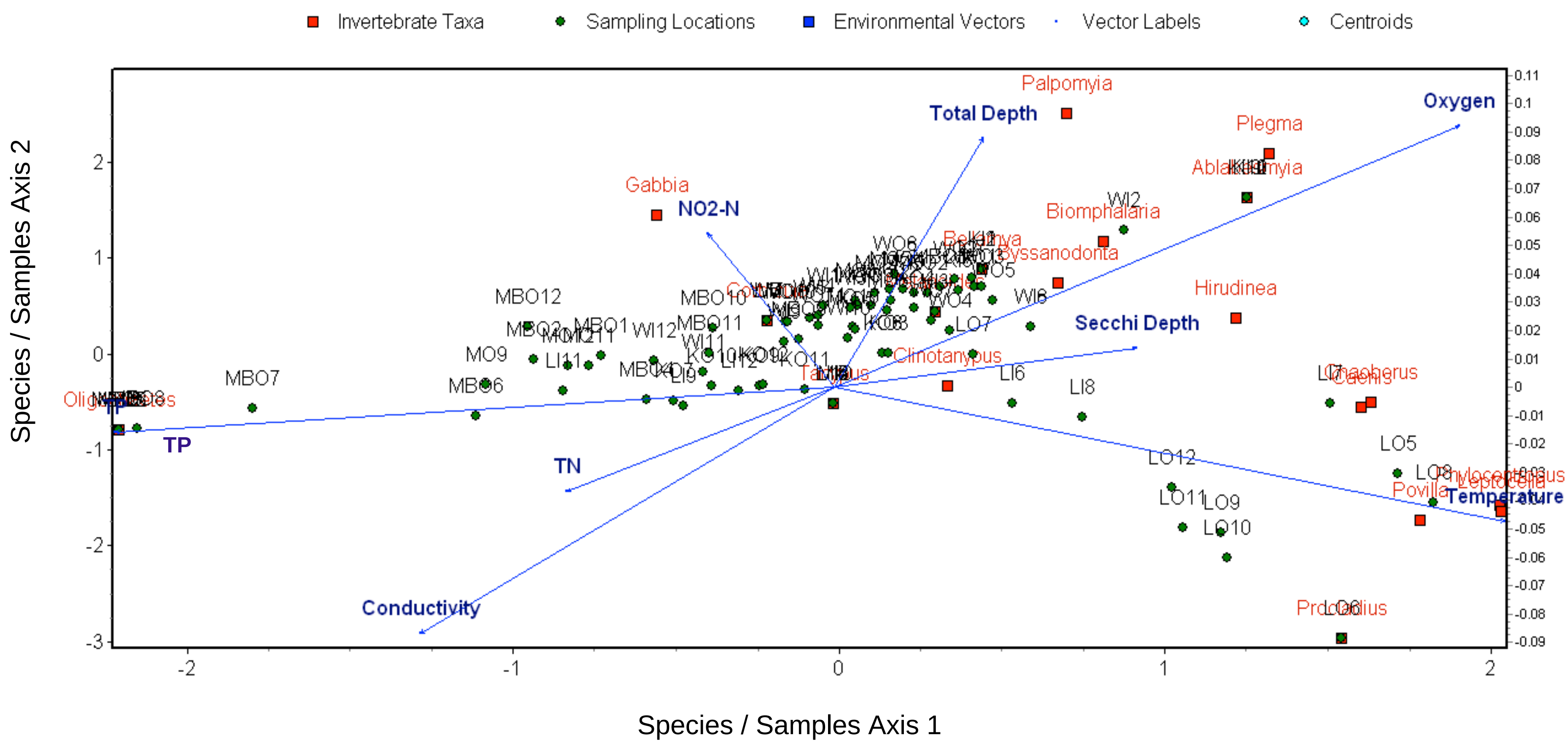


Figure 4.4 CCA plot of first and second CCA axes of invertebrate taxa, environmental variables and their corresponding sampling locations. Eigenvalues: axis 1, 1.563, axis 2, 0.2071. First 2 axes account for 48.69% of the variance. Monte Carlo test was significant ($p < 0.001$), 1000 permutations. Where sampling locations LI and LO = Lwanika Inshore and Offshore, MBI and MBO = Murchison Bay Inshore and Offshore, WI and WO = Winday Bay Inshore and Offshore, MI and MO = Masese Inshore and Offshore, KI and KO = Kirinya Inshore and Offshore. Numbers represent different sampling efforts.

4.5. Discussion

The water quality environmental data in chapter 3 and the macroinvertebrate taxonomic composition in this chapter grouped the sampling locations into three clusters, i.e., least, moderate and highly polluted. This provided a clear distinction between the effluent impacted sites and the non-effluent impacted sites which included grouping the reference site in the least impacted cluster. In this chapter multivariate techniques that included CA, PCA and CCA, and community indices which included Shannon diversity, Margalef richness and Simpson's dominance were used to explore variation in invertebrate taxa community structure in relation to deterioration in environmental water quality in the studied wetlands. This was done with the view of exploring benthic macroinvertebrates as biological indicators of water quality in selected wetlands in Uganda, and to provide a basis to make further comparisons for other sites in the equatorial region.

There were differences in the macroinvertebrate abundance and community structure between the urban effluent-impacted wetlands and the non-effluent impacted wetlands as the most sensitive macroinvertebrate taxa were absent from the highly polluted sites. Based on CCA results, high dissolved oxygen and temperature, and low conductivity were associated with the least and moderate polluted sites and most invertebrate taxa were collected from these conditions. Besides, there were variations in ecosystem integrity among stations and this was reflected in community composition and structure of resident macroinvertebrates. This chapter shows that benthic macroinvertebrate taxa have potential as meaningful biological indicators in aquatic environmental pollution assessment in wetlands in Uganda. Thus the biomonitoring potential of benthic macroinvertebrates in relation to environmental water quality in the study wetlands is discussed in this chapter based on the composition and relative abundance of invertebrate taxa in the study sites, their spatial diversity and site grouping based on invertebrate taxa characteristics, and the relationship between macroinvertebrate taxa and environmental variables.

4.5.1. Composition and relative abundances of invertebrate taxa in the study sites

The predominance in all the study sites of *Chironomus* sp and *Tanypus* taxa in the Chironomidae family and *Corbicula*, *Bellamya*, and *Melanoides* molluscs is similar to results from recent studies that were carried out in the Northern part of Lake Victoria (Ngupula and Kayanda 2010, Sekiranda et al. 2004). These results are also related to the historical perspectives of studies from the early 1990s (Mbahinzireki 1994, 1993, Mwebaza-Ndawula 1994, Witte et al. 1992). These studies also observed a predominance and ubiquity of *Melanoides* and *Bellamya*. In the present study and in a study by Sekiranda et al. (2004), numerically significant taxa of *Melanoides* and *Bellamya* were observed in all the study sites, implying that these taxa are still prevailing in the Lake environment. This suggests that variation in physicochemical conditions did not impact on these taxa and their resilience is further attributed to the reduced intensity of the grazing of haplochromines (Sekiranda et al. 2004, Mwebaza-Ndawula 1994, Witte et al. 1992). The prevailing mollusc densities have been attributed to infestation with water hyacinth (Branstrator et al. 1996). Given that these occurred in the varied water quality environments in the present study, their resistance to environmental degradation is likely to play an ecological role. This is supported by Efitre et al. (2001), who attributed the lack of mulluscs in Lake Nabugabo to the low conductivity of less than 40µS/cm as the limiting factor. It is thus hypothesised that the present study sites that registered conductivity values above 100µS/cm, provide conditions that are ideal for the calcification of mullusc shells and exoskeleton, thus favouring the abundance of these communities in the environment.

Some results differed from earlier studies thereby pointing towards ecological transformation and environmental degradation of the lake which could have occurred over the years (Muli and Mavuti 2001, Mbahinzireki 1994, Mwebaza-Ndawula 1994, Witte et al. 1992, Okedi 1990, Mothersill et al. 1980). For example, Mothersill et al. (1980) observed that gastropods *Melania* and *Bellamya* sp., the insects chironomid spp. and *Chaobotus* sp., the pelecypod *Corbiculina* sp. and oligochaetes were the dominant benthic macroinvertebrate taxa in northwestern Lake Victoria. Of these, only *Chironomus* sp, *Corbicular*, *Bellamya* and Oligochaeta were encountered in the present study and their occurrence varied in different study sites. Okedi (1990) reported that *Pisidium* sp was one of the important small species in Murchison Bay, but this taxon was not observed in any of the present study sites. A similar trend was reported by Sekiranda et al. (2004). Nevertheless Sekiranda et al. (2004) observed a predominance of *Lymnaea sp*. and *Biophalaria* at Murchison Bay's Nakivubo channel inlet point while results from the present study point to Oligochaeta as the most predominant taxon in Murchison Bay at the inshore (not far from the Nakivubo channel inlet) and the offshore locations. Although, *Biophalaria* was observed at the two locations in Murchison Bay in the present study, it was among the rare occurrence taxa (it occurred in less than 4 out of the 12 sampling times) and *Lymnaea* sp. were absent. Furthermore, Okedi (1990) found *Caelatura* sp and *Mutera* sp as important large bivalves in Murchison Bay, and Sekiranda et al. (2004) observed the two taxa but at insignificant numerical abundances, while Ngupula and Kayanda (2010) found *Caelatura* sp but not *Mutera* sp. In the present study however, *Caelatura* sp and *Mutera* sp were not observed in any of the study sites. These differences could be attributed to the ecological transformation of the lake, possibly due to the increasing degradation of the aquatic environment.

The sampling locations in the least and medium polluted clusters, as was classified in chapter three and in this chapter, had the highest numbers of invertebrate taxa as was reflected in Winday Bay inshore and Lwanika offshore with 20 taxa each followed by Winday Bay offshore and Masese offshore with 19 taxa each and Masese and Lwanika inshore locations with 18 taxa each. The relatively poor-taxa invertebrate assemblage in Murchison Bay and Kirinya comprising *Chironomus* sp, *Corbicula* and Oligochaeta could be attributed to the high tolerance of these taxa to anoxic and eutrophic conditions (Revenga and Kura 2003). Pollution-sensitive groups of Emphemeroptera and Trichoptera were absent from Murchison Bay, Kirinya and Masese, but two taxa of each of these groups were recorded in Lwanika and Winday Bay. The absence of these groups in the highly polluted clusters may be explained by the fact that the groups are known to express less tolerance to degradation in water quality, such as low dissolved oxygen levels, a condition that was found in the highly polluted cluster (Revenga and Kura 2003). However, the higher number of individuals/m^2 in Murchison Bay suggests a high proliferation of the low oxygen and pollution tolerant taxa such as Oligochaetes, molluscs, especially *Corbicular* and diptera such as *Chironomus* sp, *Chaoborus*, and *Procladius*. These observations taken together imply that water quality degradation influenced the taxa abundances in the study sites. In Uganda, this is the first time that data show to what extent environmental changes influence invertebrate biodiversity.

The higher abundance of macroinvertebrates in the offshore than inshore sites was probably attributable to depth. The average inshore depths ranged from 1 to 1.4 m while that of the offshore depths ranged from 2.2 to 3.9 m because the present study was restricted to the wetland zones of the lake shallow bays which should not exceed 6 m by definition of the coastal wetland (Kasimbazi 2007, RAMSAR 1971). This may seem to contradict results from other studies that reported a higher abundance of macroinvertebrates in the inshore sites (1 – 10 m) as compared to the offshore sites (above 10 m), but the present study's inshore and offshore locations (below 5 m) both fall within the inshore locations of these other studies, making the results comparable (FDEP 2007, Pamplin et al. 2006, Solimini et al. 2006, Efitre et al. 2001, Muli and Mavuti 2001). Depth seemed to play an important role in taxa richness and this was confirmed by the CCA plot (Figure 4.4) where most of the taxa were highly associated with higher total depth of above 1.5 m and below 5 m as was associated with the offshore locations in this case. The higher taxa richness and densities in the inshore sites of less than 10, including this study, is supported by the high diversity of macrophytes in the shoreline habitats, that may include different vegetation types, dead wood, etc. (Efitre et al. 2001).

The lack of seasonal and temporal variation observed in this study has also been reported in previous studies conducted in the Lake Victoria basin that suggested lack of seasonal and temporal variation (Efitre et al. 2001, Muli and Mavuti 2001). The irregular and un-harmonised rainfall patterns that were observed in the different study sites during the study period could have obscured any seasonal trends, thus influencing the degree of environmental differences between the dry and wet seasons. Efitre et al. (2001) observed high rainfall patterns during the dry and wet season making seasonal differences in benthic macroinvertebrate communities of Lake Nabugabo, Uganda less distinct. These authors argued that seasonal changes in most environmental characters were modest and did not incur any strong seasonal trends in macroinvertebrate numbers.

4.5.2. Spatial diversity and site grouping based on invertebrate taxa characteristics

The CA plot and the PCA ordination, confirmed the previous section's findings that the highly polluted sampling locations of Murchison Bay and Kirinya were associated with stenotopic and eutrophic populations of pollution-tolerant groups that included three Chironomidae taxa, such as *Chironomus sp, Clinotanypus* and *Tanypus*; molluscs such as *Corbicula*, *Melanoides*, *Sphaerium*, *Bellamya* and *Bulinus*, and Oligochaeta. This is similar to findings by Sekiranda et al. (2004), who observed similar groups in the Murchison Bay offshore locations. In contrast to Sekiranda et al. (2004), this study observed Oligochaeta as the most predominant taxa in the inshore of Murchison Bay as compared to *Lymnaea sp*. and *Biophalaria* reported by Sekiranda et al. (2004). The difference could be attributed to the sampling locations, whereby Sekiranda et al. (2004) sampled right at the Naikivubo cannel mouth, while the present study's inshore locations were slightly away from the exact opening. The results are also similar to those from pollution studies carried out in other aquatic systems. For instance Parsons et al. (2010) registered an increase in Chironomidae and Oligochaeta in the Athabasca Oil Sands Region in Alberta, when the lake was subjected to sulphur and nitrogen emissions. Similar trends were observed in rivers (Arimoro 2009, Aguiar et al. 2002, Coimbra et al. 1996) and streams (Pamplin et al. 2006, Griffith et al. 2001) subjected to effluent pollution. This demonstrates to what degree pollution of the aquatic environment with urban effluent can induce proliferation of the pollution-resistant Chironomidae and mollusc taxa, and disappearance of sensitive taxa in the Empemeroptera and Trichoptera groups.

Another important finding was that the least impacted cluster that comprised of the reference site, was highly associated with the two Ephemeroptera taxa *Caenis* and *Povilla*, the two Trichoptera taxa, *Leptocella* and *Phylocentropus*, and two diptera taxa, *Procladius* and *Chaoborus*. This corroborates the findings by other studies that reported presence of pollution sensitive taxa in the less impcated reference sites (Arimoro 2009, Sekiranda et al. 2004, Thorpe and Lloyd 1999, Coimbra et al. 1996). In addition, Shannon diversity and Margalef species richness indices were highest at the references site of Lwanika and Winday Bay. In other words the two sites that were not directly subjected to urban effluent discharges registered the highest species richness and diversity while the highly impacted effluent recipient Murchison Bay and Kirinya recorded the lowest indices. This suggests that urban wastewater discharges that include industrial, domestic and storm-water effluent negatively influenced the physicochemical variables consequently impacting on the macroinvertebrate diversity in the affected environments. Some studies also reported such findings for polluted environmnts (Arimoro 2009, Sekiranda et al. 2004, Coimbra et al. 1996)

Given that the more diverse the community is the better the water quality conditions, Winday Bay and Lwanika, the two sites that do not receive urban effluent, registered the highest diversity indices at both the inshore and offshore locations. However, the inshore values were significantly lower than those of the offshore sampling locations in all the study sites because of the difference in depth between the two locations. This cautions that habitat factors should be taken into consideration otherwise the indices alone may be misleading and may likely fail to classify the sites according to their ecological and water quality integrity. The significance difference in the indices between sites, even sites that were in the same clusters makes the macroinvertebrates indices not to be straight forward predictors of water quality and they should be used cautiously for this purpose. This supports the concept that during sampling care should be taken to ensure that the habitat factors driving natural variability are well understood. For example, comparison should be between sites of similar characteristics, in terms of depth level and distance from the shore line.

Finally, the three clusters observed in this chapter are relatively comparable with the three clusters observed in the previous chapter. This not only confirms the previous findings on water quality deterioration in the effluent-impacted sites but also contributes data that assist in our understanding of the connection between the invertebrate community structure and the water quality status in the studied sites. These findings suggest that benthic macroinvertebrates are useful indicators of environmental water quality in urban wetlands.

4.5.3. Relationship between macroinvertebrate taxa and environmental variables

The distribution and abundances of the invertebrate taxa was strongly influenced by oxygen levels, temperature, total depth, and nutrient levels especially total phosphorus concentration, with more invertebrate taxa associated with sites with total depth of above 1.5 m in the offshore locations (1.6 to 5 m), high dissolved oxygen and temperature, and low conductivity as observed in the CCA plot (Figure 4.4.). These results are similar to other studies that observed the distribution and abundance of the macroinvertebrates to be highly influenced by oxygen, conductivity and nutrients especially phosphorus and nitrate (Ngupula and Kayanda, 2010, Arimoro 2009, Pamplin et al. 2006, Solimini et al. 2006, Sekiranda et al. 2004, Efitre et al. 2001, Coimbra et al. 1996) and depth (FDEP 2007, Pamplin et al. 2006, Solimini et al. 2006, Efitre et al. 2001, Muli and Mavuti 2001). The few studies that suggested depth as an influencing factor were conducted on lake-associated systems (FDEP 2007, Pamplin et al. 2006, Solimini et al. 2006, Efitre et al. 2001, Muli and Mavuti 2001). Given that depth played a role for the distribution of the benthic macroinvertebrates in the present study, these findings confirm the remarks by Solimini et al. (2006), i.e., that benthic invertebrate diversity showed considerable spatial variation with lake depth, across habitats, and across lakes. Thus benthic macroinvertebrates could be used for a holistic indication system for lake ecosystem health because they are not only sensitive to pollution, but also to other human and natural impacts, such as habitat change, hydrology, and climate change.

Another important observation was that oligochates were highly associated with sites with high levels of total phosphorus, further supporting the fact that this taxon is resistant to eutrophication and low oxygen levels. This is consistent with many studies that reported proliferations of Oligochates in highly polluted sites (Parsons et al. 2010, Arimoro 2009, Pamplin et al. 2006, Sekiranda et al. 2004, Aguiar et al. 2002, Thorpe and Lloyd 1999, Coimbra et al. 1996).

4.6. Conclusion and recommendations

This chapter has given an account of the potential of using benthic macroinvertebrates as biological indicators of water quality in the studied wetlands in Uganda. Unlike the previous studies, this study is among the few studies in the region that allow quantification of changes in benthic macroinvertebrate diversity in relation to environmental changes. The findings suggest that benthic macroinvertebrates are useful indicators of environmental water quality in urban wetlands. The results suggest that the composition, distribution and abundance of the invertebrate taxa were influenced by the site's depth and the water quality physicochemical variables. This study therefore recommends that in addition to the physicochemical variables, further investigations on the habitat and biological effects should be considered, and a wider spatial coverage also taken into account to strengthen the already available results including the findings from this study. The predominance of the oligochaete and some dipteran taxa associated with eutrophication and low dissolved oxygen levels in the highly polluted sites on Murchison Bay and Kirinya; and the absence of some of the taxa observed by earlier studies in the same sites such as, *Caelatura* sp, *Mutera* sp., *Lymnaea* sp and *Biophalaria* leads to the hypothesis that effluent-induced environmental changes may have led to ecological transformation and environmental degradation of the lake, thereby rendering the environment unfavourable to some macroinvertebrates.

Another significant finding was that, of the endpoints used to describe the invertebrate community structure and variation across study sites, CA, PCA and CCA gave a better indication of aquatic environmental status, than the community indices (Shannon diversity and Margalef richness). CA, PCA and CCA indicated that the invertebrate community structure and function varied across sites thereby separating the sites into three clusters according to their levels of environmental degradation, into highly polluted, medium polluted and least polluted. The clusters were consistent with the three clusters observed in the previous chapter when the sites were classified according to the water quality physicochemical variables. These responses provide an indication that benthic macroinvertebrate are good candidates for assessing ecosystem integrity and should be considered in the development of biomonitoring networks and programmes to assess water quality in urban wetlands. The study also affirms that urban effluent impacts on the shoreline aquatic biodiversity and recommends that mitigation measures be instituted for treatment and or biodegradation of the urban effluent before discharging into the lake. In addition the encroachment and degradation of the coastal wetland and lake catchment should be sustainably regulated.

CHAPTER 5

Fish as biological indicators of water quality in selected wetlands, Uganda

5.1. Introduction

Fish have been widely documented as useful indicators of environmental water quality because of their differential sensitivity to pollution (Mora et al. 2008, Das and Chakrabarty 2007, Sekiranda 2006, Mora and Robertson 2005, Gratwicke and Speight 2005, Gratwicke 2004, Mora et al. 2003, Barrella and Petrere 2003, Smith et al. 1999, Hughes et al. 1998, Ham et al. 1997, Taylor et al. 1993). Estimating the number of species occurring in a particular area is central to biodiversity studies and remains the fundamental theme of ecology. Measures of diversity are frequently seen as indicators of the wellbeing of ecological systems and are important in understanding the mechanisms and effects of certain ecological phenomena such as environmental disturbances like pollution (Mora et al. 2008, McGill et al. 2007, Tokeshi and Arakaki 2007). Estimation of the species diversity of a habitat is also useful for detecting trends, impacts, or recovery of ecosystems, and quantification of extinction risks and thus prioritisation of conservation efforts in biodiversity hot-spot areas (Mora et al. 2008). On a global scale fishes form the most important wetland product providing the primary source of protein and essential fatty acids for nearly one billion people and food security for many more (FAO 2009). The Lake Victoria wetlands, swamps, satellite lakes and shallow bays have been reported to be critical habitats for the recovering fish biodiversity following the decline and extinction of many fish species in the lake (Balirwa et al. 2003, Chapman et al. 2002).

Lake Victoria is famous for its abundance of fish and plays an important role in providing food for the millions of people that inhabit the basin. The Lake Victoria basin is today one of the most densely populated areas in the world and the increasing demand for natural resources by the human population has had a tremendous impact on the ecosystem biodiversity. For example the decline of fish stocks and fish species diversity in Lake Victoria has been reported by several authors (Ogutu-Ohwayo and Balirwa 2004, Balirwa et al. 2003, Ogutu-Ohwayo et al. 1997). The authors also add that the decline seems to be largely due to overexploitation, use of destructive fishing gears and methods, and environmental degradation, all exacerbated by the inadequate to total absence of effective management regimes. The decline has also been attributed to the introduction of the non indigenous fishes, especially the Nile perch during the 1950s. This is largely because a drastic increase in the stocks of Nile perch coincided with a decline in many populations of indigenous species causing disturbances in the ecosystem (Witte et al. 2007, Balirwa et al. 2004b, Ogutu-Ohwayo and Balirwa 2004, Balirwa et al. 2003, Kaufman et al. 1997). Initially there were increasing catches of Nile Perch, but the species is over fished and the population size has dropped significantly. The over fishing has caused resurgence of several endemic species that were on the brink of extinction, occurring in Lake Victoria wetlands, swamps, satellite lakes and shallow bays (Balirwa et al. 2003, Chapman et al. 2002, Witte et al. 2000).

However there is an increase in the environmental degradation of the catchments of these habitats, especially in the urban areas (GoU 2010, Nyenje et al. 2010, Banadda et al.

2009, Munabi et al. 2009, Kayima et al. 2008, Oguttu et al. 2008, Cózar et al. 2007, Muwanga and Barifaijo 2006, GoU 2006a, Matagi 2002). This poses a critical question as to whether the resultant environmental conditions may allow for ecological diversity to thrive in urban wetlands and shallow bays receiving industrial and municipal effluents. In order to address this complex socio-ecological challenge of declining fish stocks and species diversity, and ecosystem degradation, long-term recommendations for management were made and they include putting in place effective research and management programmes, strong policy and legal frameworks and information systems for management of the fisheries and fish habitats (Ogutu-Ohwayo and Balirwa 2004, Balirwa et al. 2003, Machiwa 2003). Furthermore, immediate interventions included control of fishing effort and law enforcement with regard to the use of illegal fishing gear and methods (Ogutu-Ohwayo and Balirwa 2004), alongside re-stocking programmes and aquaculture which is also still on a small scale (FAO 2006) with little attention given to the assessment of the environmental impacts on water quality and biodiversity, and environmental impact mitigation. Sound conservation planning and management of the environmental impact on water quality and biodiversity requires not only a comprehensive inventory of the species diversity but also strategic assessment of the catchment’s anthropogenic impacts on the aquatic fauna (Darwall et al. 2005). The activities carried out in the catchment have a great impact on the biodiversity in the aquatic systems within the respective catchment domain. It is therefore important to monitor changes in the different aspects of the fishery so that policies can be adjusted to match the changes.

Fishes are ideal animals for such ecological monitoring because they are readily identified using field guides, they are species-rich and some species are very abundant. Additionally, warm tropical waters with high visibility are conducive to direct observations, unlike many other places that are often too cold and / or have very low visibility (Gratwicke 2004). A diverse assemblage of cichlid and non-cichlid fishes inhabit the Lake Victoria basin (Darwall et al. 2005, Lundberg et al. 2000a, Lévêque 1997, Rosenberger and Chapman 1999, Kaufman et al. 1997, Kaufman 1992, Greenwood 1984, 1980). The authors also note that the species of endemic haplochromine cichlids in the basin of East Africa is well known as an example of the most diverse and extensive radiation of vertebrates. This renders Lake Victoria and its catchment ideal for fish community studies as indicators of water quality deterioration. The use of water quality indicator organisms is critical to conservation of not only the cichlid fauna but also for setting a regulatory mechanism in place to avoid further loss of biodiversity. This chapter tests the hypothesis that fish are useful biological indicators of water quality deterioration in wetlands in Uganda. To test this hypothesis, the fish community structure in relation to the water quality physicochemical variables was investigated in five wetlands variably impacted by urban effluent.

5.2. Literature review

5.2.1. Spatial diversity and occurrence of fish species in relation to water quality

The relationship between water quality and fish assemblages has been poorly documented in Lake Victoria, despite being important for water management and ecological monitoring. Lake Victoria has diverse ecological zones and the impacts of environmental degradation on water quality are complex but the extent of this impact is not fully understood. Studies conducted on Lake Victoria and its satellite lakes emphasised spatial and temporal fish biodiversity profiles in relation to Nile perch population growth (Witte et al. 2007, Darwall et al. 2005, Namulemo 2004, Okaronon 2004, Wandera 2004, Balirwa et al. 2003, Chapman et al. 2003, 2002, 1995, Lundberg et al. 2000b, Rosenberger and Chapman 2000, 1999, Lévêque 1997, Seehausen et al. 1997b, Chapman and Chapman 1996, Lowe-McConnel 1993, Ogutu-Ohwayo 1990); with no reference to water quality. Studies carried out elsewhere that addressed the relationship of water quality and fish assemblages suggest that spatial variation in fish community structure is related to the environmental pollution with the most polluted sites registering lower diversity and abundances than the less polluted sites (Mondal et al. 2010, Shahnawaz et al. 2010, Das and Chakrabarty 2007, Sutin et al. 2007, Carol et al. 2006, Sekiranda 2006, Ye et al. 2006, Barrella and Petrere 2003, Bhata 2003, Smith et al. 1999).

For example, fish diversity with relation to water quality of Bhadra River of Western Ghats in India was examined and results showed low species richness and diversity at highly impacted sites (Shahnawaz et al. 2010). Still in Western Ghats, four rivers, Sharavati, Aghanashini, Bedti and Kali, of the central Western Ghats were studied for their fish diversity and composition, with Sharavati and Kali rivers subjected to habitat destruction and alteration due to industrial development projects that included hydroelectric power plants and small industries (Bhata 2003). These developments were causing severe damage to the natural habitat and water quality of the aquatic fauna and some of the species which are commonly distributed in Aghanashini and Bedti were not found in Sharavati and Kali. In other river systems in India, Das and Chakrabarty (2007) studied fish and water quality community structures of two rivers with varying degrees of pollution in West Bengal and observed that fish species appeared to have been eliminated from the polluted Churni River as opposed to the less polluted Jalangi river. In a related study on the Tietê and Paranapanema Rivers in Brazil, a decline in species richness was detected in the polluted stretches of Tietê as compared to the less polluted Paranapanema (Barrella and Petrere 2003). The Tietê River crosses the metropolitan region of São Paulo and receives a large amount of pollution, in contrast to the Paranapanema River which runs in a less populated area.

Water quality and freshwater fish diversity data from nine waterfalls at Khao Luang National Park, Thailand showed that the surrounding undisturbed area of Khao Luang National Parks was important in maintaining fish stocks (Sutin et al. 2007). The fishes of the Khao Luang National Parks in the upper rivers were dominated by the family Cyprinidae, with many of its species having evolved a variety of mechanisms to adapt to living in swift-flowing mountain rivers. In a related study, fish assemblages were significantly different between the control and outfall locations following the discharge of sewage effluent from a deepwater outfall. The differences were attributed to the decline in relative abundance of several commonly occuring, resident species of reef fish at the contaminated site (Smith et al. 1999). The studies showed lower species diversity and richness in polluted rivers, than in the less polluted rivers.

Similar to studies conducted in river systems, a higher diversity and abundance of fish was reported in less contaminated sites in lakes and reservoir systems (Mondal et al.

2010, Carol et al. 2006, Sekiranda 2006, Ye et al. 2006). For example, a three-year study conducted in two floodplain lakes to evaluate changes between water quality and finfish diversity indices, reported a significant impact of environmental change on diversity indices (Mondal et al. 2010). The authors recommended that fish diversity and water quality should be taken into consideration when designing policies to increase the long-term sustainability of fishing activities in the lakes. Sekiranda (2006) employed GIS techniques to assess water quality and fish community structure in three shallow bays along Lake Victoria and reported the highest values of mean species abundances, diversity, evenness and equitability in the less impacted Hannington bay, followed by the medium impacted Fielding bay and lowest in the highly impacted Murchison bay. Given that water quality negatively impacts on fish species diversity and abundances, the present study quantified changes in fish diversity in relation to environmental changes.

5.2.2. Role of wetlands and shallow bays in fish biodiversity conservation in Lake Victoria

Research on the Lake Victoria wetlands and coastal shallow bays assented to the role of wetlands in the conservation of fish biodiversity (Balirwa et al. 2003, Chapman et al. 2003, Chapman et al. 2002, Rosenberger and Chapman 1999, Chapman and Chapman 1996). These researchers pointed to wetlands, swamps, satellite lakes and shallow bays as critical habitats for the recovery and resurgence of some of the cichlid species that were thought to be extinct. Particularly rocky and shallow inshore habitats with fringing macrophyte cover have been reported to be important refugia for the endangered native fish species of Lake Victoria (Balirwa et al. 2004a, Namulemo 2004, Balirwa et al. 2003, Chapman et al. 2002, Rosenberger and Chapman 1999, Seehausen et al. 1997b). The authors argue that these heavily vegetated wetlands serve as structural and low-oxygen refugia for fishes that can tolerate such conditions. They also function as barriers to the dispersal of Nile perch, thereby protecting the cichlids from Nile perch predation.

For example, fish distribution in three wetland habitats was compared with previous surveys which had reported the disappearance of 16 indigenous species from the open waters of Lake Nabugabo, a satellite lake of Lake Victoria (Chapman et al. 1995). The authors observed that 9 of the 16 species (which were not recovered in the open waters in the earlier surveys) were found in the wetland ecotones or beyond the margins of the lake in wetland lagoons and tributaries. In two related studies conducted in a wetland tributary of Lake Nabugabo, the composition and stability of fish assemblages was quantified (Rosenberger and Chapman 1999, Chapman and Chapman 1996). The authors reported a rare incidence of Nile perch and the presence of nine species that had not been recorded in the open waters. This led to the conclusion that the low oxygen concentrations in the wetland tributary may permit persistence of only hypoxia-tolerant species. These wetland conditions seemed to limit the presence of Nile perch, thus providing a critical refuge for a subset of the basin fauna. Subsequent studies have revealed that with the overfishing of Nile perch some of the species that were initially limited to refugia, have been observed in the open waters (Chapman et al. 2003).

On the other hand, a survey on composition, biomass, distribution and population structure of the fish stocks in the sub-litoral zone (6 m to 20 m deep) on the Ugandan part of Lake Victoria from January 1999 to December 2000, revealed an overall decline of fish species diversity and abundances in the lake (Okaronon 2004). The author also reported that *Lates niloticus* and haplochromines occurred in all areas sampled while *Oreochromis niloticus* and other tilapiine species were restricted to shallower waters of less than 20 m depth. In addition a number of studies have reported the dominance of the introduced *O. niloticus* in the shallow waters of Lake Victoria (Njiru et al. 2008b, 2006, Balirwa et al. 2004b). These authors attribute the high reproductive productivity of *O. niloticus* in Lake Victoria to coastal zone factors and abundant food sources. *O. niloticus* is also reported to survive in a wide range of physical and chemical conditions including environments with low oxygen concentrations (Njiru et al. 2008b, 2006, Chapman et al. 1995, Lowe-McConnell 1993). Inshore waters and closed bays are also nurseries for *Rastrineobola argentea* and many other fish species (Wandera 2004).

These studies highlight the importance of wetlands and shallow bays for fish biodiversity conservation in Lake Victoria. The continued survival of the fish species in such habitats is threatened by human impacts in the catchments. The impacts include drainage of fringing swamps for agriculture, habitat loss and deforestation, overexploitation, use of destructive fishing gears, collection of ornamental fish for the aquarium trade and ecosystem degradation though pollution (Darwall et al. 2005, Namulemo 2004, Revenga and Kura 2003). Although environmental degradation has been reported as a problem in Lake Victoria (Balirwa et al. 2003), coupled with the fact that degradation through industrial and domestic effluent is among the major human impacts on the habitats located in urban centres, not much research has focused on this aspect. Environmental changes as a result of industrial and domestic wastewater effluent may reduce fish access to the refugia grounds in the urban waters. Therefore this study assessed urban wetland fish biodiversity that is threatened by pollution from industrial and domestic effluent.

5.2.3. Relationship between fish communities and water quality physicochemical variables

The distribution and abundance of fish species in water bodies such as freshwater lakes, rivers and reservoirs is influenced by physicochemical conditions (Mondal et al. 2010, Carol et al. 2006, Ye et al. 2006, Mrosso et al. 2004). However, no study was found that explored this relationship on the Ugandan part of Lake Victoria. The majority of studies were conducted on aquatic systems outside the African continent. In a closely related study conducted on the Tanzanian part of Lake Victoria, the relationship between water transparency and species richness of surviving haplochromines in selected habitats in Mwanza gulf - Lake Victoria, indicated that species richness was significantly correlated with water transparency / secchi readings (Mrosso et al. 2004). The low species richness even in rocky habitats was attributed to the decreasing water transparency.

In two floodplain lakes of India, water quality and fish biodiversity indices as measures of ecological degradation were studied and there was a significant impact of depth, conductivity and salinity of water on diversity indices (Mondal et al. 2010). The authors recommended consideration of the relationship between water quality parameters and fish biodiversity when designing policies to increase the long-term sustainability of fishing activities in the lakes. The spatial and seasonal variations in community structure of small fishes in relation to key environmental factors were investigated in Niushan Lake, a shallow macrophytic lake along the middle reach of the Yangtze River in China (Ye et al. 2006). Canonical correspondence analysis showed that the spatial distribution of most small fishes was associated with complex macrophytic cover condition. However, unlike in the case of Mondal et al. (2010), water depth had no significant effect on species diversity and distribution of the small fishes in Niushan Lake. This implies that for different lake systems, there are different specific factors that influence fish biodiversity and the factors should be defined for the respective systems in order to implement proper management procedures.

In the river systems, fish assemblages in the upper Red River system of Southwestern Oklahoma (USA) were predictable as a function of environmental gradients. Conductivity was found to be the most important variable predicting structure of fish assemblages, followed by stream size, alkalinity, woody debris, and water clarity (Taylor et al. 1993). The effects of limnological variables on fish assemblages of 14 Spanish reservoirs were investigated and multivariate analysis using ordination methods and generalised additive models showed that altitude and trophic state (indicated by chlorophyll or nutrient concentrations) independently explained most of the variation of fish assemblages in these reservoirs (Carol et al. 2006). The most eutrophic reservoirs were dominated by common carp (*Cyprinus carpio*) whereas oligotrophic reservoirs presented other fish species intolerant to pollution and mainly native, such as brown trout, *Salmo trutta*. The absolute and relative abundance of common carp was strongly related to the trophic state of the reservoir and 40% of its variation was explained by total phosphorus concentration (Carol et al. 2006).

In Lake Victoria, eutrophication manifests as a result of increased concentration of total P and N accompanied by severe silica depletion and proliferation of cyanobacteria (Kling et al. 2001, Hecky 1993). Phosphate will stimulate the growth of plankton and aquatic plants which provide food for larger organisms, including zooplankton, fish, humans, and mammals. Initially, this increased productivity will cause an increase in the fish population and overall biological diversity of the system. However, as the phosphate loading continues and as there is a build-up of phosphate in the lake or surface water ecosystem, the aging process of the`lake or surface water ecosystem will be accelerated leading to an imbalance in the nutrient and material cycling process (Mugidde 2001). For Lake Victoria, eutrophication initially resulted in increased phytoplankton biomass and primary productivity which supported fish yields that rose 4 to 5-fold since the 1950s (Ogutu-Ohwayo et al. 1997) but has since had several negative consequences that include elevated algal biomasses and proliferation of the water hyacinth that thrives in the shallow eutrophic bays receiving nutrient-rich influents from rural and urban watersheds (Mugidde 2001). Establishing the relationship of eutrophication and other water quality variables to fish community structures is necessary. The objective of this chapter therefore was to investigate the fish community structure in relation to the physicochemical variables to explore fish as biological indicators of water quality deterioration in selected Ugandan wetlands. As observed in chapter four, multivariate techniques such as Cluster Analysis (CA), Principal Component Analysis (PCA), and

Canonical Correspondence Analysis (CCA), and richness, diversity and dominance indices were used to explore changes in invertebrate community structure in relation to deterioration in environmental water quality in the studied wetlands.

5.3. Specific Methods

5.3.1. Field sampling

Fish were obtained by the use of multiple-size monofilament gill nets set overnight at two locations, i.e. at the shoreline (inshore site) and 60 m off the shoreline (offshore site). All fish from each net were identified to species level and counted. At each sampling location physicochemical variables were measured at different depths. Where the total depth was more than 0.5m but less than one metre, measurements were taken at surface and at 0.5 m. The variables were: pH, conductivity, temperature, dissolved oxygen concentration, secchi disk readings, total phosphorus (TP), total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-), ammonium-nitrogen (NH_4^+), and chlorophyll a (Chl-a). Detail of physicochemical methods and measurement are described in chapter 3.

5.3.2. Statistical analysis

The means, standard deviations and ranges for each variable in the study sites were determined. ANOVA was used to compare differences between mean water quality values for the five sites on Log 10 (x+1) transformed data. Posthoc comparisons were used to identify significant differences between means of different sites ($p \leq 0.05$) using Tukey HSD test. Water quality variables were subjected to principal component analysis

(PCA) so as to group the sites according to their water quality using the Community Analysis Package 1.52 (Henderson and Seaby 2000a). The spatial variation of fish species community structure was assessed along their species accumulation and rank abundance curves, Margalef species richness, Shannon diversity and Simpson dominance indices. The Species Diversity and Richness (SDR Version 2.65) programme was used to calculate and compare the fish community indices in the different study sites (Seaby and Henderson 2000). Comparison of indices for significant differences between sites was done using the randomisation test of Solow (1993).

The species accumulation curves were plotted to establish the accumulation of fish species over time in the sampled area (Ugland et al. 2003). Species accumulation curves chart the increase in recovery of additional species as a function of sampling effort (Dove and Cribb 2006). Species abundance was graphically presented using Rank Abundances on logarithmic data. The rank abundance plot summarises both equitability in abundance between species and species richness. The study used a log-log plot, because species varied in abundance over a number of orders of magnitude (Seaby and Henderson 2006). In this plot the number of individuals of each species per site is arranged in descending order and the proportion of the total number of individuals in each species is plotted against the species rank on a logarithmic scale.

Species richness was estimated using the Margalef Index. Species richness represents a diversity measure of prime importance in community ecology which is often relied upon as basic information for implementing habitat protection and conservation measures

(Mora et al. 2008, Tokeshi and Arakaki 2007). The authors argue that the Margalef Index has a good discriminating ability to measure the number of species present for a given number of individuals. Species diversity was estimated using the Shannon diversity index. The Shannon diversity is a very widely used index for comparing diversity between habitats (Clarke and Warwick 2001). The Simpson's species dominance index describes the probability that a second individual drawn from a population should be of the same species as the first (Seaby and Henderson 2006). The Simpson's species dominance index is reported as one of the most meaningful and most robust diversity measures available as it captures the variance of the species abundance distribution (Magurran 2004).

Canonical correspondence analysis (CCA) was performed to evaluate the relationship between fish communities and environmental variables using the Environmental Community Analysis Package 2 (ECOM) by Henderson and Seaby (2000b). CCA is a useful tool for simplifying complex data sets, and, being a direct gradient analysis, it allows integrated analysis of both taxa and environmental data (Arimoro 2009, ter Braak and Smilauer 2002). It detects variations in a community composition, then undertakes an auxiliary analysis to identify and plot the environmental variables that correlate best to the ordination axes and displays these as a graphical plot of species, sampling sites and environmental vectors (ter Braak 1986). A Monte Carlo test with 1000 randomisations was run to test for the probability of the observed pattern being due to chance. It is also recommended that it is more sensible to characterise the main body of the community than to give too much weight to species that are almost never encountered, in order to minimise on bias and narrow the error of estimation (Arimoro 2009, ter Braak and Smilauer 2002, ter Braak 1986). Sampling was carried out 12 times, by the 5th sampling (10 months) the maximum number of species in the less impacted sites had been achieved and by the 8th sampling (16 months) that of the effluent impacted sites had been achieved. Sampling efforts thereafter did not add more species. Species that occurred less than four times by the end of the 12th sampling (18 months) were thus considered irregular.

5.4. Results

5.4.1. Composition and relative abundance of fish species in the study sites

A total of 29 fish species belonging to five families were recorded, with 83% belonging to the Cichlidae. The other four families were Alestiidae, Centropomidae, Protopteridae and Mochokidae (Table 5.2). There was no significant seasonal variation in fish assemblages during the study. Before excluding the rare occurrences, Winday bay registered the highest number of species (22) followed by Lwanika and Masese with 20 each (Table 5.1 and 5.2). However, on exclusion of the rare occurrences, Lwanika (the reference site) registered the highest number of species (12) followed by Winday Bay with 11 species (Table 5.1 and 5.3). Also to note is that the inshore sampling location registered the highest number of species before excluding the rare occurrences, but the numbers seemed to even out on exclusion of the rare occurrences (Table 5.1 and 5.3).

All together 13 common occurrence species were recorded and their abundance distributions calculated using Simpson’s species dominance index were ranked and summarised for the 10 sampling locations in table 5.3. Five species occurred in all the sampling locations and these included *Oreochromis niloticus, Lates niloticus, Tilapia zillii*, *Brycinus sadleri* and *Paralabidochromis* "*redtail*" (Table 5.3). Three of these species which included *O. niloticus, L. niloticus*, and *T. zillii* were introduced into Lake Victoria while *B. sadleri* and *P.* "*redtail*" are endemic. The highly adaptive and resilient *O. niloticus* was the most dominant species in 7 of the 10 sampling locations with the exception of Lwanika offshore and Kirinya offshore where *P.* "*redtail*" dominated and Winday Bay offshore dominated by *L. niloticus*. The effluent impacted Murchison Bay supported fewer fish species and all these species were recorded in the other study sites. A number of native *Haplochromine species* observed in the less impacted sites were not observed in Murchison Bay and these included Astatotilapia species such as *A. nibula* and *A. latifasciata*; *P. chilotes*; *H. victorianus*; *A. alluaudi*; *X. phytophagus*; and *Y. fusiformis*. Other species included *B. jacksonii*. Also to note are the rare occurrences for examples, *O. variabilis* and *P. rockkribensis* that was observed only in Winday Bay. *Ptyochromis sp* was only found in Kirinya, *L. melanopterus* in Masese and *P. "Long Shaft"* in the reference site (Lwanika). Except for *O. variabilis*, most of these species were one time occurrences and were recorded in small numbers (Table 5.2).

Table 5.1 Number of fish species observed in the five wetlands before and after excluding the rare occurrence species (n = 12).

Site	Including rare occurrences						Excluding rare occurrences		
	Number of species			Number of fish			Number of species		
	Total	Inshore	Offshore	Total	Inshore	Offshore	Total	Inshore	Offshore
Kirinya	19	18	15	1028	683	345	10	10	10
Masese	20	16	12	996	737	259	9	9	8
Winday Bay	22	18	17	2115	1467	648	11	11	11
Murchison Bay	15	13	11	1076	487	589	7	7	7
Lwanika	20	18	12	1611	805	806	12	12	10

Table 5.2 Relative abundance (in percentage) of fish species in the study wetlands. Sampling locations L In and L Off = Lwanika Inshore and Offshore (Reference sites), M In and M Off = Masese Inshore and Offshore, W In and W Off = Winday Bay Inshore and Offshore, K In and K Off = Kirinya Inshore and Offshore, MB In and MB Off = Murchison Bay Inshore and Offshore (n = 12).

Family	**Genus**	**Species**	**K-In**	**K-Off**	**M-In**	**M-Off**	**W-In**	**W-Off**	**MB-In**	**MB-Off**	**L-In**	**L-Off**
Cichlidae	Oreochromis	*O. leucostictus*	8.20	2.03	12.62	0.00	3.27	0.00	35.73	30.39	0.85	0.00
		O. niloticus	13.32	42.32	6.38	25.48	9.20	0.27	30.18	30.05	17.22	26.30
		O. variabilis	0.00	0.00	0.00	0.39	25.02	0.09	0.00	0.00	0.00	0.00
	Paralabidochromis	*P. "red tail"*	23.43	9.28	14.93	5.79	33.81	0.16	6.37	13.92	28.08	16.25
		P. crassilabris	5.56	1.45	6.38	1.93	2.79	0.06	0.41	0.34	7.57	15.01
		P. "big eye"	0.00	0.00	0.00	0.39	2.18	0.04	2.67	2.55	0.00	0.00
		P. chilotes	0.15	0.29	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
		P. rockkribensis	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00
		P. "Long Shaft"	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.61	0.50
	Harpagochromis	*H. victorianus*	4.54	0.00	0.00	0.00	0.68	0.01	0.00	0.00	3.05	6.70
		H. serranus	0.29	0.58	0.00	3.47	0.00	0.00	0.21	0.00	0.37	0.00
		H. quiarti	0.59	0.00	1.49	0.00	0.00	0.00	0.00	0.34	1.22	0.00
	Astatotilapia	*A. nubile*	0.15	0.58	0.14	0.00	0.07	0.00	0.00	0.00	0.37	0.00
		A. latifasciata	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
		A. brownie	4.69	0.87	0.27	0.00	2.59	0.05	5.34	3.90	8.55	4.09
	Psammochromis	*P. riponianus*	8.64	8.70	13.84	0.77	4.23	0.06	0.00	0.68	5.86	9.93
	Tilapia	*T. zillii*	11.57	4.06	4.07	29.34	5.93	0.02	1.23	5.43	11.48	1.24
	Xystichromis	*X. phytophagus*	0.73	0.00	0.95	0.00	0.00	0.01	0.00	0.00	1.47	2.73
	Yssichromis	*Y. fusiformis*	0.00	0.00	1.63	0.00	0.00	0.00	0.00	0.00	0.24	0.00
	Astatoreochromis	*A. alluaudi*	5.27	1.45	26.19	0.77	3.00	0.00	0.00	0.00	5.13	0.00
	Lipochromis	*L. melanopterus*	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Ptyochromis	*Ptyochromis sp*	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5.2 Continues

Relative abundance (in percentage) of fish species in the study wetlands. Sampling locations L In and L Off = Lwanika Inshore and Offshore (Reference sites), M In and M Off = Masese Inshore and Offshore, W In and W Off = Winday Bay Inshore and Offshore, K In and K Off = Kirinya Inshore and Offshore, MB In and MB Off = Murchison Bay Inshore and Offshore (n = 12).

Family	**Genus**	**Species**	**K-In**	**K-Off**	**M-In**	**M-Off**	**W-In**	**W-Off**	**MB-In**	**MB-Off**	**L-In**	**L-Off**
Clariidae	Clarias	*C. gariepinus*	0.00	1.16	0.00	0.00	0.14	0.00	0.41	0.00	0.00	0.00
Centropomidae	Lates	*L. niloticus*	11.42	26.67	5.29	28.57	4.98	0.19	7.19	6.28	4.40	8.44
Alestiidae	Brycinus	*B. sadleri*	1.02	0.29	5.02	2.32	1.91	0.01	9.65	6.11	2.44	8.31
		B. jacksonii	0.00	0.00	0.00	0.77	0.07	0.00	0.00	0.00	0.24	0.00
Mochokidae	Synodontis	*S. afrofisheri*	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.00	0.85	0.00
Protopteridae	Protopterus	*P. aethiopicus*	0.29	0.29	0.54	0.00	0.00	0.00	0.21	0.00	0.00	0.50

Table 5.3 Ranks of species based on variance of the species abundance distributions calculated using Simpson's species dominance index for the common occurrence species (n = 12).

Rank	Lwanika Inshore (Ref)	Lwanika Offshore (Ref)	Winday Bay Inshore	Winday Bay Offshore	Murchison Bay Inshore	Murchison Bay Offshore	Masese Inshore	Masese Offshore	Kirinya Inshore	Kirinya Offshore
1	*O. niloticus* (6.409)	*P. "redtail"* (6.705)	*O. niloticus* (8.344)	*L. niloticus* (7.031)	*O. niloticus* (5.230)	*O. niloticus* (6.817)	*O. niloticus* (10.295)	*O. niloticus* (8.412)	*O. niloticus* (9.202)	*P. "redtail"* (12.098)
2	*A. brownie* (5.182)	*L. niloticus* (5.979)	*T. zillii* (7.604)	*O. niloticus* (6.542)	*P. "redtail"* (2.704)	*T. zillii* (6.359)	*T. zillii* (9.063)	*L. niloticus* (7.127)	*L. niloticus* (7.461)	*O. niloticus* (9.756)
3	*O. leuco.* (4.500)	*O. niloticus* (5.818)	*P. "redtail"* (6.219)	*P. "redtail"* (6.339)	*O. leuco.* (2.458)	*O. leuco.* (4.562)	*L. niloticus* (7.639)	*T. zillii* (6.013)	*O. leuco.* (6.871)	*P. crass.* (5.000)
4	*P. riponian.* (3.930)	*B. sadleri* (4.013)	*L. niloticus* (5.814)	*P. riponian.* (4.309)	*T. zillii* (1.977)	*P. "redtail"* (3.992)	*O. leuco.* (6.404)	*P. crass.* (5.000)	*A. alluaudi* (3.580)	*L. niloticus* (4.521)
5	*P. crass.* (3.836)	*T. zillii* (3.750)	*P. crass.* (4.100)	*O. variabilis* (3.072)	*B. sadleri* (1.969)	*B. sadleri* (3.366)	*A. alluaudi* (5.397)	*B. sadleri* (5.000)	*B. sadleri* (3.500)	*A. alluaudi* (3.333)
6	*L. niloticus* (3.539)	*X. phyto.* (3.726)	*A. alluaudi* (4.026)	*H. Victorian.* (2.500)	*A. brownie* (1.890)	*L. niloticus* (3.073)	*P. "redtail"* (5.319)	*P. "redtail"* (4.200)	*T. zillii* (3.443)	*T. zillii* (2.677)
7	*B. sadleri* (3.333)	*P. crass.* (3.625)	*P. riponian.* (3.178)	*A. brownie* (2.200)	*L. niloticus* (1.500)	*A. brownie* (2.091)	*P. crass.* (5.051)	*A. alluaudi* (2.000)	*P. riponian.* (2.819)	*P. riponian.* (2.377)
8	*H. Victorian.* (3.261)	*P. riponian.* (2.708)	*O. variabilis* (3.128)	*T. zillii* (1.964)			*P. riponian.* (2.403)	*P. riponian.* (1.000)	*A. brownie* (2.351)	*O. leuco.* (2.333)
9	*T. zillii* (3.067)	*H. victorian* (2.524)	*B. sadleri* (2.291)	*P. crass.* (1.794)			*B. sadleri* (1.521)		*P. "redtail"* (2.202)	*A. brownie* (1.000)
10	*P. "redtail"* (2.264)	*A. brownie* (1.970)	*H. Victorian.* (1.607)	*A. alluaudi* (1.000)					*P. crass.* (1.980)	*B. sadleri* (1.000)
11	*X. phyto.* (2.200)		*A. brownie* (1.400)	*B. sadleri* (1.000)						
12	*A. alluaudi* (2.065)									

5.4.3. Spatial diversity and site grouping based on fish species characteristics

Shannon diversity and Margalef species richness indices varied significantly among sampling locations ($p < 0.05$). The two indices were highest at the reference site and lowest at the highly impacted Murchison Bay for the inshore locations (Figure 5.1). For the offshore locations, the Shannon diversity was highest at the reference sites and lowest at Masese; while the Margalef richness index was highest at Winday Bay and lowest at Murchison Bay (Figure 5.1). With the exception of the reference site, the offshore sampling locations registered significantly higher Margalef indices than the inshore locations (Figure 5.1). On the other hand, there was no significant difference in Shannon diversity between the inshore and offshore locations at the reference site (2.0854 and 2.0511) and Winday Bay (1.937 and 1.927). For Kirinya and Masese, the Shannon diversity was significantly higher at the inshore sites with 2.0854 and 2.0117 than the offshore with 1.5559 and 1.4958 respectively. On the contrary, the offshore location of Murchison Bay registered a significantly higher Shannon diversity than the inshore.

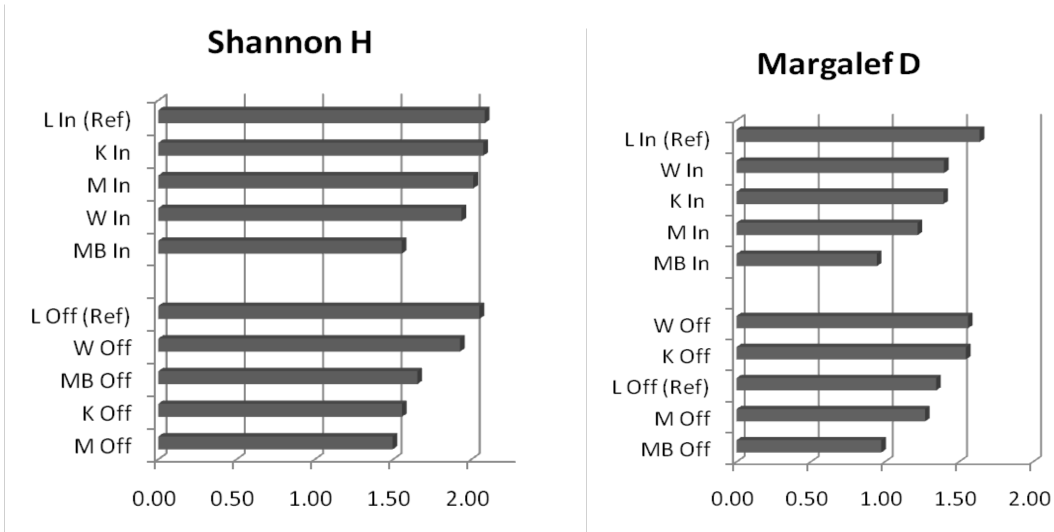


Figure 5.1 Shannon diversity H and Margalef richness D indices for fish species in the study sampling locations. Where: L In = Lwanika Inshore (reference site), W In = Winday Bay Inshore, M In = Masese Inshore, MB In = Murchison Bay Inshore and KI Kirinya Inshore. L Off = Lwanika Offshore (reference site), W Off = Winday Bay Offshore, M Off = Masese Offshore, MB Off = Murchison Bay Offshore, K Off = Kirinya Offshore.

Hierarchical cluster analysis grouped the 10 sampling location into three clusters based on the similarity of fish species characteristics. The reference site (Lwanika) and Winday Bay were grouped in the less polluted cluster 1 (Figure 5.2). This cluster was mainly associated with haplochromine species which included *P* “*red tail*”, *P. crassilabris*, *H. victorianus* and *P. riponianus* (Figure 5.3). The medium polluted cluster 2 included the inshore and offshore sampling locations of Masese and Kirinya (Figure 5.2). This cluster was mainly associated with *T. zilli* and *A. alluaudi* (Figure 5.3). The Murchison bay inshore and offshore locations were grouped in the highly polluted cluster 3 mainly associated with *B. sadleri*, *O. niloticus* and *O. leucostictus* (Figures 5.2 and 5.3). The three clusters as displayed in the dendrogram (Figure 5.2) were relatively similar to the clusters obtained based on the water quality physicochemical variables (chapter three Figure 3.2), and the invertebrate taxa characteristics (chapter four Figure 4.1). In all the three variables (water quality, invertebrate taxa and fish species characteristics), the reference site was grouped under the least polluted cluster, Masese was grouped under the medium polluted cluster while Murchison Bay was grouped under the highly polluted cluster. Winday Bay on the other hand varied between the least polluted (by fish species characteristics) and the medium polluted (based on water quality and invertebrate taxa characteristics) while Kirinya fluctuated between the medium (by fish species characteristics) and highly polluted (by water quality).

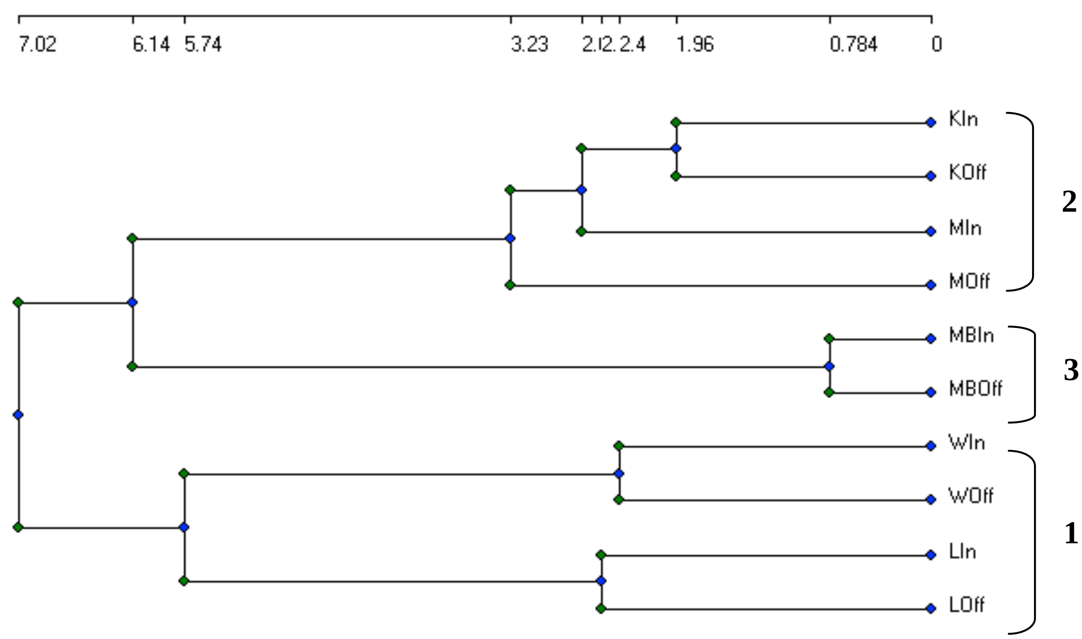


Figure 5.2 Hierarchical Cluster Analysis dendrogram showing three clusters of site composition, each cluster indicates sites with similar fish species composition. Homogeneity within clusters was based on Euclidean distance and the heterogeneity between clusters was based on Ward's method. Sampling locations L In and L Off = Lwanika Inshore and Offshore, W In and W Off = Winday Bay Inshore and Offshore, MB In and MB Off = Murchison Bay Inshore and Offshore, M In and M Off = Masese Inshore and Offshore, and K In and K Off = Kirinya Inshore and Offshore.

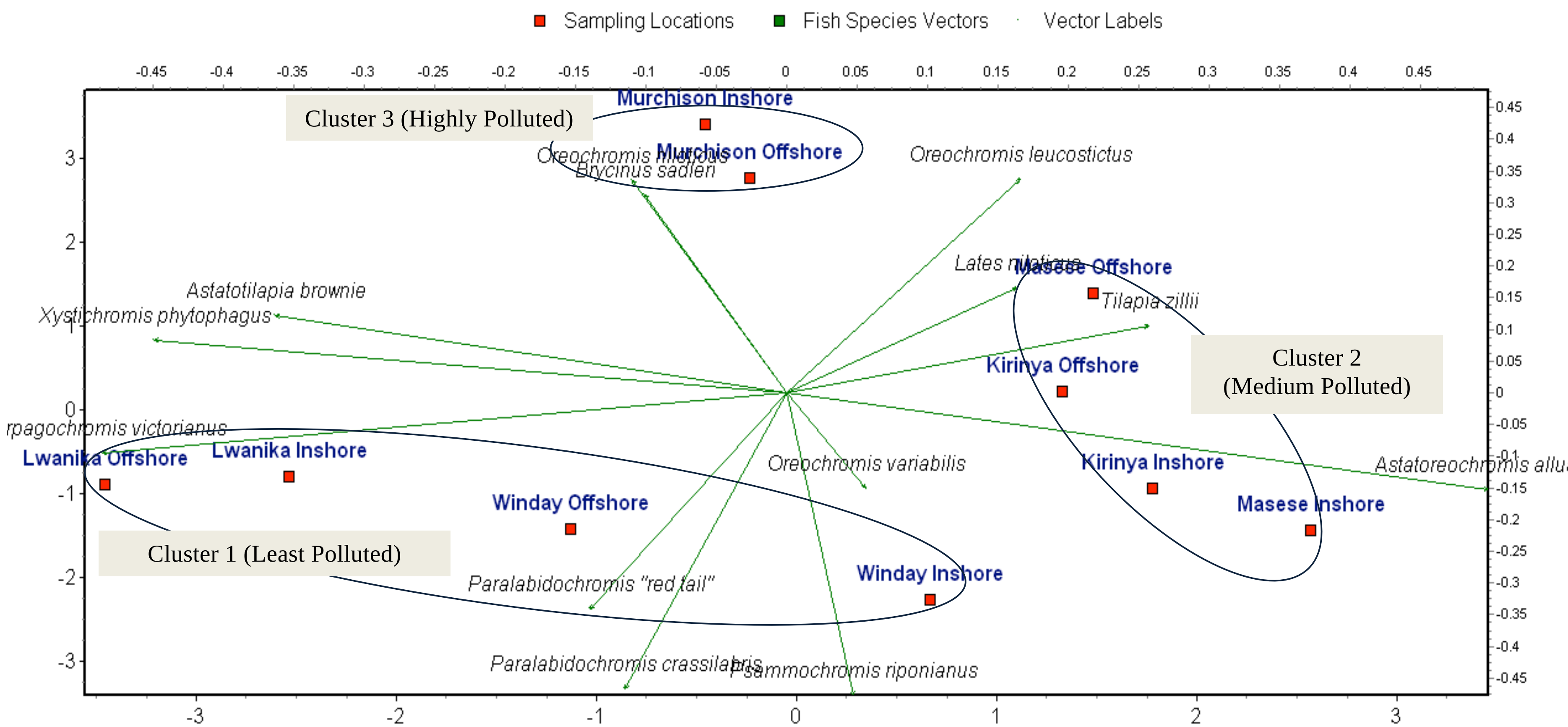


Figure 5.3 PCA ordination biplot of the ten sampling locations based on the fish species variables for plot component one (X axis) and plot component two (Y axis). Note the 3 groups (clusters) of the higly polluted Murchison bay, the medium polluted Kirinya and Masese and the least polluted reference site (Lwanika) and Winday bay.

5.4.4. Relationship between fish communities and environmental variables

The Canonical Correspondence Analysis (CCA) ordination showed a good relationship between fish species distribution and environmental variables as reflected by the high species-environmental correlation coefficient associated with each axis (Table 5.4). The strongest explanatory factors were total conductivity, total phosphoros, total nitrogen, secchi depth and oxygen. Murchison Bay inshore and offshore locations (MBI and MBO) and some of the Kirinya inshore (KI) were grouped closely and were associated with high nutrient levels and high electrical conductivity. *O. leucostictus* occurred under these conditions (Figure 5.4). On the other hand, the reference site (Lwanika, denoted by LI and LO) and Winday Bay (WI and WO) were associated with high secchi depth, temperature and oxygen; and most of the fish species occurred under these conditions (Figure 5.4). Unlike in the previous chapter on the invertebrate taxa, total depth (with a shorter arrow) did not highly influence the fish species distribution. The first two axes accounted for 34.6% of the total species variance (Table 5.4) explained in the observed patterns in the CCA plot (Figure 5.4).

Table 5.4 Summary of CCA axis length showing axis eigenvalues, correlation between species and the environmental gradients, and variance of species, following canonical correspondence analysis of fish species abundance data in the study sites

	CCA Axis 1	CCA Axis 2	CCA Axis 3
Canonical eigenvalue	0.601	0.427	0.189
Cumulative percentage variance	20.2	34.6	41
Species environmental correlation	0.954	0.933	0.764
Number of species (response variable)	13		
Number of environmental variables	12		
Total variance in species data	2.97		

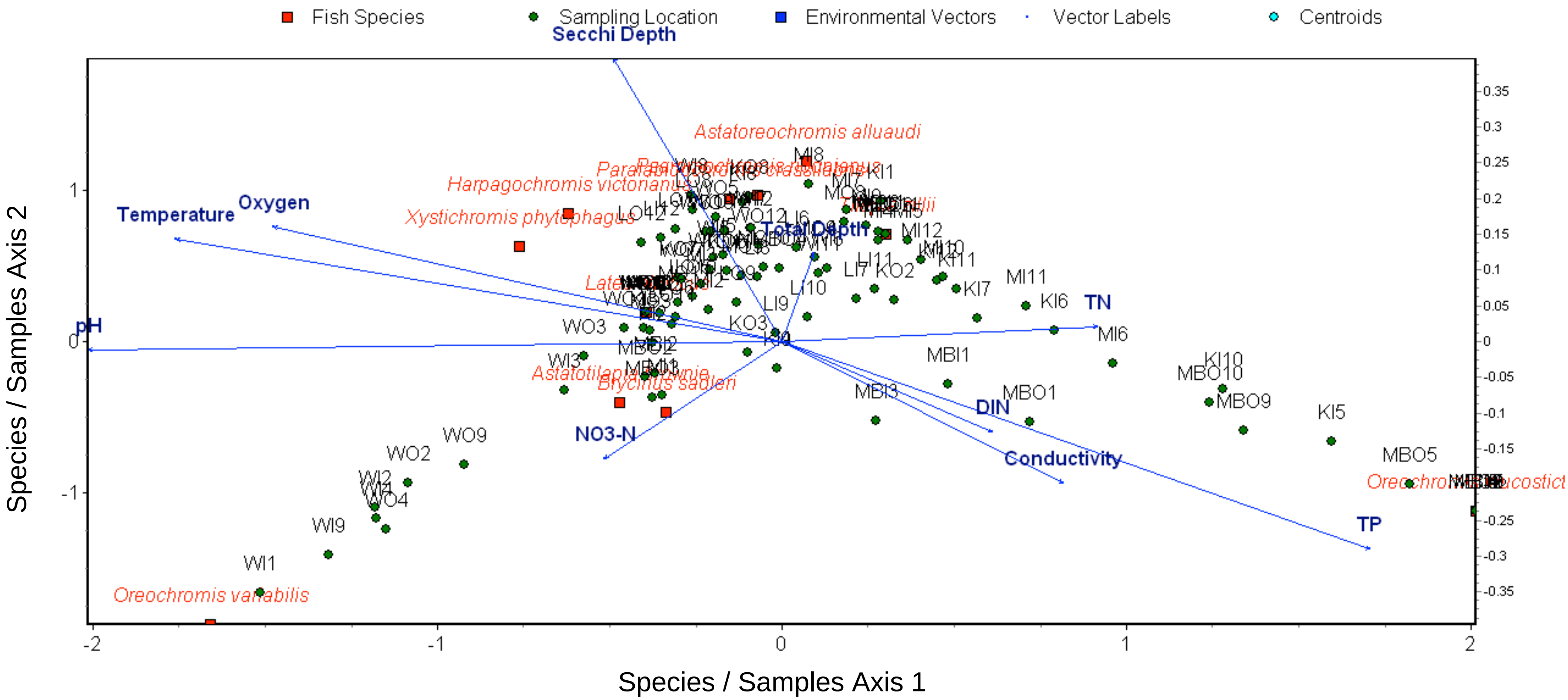


Figure 5.4 CCA plot of first and second CCA axes of fish species, environmental variables and their corresponding sampling stations. Eigenvalues: axis 1, 0.60, axis 2, 0.43. First 2 axes account for 34.6% of the variance. Monte Carlo test was significant ($p < 0.001$), 1000 permutations. Where sampling sites L = Lwanika, MB = Murchison Bay, W = Winday Bay, M= Masese, K = Kirinya. Numbers represent different sampling efforts.

5.5. Discussion

5.5.1. Composition and relative abundance of fish species in the study sites.

Eighty three percent (83%) of the fish species observed during the study were Cichlids. Cichlids are the most abundant and dominant group in Lake Victoria and other lacustrine environments in the East African Great Lakes (Witte et al. 2007, Darwall et al. 2005, Balirwa et al. 2003, Lundberg et al. 2000b, Rosenberger and Chapman 2000, Lévêque 1997, Seehausen et al. 1997b, Kaufman et al. 1997, Lowe-McConnel 1993, Kaufman 1992, Greenwood 1984, 1980). The other four families were Alestiidae, Centropomidae, Protopteridae and Mochokidae which is similar to reports that some non-cichlid species inhabit the lake (Namulemo 2004, Seehausen et al. 1997b). It is important to note that the inshore sampling location registered the highest number of species before excluding the rare occurrences as compared to the offshore sampling locations. Although these species were mainly rare occurrences, their occurrence strengthens the explanations of the role of the wetlands, swamps, satellite lakes and shallow bays in the life cycle of many fishes, as well as the sites serving as critical habitats for the recovering biodiversity in Lake Victoria (Balirwa et al. 2003, Chapman et al. 2003, Chapman et al. 2002, Witte et al. 2000, Rosenberger and Chapman 1999, Chapman and Chapman 1996). In marine coastal areas, this is linked to the preferential settling of pelagic fish larvae during tidal water exchanges between the bays and reefs (Gratwicke and Speight 2005). The lack of significant seasonal / temporal variation confirms why for a long time, seasonal changes in fish assemblages has not been considered in the assessment of effects of anthropogenic perturbations (Bozzetti and Schulz 2004, Karr 1981).

Overall, five major species were collected in all study sites and these included *Oreochromis niloticus*, *Lates niloticus*, *Paralabidochromis* "*redail*", *Brycinus sadleri* and *Tilapia zilli*. Three of these species (*O. niloticus, L. niloticus,* and *T. zilli*) were introduced, while *B. sadleri* and *P.* "*redtail*" are native, with *O. niloticus* being the most prevalent in all study sites. These findings concur with earlier studies that reported the dominance of *O. niloticus* in Lake Victoria waters (Njiru et al. 2008b, 2006, Okaronon 2004, Balirwa et al. 2004b).

The dominance and wide distribution of *O. niloticus* compared to other tilapiines, is mainly attributed to this species' ability to feed on a variety of food items, high fecundity, ability grow to a large size and capacity to survive in a wide range of physical and chemical conditions in the lake (Njiru et al. 2008b, 2006, Okaronon 2004, Balirwa et al. 2004b). The authors further show that ecology and feeding biology of *O. niloticus* has changed, probably in response to changes occurring in the ecosystem. This explains why *O. niloticus* was not only the dominant species in this study but was also observed in every site during each sampling time. These findings agree with those of Sekiranda (2006) who observed *O. niloticus*, *L. niloticus* and *B. sadleri* among the major species observed in three shallow bays of Lake Victoria.

The other predominant species among the common occurrences were *L. niloticus*, *T. zilli* and *P.* "*redtail*". *L. niloticus* was the most prevalent in Winday Bay offshore and the second most prevalent in Lwanika, Masese and Kirinya offshore. The presence of *L. niloticus* among the highly prevalent species confirms that wetlands and shallow bays play a role in the life cycle of Nile perch. *L. niloticus* though a non-wetland species, the juveniles are only found in shallow waters, usually close to the shore. On the other hand the low frequency of *L. niloticus* in Murchison bay could be attributed to the low oxygen levels in this bay. It is hypothesised that the high oxygen requirements of *L. niloticus* limit its distribution. This explanation is supported by studies that recorded low abundance of *L. niloticus* in hypoxic environments (Balirwa et al. 2004a, Namulemo 2004, Balirwa et al. 2003, Chapman et al. 2002, Rosenberger and Chapman 1999, Seehausen et al. 1997b). Physiological studies suggest that Nile perch are relatively intolerant of low oxygen conditions (Schofield and Chapman 1999, Fish 1995). Well oxygenated shallow shorelines are therefore beneficial for the survival of juvenile Nile perch, implying that degradation of the shoreline does not only impact on the wetland species composition and survival but also on species whose life cycle includes wetlands, such as the Nile perch. Fish (1995) found that Nile perch require water with high dissolved oxygen of above 5 mg/l because their blood has low dissolved oxygen affinity. In addition, both the metabolic rate and the critical oxygen tension of Nile perch are high, which may limit their use of low-oxygen habitats.

Haplochromine cichlids and non-cichlid species in the Lake Victoria basin have been reported to have a relatively high tolerance to hypoxia (Rosenberger and Chapman 2000, 1999, Chapman and Liem 1995). At an average of 3.8 and 5.0 mg/l O_2, some fish species could be found in the hypoxic Murchison Bay and Kirinya wetlands respectively. *Harpagochromis Victorianus* was observed on several sampling times in Lwanika and Windy Bay while *Oreochromis variabilis* occurred only in Winday Bay and was among the most abundant at this site. *O. variabilis* population size is reported to be declining or to have disappeared in many areas of Lake Victoria (Namulemo 2004, Ogutu-Ohwayo 1990). This species was classified as critically endangered by IUCN. Its occurrence in only Winday Bay provides evidence that Winday Bay could be an apparent hot-spot for this species.

The well oxygenated rocky habit of Winday bay also makes it a favourable environment for the rock dwelling cichlids, and makes this site critical for fish biodiversity conservation. Rocky crevices could have served as shelters for the endemic haplochromine species from Nile perch predation, making the species live in the same environment. These observations are in agreement with Seehausen et al. (1997b) and Rosenberger and Chapman (2000) who reported the highest diversity of haplochromines in littoral rocky habitats of southern Lake Victoria and Lake Victoria's Nabugabo satellite lake, respectively. This implies that littoral rocky habitats are critical areas in haplochromine conservations strategies, in particular Lake Victoria where predation from Nile perch is a threat to many cichlid species. In Uganda, Winday Bay could serve an important role in the conservation of indigenous cichlid species. The bay should therefore be treated as one of the sites relevant to conservation planning for the valuable Lake Victoria's indigenous fish fauna.

The observations in Winday bay affirm further the importance of some wetlands and shallow bays in the conservation of fish biodiversity and the resurgence of many species that were considered extinct. This is in agreement with research on the Lake Victoria wetlands and coastal shallow bays that has been carried out in the past three decades with many researchers assenting to the role of wetlands and the associated rocky refuges in the conservation of fish biodiversity (Balirwa et al. 2003, Chapman et al. 2003, 2002, 1995, Rosenberger and Chapman 1999, Chapman and Chapman 1996). In addition, these researchers have reported that there is a resurgence of some cichlid species. They point to wetlands, swamps, satellite lakes and shallow bays as the critical habitats for the recovering biodiversity (Balirwa et al. 2003, Chapman et al. 2003, 2002, 1995, Rosenberger and Chapman, 1999). Heavily vegetated wetlands act as refugia and may protect cryptic species from Nile perch predation

(Balirwa et al. 2004a, Namulemo 2004, Balirwa et al. 2003, Chapman et al. 2002, Rosenberger and Chapman 1999, Seehausen et al. 1997b).

The findings of this study caution that these vegetated wetlands' role as refugia can only lead to sustainable resurgence of biodiversity if accompanied by regulation of human activities that lead to eutrophication and deoxygenation of the lake. As a result there is a need to identify and conserve wetlands that serve as species hot-spots through implementation of environmentally friendly activities that embrace cleaner use of resources in the catchments.

5.5.2. Spatial diversity and site grouping based on fish species characteristics

Shannon diversity and Margalef species richness indices were highest at the references site (Lwanika) and Winday Bay and lowest at the effluent impacted sites of Murchison Bay and Masese. Thus the reference site and Winday Bay that did not receive urban effluent discharges registered the highest diversity and richness; while the highly impacted effluent recipient Murchison bay recorded the lowest indices. This suggests that urban wastewater discharges that include industrial, domestic and storm-water effluent may have negatively influenced the physico-chemical variables consequently impacting on the fish species diversity. Similar trends were observed from other water bodies (Mondal et al. 2010, Shahnawaz et al. 2010, Das and Chakrabarty 2007, Sutin et al. 2007, Carol et al. 2006, Sekiranda 2006, Ye et al. 2006, Barrella and Petrere 2003, Bhata 2003, Smith et al. 1999). Although mainly studies were conducted on rivers (Das and Chakrabarty 2007, Sutin et al. 2007, Barrella and Petrere 2003, Bhata 2003), there are few recent studies of lakes and reservoirs (Mondal et al. 2010, Carol et al. 2006, Sekiranda 2006, Ye et al. 2006). For example, fish diversity with relation to water quality of Bhadra River of Western Ghats in

India was examined and results showed low species richness, diversity and dominance at the highly impacted sites (Shahnawaz et al. 2010).

Another significant observation was that, either there was no significant difference in Shannon diversity between the inshore and offshore sites (Lwanika and Winday Bay), or the indices were significantly higher at the inshore than the offshore (Kirinya and Masese), except for Murchison Bay where the offshore diversity was higher. The higher diversity at the inshore could be attributed to one time occurrences that occurred intermittently in low numbers. The low Shannon diversity at the inshore location for Murchison Bay could be as a result of the poor water quality at the bay's shoreline making the environment less habitable by many fish species. For species richness on the other hand, the Margalef indices were higher at the offshore locations except for the reference site. This implies that although there was a higher diversity at the inshore, these sites had low species richness; while at the offshore locations, the diversity was low but the species richness was high.

Sampling locations were grouped into three clusters based on the similarity of fish species characteristics. The sites with no effluent discharge, i.e. the reference site and Winday Bay were grouped in the less polluted cluster while the highly impacted Murchison Bay inshore and offshore locations were grouped in the highly polluted cluster. The highly polluted cluster was mainly associated with the highly adaptive and resilient *O. niloticus*, *B. sadleri*, and *O. leucostictus*; while the least polluted cluster was mainly associated with haplochromine species which included *P "red tail*", *P. crassilabris*, *H. victorianus* and *P. riponianus*.

5.5.3. Relationship between fish communities and environmental variables

The distribution and abundance of fish species was influenced by water transparency, dissolved oxygen concentration, electrical conductivity and nutrient levels in the water especially total phosphorus concentration, with more fish species associated with sites with high dissolved oxygen concentration, high secchi depth readings and low electrical conductivity. Similarly, distribution and abundance of the fish species was influenced by water quality (Mondal et al. 2010, Carol et al. 2006, Ye et al. 2006, Mrosso et al. 2004, Taylor et al. 1993). The closest study conducted on the Tanzanian part of Lake Victoria revealed that fish species richness was significantly correlated with water transparency / secchi readings (Mrosso et al. 2004). The low species richness even in rocky habitats was attributed to decreasing water transparency. The present study and the findings by Mrosso et al. 2004, agree with those of Seehausen et al. (1997a) who indicated that water transparency / clarity is one of the strongest environmental predictors of fish species biodiversity especially in the haplochromines.

It is been argued that the loss of water clarity causes loss of genetic and ecological differentiation among haplochromine species, and the decrease in water transparency associated with eutrophication has probably contributed to the loss of species diversity among cichlids (Mrosso et al. 2004, Seehausen et al. 1997a, Taylor et al. 1993). The authors associated the loss of transparency with eutrophication. This association was also observed in this study, as sites with high eutrophication were correlated with low secchi depth readings and low oxygen levels. Water transparency is reported to be highly correlated with dissolved oxygen concentration, as transparency enables light penetration which is required in the photosynthesis process. In this study 11 out of the 13 common occurrences were strongly associated with high secchi depth readings and high oxygen concentrations and were predominantly found in the reference site and Winday Bay with significantly higher secchi readings and oxygen levels than the Murchison Bay and Kirinya.

Electrical conductivity was also reported to be an important variable that could predict the structure of fish assemblages in addition to water transparency and other factors such as alkalinity and macrophyte conditions (Mondal et al. 2010, Ye et al. 2006, Taylor et al. 1993). Mondal et al. (2010) also reported that water depth influenced fish diversity. However in the present study, water depth had no significant impact on fish species distribution. In a related study, water depth had no significant effect on species diversity and distribution of the small fishes in Niushan Lake (Ye et al. 2006). The present study and the findings in other studies suggest that water transparency and electrical conductivity are good environmental predictors of species biodiversity in this habitat (Mondal et al. 2010, Carol et al. 2006, Ye et al. 2006, Mrosso et al. 2004, Taylor et al. 1993). The present study also showed that sites with high nutrient levels especially total phosphorus concentration, registered low fish diversity and richness indicating that eutrophication reduced fish biodiversity. These findings agree with those of Carol et al. (2006) who observed that trophic state indicated by chlorophyll or nutrient concentrations independently explained most of the variation of fish assemblages in 14 Spanish reservoirs.

Sites with poor water quality had low species richness and abundance. As follows only one fish species *O. leucostictus* seemed to be highly associated with and tolerant to eutrophication and as a result thrived in the hyper-eutrophic Murchison Bay. Another closely associated species but to a less extent was *O. niloticus*. This implies that eutrophication and poor water quality had a negative impact on species diversity; and is in agreement with reports that eutrophication negatively impacts on fish biodiversity (Nyenje et al. 2010, Hecky et al. 2009, Witte et al. 2007, Mugidde and Hecky 2004). These studies and the present study agree with the fact that there is a relationship between urban effluent discharge and eutrophication; and this compromises the ecological integrity of surface waters, causing depletion of oxygen and negatively impacting on fish populations and species diversity.

5.6. Conclusions and recommendations

In conclusion the distribution and abundances of the fish species was strongly influenced by water quality. The evidence presented in this chapter suggests that gradual anthropogenic changes due to the ongoing urbanisation in the catchment such as increased discharges of sewage, industrial and municipal wastewater in the environment has resulted into eutrophication which exerted its influence on water quality, nutrient levels, oxygen levels and electrical conductivity consequently affecting fish diversity. Low diversities in the fish community structure were registered in the highly impacted sites as compared to the less impacted sites. Thus fish diversity may be a useful biological indicator of water quality and this could be used in biomonitoring network and programmes to assess water quality and in mapping out fish species hot-spot areas. Winday Bay should be treated as one of the sites relevant to conservation planning for Lake Victoria’s indigenous fishes. This study also shows that the state of the aquatic environment as influenced by many societal activities in the catchments affects fish and therefore the fisheries. The study further recommends that in order to promote the wise use and conservation of fish stocks, biodiversity and water quality; conservation efforts should embrace changes in societal activities in the catchments that improve environmental quality. For example promotion of treatment and biodegradation of the effluents and sewage from point source polluters, such as industries, factories and hotels before discharging into the lake.

CHAPTER 6

Fish histopathology as a biomarker of aquatic environmental quality in wetlands receiving urban runoff in Uganda

6.1. Introduction

The increasing emphasis on the assessment and monitoring of aquatic ecosystems has highlighted the need to develop appropriate biological indices in environmental quality assessments. As elaborated in chapter one, biomarkers offer the advantage of application in the field where complex spatial and temporal changes in pollutants exposure or biological effects may be measured (Handy and Depledge 1999, den Besten 1998, McCarthy and Shugart 1990). Particularly, fish diseases and pathologies with a broad range of aetiologies are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of prior exposure (van Dyk et al. 2009b, Zimmerli et al. 2007, Bernet et al. 2004). Histopathology in animal tissues has long been applied as a diagnostic tool in human and animal medicine. In the past two decades however, histopathologic characteristics have become potent indicators of prior exposure to environmental stressors in fisheries science (van Dyk et al. 2009a, 2009b, Zimmerli et al. 2007, Au 2004, Van der Oost et al. 2003, Myers and Fournie 2002, Wester et al. 2002, Hinton 1994, Hinton et al. 1992, Hinton and Laurén 1990a). The identification of elevated levels of fish disease and pathology, whether caused by infectious agents, environmental factors or xenobiotics, are considered to be highly relevant indicators of ecosystem health (Lyons et al. 2006, 2004, Handy et al. 2002, Stentiford et al. 2003).

Although there are physiological and biochemical procedures of determining pathological change (Schlenk and Di Giulio 2002, Barton et al. 2002); histopathological manifestations have received increasing interest as biomarkers because they integrate the cumulative effects of alterations in physiological and biochemical systems in an organism (Van der Oost et al. 2003, Myers and Fournie 2002, Hinton and Laurén 1990a). This offers a unique advantage to histopathology because the observed histopathological manifestations can be linked to effects seen at other levels of biological organization. Histopathology also allows effects to be visible at lower or sub-lethal exposure levels than many other integrative endpoints, such as mortality, behaviour, growth, reproductive, and changes in biodiversity (Handy et al. 2002, Wester et al. 2002, Bernet et al. 1999). Histopathological characteristics stem from alterations at lower levels of biological organization such as molecular level and sub-cellular (organelles) levels. It also occurs earlier than reproductive, growth, behavioural and diversity changes which are detectable at organism, community or ecosystem levels, rendering histopathology more sensitive than these endpoints (Van der Oost et al. 2003, Wester et al. 2002, Hinton and Laurén 1990a). This location in the hierarchy of biological organization makes histopathologic manifestations useful as early warning signals of environmental quality deterioration (Handy et al. 2002, Bernet et al. 1999)

In many aquatic systems however, exposure of organisms to toxicants or other stressors is sublethal to the organism, causing damage that may be detected in cells and tissues. Such changes can be identified and quantified in aquatic organisms inhabiting the environment being investigated (Myers and Fournie 2002, Bernet et al. 1999). As an indicator of exposure to contaminants, histopathology thus represents a useful tool to assess the impact of pollution, particularly for sub-lethal and chronic effects as is often the case with most contaminants in natural environments (Leusch et al. 2006, Handy et al. 2002, Myers and Fournie 2002, Bernet et al. 1999). Histopathologic biomarkers also incorporate pathogenic and biotic factors in addition to water quality thus enabling a more holistic view of the environmental deterioration and its effects on organisms such as fish (Au 2004, Handy et al. 2002, Schmidt-Posthaus et al. 2001, Hinton et al. 1992). This is of high ecological relevance as it eliminates the alternative of using animals in laboratory studies, and the need for extrapolating results to different species in the natural environment (Myers and Fournie 2002). Consequently, histopathological techniques have been recommended for field assessments of aquatic environmental quality as some of the easiest and fastest methods of assessing both short- and long-term toxicant effects of exposure to a multitude of pollutants (Van der Oost et al. 2003, Handy et al. 2002, Myers and Fournie 2002, Bernet et al. 1999, Hinton et al. 1992, Moore and Simpson 1992, Wester and Canton 1991, Hinton and Laurén 1990a).

Histopathological techniques allow for identification and localisation of injury to specific organelles, cells, and tissues in multiple organs that have been affected in vivo (Myers and Fournie 2002, Hinton et al. 1992, Moore and Simpson 1992, Wester and Canton 1991, Hinton and Laurén 1990a). Accordingly, histopathological biomarkers in field studies are lesions that signal effects resulting from prior or ongoing exposure to one or more toxic agents, their synergistic and antagonistic effects and biotic factors (Hinton et al. 1992, Moore and Simpson 1992, Wester and Canton 1991, Hinton and Laurén 1990a). The lesions appear as they existed prior to sacrifice or fixation of the tissue, permitting the visual localisation of the *in situ* relationship within organs (Myers and Fournie 2002, Hinton and Laurén 1990a). Similar to biochemical and physiological methods, the approaches to using histopathology share the problem that many environmental pollutants act through a limited number of toxic mechanisms to produce a restricted array of effects on biological target systems (Hinton and Laurén 1990a). However, because of the integrative nature of histopathological alterations, links between such pathologies and contaminant exposure are not definitive in most field conditions. Only broad generalizations regarding the possible specific aetiologic agent(s) responsible for such lesions can be made (Hinton et al. 2001, Schmidt-Posthaus et al. 2001).

The ability to determine the magnitude of toxic impairment through histopathology strengthens efforts to predict eventual impact of current or prior exposure; on the survival of the affected individual and in some instances the population (Myers and Fournie 2002). Such surveillance not only demonstrates that toxicants have entered organisms, have been distributed between tissues, and are eliciting effects at cellular and organ level (Van der Oost et al. 2003, McCarthy and Shugart 1990), but it also provides useful insights into individual, population and overall ecosystem quality and health (van Dyk et al. 2009b, Zimmerli et al. 2007). Despite their high mobility, fish are considered to be the most feasible organisms for pollution monitoring in aquatic systems (Van der Oost et al. 2003). They can be found in most aquatic environments. Understanding the environmental responses in fish may therefore have a high ecological and public health relevance (Van der Oost et al. 2003). The objective of this chapter was to investigate fish histopathology in relation to urban effluent exposure in the study wetlands with a view to explore fish histopathology as a biomarker of water quality deterioration in selected Ugandan wetlands. Specifically the study assessed the histological alterations in the liver, gills, gonads and spleen of *O. niloticus* from five variably impacted wetlands in Uganda. The study sites were evaluated and compared in terms of the fish histopathology indices and lesion prevalence.

6.2. Specific Methods

6.2.1. Sampling protocol and sample preparation

Sampling included collecting data from five wetland ecotones along the shoreline of Lake Victoria (see general methods chapter two). Initially all species that were collected alive were processed for histological examinations but over time only *O. niloticus* was consistently found in all study sites. *O. niloticus*' ubiquitous nature and availability in all the five study sites rendered it a suitable bioindicator species for the histopathology study. Subsequently only *O. niloticus* was sampled. Live fish were dissected to remove the liver, gills, spleen and gonads for histology processing (see general methods chapter two).

6.2.2. Histological assessment

Microscopic observation involved light microscopy for the examination of the tissues. Histological alterations were qualitatively assessed in all processed organs. The results were semi-quantitatively evaluated according to a protocol proposed by Bernet et al. (1999), which was slightly modified in nomenclature and with the inclusion of gonadal and splenic histopathology that were not included in the Bernet protocol. For each organ investigated, the pathological changes were classified into five reaction patterns and these included: Circulatory disturbances (C), regressive changes (R), progressive changes (P), inflammation (I) and neoplasms (N). Following the Bernet et al. (1999) protocol, the lesions under each reaction pattern were identified and scored. Accordingly, circulatory disturbances were changes that resulted from a pathological condition of blood and tissue fluid flow such as congestion. Regressive changes were processes which could terminate in a functional reduction or loss of an organ such as atrophy, degenerative changes and necrosis. Progressive changes were processes which lead to an increased activity of cells or tissues. Inflammatory changes were often associated with processes belonging to other reaction patterns such cellular infiltration and exudates. Lastly a tumour or neoplasm depicted an uncontrolled cell and tissue proliferation (autonomous proliferation).

Each alteration (lesion) was assigned an importance factor of 1 to 3 depending on its pathological importance in affecting the organ function and ability of the fish to survive, with 1 assigned to a lesion easily reversible such as haemorrhage and plasma alterations and 3 to a lesion that may lead to partial or total loss of the organ, for example necrosis (Bernet et al. 1999). Every lesion was then assessed using a score ranging from 0 to 6, depending on the degree of alteration whereby (0) was unchanged; (2) mild occurrence; (4) moderate occurrence; and (6) severe occurrence (diffuse lesion), with intermediate values also considered (Bernet et al. 1999).

For each lesion a lesion index was calculated by multiplying the importance factor with the score value, and the reaction pattern for each organ was calculated as the sum of the lesion indices under that reaction pattern. For example for the liver, a circulatory liver index (IL-C), regressive liver index (IL-R), inflammatory liver index (IL-I) and neoplastic liver index (IL-N) were calculated. Similar reactions patterns were calculated for the gills, gonads and spleen. The sum of the reaction indices per organ yields the total organ index: IL for the liver, IGl for gill, IGd for gonads and IS for the spleen. These indices are indicative of the extent and intensity of histological alterations in the respective tissue and converted the observed qualitative histological alterations into a quantitative value (van Dyk et al. 2009b, Zimmerli et al. 2007, Bernet et al. 1999).

In order to classify the study sites according to the severity of the histological response, the organ index results were graded into four grades. The grades were a modification of a scoring scheme developed for trout in Swiss rivers by Zimmerli et al. (2007) and a system developed for *Clarias gariepinus* and *Oreochromis mossambicus* in reconstituted and polluted waters by van Dyk et al. (2009b). For this study, organ histological indices for *O. niloticus* were classified into four classes where classes 1 and 2 indices were considered normal and recoverable while classes 3 and 4 were considered pathological with class 4 depicting severe alterations. Below is a detailed description of each class.

- Class 1 of index < 15 depicted normal tissue structure with slight histological alterations.
- Class 2 (index 16 – 30) showed normal tissue structure with moderate histological alterations
- Class 3 (index 31 – 40) showed pronounced alterations of organ tissue
- Class 4 (index > 41) showed severe alterations of organ tissue.

6.2.3. Statistical analysis

Descriptive statistics was used to derive the means, standard deviations, and ranges of the different reactions pattern indices in each study organ and significant differences between sites were established. Most data showed non-normal distribution. The Kruskal-Wallis nonparametric test and the Mann-Whitney U test, Bon-Ferroni corrected, were used to compare indice values between sites. The level of significance of all statistical tests was set at 95%. The number of fish affected by a specific histological alteration in each site was presented as percentage prevalence. The four classes of organ index results, i.e. index < 15, 16 – 30, 31 – 40, and > 41, for each organ were derived and graphically presented to show the proportion (percentage) of each class among the studied fish population for every site.

6.3. Results

6.3.1. Histopathological indices and index grades in the different study sites

Organ histopathological sensitivity decreased in the order of liver > gill > gonads > spleen. The liver and the gills registered higher organ index values (summation of all the reactive indices) compared to the gonads and spleen (Table 6.1). The reference site registered lower organ indices in all the studied organs as compared to the rest of the sites (Table 6.1).

Table 6.1 Organ and reactive pattern histopathological indices in the study sites. Where: I_C, I_R, I_P and I_I = circulatory, regressive, progressive and inflammatory indices.

Site	Index	Liver	Gill	Gonad (Female, Male)	Spleen
Lwanika / Reference (n = 42)	I_C	44	79		45
	I_R	241	21	76, 228	145
	I_P	74	576	153, 54	168
	I_I	180	0	36	110
	Organ Index	**539**	**676**	**547**	**468**
Kirinya (n = 49)	I_C	123	140		66
	I_R	1328	338	347, 497	393
	I_P	62	1341	207, 136	689
	I_I	546	6	197	324
	Organ Index	**2059**	**1825**	**1402**	**1472**
Masese (n = 37)	I_C	109	103		53
	I_R	888	157	236, 263	119
	I_P	72	915	152, 112	504
	I_I	242	0	138	220
	Organ Index	**1311**	**1175**	**901**	**896**
Winday Bay (n = 60)	I_C	142	124		71
	I_R	1065	151	206, 492	243
	I_P	198	1329	305, 114	592
	I_I	376	6	75	292
	Organ Index	**1781**	**1610**	**1192**	**1198**
Murchison Bay (n = 56)	I_C	130	289		53
	I_R	1927	461	606, 430	473
	I_P	135	1413	311, 126	793
	I_I	640	20	214	375
	Organ Index	**2832**	**2183**	**1738**	**1694**

The four classes of organ index results based on the severity of the histological response for each organ were derived (Figures 6.1 to 6.4). Overall, the majority of the fish in the effluent impacted sites of Kirinya and Murchison Bay were graded under the pathological grades 3 and 4, as compared to the reference site with most fish registered under the normal and recoverable grades 1 and 2 (Figures 6.1 to 6.4). For example, in the liver, only the reference site (Lwanika), and Winday Bay registered fish in Class 1 of index < 15, depicting normal tissue structure with slight histological alteration, while 71.4 % of the fish population in the reference site fell under this category as opposed to Winday Bay with 3.3% and no fish in the effluent impacted sites (Figure 6.1). On the other hand, Murchison Bay and Kirinya, the highly impacted sites, registered the highest proportion of fish (73.2% and 51% respectively) in the Class 4 index of > 41, a class showing severe alterations of organ tissue (Figure 6.1). Whereas the highest proportions of fish in Winday Bay (48.3) and Masese (43.3) were recorded in Class 16 – 30 of normal tissue structure with moderate histological alterations and class 31 – 40, of pronounced alteration of organ tissue, respectively (Figure 6.1). In other words, 97.6% of the studied fish population in the reference site fell under the two classes 1 and 2, that are considered to be normal and recoverable; while 94.6% of the fish population in Murchison Bay and 85.5% in Kirinya fell under class 3 and 4, the two classes that are considered pathological (Figure 6.1).

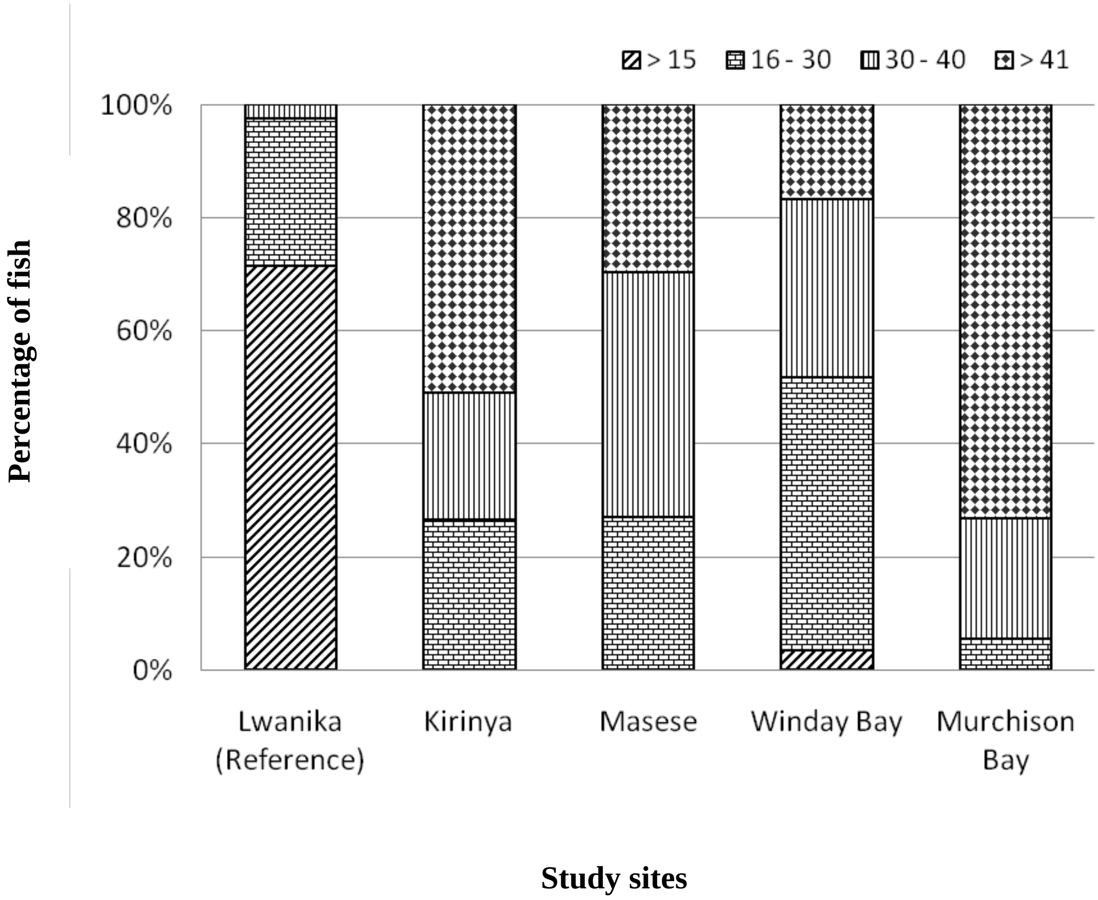


Figure 6.1 Grades of lesion severity in the study sites showing the percentage of fish in different classes of histological response based on the liver index. Where Index < 15 = normal tissue structure with slight histological alterations; Index 16 – 30 = normal tissue structure with moderate histological alterations; Index 31 – 40 = pronounced alterations of organ tissue and Index > 41 = severe alterations of organ tissue. Where Lwanika n = 42, Kirinya n = 49, Masese n = 37, Winday Bay n = 60 and Murchison Bay n = 56.

Gills:

In the gills, a similar trend was followed in the reference site with 97.6% of the fish recorded in the normal and recoverable classes 1 and 2; but with 67.8% of fish in Murchison Bay and 61.2% in Kirinya falling under the pathological classes 3 and 4 (Figure 6.2). In addition no fish was recorded in the reference site under the severe alterations class 4 of index > 41 as compared to the other sites (Figure 6.2). For the gills, Murchison Bay, Masese and Kirinya registered higher proportions of fish in Class 16 – 30, a class showing normal tissue structure with moderate histological alterations as compared to the liver index classification (Figures 6.2 and 6.1).

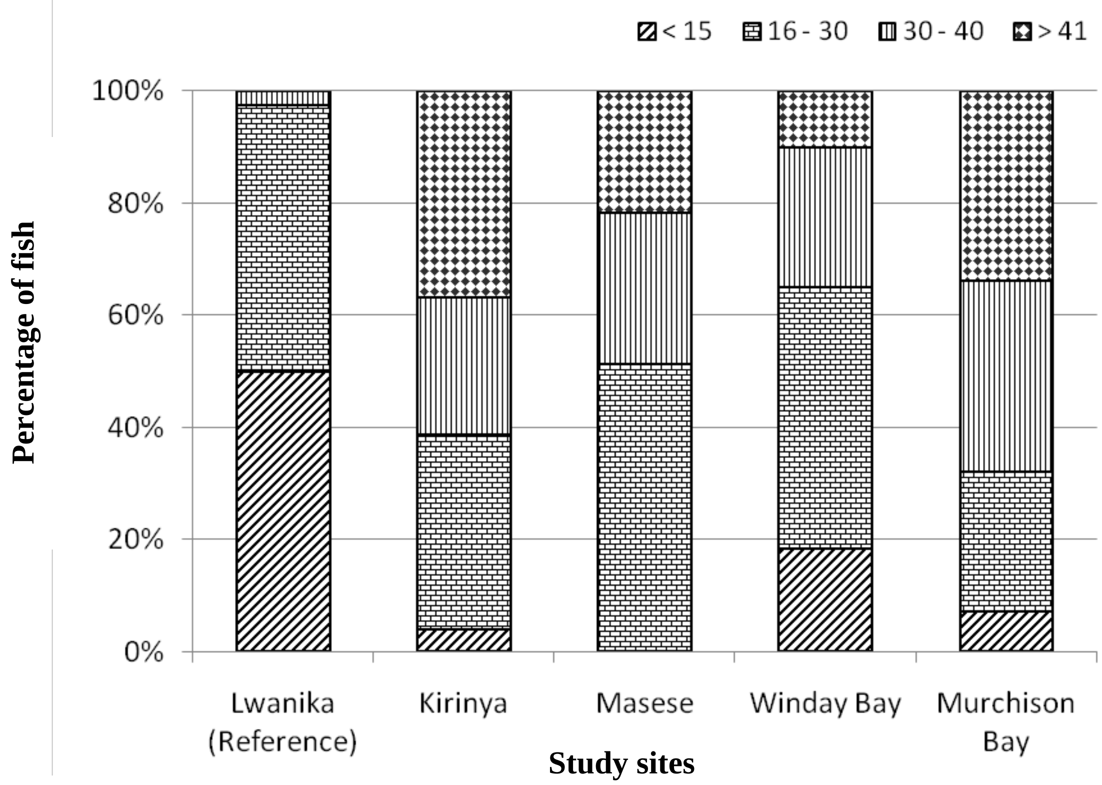


Figure 6.2 Grades of lesion severity in the study sites showing the percentage of fish in different classes of histological response based on the gill index. Where Index < 15 = normal tissue structure with slight histological alterations; Index 16 – 30 = normal tissue structure with moderate histological alterations; Index 31 – 40 = pronounced alterations of organ tissue and Index > 41 = severe alterations of organ tissue. Lwanika n = 42, Kirinya n = 49, Masese n = 37, Winday Bay n = 60 and Murchison Bay n = 56.

Gonads:

For gonadal-index-based classification, all sites registered fish under Class 1 < 15 fish category with Lwanika recording the highest proportion (71.4%), followed by Winday Bay (45%) as compared to the effluent impacted sites with less than 20% (Figure 6.3). Similar to the liver and gills, no fish were registered under class 4 index of > 41 in the reference site. The same trend was observed in Winday Bay (Figure 6.3). Notable in Murchison Bay and Kirinya was a similar trend as was observed in the gills whereby lower percentages (68% and 51%) were registered in the two pathological classes of 3 and 4 (Figure 6.3).

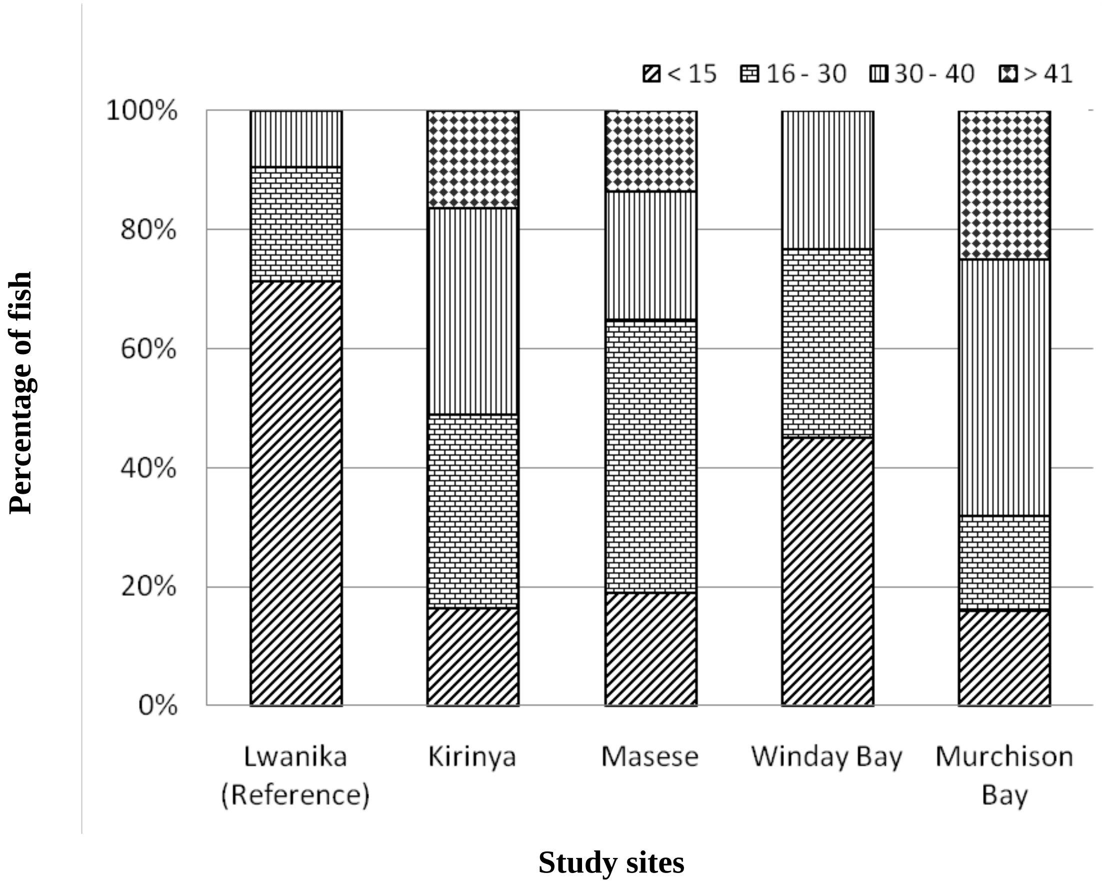


Figure 6.3 Grades of lesion severity in the study sites showing the percentage of fish in different classes of histological response based on the gonad index. Where Index < 15 = normal tissue structure with slight histological alterations; Index 16 – 30 = normal tissue structure with moderate histological alterations; Index 31 – 40 = pronounced alterations of organ tissue and Index > 41 = severe alterations of organ tissue. Where Lwanika n = 42, Kirinya n = 49, Masese n = 37, Winday Bay n = 60 and Murchison Bay n = 56.

Spleen:

The largest proportion of fish in all study sites were observed to fall within the two normal and recoverable classes 1 and 2, with the highest percentages registered in the reference site (95.3%) and Winday Bay (81.7). The other sites proportion in this category were, Kirinya with 65%, Masese, 67.5% and 62.5% in Murchison Bay. The reference site did not register fish in Class 4 index of >41. This was also observed in Masese. Kirinya on the one hand did not register fish in the < 15 index (Table 6.4).

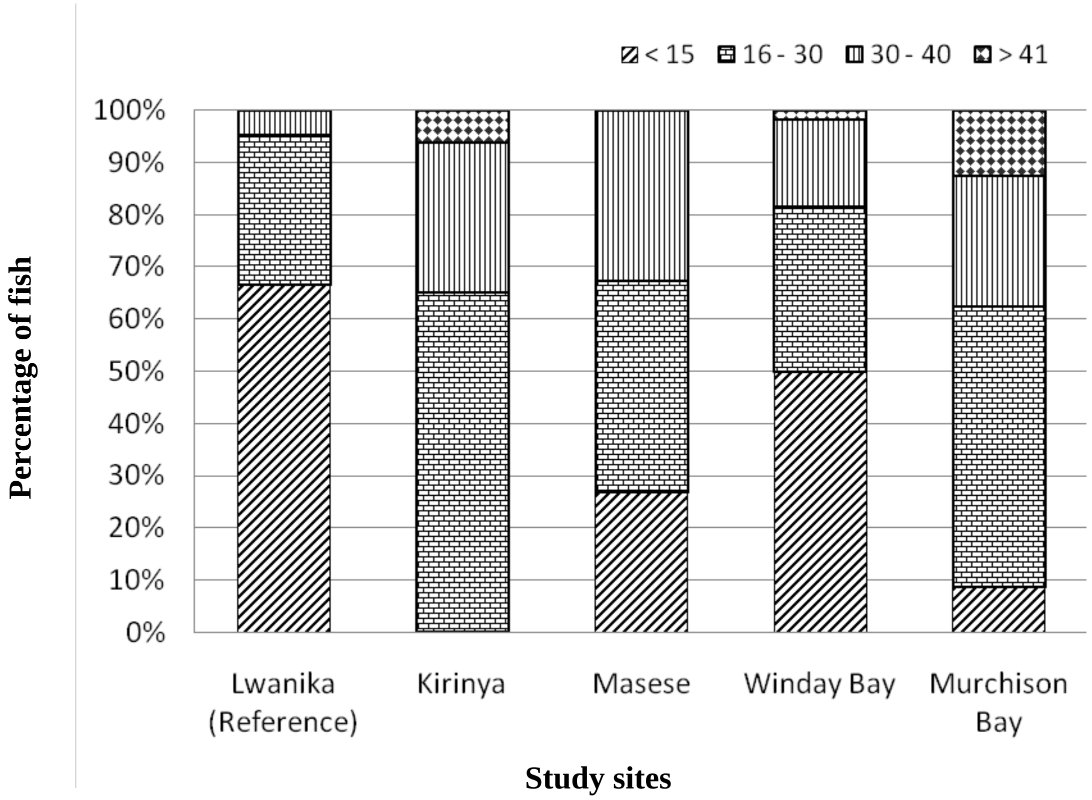


Figure 6.4 Grades of lesion severity in the study sites showing the percentage of fish in different classes of histological response based on the splenic index. Where Index < 15 = normal tissue structure with slight histological alterations; Index 16 – 30 = normal tissue structure with moderate histological alterations; Index 31 – 40 = pronounced alterations of organ tissue and Index > 41 = severe alterations of organ tissue. Where Lwanika n = 42, Kirinya n = 49, Masese n = 37, Winday Bay n = 60 and Murchison Bay n = 56.

6.3.2. Reaction indices for each organ

Liver

Alterations in the organs studied were summarised into numerical values along the five reaction patterns: circulatory (C), regressive (R), progressive (p), inflammatory (I), and neoplastic (N) changes. Neoplastic alterations were not detected in any of the fish sampled in all the study sites but the other four reaction patterns were observed. In the liver, the reference site registered significantly lower indices compared to the rest of the study sites ($P < 0.5$). This was followed by Winday Bay (Table 6.2). On the other hand Murchison Bay had the highest indices for all reaction patterns, followed by Kirinya (Table 6.2). The major liver lesions were regressive in nature (Table 6.2) and these included hepatocyte and interstitial tissue degeneration (hydropic, vacuolar and fatty degeneration) and necrosis. The main alteration under liver circulation reaction pattern was congestion of sinusoids and major blood vessels. Liver progressive changes were hypertrophy and hyperplasia of hepatocytes and interstitial tissue, while inflammatory changes included foci of cellular infiltration and melanomacrophage infiltration.

Table 6.2 Mean ± standard deviation (number of fish affected in parenthesis) of liver reaction indices of fish in the study sites. Different letters in rows denote significant differences.

Reaction index	Lwanika (Reference)	Kirinya	Masese	Winday Bay	Murchison Bay
Liver Circulation Index (ILC)	2.9±1.5[a] (15)	4.7±0.9[b] (26)	4.2±0.8[c] (26)	4.2±0.8[c] (34)	5.0±0.6[b] (26)
Liver Regressive Index (ILR)	5.7±5.9[a] (42)	27.1±10.5[b] (49)	24.0±11.0[b] (37)	17.8±8.1[c] (60)	34.4±10.5[d] (56)
Liver Progressive Index (ILP)	2.8±1.4[a] (26)	2.7±0.8[a] (23)	3.8±1.3[b] (19)	4.2±1.0[b] (47)	4.2±0.9[b] (32)
Liver Inflammatory Index (ILI)	4.5±1.[6a] (40)	11.1±6.0[b] (49)	6.5±2.2[c] (37)	6.6±2.1[c] (57)	11.4±5.5[b] (56)

Gills

In the gills, inflammatory changes were rarely observed, with regressive and progressive changes being more common. The reference site registered significantly lower indices in all the recorded reaction patterns, followed by Winday Bay and Masese (Table 6.3). A similar trend as observed in the liver was also recorded for Murchison Bay and Kirinya where significantly higher indices were recorded in the two sites (Table 6.3). However, unlike in the liver, the gills registered higher indices in the progressive reaction pattern category, making the progressive alterations the major lesions in the studied gills (Table 6.3). These alterations included hypertrophy and hyperplasia of cellular (epithelial cells, interlamellar basal cells and mucous cells) and supporting tissue. The main gill circulatory alterations included telangiectasis and oedema of sub-epithelial space, while gill regressive disturbances primarily included, architectural and structural alterations, and necrosis of epithelium and supporting tissue.

Table 6.3 Mean ± standard deviation (number of fish affected in parenthesis) of gill reaction indices of fish in the study sites. Different letters in rows denote significant differences.

Reaction index	Lwanika (Reference)	Kirinya	Masese	Winday Bay	Murchison Bay
Gill Circulation Index (IGC)	3.0±0.8^{a} (26)	4.8±1.6^{b} (29)	4.1±0.9^{b} (25)	4.3±1.4^{b} (29)	5.6±2.2^{d} (52)
Gill Regressive Index (IGR)	2.1±1.2^{a} (10)	7.2±4.8^{b} (47)	4.5±2.0^{c} (35)	4.7±2.5^{c} (32)	8.9±7.8^{b} (52)
Gill Progressive Index (IGP)	16.1±5.0^{a} (42)	27.4±10.5^{b} (49)	24.7±6.3^{bc} (37)	22.2±7.1^{c} (60)	25.2±7.6^{b} (56)

Gonads

In the gonads only three of the five reaction patterns were observed and these included regressive, progressive and inflammatory disturbances. No circulatory disturbances were observed. Nevertheless, as observed in the liver, higher indices were recorded in the regressive reaction pattern but the main alterations in the reference site were progressive in nature. The major progressive lesions observed included, hyperplasia and hypertrophy of the perifollicular oocytes in females; and hyperplasia of the testicular tissue in males. Testicular tissue hypertrophy was rarely observed. Regressive challenges on the other hand included decreased vitellogenesis, pre-ovulatory atresia, interstitial proteinaceous fluid, oocyte and interstitial necrosis in females while males displayed various degenerative changes including necrosis. Inflammatory changes in both male and female included, intracellular exudates and infiltration with fibroblasts and macrophages. As was observed in the liver and gills, the gonadal lesions also showed significantly lower index values in the reference site as compared to the effluent impacted sites (Masese, Kirinya and Murchison Bay) in all the observed reaction patterns (Table 6.4).

Table 6.4 Mean ± standard deviation (number of fish affected in parenthesis) of gonad reaction indices of fish in the study sites. Different letters in rows denote significant differences.

Reaction index	Lwanika (Reference)	Kirinya	Masese	Winday Bay	Murchison Bay
Gonadal Regressive Index – Female (F-IGdR)	9.5±5.0**a** (8)	15.8±8.7**b** (22)	15.7±6.2 **b** (15)	9.4±7.0**a** (22)	20.9±9.5**b** (29)
Gonadal Regressive Index – Male (M-IGdR)	12.0±6.6**a** (19)	20.7±8.6**b** (24)	14.6±6.6 **a** (18)	15.4±7.6**a** (32)	17.9±8.0**b** (24)
Gonadal Progressive Index – Female (F-IGdP)	7.7±3.9**a** (20)	9.4±3.2**b** (22)	10.9±4.2 **b** (14)	10.5±3.0**b** (29)	10.4±3.1**b** (30)
Gonad Progressive Index - Male (M-IGdP)	9.0±2.4**a** (6)	6.5±2.3**b** (21)	7.0±1.3**b** (16)	5.7±1.6**b** (20)	7.4±2.1**b** (17)
Gonad Inflammatory Index (IGdI)	3.6±0.8**a** (10)	6.0±4.2**a** (33)	5.5±3.0**a** (25)	4.2±1.7**a** (18)	5.9±2.8**a** (36)

The Spleen

In the spleen, four reaction patterns were recorded and these included circulatory, regressive, progressive and inflammatory disturbances. Circulatory disturbances included congestion of major blood vessels and ellipsoids. Regressive changes included splenic and supporting tissue necrosis and deposits in the ellipsoids. Progressive disturbances on the other hand included splenic, lymphoid and interstitial tissue hyperplasia and hypertrophy; while inflammatory changes included infiltration with macrophages. Lower indices were observed in the spleen compared to the other three organs with the circulatory disturbances showing no significant differences between the reference and the effluent impacted sites (Table 6.5). The reference site and Winday Bay, that do not receive urban effluent reported lower progressive and regressive indices and these were significantly different from the effluent impacted Murchison Bay, Kirinya and Masese (Table 6.5).

Table 6.5 Mean ± standard deviation (number of fish affected in parenthesis) of spleen reaction indices of fish in the study sites. Different letters in rows denote significant differences.

Reaction index	Lwanika (Reference)	Kirinya	Masese	Winday Bay	Murchison Bay
Spleen Circulation Index (ISC)	2.4±1.0[a] (19)	2.8±1.2[a] (24)	3.3±1.1[a] (16)	2.8±0.9[a] (25)	3.5±1.0[a] (15)
Spleen Regressive Index (ISR)	4.3±3.1[a] (34)	8.4±7.9[b] (47)	7.0±5.3[b] (17)	4.9±3.1[a] (50)	9.1±7.0[b] (52)
Spleen Progressive Index (ISP)	9.0±4.3[a] (41)	14.1±2.9[b] (49)	13.6±5.6[b] (37)	10.4±5.2[a] (57)	14.2±4.6[b] (56)
Spleen Inflammatory Index (ISI)	4.8±2.5[a] (23)	6.8±2.0[b] (48)	6.5±2.1[b] (34)	6.5±1.9[b] (45)	7.7±2.7[c] (49)

6.3.3. Prevalence of specific histological alteration in the study sites

Histological alterations were observed in all studied organs in all study sites but the prevalence and severity of the lesions varied between sites (tables 6.6 to 6.9). Normal liver architectural structure showed a network of hepatic cell cords with hepatocytes located among the sinusoids with centrally located nucleus (Plates L6.1a and b). In the liver, alterations were necrosis of hepatocytes (Plate L6.4a and b) which was highly prevalent in Murchison Bay (86%) and Kirinya (65%), as compared to the reference site (Lwanika) with 2% (Table 6.6). Also to note was foci of cellular infiltrations which although occurred at low prevalence overall, this lesion was not observed in the reference site but was observed in the effluent-impacted sites (Table 6.6). The highly basophilic infiltrating cells were not typical liver cells but are suggested to be inflammatory cells (Plate L6.2). Other significant liver lesions included hepatocyte nuclear pleomorphism (Plate L6.3) and the infiltration of melanomacrophages that created multiple focal melanomacrophage centres in the liver tissue (Plate L6.5). These lesions could serve as significant biomarkers of environmental health. Other degenerative changes observed in the liver included, hydropic, vacuolar and fatty degeneration; congestion of major blood vessels and sinusoids (Plate L6.6), hyaline deposition in hepatocytes and structural / architectural alterations.

Overall, congestion of sinusoids was highly prevalent in all grade 3 (31 to 40 organ index lesion grades), a grade that showed pronounced alterations of organ tissue, especially in the highly polluted sites of Kirinya and Murchison Bay. Fatty degeneration, extended melanomacrophage centres, hepatic necrosis and nuclear pleomorphisms were highly prevalent in the grade 4 organs (organ index above 41), indicating severe alterations of organ tissue. These lesions were highly prevalent in all the study sites (Table 6.6). In Murchison Bay, the liver of most fish was characterised by heavy and diffuse melanomacrophage centres and hepatocellular degenerative changes and necrosis. Near neoplastic lesions were found in a few Murchison Bay livers, where necrotic inclusions were observed in the hepatocyte cytoplasm which denotes early stages of hepatomas.

Table 6.6 Prevalence of the major histological alterations identified in the liver tissue of *O. niloticus* in the study sites

Histological Alteration	**Lwanika** (Reference) (n = 42)	**Kirinya** (n = 49)	**Masese** (n = 37)	**Winday Bay** (n = 60)	**Murchison Bay** (n = 56)
Congestion of sinusoids and major blood vessels	36	53	70	57	46
Architectural and structural alterations	5	78	84	55	88
Hepatocyte fatty degeneration	12	57	30	32	45
Hyaline deposition in hepatic cytoplasm	2	47	19	13	66
Hepatocyte vacuolar degeneration	29	57	78	85	84
Hepatocyte hydropic degeneration	60	41	62	77	38
Hepatocyte nuclear pleomorphism	5	65	65	27	74
Hepatic necrosis	2	67	54	28	86
Fibrosis of perivascular tissue	0	54	62	25	54
Hypertrophy of hepatocytes	62	47	51	78	57
Foci of cellular infiltrations	0	43	22	3	59
Melanomacrophage infiltration	48	98	97	95	98

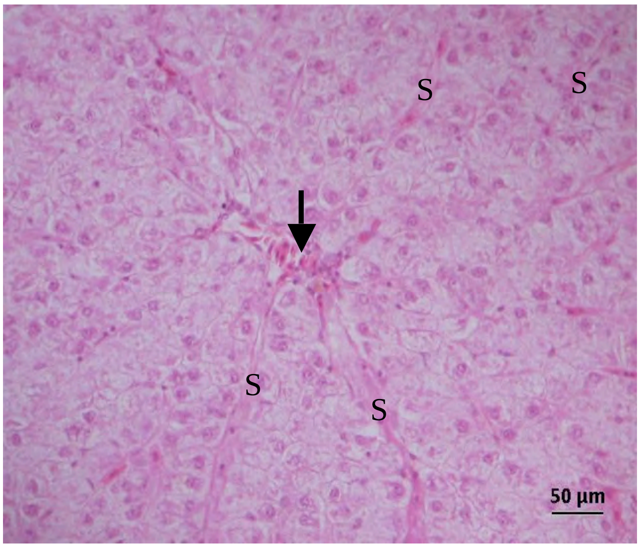


Plate L6.1a Section of liver showing normal architectural structure with a central blood vessel (arrow) and a network of sinusoids (S)

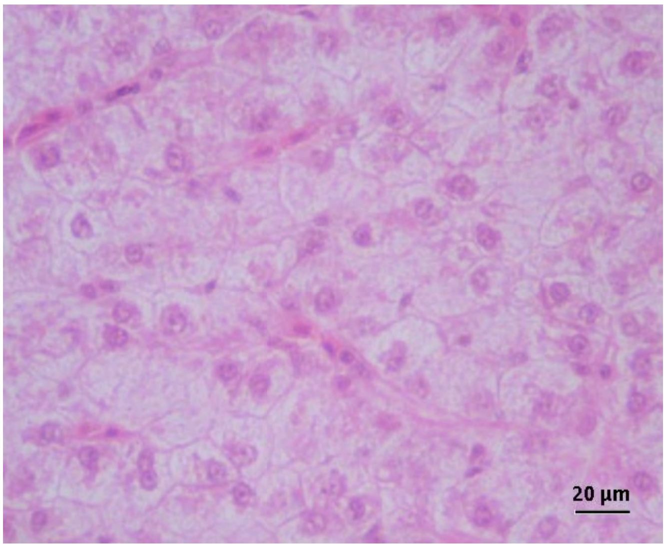


Plate L6.1b Section of normal liver showing normal architectural structure with uniformly sized hepatocytes and hepatocyte nuclei

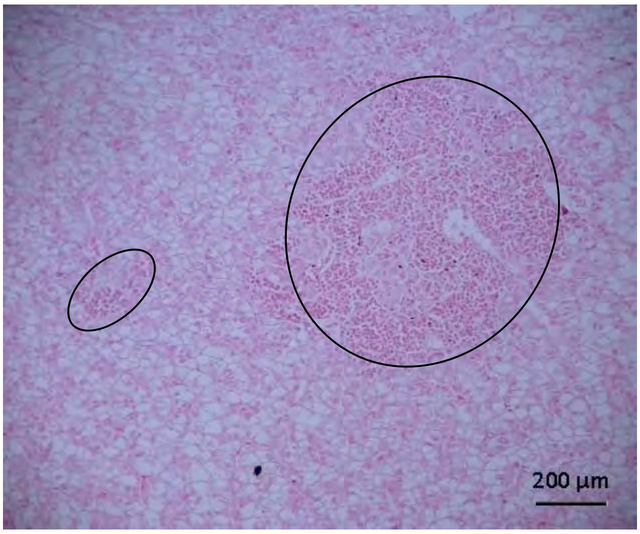


Plate L6.2 Section of liver tissue showing focal cellular infiltrations (circles)

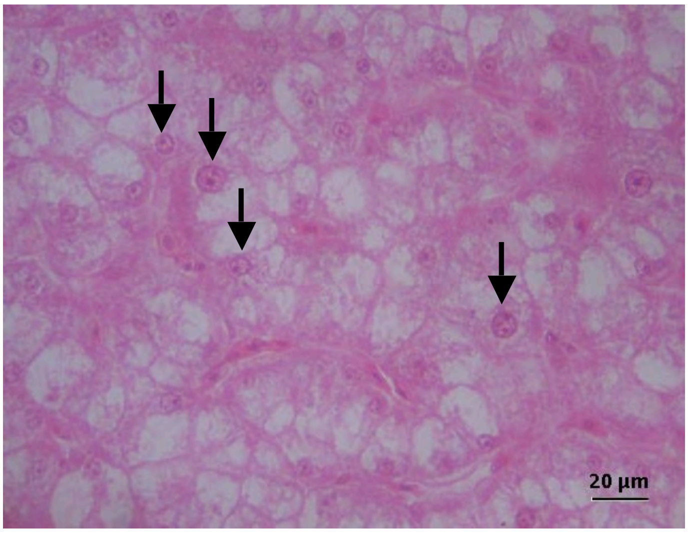


Plate: L6.3 Liver tissue showing nuclear pleomorphism (arrows)

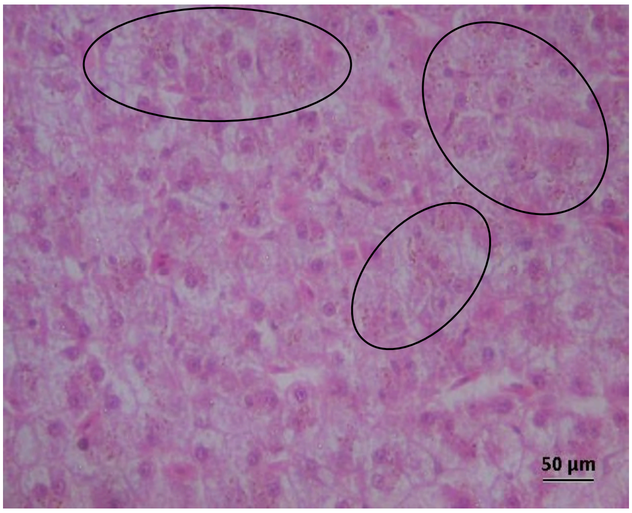


Plate L6.4a Liver tissue showing early stages of diffuse necrosis (circles). Tissue structural integrity not completely lost.

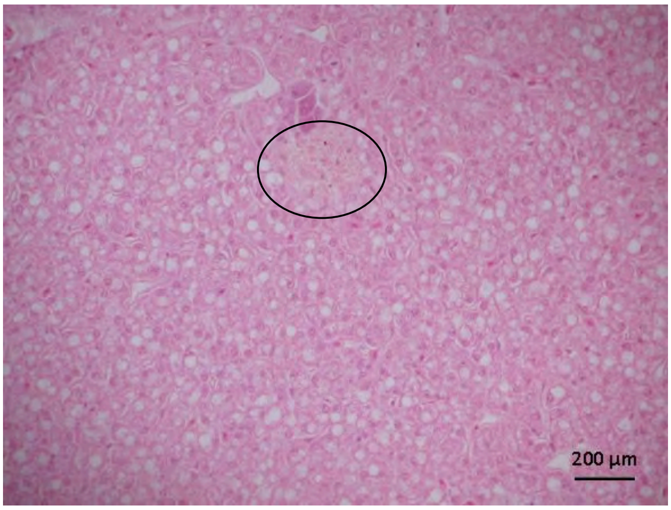


Plate L6.4b Section of liver showing focal necrosis (circle). Liver under going diffuse fatty degeneration and structural loss.

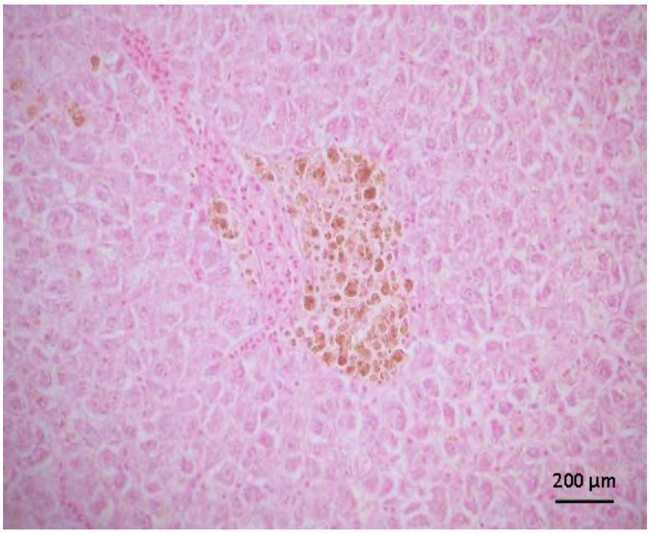


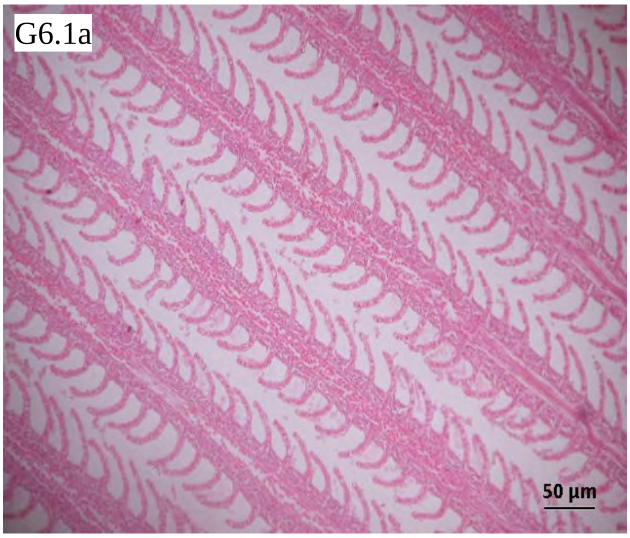
Plate L6.5 Sections of Liver tissue showing a focal melanomacrophage centre around a congested blood vessel.

Plate: L6.6 Severe congestion of major blood vessels (BV) and sinusoids (S).

Gills

Most of the fish in the reference site showed a relatively normal gill structural arrangement. The primary lamellae appeared normal with a central core of cartilage, chondrocytes and erythrocytes extending from base to tip in variable thickness. The secondary lamellae were lined by simple squamous epithelial cells and they were filled with erythrocytes and pillar cells (Plates G6.1a and b). The lesions in the reference site and Winday Bay were mild and registered low reaction indices in grade 1 and 2, with organ index < 15 and 16 – 30, as was observed in section 6.3.1 above. However a number of lesions were highly prevalent in the gills and could serve as important biomarkers of environmental quality. These included hyperplasia of interlamellar basal cells, supporting tissue and epithelial cells (Plates G6.2a and b). These lesions were highly prevalent (above 95%) in the effluent impacted sites as compared to the reference site (Table 6.7). Also noted in the effluent impacted sites was telangiectasis and oedema of the sub-epithelial spaces, also known as lamellar oedema (Table

6.7). In some cases there was mucous cell hypertrophy and complete oedematous separation of the respiratory epithelium of the primary lamellae (Plate G6.3). These lesions were diffuse and ranged from moderate to severe in the effluent-impacted sites (grades 3 and 4 with organ index of 31 – 40 and > 41). Other lesions that occurred at low prevalence in the effluent-impacted sites were plasma alterations and deposits and inflammatory cellular infiltrations.



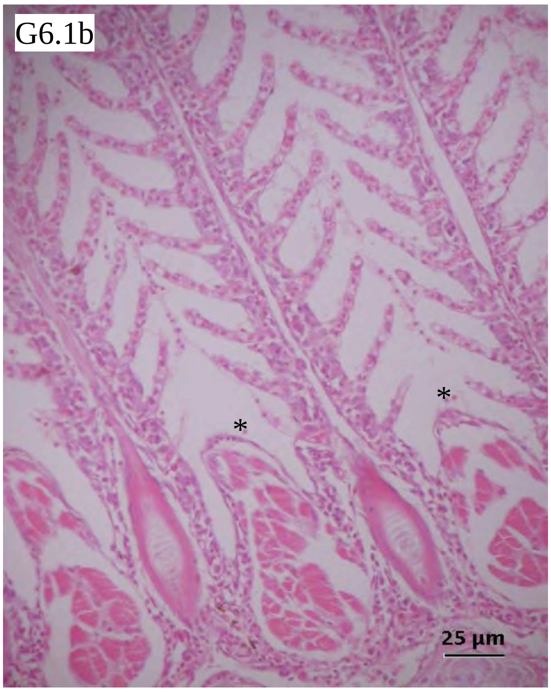


Plate G6.1a Section of a gill showing normal primary lamellae

Plate G6.1b Section of a gill showing normal inter lamellae base (*)

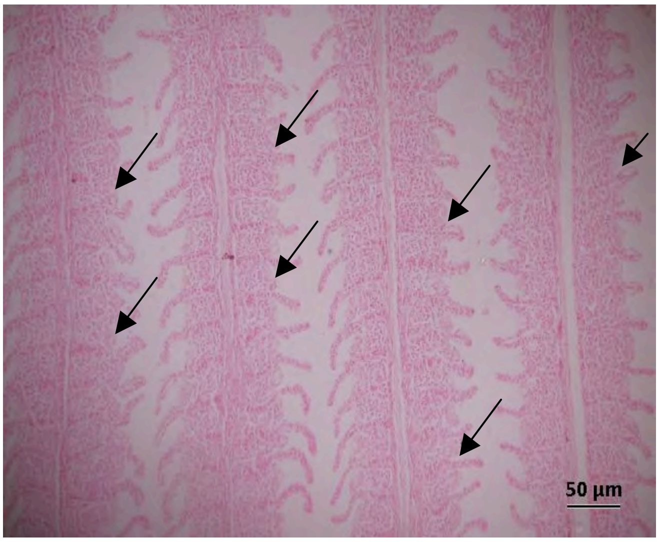


Plate G6.2a Section of gill showing interlamellar cells hyperplasia (arrows)

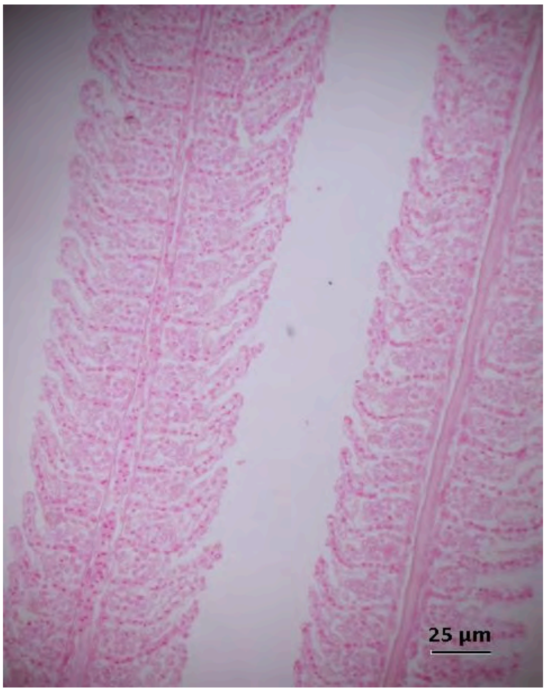


Plate G6.2b Section of gill showing complete lamellar fusion due to severe epithelial cell hyperplasia

Table 6.7 Percent prevalence of the major histological alterations identified in the gill tissue of *O. niloticus* in the study sites

Histological Alteration	**Lwanika** Ref. site (n = 42)	**Kirinya** (n = 49)	**Masese** (n = 37)	**Winday Bay** (n = 60)	**Murchison Bay** (n = 56)
Telangiectasis	0	16	3	3	43
Oedema of sub-epithelial space	32	53	68	48	73
Architectural/structural alterations	24	94	95	53	100
Epithelial necrosis	0	12	11	2	29
Supporting tissue necrosis	0	4	0	8	25
Epithelial cell hypertrophy	40	90	57	62	45
Interlamellar basal cell hypertrophy	48	67	54	52	38
Mucous cell hypertrophy	88	67	30	40	34
Epithelial cell hyperplasia	50	96	100	83	100
Interlamellar basal cell hyperplasia	43	96	100	85	100
Mucous cell hyperplasia	54	49	54	57	41
Hypertrophy of supporting tissue	43	0	27	0	0
Hyperplasia of supporting tissue	0	20	38	10	59

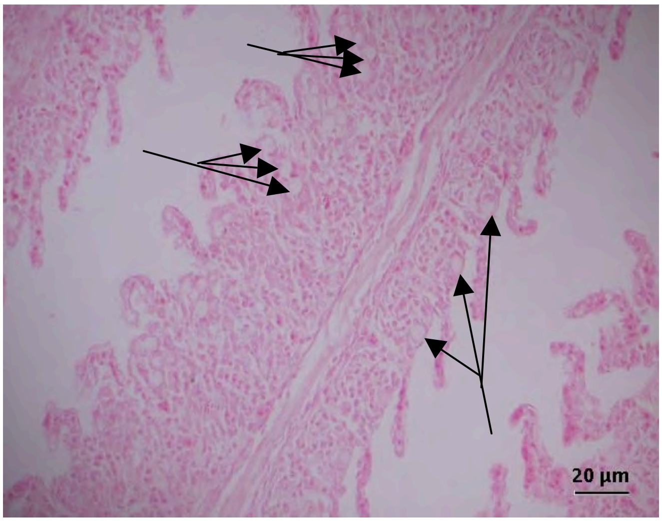


Plate G6.3a Section of gill showing mucous cells hyperplasia and hypertrophy (arrows); and interlamellar cell hyperplasia

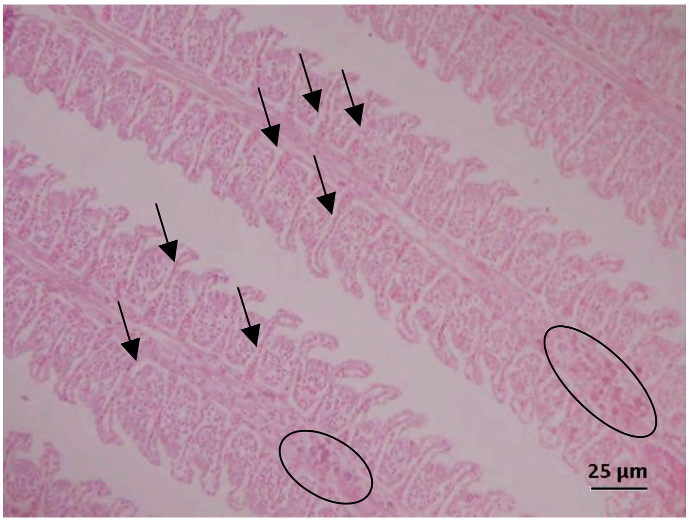
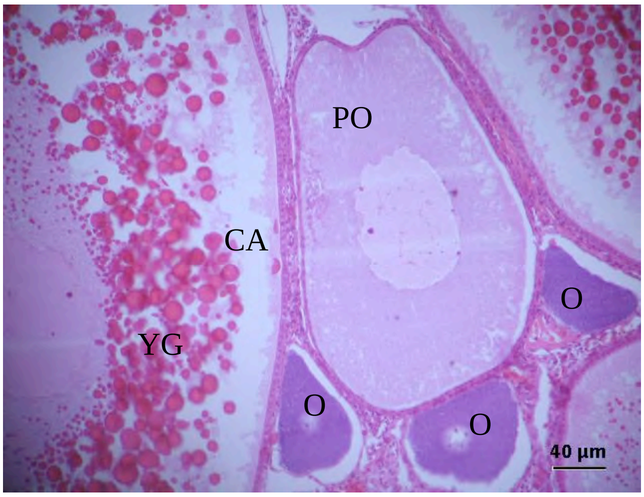


Plate G6.3b Section of gill showing diffuse oedema and complete lifting of epithelium (arrows); cellular infiltration in the central core (circles); and interlamellar cell hyperplasia

Gonads

In the normal female gonads, the oogonia and oocytes appeared normal with normal interstitial and perifollicular cells (Plate Gd6.1a and b). However a number of lesions were observed in the female gonads and these included, oocyte necrosis, oocyte interstitial degeneration, atresia of pre-ovulatory oocytes (Plate Gd6.2) and hyperplasia of perifollicular cells, i.e. granulosa and theca cells (Plate Gd6.3). Oocyte necrosis and interstitial degeneration were highly prevalent in the effluent-impacted sites of Murchison Bay, Kirinya and Masese (Table 6.8). Although hyperplasia of perifollicular cells was prevalent in Winday and Lwanika, the degree and extent of alteration of this lesion was mild, as compared to the other sites. Other alterations in the female gonads were not registered in the reference site but occurred at low prevalence in other sites were decreased vitellogenesis and pre-ovulatory atresia (Table 6.8).

In case of the normal male gonads, the testicular germ cells and lobular architectural structure appeared normal, with normal interstitial (Leydig) cells (Plate Gd6.4a and b). However, male gonadal alterations included focal testicular germ cell and interstitial tissue degeneration (Plate Gd6.5), testicular necrosis (Plate Gd6.5) and hyperplasia of testicular tissue (Plate Gd6.6). These lesions were more prevalent in the effluent impacted sites of Murchison Bay, Kirinya and Masese than in the reference site (Table 6.8). A number of lesions were highly prevalent in Winday Bay, the urban bay that is not subjected to direct effluent. Also observed in the male gonads in Murchison Bay and Kirinya but at low prevalence was intersex/ovitestis (Plate Gd6.7). Focal testicular germ cell and interstitial tissue degeneration, testicular necrosis and hyperplasia of testicular tissue were the major alterations highly prevalent in the effluent-impacted sites as compared to the reference site.



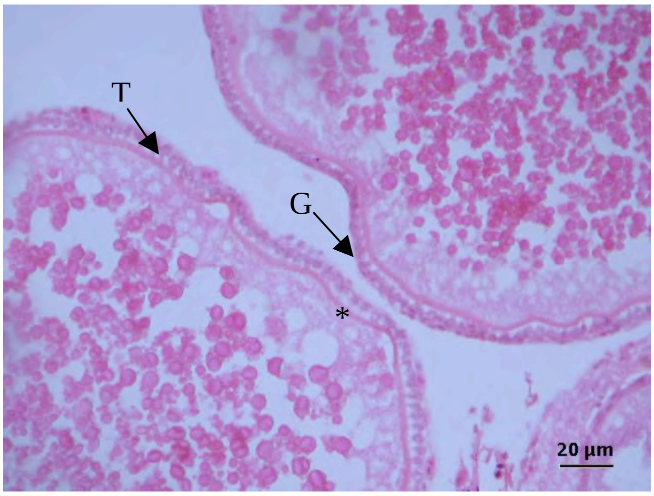
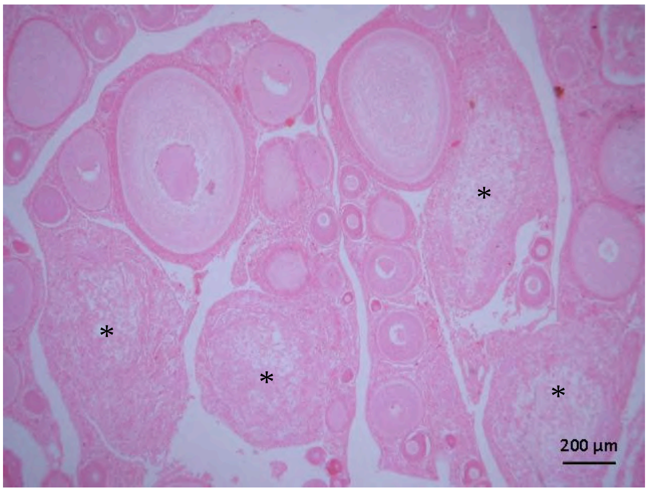


Plate Gd6.1a Section of female gonad showing normal development of different oocyte stages. YG = Yolk Globules and CA = Cortical Alveoli of a secondary oocyte, PO = Primary oocyte and O = Oogonia.

Plate Gd6.1b Section of female gonad showing normal tertiary oocyte perifollicular cells as a thin layer of granulosa (G) and theca (T) cells. * = Zona radiata.



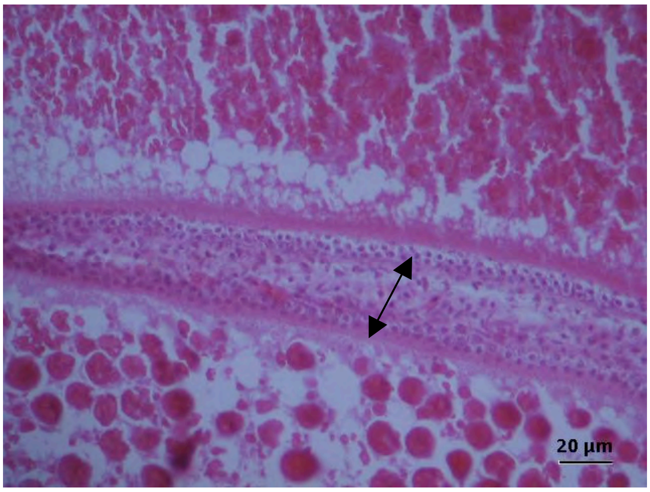


Plate Gd6.2 Section of female gonad showing pre-ovulatory oocyte atresia (*) and interstitial cell degeneration.

Plate Gd6.3 Section of female gonad showing hyperplasia of perifollicular cells completely joining two adjacent tertiary oocytes (double arrow).

Table 6.8 Percent prevalence of the major histological alterations identified in the gonadal tissue of *O. niloticus* in the study sites

Female	**Lwanika (Reference) n = 22**	**Kirinya n = 23**	**Masese n = 17**	**Winday Bay n = 29**	**Murchison Bay n = 32**
Decreased vitellogenesis	0	43	41	38	44
Pre-ovulatory atresia	0	35	18	17	47
Interstitial proteinaceous fluid	32	43	12	52	66
Oocyte necrosis	0	74	71	38	78
Oocyte interstitial degeneration	27	70	88	34	91
Hyperplasia of perifollicular cells	46	91	82	73	88
Hypertrophy of perifollicular cells	57	61	59	60	78
Male	**n = 20**	**n = 26**	**n = 20**	**n = 31**	**n = 24**
Focal testicular germ cell degeneration	56	85	85	94	83
Testicular interstitial degeneration	60	88	65	62	92
Testicular necrosis	10	69	60	48	67
Hyperplasia of testicular tissue	30	81	80	65	71
All	**n = 42**	**n = 49**	**n = 37**	**n = 60**	**n = 56**
Exudate (Intracellular and exudate)	12	53	46	13	49
Infiltration (fibroblasts and macrophages)	7	45	38	22	51
Intersex / Ovitestis	0	8	0	0	25

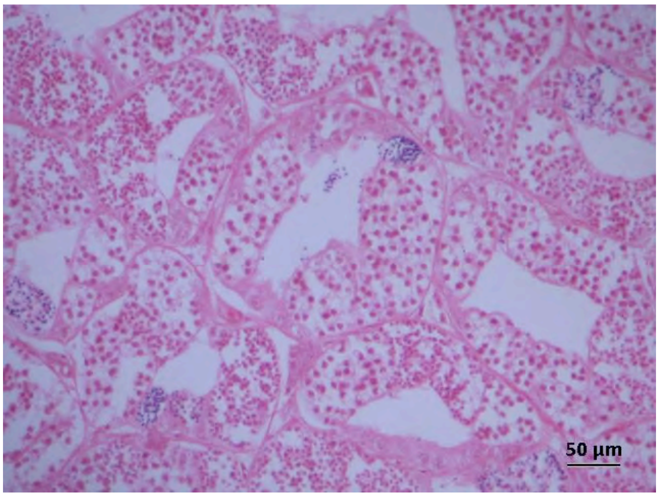


Plate Gd6.4a Section of male gonads showing normal lobular architectural structure with normal interstitial and testicular germ cells

Plate Gd6.4b Section of male gonad showing normal interstitial (leydig) cells (surrounded by arrows) and testicular germ cells, i.e. Primary Spermatocytes (PS), Secondary Spermatocytes (SS), Spermatids (ST) and E = Erythrocytes in the interstitial tissue

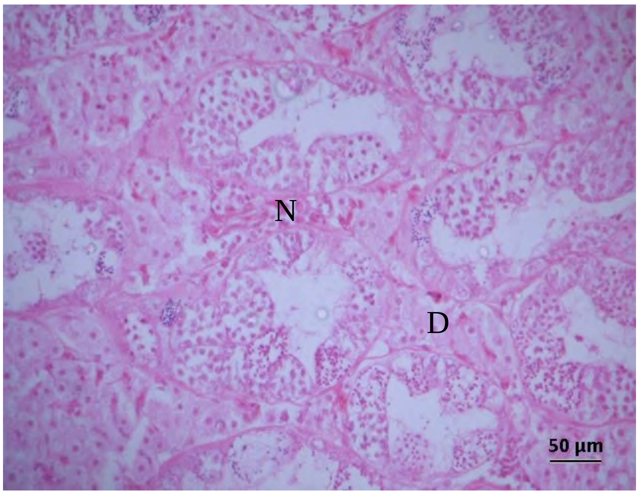


Plate Gd6.5 Section of male gonad showing degeneration (D) and necrosis (N) of hyperplastic interstitial (leydig) cells

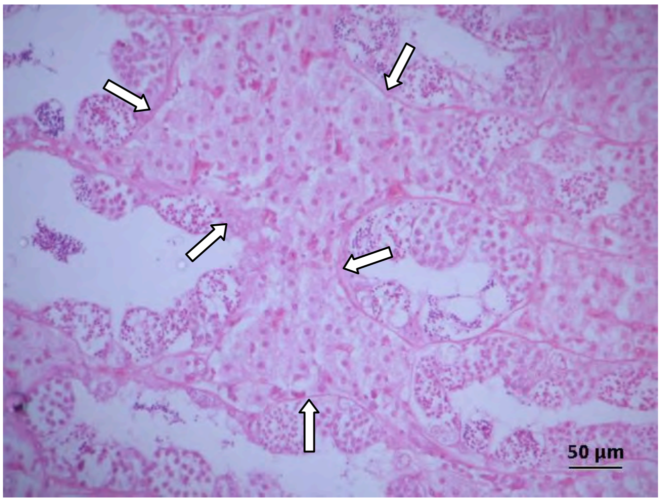


Plate Gd6.6 Section of male gonad showing severe hyperplasia of interstitial (leydig) cells (arrows)

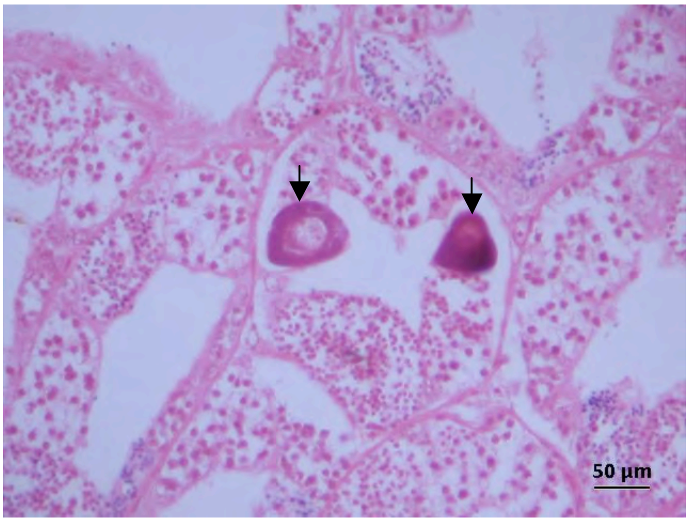


Plate Gd6.7 Section of male gonad showing ovitestis
(arrows show ova embedded in testicular tissue)

Spleen

The normal spleen showed minimal heamosiderin deposits in interspersed red and white pulp, and normal ellipsoids (Plate S6.1a and b). The most significant lesions in the spleen included macrophage infiltrations and heamosiderin deposits (Plate S6.2 and S6.3), necrosis on the splenic and interstitial cells, and hyperplasia of the splenic and interstitial tissue. Hyperplasia, heamosiderin deposition and macrophage infiltrations were highly prevalent in the three effluent impacted sites, of Murchison Bay, Kirinya and Masese followed by Winday Bay and least prevalent in the reference site (Table 6.9). Overall splenic and interstitial necrosis occurred at low prevalence and was rarely observed in the reference site as compared to the impacted sites (Table 6.9).

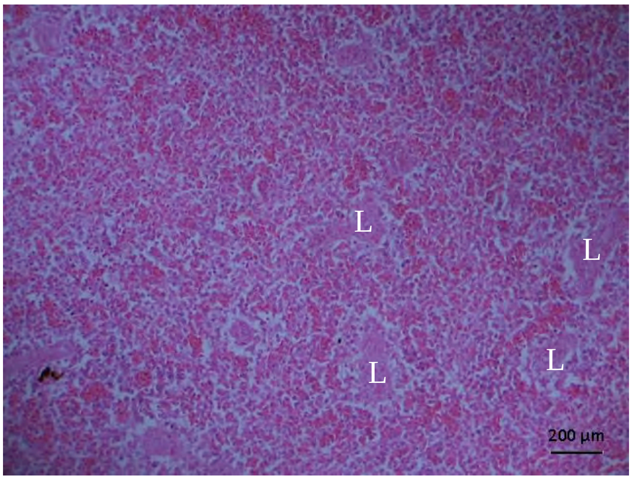


Plate S6.1a Section of normal spleen showing walls of the ellipsoids (L), interspersed in the red and white pulp

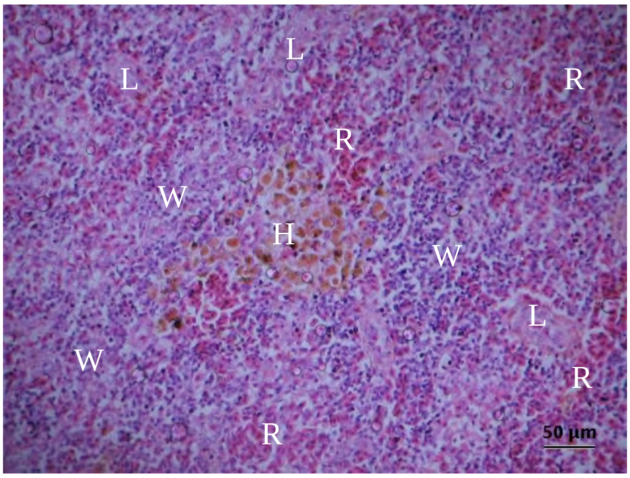


Plate S6.1b Section of spleen showing minimal haemosiderin deposits (H) in the red pulp and dominance of different sized lymphocytes (highly basophilic cells) in the white pulp (W), L = Elliposids, R = Red pulp

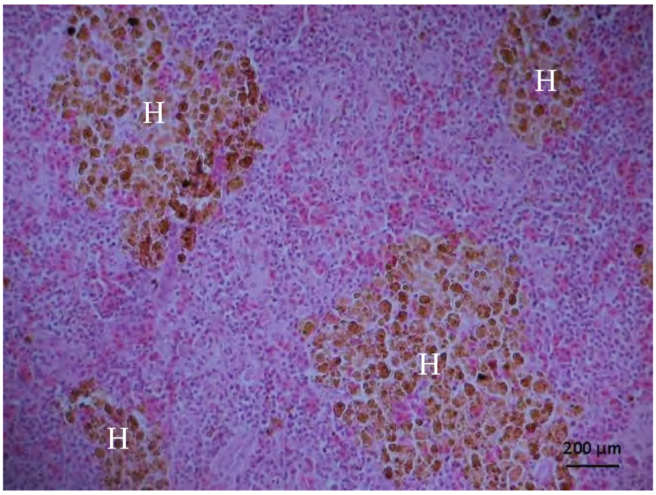


Plate S6.2 Section of spleen showing severe haemosiderin deposition (H)

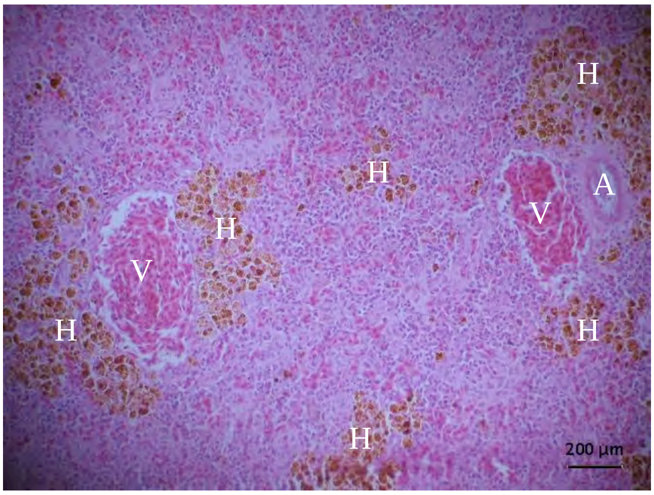


Plate S6.3 Section of spleen showing congestion of the venous blood vessels (V) surrounded by severe haemosiderin deposition (H), A is an artery

Table 6.9 Percent prevalence of the major histological alterations identified in the splenic tissue

Histological Alteration	**Lwanika** Reference (n = 42)	**Kirinya** (n = 49)	**Masese** (n = 37)	**Winday Bay** (n = 60)	**Murchison Bay** (n = 56)
Congestion of blood vessels	45	49	43	42	27
Deposits of heamosiderin	48	90	27	83	82
Splenic necrosis	0	41	11	2	43
Interstitial necrosis	5	22	16	5	41
Plasma alterations (Vacuolation)	5	37	22	8	7
Splenic tissue hypertrophy	33	6	62	43	30
Splenic tissue hyperplasia	45	86	68	27	64
Lymphoid tissue hyperplasia	49	96	84	80	89
Interstitial tissue hyperplasia	26	67	59	28	46
Infiltration (with macrophages)	55	98	92	75	88

6.4. Discussion

This chapter involved investigating fish histopathology as a biomarker of water quality deterioration in urban wetlands. A linkage between environmental water quality deterioration due to effluent and histopathological responses of *O. niloticus* in the study wetlands was established. Organ sensitivity and spatial diversity in histopathological alterations were established. The quantitative differences in spatial histopathology index grades, severity of reaction indices and lesion prevalence identified fish collected at effluent impacted sites from the less-altered reference site. In accordance with findings of similar studies else where, histopathological investigations were found to be a valuable tool in the assessment of water quality and the effects of pollution on fish, particularly at sub-lethal / chronic exposure (Bernet et al. 2004, 1999, Handy et al. 2002, Schwaiger et al. 1997, Teh et al. 1997).

6.4.1. Organ sensitivity based on histopathology of *O. niloticus* in the study sites

The histopathology organ index grades decreased in the order of liver > gills > gonads > spleen. However the gonads had higher regressive indices than the gills. Regressive reactions patterns are advanced pathological changes that depict processes which terminate in a functional reduction or loss of the organ as compared to progressive changes that reflect increased activity of the cells or tissue (Bernet et al. 1999). The low total organ index in the gonads could be explained by the absence of the circulatory disturbances, like haemorrhage and oedema which were not observed in the gonadal tissue. Based on the severity of reaction indices and lesion prevalence, organs were affected in decreasing order of liver > gonads > gills > spleen. This made the liver the most responsive in histopathological alterations and the most sensitive organ to environmental degradation followed by the gonads and gills. For example the percentage of fish in Class 4, with organ index > 41, showing severe alterations of organ tissue, was highest in the liver in all sites. For Class 3 with index 31 – 40, showing pronounced alterations of organ tissue, the percentages of fish were highest in the liver for Masese and Winday Bay and in gonads for Murchison Bay and Kirinya. The reference site registered lower percentages in these two classes for all organs. In addition the mean reaction indices were highest in the liver followed by gonads, gills and lowest in the spleen. The liver and gonads also registered higher prevalence of regressive lesions such as necrosis than the gills and spleen. The gills of fish from Murchison Bay, Masese and Kirinya, registered higher percentages of fish in Class 16 – 30, a class showing normal tissue structure with moderate histological alterations. The gill lesions in the effluent-impacted sites were therefore less severe compared to the liver and gonads. The higher histopathological sensitivity of the liver followed by gonads and gills makes these three organs better indicators of contamination exposure than the spleen in this study.

Severe effects in the livers of fish from contaminated environments have been reported in other studies (Bernet et al. 2004, Stentiford et al. 2003, van Dyk et al. 2009b, Handy et al. 2002, Schmidt et al. 1999, Hinton and Laurén 1990b). Cellular alterations in the liver can provide a good early indication of overall fish health and water quality status and has thus been applied in biomonitoring programmes (Stentiford et al. 2003, Myers et al. 1998). Laboratory and field-based studies have established the liver as a highly sensitive organ to contaminant exposure and toxicopathic lesion formation in many fish species exposed to different environmental conditions (Stentiford et al. 2003, van Dyk et al. 2009b, Handy et al. 2002, Naigaga 2002, Schmidt et al. 1999). For example in three-spined sticklebacks *Gasterosteus aculeatus* from several rivers in England (Handy et al. 2002); *O. mossambicus* exposed to copper in the laboratory (Naigaga 2002), and brown trout *Salmo trutta* exposed to sewage (Schmidt et al. 1999). The liver's high sensitivity could be attributed to its physiological functions. The liver is a key organ of overall homeostasis, in terms of nutrition, defence against toxicants and reproductive development (Hinton and Laurén 1990b). The poor blood perfusion in the liver of fish as compared to mammals may enhance the stasis of toxicants causing more damage in the liver tissue (Metcalfe 1998, Heath 1995, Hinton and Laurén 1990b). Thus, the liver is the first organ to encounter absorbed nutrients, vitamins, metals, drugs and environmental toxicants as well as waste products of bacteria that enter the portal blood (Moslen 1996). Efficient scavenging or uptake processes extract these absorbed materials from the blood for catabolism, storage, and and/or excretion into bile (Moslen 1996). Thus, the liver is the primary organ for biotransformation and excretion of xenobiotics.

Although this study could not establish the presence or concentrations of some selected toxicants in the environment, some of the observed histological alterations have been shown to provide definite biological end points of exposure to a number of specific contaminants (van Dyk et al. 2009a, 2009b, Williams et al. 2009, Bhattacharya et al. 2008, Getsfrid et al. 2004, Leino et al. 2005, Jobling et al. 2002, 1998). For example, hepatocellular foci of cellular alterations have been suggested to represent early stages in the stepwise formation of hepatic neoplasia and are recommended as an excellent example of a histopathological biomarker for contaminant exposure (Hinton et al. 1992). Cellular and nuclear pleomorphism, previously associated with contaminant-induced hepatocellular degenerative necrosis in flat fish and Baltic flounder (Lang et al. 2006, Stentiford et al. 2005, Hinton et al. 1992) was highly prevalent in this study. This lesion has been viewed as an initial toxipathic lesion resulting from exposure to toxic and carcinogenic chemicals (Hinton et al. 1992). Decreased vitellogenesis has been linked to the presence of endocrine-disrupting compounds (EDCs) in the environment (Williams et al. 2009, Jobling et al. 2002, 1998). Ovitestis/intersex, although reported to be spontaneous and normal in some fish species such as the Japanese medaka (Getsfrid et al. 2004), this condition has also been linked to exposure of the fish to EDCs (Williams et al. 2009, Jobling et al. 2002, 1998). Ovitestis was less prevalent and it could not be ascertained whether it was a result of exposure to EDCs. However, evidence of abnormal gonadal and hormonal changes in wild fish is a significant environmental and conservation issue (Jobling et al. 2002, 1998). Research is thus needed to determine the levels of EDCs and the extent of their disruption in fish in Murchison Bay and Kirinya where this condition was observed, so as to understand the exact cause-effect relationship for the observed ovitestis.

Impaired organ structure and function can directly affect survival, growth and reproduction of the affected organism and effects manifested at population and ecosystem levels (Vajda et al. 2008, Wahli et al. 2007, Hinck et al. 2006, Leusch et al. 2006, Zha et al. 2006, Nash et al.

2004, Jobling et al. 2002, 1998, Kwak et al. 2001, Naglar and Cyr 1997, Teh et al. 1997, Kocan et al. 1996). For example, the liver degenerative changes with severe alterations as observed in some fish from Murchison Bay and Kirinya showed regressive changes like nuclear alterations, atrophy and necrosis which if not reversed may terminate into organ hypofunction or loss of the organ (Bernet et al. 1999). These changes are related to the damage to cellular organelles like mitochondria and are likely to interfere with several liver functions such as impairment of storage and interconversion of foodstuffs, impairment of absorption and excretion of waste products and impairment of function of other body systems. Similarly degeneration or malfunction of the ovaries reduces the female fecundity and reproductive potential (Rideout and Burton 2000). The histopathological changes associated with female gonadal lesions such as pre-ovulatory atresia is indicative of disruption of ovarian development and or spawning and is suggestive of the female missing a spawning opportunity implying the presence of endocrine disruptors in the environment (Leino et al. 2005). However follicular atresia may also be part of a normal process in fish such as was shown for fathead minnows (Leino et al. 2005). Although ovitestis was observed in a few fish in the effluent-impacted sites, this morphological abnormality is a pathological condition where fish contains both male and female sex cells also known as intersex (Williams et al. 2009, Jobling et al. 2002, 1998).

The most prevalent lesions in the gills in all study sites were progressive in nature depicting an increase in cellular activity and included epithelial cell and interlamellar basal cell hyperplasia leading to partial or complete fusion of the secondary lamellae. Hyperplasia of the gill epithelium is a measure to protect the gill filaments from any irritation in the surrounding water while interlamellar cells are involved in the regeneration of the secondary lamellar epithelium (Roberts 1989). A proliferation of these cells indicates an increased cellular metabolism directed towards repair of subcellular damages or detoxification (Roberts 1989). Epithelial hyperplasia and lamellar fusion were also observed in the gills of *Clarias gariepinus* from polluted South African urban aquatic systems (van Dyk et al. 2009a); rosy barb *Pethia conchonius* exposed to nonylphenol (Bhattacharya et al. 2008); brown trout *Salmo trutta* exposed to sewage effluent (Bernet et al. 2004). Fusion of the lamellae reduces the functional surface area for oxygen uptake. Lamellar oedema, a circulatory disturbance was highly prevalent in Murchison Bay. Lamellar oedema, lamellar hyperplasia and lamellar fusion are usually observed when there is a severe irritant stimulus (Roberts 1989). Regressive changes in the gills were mainly observed in the effluent-impacted sites and included epithelial cell necrosis.

One of the most outstanding observations in the spleen was that the largest proportion of fish in all the study sites was observed under the two normal and recoverable classes 1 and 2. This shows that the spleen was the least sensitive and that it was less impacted by environmental effects than the liver, gonads and gills. The most prevalent lesions in the spleen were progressive and inflammatory in nature and included lymphoid tissue hyperplasia and macrophage infiltration, respectively. Progressive changes depicted the increased activity of the splenic tissue (red and white pulp), interstitial tissues and lymphoid tissue. The lymphoid tissue was composed of dense accumulation of small round cells, with relatively large nuclei and scanty cytoplasm. It serves as a precursor to all the cellular constituents of the blood (Yoffey 1929). However, in the Murchison Bay and Kirinya, regressive changes such as cellular and tissue necrosis were observed although not highly prevalent. These changes affect the splenic ability to elicit immune responses. Deposits of heamosiderin with melanomacrophages were highly prevalent in Murchison Bay, Kirinya and Winday Bay. This lesion depicts increased phagocytosis of erythrocytes (Fänge and Nilsson 1985).

6.4.2. Spatial differences in the histopathology of *O. niloticus* in the study sites

Although there are multiple environmental stressors in the aquatic environment which can alter the structural integrity of critical tissues and organs, the results in this study could link the observed lesions to water quality aetiology. The majority of fish from the effluent impacted sites of Murchison Bay and Kirinya registered higher histopathology index grades, severe reaction indices and higher advanced lesion prevalence than the reference site. Consequently, the two sites registered the highest percentage of fish under the pathological grades 3 and 4. In addition the mean reaction indices in all the four observed reaction patterns (circulatory, progressive, regressive and inflammatory) were highest in Murchison Bay followed by Kirinya. According to the Bernet et al. (1999) protocol applied in this study, the higher indices imply that the degree and extent of alterations of lesions under the respective indices were mostly severe / diffuse lesions. Murchison Bay and Kirinya also had the highest prevalence of regressive changes which included necrosis and nuclear alterations in the study organs. Based on these results, these two sites' environmental water quality is deemed to be more degraded compared to Masese, Winday Bay and the reference site.

The severe histopathological responses observed in the effluent-impacted sites is comparable to field-based studies conducted elsewhere that reported severe histopathological characteristics in fish from highly contaminated environments (van Dyk et al. 2009a, 2009b, Paolini et al. 2005, Bernet et al. 2004, Schwaiger et al. 1997, Teh et al. 1997). This study looked at *O. niloticus*. Severe histopathological alterations have been reported in other fish species in contaminated environments. Such species include *C. gariepinus*, *C. ngamensis*, *O. andersonii* and *S. angusticeps* (van Dyk et al. 2009b); *Leuciscus cephalus* (Paolini et al. 2005); *Salmo trutta* (Paolini et al. 2005, Bernet et al. 2004, Schwaiger et al. 1997); *Lepomis* *auratus* and *Micropterus salmonidae* (Teh et al. 1997). Although the sensitivity of different species to pollution may differ, the consistence of higher lesion intensity in heavily contaminated waters was apparent. For example, trout exposed to river water supplemented with treated waste water from the sewage treatment works (STW) showed higher histopathological indices than trout caught upstream of the discharge point of the STW and that kept in river water only (Bernet et al. 2004). More severe gill pathology, reflected in a higher gill index was observed in *C. gariepinus* sampled from sites with higher heavy metals in the water and sediment (van Dyk et al. 2009a). This goes to support the suggestion that histopathological investigations are a sensitive tool in the assessment of toxic effects of pollutants in the aquatic environment.

Low histopathology indices and lesion prevalence were apparent in the reference site, Lwanika. However, *O. niloticus* from Winday Bay, a bay that is not subjected to direct effluent discharge also showed a considerable degree of organ histopathology different from that reported in the reference site. This difference could be attributed to the bay's location which happens to be in the same gulf but adjacent to Kirinya and Masese. It is likely that the impacts of the waste water coming through Kirinya and Masese impacts the entire Napoleon gulf. Additionally, considering that this study employed passive biomonitoring approaches, migration of fish could also have played a role whereby the location in which the fish was sampled (in this case Winday Bay), was not necessarily the site that the fish had inhabited for a long period. Winday Bay shares the same gulf (Napoleon gulf) with Kirinya and Masese; it is possible that the fish in the bay might have been exposed to a different water quality before getting to Winday Bay.

Based on fish histopathology indices and lesion prevalence, the sites environmental degradation decreased in the order of Murchison Bay > Kirinya > Masese > Winday Bay > Lwanika (the reference site). These results complement previous results observed in chapters three, four and five that highlighted Murchison Bay and Kirinya wetlands (mostly the inshore location) as the most degraded in terms of poor water quality (chapter three). These two sites were also associated with stenotopic and eutrophic invertebrate taxa of pollution tolerant Chironomidae and Oligochaeta (Chapter four) and low species diversity especially in Murchison Bay where many native haplochromines were absent (chapter five). It is thus observed that effects of environmental degradation were observed at community level and cellular level.

6.5. Conclusions and recommendations

In conclusion, the results of this chapter described effects of the water quality deterioration due to urban effluent on the histology of liver, gills, gonads and spleen of *O. niloticus*. The histopathological responses clearly differentiated *O. niloticus* exposed to the less polluted environments from those of the more polluted environments. Organ lesions in the reference site were mainly restricted to slight alterations which were classified under the normal grades. In the sites highly impacted with urban effluent, *O. niloticus* showed higher histopathological indices and more than 50% of the fish were classified under the two pathological grades. This indicates that either irritants in the waste water effluents (e.g. toxicants, insoluble substances), or the changed physicochemical conditions due to the effluents as was observed in chapter three (e.g. electrical conductivity, dissolved oxygen, pH, nutrients) had a detrimental impact. However, the study could not establish a causal link between histological lesions and specific contaminants in the water. Given severity of histological observations observed in Murchison Bay and Kirinya, especially in the liver and gonads, this study recommends analysis of effluent at these sites for toxicant-specific assessment especially of endocrine disruptors. The fish especially in Murchison Bay could be at risk of reproduction interruption if the pollution in this site continues. The liver, gonads and gills were good indicator organs for assessing water quality deterioration because of their higher sensitivity to environmental deterioration and lesion development. Relating the results observed in this study to the pollution assessment literature, the following lesions stand out as potential biomarkers of water quality deterioration in urban wetlands in Uganda using *O. niloticus* as an indicator species: hepatocellular foci of cellular alterations, hepatocellular and nuclear pleomorphism; ovitestis/intersex, pre-ovulatory oocyte atresia and hyperplasia of the perifollicular cells; Lamellar oedema, lamellar hyperplasia and lamellar fusion. This study recommends the use of histopathological investigations in the assessment of aquatic ecosystem contamination as part of a comprehensive biomonitoring programme.

CHAPTER 7

General discussion and application of fish and invertebrate bioindicators and fish histopathology biomarkers in water quality assessment of urban wetlands in Uganda

Pollution of aquatic ecosystems has become a global concern but nonetheless most developing nations like Uganda are still producing vast pollution loads and the trends are expected to increase with the increase in population size and urbanisation. Knowledge of pollution sources and their impacts on ecosystems is important not only for a better understanding of the ecosystem's responses to pollutants but also in formulating remediation and mitigation measures. Lake Victoria wetlands, swamps, satellite lakes and shallow bays in Uganda have registered a resurgence of several endemic fish species that were on the brink of extinction (Balirwa et al. 2003, Chapman et al. 2002, Witte et al. 2000). However, the evidence collected in this study highlights that environmental changes as a result of industrial and domestic wastewater effluent and storm water runoff may reduce fish access to the wetland refugia grounds in urban water bodies in a number of ways. Such ways include 1) Direct effects to the fish as was observed by the histopathological responses in the liver, gonads and gills which may interfere with the fish's growth and reproductive potential. 2) Poor water quality which may not be favourable for some endemic fish species as was observed in Murchison Bay where many endemic species that were once observed in the bay were missing. 3) Impacts on the food chain as was observed by the invertebrate taxa present and biodiversity changes in the highly polluted sites.

Assessment and mitigation of the environmental impacts on water quality and biodiversity have now become necessary. The past two decades have seen many researchers recommend the establishment of a biological monitoring system for water quality assessment in Uganda.

Although much research has been done in the area of bioindicator-biomarker application in water quality assessment, little has been done to develop these indicators and apply them for water quality assessment in the water bodies of Uganda. This gap in knowledge has been addressed by identification of fish and invertebrate bioindicators and fish histopathology biomarkers for use in water quality assessment. The study provided an integrated assessment of water quality in four urban wetlands variably impacted by urban effluent and one rural reference wetland in Uganda.

The aim of the study was to integrate invertebrate and fish as bioindicators and fish histopathology as a biomarker in the assessment of water quality deterioration in urban wetlands in Uganda. This is the first time that macroinvertebrate and fish bioindicators and fish histopathology biomarkers are integrated in one biomonitoring tool. Unlike previous studies which considered these aspects in isolation, the integration gives a unique perspective that counteracts the shortfall of the isolated cases giving the tool greater predictive power of the effects of environmental change.

Urban effluents were the main source of direct and continuous input of environmental contamination in the study sites. Due to the unknown, complex and often highly variable composition of xenobiotics in effluents and their antagonistic and synergistic impacts, relating the observed effects to specific pollutants or classes of pollutants remains a difficult task. In addition, pollution impacts occur at several biological levels ranging from the biochemical level, to causing effects at the individual level, community level and ultimately impacting the whole ecosystem (Wepener et al. 2005, Van der Oost et al. 2003). Under these circumstances, an approach integrating multiple endpoints at different biological organization levels should give a more detailed understanding of the effects and extent of pollutant exposure to the aquatic organisms. The approach also gives an understanding on how responses at different levels are interrelated thereby establishing a connection between effluent exposure, organ tissue response to contamination and effects at community level. This study is among the few studies in the region that allowed quantification of changes in fish and benthic macroinvertebrate diversity in relation to environmental changes, and the first to employ histopathology techniques in aquatic environmental biomonitoring.

In this study effects of effluent exposure were determined using:

1. Physicochemical variables commonly available to the water resource managers. Here, study sites were characterised based on their physicochemical properties and possible underlying factors that influenced the physicochemical variables in the study sites were identified.
2. Invertebrate taxa and fish community structure in relation to the water quality physicochemical variables. The invertebrate taxa and fish species were explored as biological indicators of water quality deterioration in wetlands in Uganda.
3. Multi-organ fish histopathology. Histopathology of the liver, gills, gonads and spleen of *O. niloticus* was evaluated and compared in terms of the fish histopathology indices and lesion prevalence in relation to environmental contamination.

Results suggested that the approach of using invertebrate and fish as bioindicators and the fish histopathology as a biomarker, in relation to water quality physicochemical variables was a useful tool in highlighting the spatial differences in environmental quality. The effluent-impacted sites differed from the less impacted reference site, highlighting Murchison Bay and Kirinya inshore as sites in need of intervention. The two sampling locations at Murchison Bay (inshore and offshore) and one sampling location at Kirinya (inshore), that were highly impacted with urban effluent, showed elevated nutrient levels, low pH, dissolved oxygen and secchi depth readings. This corresponded with low invertebrate taxa and fish species diversity and richness; and severe histopathological responses in liver, gonads and gills of *O. niloticus*.

These two sites are deemed to be highly polluted as compared to the reference site. This was underlined in the poor water quality, low invertebrate taxa and fish species diversity and richness and severe fish histopathological responses as compared to the reference site. These variables were moderately influenced in Masese and Winday Bay. Masese is impacted with domestic effluent while Winday Bay does not receive effluent discharges, and lies adjacent to Kirinya and Masese sharing the same gulf. Indicator responses in Winday Bay were obscure at physicochemical and bioindicator levels but were detectable at the histopathology biomarker level, highlighting that this approach can be an early warning signal of water quality deterioration. In contrast the reference site of Lwanika showed higher pH, dissolved oxygen and secchi depth readings, and relatively low nutrient levels, higher invertebrate taxa and fish species diversity and richness, and histological responses that fell within the normal range. The reference site was consistently classified as the least polluted among the five wetlands studied.

Proposed strategy for the integrated application of fish and invertebrate bioindicators and fish histopathology biomarkers in water quality assessment of urban wetlands in Uganda

As was discussed in chapter one, the current environmental water quality assessment in Uganda is mainly based on physicochemical variable analysis, which is also irregular due to limited financial resources. The ability of various pollutants and their interaction to exert their toxic actions complicates the process of water quality management based solely on chemical or physicochemical analysis. To help restore and maintain the biological integrity of Uganda’s urban waters, this study proposes that biological surveys should be fully integrated with physicochemical variable assessment in national water quality assessment programs. This study thus proposes the integration of bioindicators, biomarkers and physicochemical variables as a comprehensive approach to water quality management in urban water bodies. Although each of these variables can independently provide some but limited assessment of designated aquatic system impairment, their integration counteracts the shortcomings of each method and thus builds a more robust predictive tool that gives a better view of the impacts to the entire ecosystem. This is the first time such an integrative and comprehensive tool is being studied in water assessment procedures in Uganda, and the region at large.

Biomarkers show contaminant-induced impacts at sub-organism / organ level before population or community level responses become apparent, making them more sensitive as early warning signals. On the other hand, change in the community / population structure are indicative of severe stress that has already occurred thus providing ecological relevance but offering no early warning of potential effects (Adams et al. 2005, Wepener et al. 2005, Van der Oost et al. 2003). Thus biomarkers alone or community structure of macroinvertebrates or fish alone may not provide a good representation of the response of an ecosystem to anthropogenic impact. In addition physicochemical variables alone are less sensitive than structural properties such as species community structure, as they also offer uncertain biological and toxicological relevance. Therefore, the differential response characteristics of various individual measurements are a strong argument for incorporating multiple indicators to maximize response sensitivity and ecological relevance in biomonitoring program designs. A biomonitoring approach incorporating multiple indicators in one tool has not been explored in the Ugandan part of the Lake Victoria basin and the East African region in general. This study thus offers a unique advantage incorporating early detection and prediction of eventual impact of current or prior long-term exposure.

This comparative study provided an opportunity to assess the effectiveness of integrating various ecologically relevant endpoints as indicators of urban effluent stress on five Lake Victoria wetland ecosystems. Good water quality provision and consequently sustainable productivity of Lake Victoria requires a multifaceted approach in the management of its wetlands. This could be achieved through constant monitoring of the wetland physicochemical variables, fish food sources and the fish itself. The study thus proposes the following strategy of integrating the invertebrate and fish bioindicators and fish histopathology biomarkers in water quality assessment of Uganda urban wetlands. It is hypothesized that the proposed concept should be applicable to other ecosystems in this ecoregion and may be modified to other ecogeographical zones.

- Lake Victoria being highly eutrophic, and a continuous recipient of untreated sewage, runoff and storm water, nutrient levels and water quality physicochemical variables such as temperature, pH, dissolved oxygen, conductivity etc. should be assessed regularly. These variables give insight into the water quality status at a given point in time and they further influence the biodiversity. In order to indicate their impact at biological level, they should thus be complemented with biological measures at different biological organisation levels (Figure 7.1).

- Pollution contamination in Uganda is mainly sub-lethal and chronic. Fish histopathological biomarkers being sensitive at sub-lethal levels should be incorporated in biomonitoring programmes as early warning signals (Figure 7.1). Fish play an ecological role in the aquatic food-webs because of their function as a carrier of energy from lower to higher trophic levels. Therefore, the understanding of toxicant responses in fish populations may have a high ecological relevance. One of the limitations of fish histopathology in field-based studies is to obtain a suitable sample size of live fish. Given the histopathology rapid response time, if a large sample size of live fish can be obtained in a shorter time, the results can almost be immediate, especially in cases where seasonal variation is not significant. In this study the sample size ranged from 37 to 60. Fish histopathology also has the advantage of linking lower and upper levels of biological organization, thus strengthening the observations at population level.

- Macroinvertebrates are ideal components of a natural resource water quality management programme and should be considered in the assessment of Uganda's urban water bodies. Their predictable response to water quality deterioration and their limited migration patterns make them good indicators of water quality. Invertebrate bioindicators also have an advantage of providing information at community / population level thus giving insight into the ecosystem status in a relatively shorter time. In this study, the maximum number of taxa (asymptone) was achieved at the fifth time (10 months) of sampling in both effluent impacted and non effluent impacted sites. This study thus proposes a minimum of 10 months sampling time in the use of benthic macroinvertebrates as bioindicators of environmental water quality (Figure 7.1).

- Fish as bioindicators of water quality should be incorporated into biomonitoring programmes in Uganda to give an indication of the long-term impacts of pollution on an ecosystem (Figure 7.1). Fish unlike macroinvertebrates rarely establishes itself and flourish within the constraints of the anthropogenic stress (Van der Oost et al. 2003), thus making fish good indicators of water quality that complements macroinvertebrate community studies. Although sampling for this study was conducted 12 times in 24 months, fish species asymptone was achieved after 16 months (8 sampling times) in effluent impacted and 10 months (5 sampling times) in non-effluent impacted sites. This study thus recommends a minimum of 16 months sampling in using fish as bioindicators of environmental water quality.

- This study also recommends the use of *O. niloticus* as a source of histopathology and other biomarkers for environmental water quality assessment in Uganda and the region because of its abundance and wide distribution indicating its ability to thrive in varied environmental conditions.

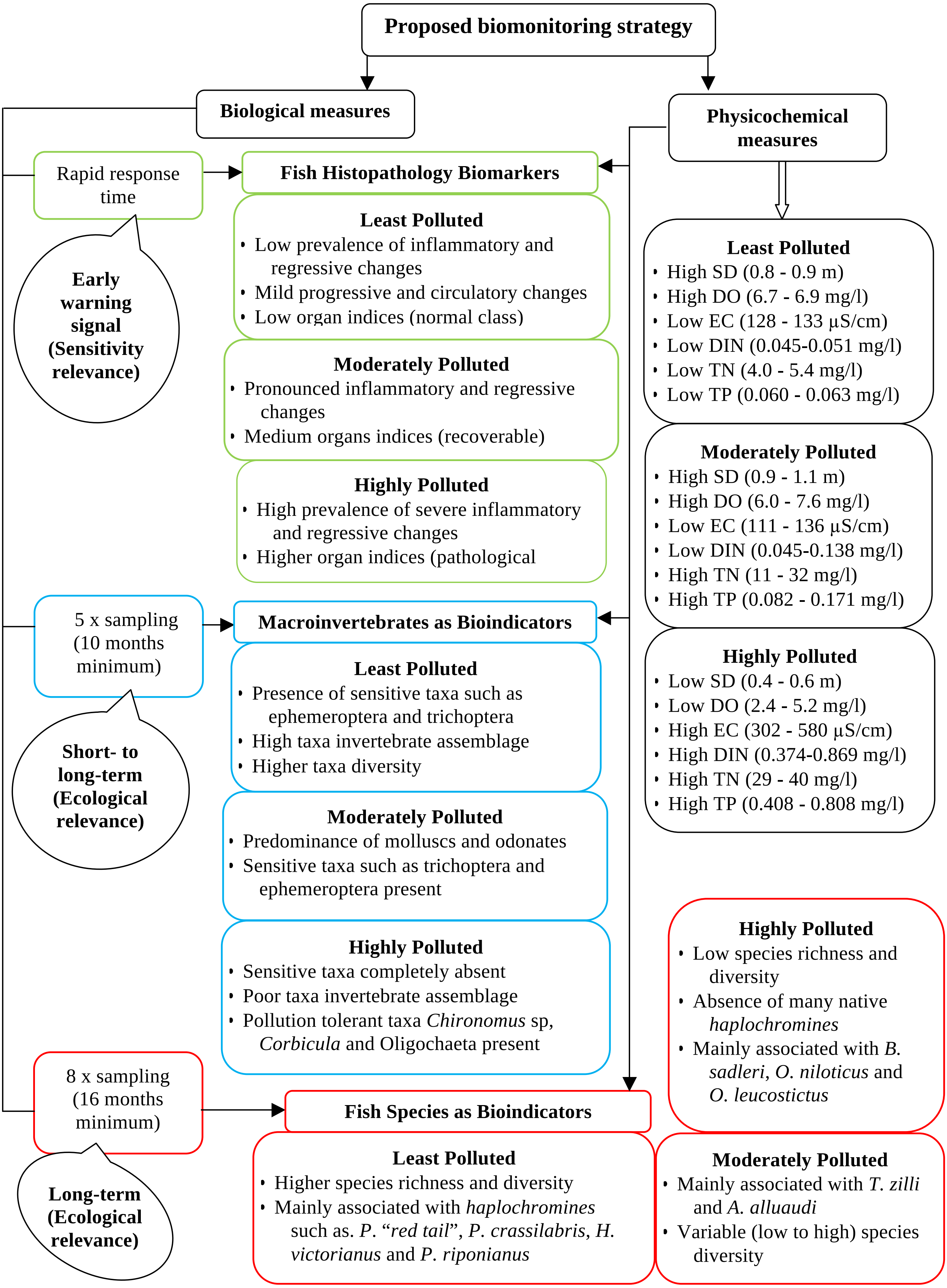


Figure 7.1 Framework for the proposed biomonitoring strategy of urban-wetland water quality assessment in Uganda

Conclusion

In conclusion, the evidence presented in this thesis suggests that, although there is wide-spread eutrophication of Lake Victoria, catchment environmental degradation with effluent and the resultant deterioration of water quality on the Ugandan side appeared to be localized. This study showed urban effluent as an important pollution source that caused deterioration in physicochemical variables, diversity and richness loss of invertebrate taxa and fish species, and severe histopathological responses in fish from effluent-impacted sites. Effluent-induced environmental changes could have led to ecological changes and environmental degradation of the lake, thereby rendering the environment unfavourable to some macroinvertebrate taxa and fish species in the effluent-impacted sites as was observed in Murchison Bay. Based on this study's assessment, Lwanika (the reference site) was considered to be the least polluted, followed by Winday Bay and Masese classified as medium polluted. The pronounced histopathological responses in the moderately polluted sites showed that these sites are being polluted and this calls for regular monitoring and mitigation strategies to abate further degradation. Murchison Bay and Kirinya were found to be highly polluted and are thus deemed to require immediate intervention so as to reduce the pollution load.

Even though the application of biomonitoring for Ugandan aquatic systems faces a setback of inadequate studies documenting bioindicators and biomarkers of water and habitat degradation, the present study sets a good basis for developing management strategies for urban water bodies. The multiple indicators used (physicochemical variables, invertebrate and fish community structure, and fish histopathology) encompassed a range of temporal and biological scales that together yielded a robust outcome with differential sensitivity and ecological relevance in spite of the study being short-term. The proposed biomonitoring strategy with further testing, modification and refinement could be applied in assessment programmes of environmental water quality deterioration in urban ecosystems.

A summary of recommendations

This study recommends biomonitoring data to become an important resource for water pollution management decisions in Uganda. In order to advance biomonitoring approaches in water quality assessment in Uganda, this study gives the following recommendations:

1. One of the major problems encountered during the study was the insufficient taxonomic knowledge for both fish and macroinvertebrates with direct consultations of experts having to be carried out in many cases. For example, unidentified fish species in the field were preserved and taken to the National Fisheries Resources Research Institute (NaFIRRI) for consultations and identification by the help of the fish experts. Efforts should be made to provide fundamental knowledge and expertise necessary to support biomonitoring approaches in Uganda. To this effect this study recommends:
    - Local invertebrate and fish fauna should thus be inventoried and described as the distribution of many invertebrate taxa and haplochromine fish species remain undescribed. Easy to use field guides and manuals with illustrated keys and photographs should be published for use by the biomonitoring technical teams so as to overcome taxonomic challenges.
    - Training and equipping of personnel in biomonitoring techniques so as to ensure availability of skilled technicians responsible for water quality monitoring who can inform management and regulatory decisions.
    - Creation of a database on the distribution, abundance, and temporal characteristics of fish species and macroinvertebrate taxa in Uganda. Results from this study have been limited to one ecoregion. Macroinvertebrate and fish bioindicators and

fish histopathology biomarker indices of different ecoregions should be determined.

2. With the increasing interest in biomonitoring in the region, protocols for biomonitoring that are being developed, including findings from this study should be tested and revised to improve their predictive power and ensure accurate applicability in different aquatic systems. For example, future biomonitoring studies should consider incorporation of functional aspects of the bioindicator assembladge composition and the influence of other factors such as macrophyte quality in structuring macroinvertebrate and fish communities for Lake Victoria's embayment wetlands. In addition, active biomonitoring using *O. niloticus* as a sentinel species should be carried out to test and confirm the predictive power of the observed histopathology biomarkers.
3. This study recommends regular monitoring of the reference site, Winday Bay and Masese for maintenance purposes, and immediate intervention in Kirinya and Murchison Bay to mitigate further degradation and restore the degraded habitats. Toxicant-specific assessment should be conducted in Murchison Bay and Kirinya especially for endocrine disrupting compounds given the histopathological responses observed in the gonads of *O. niloticus*. In the meantime proper implementation strategies and enforcement of existing wastewater discharge standards should be enforced.

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