Assessing the effects of crocodile farm effluent on benthic macroinvertebrates diversity at effluent disposal points on Lake Kariba

Michael Tonderayi Machingura
R965814C

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Supervisor: Dr C. Phiri
Co-supervisor: Mrs Z.Jiri

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DECLARATION

I, Michael Tonderayi Machingura, declare to the senate of Bindura University of Science Education that this dissertation is my original work and all other sources of material used are duly acknowledged. This work has not been submitted to any other university for any academic award.

...........................................
Signature

Michael Tonderayi Machingura
Bindura University of Science Education
Dept of Biological Sciences
Zimbabwe
June 2011
DEDICATION

This work is dedicated to my wife Erika who bore so much in my absence but soldiered on.

To my sons Nyasha, Tonderayi (Jnr), Tatenda and Tadiwa; from you, nothing less than this brings joy to your father’s heart.

To my late father for post-humously contributing to financing this endeavour.
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ABSTRACT

The proliferation of aquacultural ventures in the last decade within the Sanyati Basin of Lake Kariba has made it imperative that ecological surveys be made so as to assess the effect of these activities on the lake. The study assessed the effects of crocodile farm effluent on benthic macroinvertebrates diversity at effluent disposal points on Lake Kariba. The toxicity of crocodile farm effluent was also evaluated by means of 24 hour assays using a decapod, *Caridina nilotica*. The deep scoop net and snail scoop methods were used in the field to sample two perturbed sites and a relatively pristine site for abundances and diversity of benthic macroinvertebrates. Water physico-chemical parameters were also measured for the three sites during the four months sampling period. Principal components analysis (PCA) showed that ammonium, turbidity, orthophosphate, Total Phosphorous and conductivity were the key components that separated the three sites with respect to physico-chemical attributes. The richness index (ANOVA, $F = 29.84, P < 0.05$), the Shannon Wiener index (ANOVA, $F = 19.67, P < 0.05$) and Pielou’s evenness index (ANOVA, $F = 10.80, P < 0.05$) showed a significant difference between the two perturbed sites and the control. The post hoc pair wise test showed that the control site was significantly different from the two discharge sites ($P < 0.05$). The lethal concentration ($LC_{50}$) for whole effluent on *Caridina nilotica* was 3.11% effluent concentration. The crocodile farm effluent showed high toxicity to *C. nilotica* in the bioassays. This collaborated with the field observation of low diversity in the perturbed areas which were dominated by the pollution tolerant gastropod *Melanoides* and *Chironomidae* larvae. The study showed that crocodile farm effluent has a significant effect in shaping the benthic macroinvertebrate community within the confines of the effluent disposal points.
# TABLE OF CONTENTS

- Declaration ........................................................................................................... i
- Dedication ............................................................................................................. ii
- Acknowledgements ............................................................................................... iii
- Abstract ................................................................................................................ iv
- Table of Contents .................................................................................................. v
- List of Tables ....................................................................................................... vii
- List of Figures ..................................................................................................... viii
- List of Appendices ............................................................................................... ix

## Chapter I Introduction

1.1 Background ........................................................................................................ 1
1.2 Assumptions ....................................................................................................... 3
1.3 Hypotheses ......................................................................................................... 3
1.4 Research Questions ........................................................................................... 4
1.5 Rationale ........................................................................................................... 4
1.6 Aim .................................................................................................................... 5
1.6.1 General Objective ......................................................................................... 5
1.6.2 Specific Objectives ....................................................................................... 5
1.7 Definition of Terms .......................................................................................... 6

## Chapter II Literature Review

2.1 Water Pollution ................................................................................................. 10
2.2 Aquaculture Pollutants ..................................................................................... 10
2.3 Eutrophication .................................................................................................. 13
2.4 Physico-chemical Parameters .......................................................................... 14
2.5 Effluent Management ....................................................................................... 17
2.6 Biomonitoring .................................................................................................. 19
2.7 Bioassessment Systems .................................................................................... 21
2.8 Use of Benthic Macroinvertebrates ................................................................ 25
2.9 Laboratory Toxicity tests ................................................................................ 27
2.10 Aquaculture on Lake Kariba ........................................................................... 31
2.11 Related Studies ............................................................................................... 32
LIST OF TABLES

Table 1 Sites description and codes......................................................36
Table 2 Summary of physico-chemical variables measured during this study......44
Table 3 Loadings for environmental variables...........................................47
Table 4 Percent relative abundances of taxa collected during the study...........48
Table 5 One-way ANOVA results for community indices.........................49
Table 6 T-test results of settling pond physico-chemical parameters............54
LIST OF FIGURES

Figure 1 Location map of study area and sites ..................................................35
Figure 2 PCA scatter plot for environmental variables .................................46
Figure 3 Community indices ...........................................................................50
Figure 4 Multidimensional scaling plot .........................................................51
Figure 5 Hierarchical clustering of macroinvertebrate assemblages ...............52
LIST OF APPENDICES

APPENDIX A – Physico-chemical variables ANOVA, Tukey post test results……74
APPENDIX B - Community indices values.....................................................75
APPENDIX C – Community indices ANOVA, Tukey post test results.........76
APPENDIX D - Mortality data of Caridina nilotica.........................................77
APPENDIX E - Probit analysis print out..........................................................78
CHAPTER 1

1 INTRODUCTION

1.1 Background

Pollution of surface freshwaters is one of the greatest environmental issues in the world (Lobo et al, 2004). Eutrophication is the most widespread form of lake pollution and has many deleterious impacts on aquatic systems (Harper, 1992 in Poulkova et al, 2004). Aquatic pollution has thus become a global concern, but even so, most developing nations are still producing huge pollution loads, and the trends are expected to increase. Pollution from both domestic and agricultural sources is on the increase. Agricultural activities are reported to contribute about 50% of the total pollution source of surface water by means of higher nutrient enrichment (Islam and Tanaka, 2004).

Knowledge of the pollution sources and impacts on ecosystems is important not only for a better understanding of the ecosystem responses to pollutants but also for the formulation of preventive measures. It has become common practice to use aquatic biota to assess the impact of human activities on freshwater resources (Woodcock and Huryn, 2007). The reason for this is that animals and plants can provide a long–term integrated reflection of water quality, quantity, habitat quality and other environmental conditions. In all countries, biological monitoring can provide an estimate of all deleterious influences on aquatic habitats, but it may be particularly useful in developing areas as it frequently has low cost and technical requirements. In recent years, there has been
increased in interest in rapid assessment techniques for the biological monitoring of water quality in several developed countries. These methods emphasize a low cost approach based on reduced sampling, and more efficient data analysis, making them attractive for use in developing countries (Thorne and Williams, 1997). Benthic macroinvertebrates (BMI) are considered valuable indicator organisms of the biotic integrity of water bodies (Galdean et al, 2000). Biological monitoring complements conventional physico-chemical analyses.

In Zimbabwe, water is one of the key natural resources. It is one of the major limiting factors when it comes to economic growth and social development. It is a scarce resource that is unevenly distributed both temporally and spatially. Lotic systems, like the Mukuvisi and Manyame Rivers, have been negatively impacted by pollution in Zimbabwe (Magadza, 1997; Phiri, 2000; Moyo and Phiri, 2002). Lentic systems have also been affected, with the Lake Chivero case well documented (Moyo, 1997; Nhapi, 2009). As the demand for water increases, with increasing human populations and economic development, so does the pollution of the aquatic ecosystems. In Zimbabwe, water quality control and biomonitoring has been more of a fire fighting approach, with action being taken only after a mishap like the infamous fish kills at Lake Chivero (Moyo, 1997).

Lake Kariba, along with its social and economical importance, has immense ecological importance, as it holds great biodiversity, including endangered biota. The main anthropogenic impacts on the lake include fisheries, boating and aquaculture in form of
fish farming (Troell and Berg, 1997) and crocodile ranching. Crocodile farming activities on the lake have undergone drastic expansion over the last two decades, and there is great possibility of expansion in the activity within this decade. No water quality and effluent disposal monitoring system is in place at the crocodile farms. The crocodile effluent is likely to cause eutrophication within the confines of the area of discharge in the lake. To effectively manage and conserve Lake Kariba, it is essential to monitor and assess the effect of crocodile farm effluent disposal on water quality and aquatic biota. Field observatory assessment, coupled with a laboratory bioassay on the species *Caridina nilotica* that was absent in effluent prone zone but abundant in the reference area, formed the basis of this study.

1.2 Assumption

It was assumed that benthic macroinvertebrates will decrease in crocodile effluent prone areas due to the resultant nutrient increase in these areas (paradox of enrichment) (Sabater *et al.*, 2006), while other aquatic organisms will be attracted to the nutrient bloom and flourish as pollution tolerant species.

1.3 Hypotheses

Crocodile farm effluent has an effect on abundance, evenness and diversity of benthic macroinvertebrate community structure in Lake Kariba.

Crocodile farm effluent has an effect on water quality in Lake Kariba.

Crocodile farm effluent has an effect on the mortality of *Caridina nilotica*. 
1.4 Research Questions

Is crocodile farm effluent affecting benthic macroinvertebrate populations? If so, how and to what extent is it affecting benthic macroinvertebrate diversity along the shoreline of Lake Kariba?

1.5 Rationale

Aquaculture in Lake Kariba started with crocodile farming at a small scale before the commencement of bream (*Oreochromis niloticus*) fish farming. Bream farming is mostly based on net pen culture on the lake, though land based pond activities exist at a smaller scale. The waste excreted by the fish in floating cages is assimilated by the biota in the surrounding body of water (Troell and Berg, 1997). The crocodile farms have been erected on dry ground along the shoreline of the lake, with water pumped in to fill shallow ponds in crocodile enclosures. This water is then directly drained back into the lake in form of untreated effluent. This water carries residue of crocodile feed and fecal deposits, and is not treated prior to discharge into the lake, save for solids settling in settling ponds. The untreated effluent pollutes the lake at the point of discharge, with ammonia, nitrates and suspended solids. These substances may contribute to the increase in biological oxygen demand, pH and conductivity in the lake, which in turn may have a possible impact on the ecological integrity of the system. The effect of crocodile farm effluent disposal into the lake, especially the shallow marginal waters, has largely been ignored. An uncontrolled discharge of waste into aquatic ecosystems is detrimental to
their integrity, and may negatively affect benthic macroinvertebrates, as enrichment does not always increase macroinvertebrate density or biomass (Sabater et al, 2006). Hence there is need for an assessment of the effects of crocodile farm effluent on the benthic macroinvertebrate diversity along the shoreline of Lake Kariba.

1.6 Aim

To assess the effects of aquaculture effluent disposal on aquatic biota.

1.6.1 General Objective

To assess the effect of crocodile farm effluent on benthic macroinvertebrate community structure along the shoreline of Lake Kariba.

1.6.2 Specific Objectives

-To determine the effect of crocodile effluent on water quality at the effluent disposal points.

-To determine the effect of crocodile effluent disposal on benthic macroinvertebrate abundance, evenness and diversity.

-To determine toxicity of whole crocodile effluent to a freshwater shrimp, Caridina nilotica.
To determine the effectiveness of the settling pond in pretreatment of crocodile effluent before disposal into the lake.

1.7 Definition of Terms

Acute toxicity test establishes the concentration required to kill a predetermined proportion of test organisms within a relatively short period of time, typically 4 days or less.

Aquaculture is the science of rearing aquatic animals or cultivating aquatic plants for food, aquarium, or scientific purposes, generally as a commercial venture. It is human cultivation of organisms in water (Asche and Khatun, 2006).

Benthic macroinvertebrates are animals without backbones that are larger than half a millimeter and are generally visible to the naked eye. These animals live on rocks, logs, sediment, debris and aquatic plants during some period in their life. The benthos include crustaceans such as fresh water shrimps, molluscs such as clams and snails, aquatic worms and the immature forms of aquatic insects such as stonefly and mayfly nymphs.

Bioaccumulation is the accumulation of contaminants by species in concentrations that are orders of magnitude higher than in the surrounding environment. Bioaccumulation is the sum of two processes: bioconcentration and biomagnification. Bioconcentration is the direct uptake of a substance by a living organism from the medium (e.g., water) via skin,
gills, or lungs, whereas biomagnification results from dietary uptake (MacDonald et al, 2003).

**Bioassay** is a test method employed in measuring response of living animal or plant tissue to the toxicity of chemical contaminants. In a bioassay, certain number of individuals of a sensitive species is exposed to specific concentration of the contaminant for a specific period to examine the toxic effects.

**Bioassessment** is the characterization of environmental conditions through the use of biological organisms (MacDonald et al, 2003).

**Bioindicators** are organisms whose presence, numbers, or intensity of development serves as an indication of some natural processes or environmental conditions.

**Biomonitoring** is the use of biological variables to survey the environment (Bonada et al, 2006). Organisms are used to assess or monitor environmental conditions.

**Biotic indices** are numerical expressions coded according to the presence of bioindicators differing in their sensitivity to environmental conditions, more or less a scale for showing the quality of an environment by indicating the types of organisms present in it.

**Chronic toxicity test** is a method used to determine the concentration of a substance that produces an adverse effect from prolonged exposure of an organism to that substance. A chronic toxicity test reveals the effects of a sublethal concentration applied throughout all
or part of the life cycle. In this test, mortality, number of young per female and growth are used as measures of chronic toxicity.

**Diversity index** is a statistic used in ecology to measure biodiversity in an ecosystem. It gives the relationship of the number of taxa or richness to the number of individuals per taxon or abundance for a given community.

**Effluent** is liquid or semisolid waste or material such as slurry or silage from a farm.

**Eutrophication** is a process where water bodies receive excess nutrients that stimulate excessive plant growth.

**Hypoxia** is a condition where there is insufficient oxygen.

**Lentic** system is a non-flowing or standing body of fresh water, such as a lake or pond.

**Lotic** system is a flowing body of fresh water, such as a river or stream.

**Monomictic** lake mixes from top to bottom during one mixing period each year.

**Oligotrophic** lake contains a low supply of nutrients in its waters.

**Sentinel species** are species whose disappearance or disturbance gives early warning of the degradation of an ecosystem.
Water quality describes the physical, chemical, biological and aesthetic properties of water, which determine its fitness for use and its ability to maintain the health of farmed aquatic organisms.

Whole effluent toxicity test is the total toxic effect of an effluent measured directly with a toxicity test.
2 LITERATURE REVIEW

2.1 Water pollution

Water pollution occurs when some substance degrades a body of water to such a degree that water cannot be used for a specific purpose (Garg, 2004). Sources of water pollution can be regarded as point sources and non-point sources, with the former including sewage-treatment plants, industrial plants, and animal feedlots (Boyd, 2003). Non-point source (NPS) pollution, unlike pollution from industrial and sewage treatment plants, comes from many diffuse sources. NPS pollution is caused by rainfall or snowmelt moving over and through the ground. As the runoff moves, it picks up and carries away natural and human-made pollutants, finally depositing them into lakes, rivers, wetlands, coastal waters, and even underground sources of drinking water. The most common NPS pollutants are sediment and nutrients. These wash into water bodies from agricultural land, small and medium-sized animal feeding operations, construction sites, and other areas of disturbance. Other common NPS pollutants include pesticides, pathogens, salts, hydrocarbons, toxic chemicals, and heavy metals (Garg, 2004).

2.2 Aquaculture pollutants

Aquaculture pollutants come mainly from food residues, fecal matter, antibiotics, detergents and soluble metabolites (Boyd, 2003). The effluent can increase nitrogen and phosphorus concentrations, dissolved oxygen demand, temperature, bacterial
concentrations, alkalinity, hardness, and organic and inorganic solids into the receiving water body. Variables that affect the amount of pollution from a farm include the culture species, diet, temperature, and management practices on the farm (Dallas and Day, 2004). The nutrient waste from crocodile farms is mainly released in the form of sludge.

Nitrogen, particularly in the form of ammonium (NH$_4^+$), is one of the most common characteristics of aquaculture effluents. Nitrogen is an essential plant nutrient and although ammonia is only a small component of the nitrogen cycle, it contributes to the trophic status of a body of water. Excess ammonia contributes to eutrophication, which is acceleration in algae and plant growth of water bodies. The resultant prolific algal growths have deleterious impacts on the aquatic life, drinking water supplies, and recreation. Ammonia at high concentrations is toxic to aquatic life, and aquaculture is a major anthropogenic source of ammonia. The criteria set for ammonia to protect aquatic life are dependent on the temperature and pH of the water. The matrix is too extensive, however, as an example, at pH 7.0 and a water temperature of 15ºC, the maximum concentration should not exceed 19.7 mg/L, and the average over 30-days should not exceed 1.77mg/L. At 0ºC, these values would be 23.2mg/L and 2.08 mg/L, respectively (R.I.C., 2000).

Nitrate is the principle form of combined nitrogen found in natural waters. It results from the complete oxidation of nitrogen compounds. Without anthropogenic inputs, most surface waters have less than 0.3mg/L of nitrate (R.I.C., 2000). Nitrate is a primary form of nitrogen used by plants as a nutrient to stimulate growth. Nitrite is a measure of a form
of nitrogen that occurs as an intermediate in the nitrogen cycle. It is an unstable form that is either rapidly oxidized to nitrate during nitrification or reduced to nitrogen gas during de-nitrification. This form of nitrogen can also be used as a source of nutrients for plants. Since nitrite is also a source of nutrients for plants, its presence encourages plant proliferation. Nitrite is toxic to faunal aquatic life at relatively low concentrations. Total nitrogen is a measure of all forms of nitrogen that is organic and inorganic. The importance of nitrogen in the aquatic environment varies according to the relative amounts of the forms of nitrogen present, be it ammonia, nitrate, nitrite, or organic nitrogen.

The other nutrient common in aquaculture effluents is phosphorus, in the particulate form or the dissolved form. It is an essential plant nutrient, and is often the most limiting nutrient to plant growth in fresh water (R.I.C., 2000). Since phosphorus is generally the most limiting nutrient, its input to fresh water systems can cause extreme proliferations of algal growth (Elenbaas, 1994). It is rarely found in significant concentrations in surface waters. Inputs of phosphorus are the prime contributing factors to eutrophication in most fresh water systems. While particulate phosphorus accumulates as sludge in the settlement ponds, the soluble form directly affects water quality, and can lead to eutrophic conditions. The particulate form can range from 7-64% in aquaculture. Phosphorus loading into Lake Kariba was found to be low by Marshall, (1984) in Troell and Berg (1997). A general guideline regarding phosphorus and lake productivity is: <10µg/L phosphorus yields is considered oligotrophic, 10-25 µg/L phosphorus will be found in lakes considered mesotrophic, and >25µg/L phosphorus will be found in lakes
considered eutrophic. The total phosphorus concentrations in most lakes not affected by anthropogenic inputs are generally less than 0.01mg/L (10µg/L) (R.I.C., 2000).

As the main sources of pollution from aquaculture, particularly crocodile ranching, are in the crocodile waste and uneaten food, the nitrogen and phosphorus content of these products is important. This may probably lead to an increase in algal growth because of them being released into the water, so causing eutrophication.

### 2.3 Eutrophication

Eutrophication is the process of gradual enrichment of water bodies with plant food, mainly nitrogen and phosphorus compounds as the nutrients. The process is accompanied with an excessive primary vegetation production (growth of aquatic plants) while no secondary production is observed (Balcerzak, 2006). Many ponds might be described as eutrophic for quite natural reasons, and can be fairly stable ecosystems. Provided that nutrient input is constant from year to year, detritus food chains will maintain equilibrium. Aquatic ecosystems with a shortage of plant nutrients termed oligotrophic systems, show low biological productivity and good water clarity. Ecosystem trophicity increases along with nutrient inflow to the system; the ecosystem passes through a mesotrophic phase to a eutrophic phase, with a sufficient supply of nutrients, and then finally it reaches the polytrophic phase with an abundance of nutrients. This conversion, which has been naturally occurring in nature for many years, picked up the pace in recent years and currently the main cause of water contamination with nutrients is anthropogenic eutrophication. At the very beginning, eutrophication was limited to lakes
only, where due to a low flow velocity, excessive biomass growth was observed. Nowadays eutrophication also affects rivers, though not to such an extent (Balcerzak, 2006). Eutrophication can be an ecological villain when it is rapid, usually as a result of human activity, including sewage disposal and agriculture. The rapid influx of extra nutrients upsets the balance and throws the ecosystem into chaos (Hammer and Hammer, 2001). Floating plants can affect the water beneath them by forming thick mats which eliminate submerged plants and algae, prevent photosynthesis and block oxygen diffusion from air causing it to become anaerobic (Gopal, 1987 in Gratwicke and Marshall, 2001). Occurrence of water hyacinth, *Eichhornia crassipes*, in the inshore waters of the Sanyati Basin may be a possible indicator of localized eutrophication in the inshore waters (Mhlanga, 2001). Aquaculture effluents are being considered as a potential contributor to eutrophication of receiving streams from associated feed-derived nutrients (Stephens and Farris, 2004).

**Physico-chemical parameters**

Temperature is a measurement of the intensity of heat stored in a volume of water. Surface water temperatures naturally range from 0°C under ice cover to 40°C in hot springs. Temperature is important as it affects the solubility of many chemical compounds and can therefore influence the effect of pollutants on aquatic life (R.I.C., 2000). Increased temperatures elevate the metabolic oxygen demand, which in conjunction with reduced oxygen solubility, impacts many species (De Meester, 2001).
Vertical stratification patterns that naturally occur in lakes affect the distribution of dissolved and suspended compounds.

Natural fresh waters have a pH range from 4.0 to 10.0 (De Meester, 2001). pH is important as high pH values tend to facilitate the solubilization of ammonia, heavy metals and salts (R.I.C., 2000). Low pH levels tend to increase carbon dioxide and carbonic acid concentrations (De Meester, 2001). Lethal effects of pH on aquatic life occur below pH 4.5 and above pH 9.5.

Dissolved oxygen (DO) is a measure of the amount of oxygen dissolved in water and is essential to the metabolism of all aerobic aquatic organisms (De Meester, 2001). Typically, the concentration of dissolved oxygen in surface water is less than 10 mg/L. The DO concentration is subject to diurnal and seasonal fluctuations that are due, in part, to variations in temperature and photosynthetic activity. The maximum solubility of oxygen (fully saturated) ranges from approximately 15 mg/L at 0°C to 8 mg/L at 25 °C (at sea level). Natural sources of dissolved oxygen are derived from the atmosphere or through photosynthetic production by aquatic plants. DO is essential to the respiratory metabolism of most aquatic organisms. It affects the solubility and availability of nutrients, and therefore the productivity of aquatic ecosystems (R.I.C., 2000). Low levels of DO facilitate the release of nutrients from sediments. Oligotrophic lakes tend to have increased concentrations of dissolved oxygen in the hypolimnion relative to the epilimnion, defined as orthograde oxygen profiles. Eutrophic lakes tend to have
decreased concentrations of dissolved oxygen in the hypolimnion relative to the epilimnion, defined as clinograde oxygen profiles (R.I.C., 2000).

Specific conductivity is the measurement of the ability of water to conduct an electric current; the greater the content of ions in the water, the more current the water can carry. Natural waters are found to vary between 50 and 1500 µS/cm (R.I.C., 2000). Specific conductivity may be used to estimate the total ion concentration of the water, and is often used as an alternative measure of dissolved solids (Dallas and Day, 2004). It is often possible to establish a correlation between conductivity and dissolved solids for a specific body of water. Due to its natural variability, there is no criterion recommended for this variable.

Turbidity is a measure of the suspended particulate matter in a water body, which interferes with the passage of a beam of light through the water (Dallas and Day, 2004). Materials that contribute to turbidity are silt, clay, organic material, or microorganisms. Turbidity values are generally reported in Nephelometric Turbidity Units (NTU). This variable is important in that high levels of turbidity increase the total available surface area of solids in suspension upon which bacteria can grow. High turbidity reduces light penetration; therefore, it impairs photosynthesis of submerged vegetation and algae (R.I.C., 2000). In turn, the reduced plant growth may suppress BMI productivity.
1.1.1 Effluent management

The effective management of a water resource receiving effluent requires information concerning the effluent source, quantities and distribution (Nhapi, 2004). The effects of the effluent within the aquatic environment and trends in concentrations and effects, and the causes of these changes should be determined. How far these inputs, concentrations, effects and trends can be modified and by what means, at what cost should also be analysed. The first stage in this management is to carry out a survey programme of measurements that define the patterns of variations of selected parameters, for example biochemical oxygen demand, temperature, nutrients, pathogens, suspended solids or acidity in space and time. Concentrations are measured at a number of stations, together with sampling of the fauna and flora at these stations. The survey will only inform one of the situation at one point in time.

The second stage will be surveillance and research, which will enable one to learn more about a problem before any policy decisions are made. Surveillance is the repeated measurement of a variable in order that a trend may be detected. Water variables are measured weekly or fortnightly to establish how the variables change. Animals and plants are sampled to establish if the original observations are repeated. The research function will be to examine the pollution process in more detail, using experimental and analytical techniques. The survival of a suitable organism in concentrations of the pollution could be studied in the laboratory or on experimental zones in the field, a process known as active biomonitoring (Dallas and Day, 2004). Smolders et al, (2003) describe active
biomonitoring as the translocation of organisms from one place to another and quantifying their biochemical, physiological and/or organism responses for the purpose of water quality monitoring. The tolerance of organisms to concentrations of pollution lower than the effluent concentration may be studied as a guide to fixing a standard for the effluent discharge concentration (Dallas and Day, 2004).

From the surveillance and research programme, and taking into account economic consideration, a policy for managing the pollution might be decided, for example by installing a treatment plant (Nhapi, 2004). It will be necessary to see that a reduction has the desired effect, that is an improvement in the state of the receiving water body, and also to ensure that the effluent’s quality is maintained. These observations on performance in relation to standards are known as monitoring. Rosenberg et al, (1998) refers to this type of biomonitoring as compliance and is used to ensure that water quality standards are met and maintained. The strategy outlined for examining pollution involves both the experimental and observational approaches (Nhapi, 2004). The experimental approach involves the simulation of pollutant’s behaviour and its action on biotic resources in experimental systems, whereas the observational approach examines the distribution of pollutants and their pattern of effects on natural resources. The integration of these two approaches is essential if a management policy for a particular pollutant or pollutional source is to be effective. Both approaches have their advantages. The experimental simulation does not take into account the complexity of normal pollutional situation in which a variety of factors influence the way a pollutant affects its target. This
very complexity however makes the interpretation of observational data exceedingly difficult.

Pollutant levels can be measured at the sites of discharge and the sites of abstraction from the watercourse; the concentrations of chemicals can be measured accurately and repeatedly using standard methods. Traditionally, physico-chemical monitoring forms the backbone of water quality monitoring in Zimbabwe (Magadza, 2003; Ndebele, 2009), and coupled with biomonitoring, may contribute more to water management. Assessment of the common physical attributes and chemical constituents of water, although essential for determining the type and concentration of pollutants entering a water body, is limited to the period of sample collection and to the physical and chemical analyses done (Dallas and Day, 2004).

2.4 Biomonitoring

Biomonitoring consist of different components, namely bioassays and bioassessments. Bioassays are laboratory-based monitoring, and are mainly carried out in form of toxicity testing using sentinel or indicator species. Bioassessments are field-based monitoring techniques, which may combine active and passive biomonitoring. Biological methods have an important role to play in the integrated management of water resources, and have several advantages over physico-chemical methods (Hellawell, 1986 in Thorne and Williams, 1997). Bioassessment assesses ecological integrity of a water body by measuring attributes of the assemblage of organisms inhabiting the water body. Common assemblages of aquatic organisms used for bioassessment include fishes,
Macroinvertebrates and algae. However, population characteristics of single sentinel species are also used as biological indicators of ecological integrity (MacDonald et al., 2003).

Animal and plant communities respond to intermittent pollution, which may be missed in a chemical sampling programme. For example, chemicals discharged intermittently may not be detected at some periods when they would have been diluted or carried downstream. Biological sampling when done monthly alongside the chemical survey will record an unexpected depression in the diversity of the biological community, as some species may be eliminated and many individuals killed by the pollutants. Some of the species missing may be known to be especially sensitive to the pollutants, thus they act as bioindicators (Buluta et al., 2010). Biological communities may also respond to new or unsuspected pollutants in the environment which chemical survey might miss since chemical analysis is predetermined for target pollutants. Biomagnification, which is the accumulation of chemicals through food chains, can also reflect the environmental pollution levels, which chemically would be too low to be significant. Bioassays may use organisms in surveillance programmes measuring growth responses for example of algae in nutrient rich water, or mortality, for example fish placed in effluent. Rapid bioassessment protocols were originally developed in the 1980s to provide cost-effective, efficient biological survey techniques. Biological monitoring, although a strategy that is increasingly advocated in the literature is still a relatively new concept in Zimbabwe. There have been few real attempts to use the technique for assessing the health of aquatic ecosystems in Zimbabwe (Moyo and Phiri, 2002).
2.5 Bioassessment systems

Bioassessment systems make use of taxonomic composition of biotic systems. However, no one system provides a complete quantitative analysis of all the organisms present in a water body (Dallas and Day, 2004). The Saprobian system is a biotic index used since 1902 in European countries. It is used primarily to indicate oxygen deficits caused by biologically decomposable organic pollution in running waters. Aquatic invertebrates are used to score clean and polluted sites. This system has major setbacks in that it requires great taxonomic effort in identifying all specimens to species level and examination of all trophic levels. It is also geographically specific and appropriate for organic pollution (Bonada et al, 2006).

Biotic indices use one or more components of the biota to provide a measure of the biological condition of a site and present it as a numerical value (Dallas and Day, 2004). These are more user-friendly and appealing even to non-biologists in practice and in policy making. Biotic indices rely on the assignment of tolerance values to various macroinvertebrates taxa. Many are designed to use family level data, but their utility in countries other than those for which they were originally designed may be limited, as family tolerances may not be reliably transferred between continents and climates and because different families may be encountered (Thorne and Williams, 1997). The Biological Monitoring Working Party (BMWP) score and its average score per taxon (ASPT) were developed in Britain, but have been modified for use in several countries like India, Spain and Australia (Thorne and Williams, 1997). The Australian National
River Health Programme (AusRivAS), Australian SIGNAL (Stream Invertebrate Grade Number Average Level) and SASS (South African Scoring System) are such biotic systems. However these are mainly for use in rivers.

Diversity indices are mathematical expressions of three components of community structure, namely richness, evenness, and abundance which is the total number of organisms present, that can be used to describe the response of a community to the quality of its environment (Dallas and Day, 2004). Diversity indices have a long history in pollution studies, although their utility has often been questioned, as mild pollution can be associated with an increase in diversity, before the characteristic decline with more severe pollution (Thorne and Williams, 1997), thus subscribing to the intermediate disturbance hypothesis (Cornell, 1926 in Stiling, 1999).

Richness (S), which is the number of taxa present, can be calculated at the family level to indicate the number of macroinvertebrate families. Simpson's index is a simple measure of the character of a community that considers both the abundance patterns and the taxa richness. Simpson’s index of dominance (D) represents the probability that two randomly selected individuals in the community belong to the same category by the equation:

\[ D = \sum (P_i^2) \]

where \( P_i \) is the ratio of individuals of one family to the total number of individuals (Krebs, 2001). Simpson’s diversity index ranges from 0 to 1 and is given by:
Shannon-Wiener index (H) measures habitat quality that may be degraded by human activity and is expressed as:

\[ H = -\sum (P_i \ln P_i). \]

Evenness (J) which is the uniformity in the distribution of individuals among species, measures similarities in the abundance of different families where

\[ J = H'/\ln S \]

The Shannon-Wiener index is used to derive this measure of sample evenness, which ranges from 0 to 1 (Pielou 1975, 1977 in Waite 2000).

The Margalef index is a measure of species richness and is rarely used in water quality assessment (Washington, 1984 in Phiri, 2000) although its discriminant ability is good. Its magnitude is also influenced by the level of identification and the index is given by:

\[ D = (S-1)/\ln n \]
Where $S = \text{no. of families in a sample}$, $n = \text{the number of individuals in a sample}$, $n_i = \text{the number of individuals in a sample of the } i \text{ family}$.

Use of the Shannon-Weiner index in pollution studies is based on the premise that species diversity decreases as human-induced ecological stress increases (Kırkagaç et al., 2004). This may not be so in cases where pollution may be masked by a change in species composition (from sensitive to tolerant species) that would not be reflected in any species diversity index. Sampling, sorting and identification demands in the use of these methods may make comparisons between studies seldom statistically valid (Dallas and Day, 2004). However these methods when coupled with other indices may approximate well on ecosystem integrity. The Shannon-Wiener index has proved to be a good robust general index of diversity, its values being influenced by species evenness and richness. When the sample contains only one species ($S = 1$); $H' = 0$. The value of $H'$ increases with $S$ but they rarely exceed 5.0. For any sample the theoretical maximum of $H'$ is equal to $\ln S$. This corresponds to the values of $H'$ obtained when all species are equally abundant, such as when the sample has maximum evenness (Waite, 2000).

Bioassessment is performed on several spatial scales (Dallas and Day, 2004). At the smallest scale, point source perturbations such as discharge from a pipe are evaluated. On a larger scale, non-point source perturbations are evaluated such as sediment derived from multiple sources of differing magnitude within a drainage system. Bioassessment may be restricted to one assessment to evaluate water body condition at one point in time, or it may be conducted at regular intervals to evaluate
change through time. Biomonitoring may reveal trends in water body condition as a result of many factors such as changes in water year types, pollution abatement practices, or habitat restoration activities (MacDonald et al., 2003). In addition, bioassessment may be conducted before and after an expected perturbation to evaluate its effect (Ziglio et al., 2006). For these assessment strategies to be successful, standardization of sampling and sample processing is crucial. Standardization saves time and cost by facilitating the integration of historic data sets derived from standardized procedures. Consequently, maintaining long term, standardized sampling and sample-processing procedures through broad geographic regions greatly enhances the power of bioassessment as a tool for evaluating aquatic ecological condition (Dallas and Day, 2004).

2.6 Use of benthic macroinvertebrates

Macroinvertebrate-based bioassessment predominantly use BMI to assess water body condition (Bonada et al., 2006). BMI are considered valuable indicator organisms of the biotic integrity of water bodies and have been used as bioindicators to complement physico-chemical evaluation of water quality after environmental perturbations (Galdean et al., 2000). BMI have been grouped into very sensitive, moderately intolerant, fairly tolerant and very tolerant of pollution. Taxa that have been found to be indicators of good water quality include Ephemeroptera, Trichoptera and Plecoptera (CIESE, 2010). Indicators of fair water quality include the order Odonata with representatives from both Anisoptera and Zygoptera (CIESE, 2010). Indicators of poor water quality include Chironomidae, Hirudinae and Syrphidae (CIESE, 2010). The value of BMI in biological monitoring is well documented (Rosenburg et al., 1998; Bonada et al., 2006) and they
remain a widely used group for this purpose in developed countries. Sampling techniques are simple and require only rudimentary equipment (Thorne and Williams, 1997). The taxonomy of BMI in many developing countries is however at best patchy. In order to save time and resources, family level identification has been successfully adopted in many biological monitoring programmes in developed countries. The identification of macroinvertebrates to family level is possible in developing countries and therefore this may be an appropriate level for the development of a multimetric system of assessment (Thorne and Williams, 1997).

BMI represent the second largest group of organisms in aquatic ecosystems and respond quickly to perturbation, provoking change in the local community structure and reducing richness to a few tolerant and generalist groups (Bonada et al, 2006). Rosenberg et al, (1998) suggested that the high density, diversity, small body size and short life cycle of BMI when compared to other organisms favour their use in aquatic-ecosystem monitoring, complementing the physical, chemical and physico-chemical evaluation of the environment. Because of BMI abundance, taxonomic diversity, residence time, and range of response to changes in their aquatic environment, they are commonly the resident biota used to monitor the quality of water resources in many countries like South Africa, Australia and United States (Dallas and Day, 2004).

Macroinvertebrates are largely non-mobile in their aquatic phase and are thus representative of the location being sampled, which allows effective spatial analyses of disturbance (Dallas and Day, 2004). They also are responsible for the maintenance of
aquatic ecosystems because they are important components of the nutrient cycle by feeding on algae, organic detritus and by preying on a wide range of small organisms. BMIs are an important food resource for fishes, amphibians, reptiles, birds and mammals thus they constitute an essential component of the food web in aquatic habitats. A limitation observed in the use of BMI is that their heterogeneous distribution and patchiness may result in natural spatial and temporal variability in BMI assemblages. While bioassessment has proven to be useful for identifying gradients of ecological condition, it is not yet developed enough to consistently isolate factors that influence the gradients. This problem is evident with respect to separating effects of natural gradients, such as those associated with elevation change, from effects of anthropogenic gradients, such as extent of impervious surface (Dallas and Day, 2004).

2.7 Laboratory toxicity tests

In developed countries a number of laboratory toxicity tests are now being used to monitor and evaluate the quality of effluents so as to establish environmental standards and practices that will protect the environment, and ensure compliance with regulations (Sponza, 2002a, and b). The methods involve the use of whole effluent toxicity (WET) testing, which describes the negative impact or toxicity to a population of organisms caused by exposure to an effluent (SETAC, 2004). A toxicity test is a procedure that involves the exposure of organisms to complex environmental samples (water, sediment, or sediment extract) under controlled conditions to determine if adverse effects occur. Test samples usually contain unknown amounts of mixtures of contaminants. This procedure is sometimes referred to as a bioassay, but toxicity test is the more appropriate
term because a bioassay is a test to determine the toxicity threshold of a specific substance, while this test is used to determine the toxicity of a whole sample, not its chemical components (MacDonald et al, 2003). Since living organisms give a complete biotic response to the effect of a pollutant, toxicity tests have become important tools in assessing the possible impacts of different effluents. The tests provide information on the initial levels of damage and assist in developing precautionary measures and strategies for environmental protection and management. Thus, in evaluating and enacting wastewater policy, the inclusion of acute and complementary chronic toxicity tests with various organisms is necessary for the protection of freshwater ecosystems.

A number of characteristics have been considered in toxicity tests to determine the effects of effluents on aquatic organisms and these include death and survival, decreased reproduction and growth, gill ventilation rate, heart rate, blood chemistry, histopathology, and morphological deformities. As it is not possible to measure all effects of effluent on a routine basis, observations in toxicity tests generally have been limited to only a few effects, such as mortality, growth, reproduction and morphological deformities (USEPA 2002a, b, c). The effects of a toxin on test organisms can be measured in a variety of ways, the ultimate effect being death of the organism. Other common measures include effects on growth rate, egg production, oxygen consumption, chlorophyll production, and photosynthetic rate amongst others (Dallas and Day 2004).

Constituent specific water quality criteria developed in South Africa for toxic constituents use the Chronic Effect Value (CEV) and the Acute Effect Value (AEV) as
biocriteria (Dallas and Day 2004). The CEV is defined as that concentration or level of a constituent at which there is expected to be a significant probability of measurable chronic effects in up to 5% of the species in the aquatic community. The AEV is defined as that concentration or level of a constituent at which there is expected to be a significant probability of acute toxic effects in up to 5% of the species in the aquatic community (Dallas and Day 2004). Acute toxicity tests are experiments that generally run for four days or less and where mortality is usually the response measured. Chronic toxicity tests last for a longer time than acute tests, depending on the reproductive cycle of the test animal. Sub-lethal endpoints such as those associated with reproduction and growth in addition to survival are generally measured in chronic toxicity tests.

Toxicity data may also be expressed as the following: The median effective concentration (EC\textsubscript{50}) which is the concentration at which a specified effect is observed in half of the test population, The median lethal concentration (LC\textsubscript{50}) is a time dependant variable which is defined as the concentration at which half the test population dies at a given exposure period, The median tolerance limit (TL\textsubscript{M}) which is defined as the concentration at which half the test population dies, The median effective time (ET\textsubscript{50}) which is defined as the time taken for an observed effect to occur in half of the test population, The median survival time (LT\textsubscript{50}) which is defined as time taken for half of the test population to die and The incipient lethal time (ILL) which is defined as the highest concentration that organisms can tolerate indefinitely (Dallas and Day 2004).
The main advantage of toxicity testing is repeatability and providing a quantifiable measure of the potential for the occurrence of bioeffects. Because toxicity tests measure the relative toxicity of a mixture of chemicals, any synergistic or antagonistic effect is automatically taken into account (MacDonald et al, 2003). On the other hand, single-species toxicity tests though considered to be a good measure of the potential for adverse environmental effects, are rarely designed to precisely mimic natural exposure (MacDonald et al, 2003) and thus suffer from low environmental realism, although they can be effective if the underlying mechanisms of environmental change are known (Scrimgeour and Wicklum, 1996 in Dallas and Day, 2004). Single-species tests shortcomings also include the fact that they do not directly measure biotic community responses and neither do they encompass the range of species sensitivities or function responsive to toxic materials that occur in biological communities. Delayed impacts and effects due to bioaccumulation and bioconcentration are neither measured. The highly controlled exposure regimes in laboratory tests do not reflect the multivariate and complex condition in aquatic ecosystems thus results may underestimate biotic community responses to chemicals because of multiple stressors (de Vlaming et al, 2000). In a bid to reduce the shortcomings, adoption of multi-species testing, the use of micro and mesocosms, the use of indigenous organisms, and the examination of site-specific conditions have brought significant advances in toxicity testing, making it more useful in the management of aquatic ecosystems (Dallas and Day 2004).
2.8 Aquaculture on Lake Kariba

Lake Kariba (28.74778° E, 16.51222°S,) is a man-made lake situated on the Zambezi River between Zambia and Zimbabwe. It was constructed in the period 1955–59 mainly for hydroelectric power generation. The lake consists of five basins and is 280 km long with a surface area of 5580 km² and a volume of 185 km³ at full capacity (WCD, 2000). The mean and maximum depths are 29.18 and 97 m, respectively. It is monomictic with a mean surface temperature of about 25°C and a turnover of the water mass in winter. The theoretical water retention time is 3.3 years (Magadza, 2006) and the water level fluctuates by an amplitude of 3m annually. The lake has gone through some drastic ecological changes since its formation and is now described as a nutrient-poor soft water lake. During the maximum stagnant period (December–April) nutrients accumulate in the hypolimnion, whereas dissolved oxygen concentration is reduced. The suspended load into the lake is most profound during the period following the rainy season.

Crocodile farming along the shores of Lake Kariba has of late been very prevalent, with four farms having been established within two decades in the Sanyati Basin alone. Intensive farming of *Crocodylus niloticus* was established along the shores of Lake Kariba, Zimbabwe in 1987. The company has grown over the years with 20 000 crocodiles being culled for skin and meat annually. The company has a total stock of 60 500 crocodiles within the farm. This farm is the biggest producer of *Crocodylus niloticus* in Africa, contributing 80% of crocodile skins and meat from Zimbabwe, and 60% in Africa. This company’s layout does not have settling ponds along its sewerage drains but discharges untreated effluent directly into the Lake Kariba.
The other farm is a crocodile ranching venture being carried out alongside other aquaculture investments on the lake. The company has a stock of approximately 20 000 crocodiles. The farm’s layout includes a settling pond that attempts at settling suspended solids, before the liquid effluent is also discharged directly into the lake without any further treatment.

2.9 Related Studies

The effects of aquaculture on the benthos of aquatic ecosystems with net-pen and cage culture on Lake Kariba has been documented by Troell and Berg, (1997) and they concluded that intensive fish farming in the tropics can generate similar eutrophication effects that are observed in temperate regions. However the results also indicated that a tropical lake system might be able to process local deposition of organic wastes better than a temperate one suggesting that microbial decomposition may be a rapid and prominent process. Stephens and Farris, (2004) on commercial catfish industry in Arkansas found that taxa richness of benthic macroinvertebrates was unaffected by pond discharge at all sites. Ephemeroptera, Plecoptera, and Trichoptera taxa (EPT) actually responded with increased abundance in receiving streams below the pond discharges. Taxa richness of fish was also unaffected in relation to upstream locations and downstream of the facility effluent release. Their findings suggested minimal detrimental instream effects result from the introduction of aquaculture effluents into receiving waters. Bredenhand, (2005) evaluated macroinvertebrates as bioindicators of water quality of the Klein Plaas dam on the Eeste River where an experimental cage culture of
trout farming is being done in South Africa. The study observed that pollution-tolerant taxa like Chironomidae, Simuliidae, Oligochaeta and Sphariidae had higher abundances just below the outfalls indicating that the trout farm might be impacting on the reservoir. Sarang and Sharma (2006) studied seasonal variations in benthic fauna and selected limno-chemical parameters of Kishore Sagar Lake, for a period of one year. This lake was found to be affected by pollution from domestic and industrial effluents as indicated by low dissolved oxygen. Despite this, the biodiversity of macrobenthic invertebrates was appreciably high, with recorded 34 species. Most dominant benthic species encountered were *Bellamya bengalensis*, *Chironomus* larvae, *Melanoides tuberculata*, *Lymnaea acuminata* and *Indoplonorbis exustus*.

Yucel-Gier *et al*, (2007) researched on a sea bass (*Dicentrarchus labrax* L., 1758) farm, in Turkey, and they observed that areas with higher organic enrichment showed lower diversity with increasing abundance of polychaeta. Organic enrichment and particle size of sediments were closely associated with faunal groups, particularly with polychaeta and mollusca. Roberts *et al*, (2009) examined the direct effects of a Virginia trout farm on benthic macroinvertebrates, and in the multispecies field tests carried out, a clear decrease in total invertebrate abundance and EPT abundance was seen in the effluent treatments compared to the spring water treatments. Roozbahani *et al*, (2010) evaluated the seasonal diversity of benthic macroinvertebrates in the Northwest of the Persian Gulf using diversity and evenness indices and concluded that the gulf was moderately polluted.
A lot of bibliography on aquaculture effluent management has been compiled, and most of it is on fish farming and other aquatic animals, however effluent management from crocodile farms has scantily been attended to. The effects of crocodile aquaculture effluents on the aquatic environment, particularly macroinvertebrates, have not generated a lot of attention in the past in Zimbabwe. Receiving waters of aquaculture effluent dilute a wide range of pollutants, such as nutrients and suspended solids, however this aspect on dilution in lentic waters has not been widely documented, but few studies on streams below fish farms document effects in the biotic community (Kirkagac et al., 2004; Couceiro et al., 2006). Most studies of the effects of aquaculture on macroinvertebrates have involved the basic design of sampling the community upstream and downstream of the fish farm in question, and few have used toxicity tests with benthic macroinvertebrates. The impact of crocodile farm operations on water quality has not been monitored and its effects have not been studied in detail. The activities related to this aspect of aquaculture are the major concern in the study.
CHAPTER 3

3  MATERIALS AND METHODS

3.1  Study Area

The study was carried out along Charara Bay (Basin 5) of Lake Kariba, which lies east of Nyamhunga Township, Kariba Town, Zimbabwe. Three sites, purposefully chosen, were sampled during the study, Site 1 (S16º31.837’; E028º51.233’), Site 2 (S16º32.629’; E028º52.593’) and Site 3 (S16º33.125’; E028º53.724’) (Fig 1). Table 1 gives the description of the sites.

Fig 1: The location of the sampling sites (1-3) along the shoreline of the Sanyati Basin of Lake Kariba. Insert The location of Lake Kariba in Zimbabwe.
3.1.2 Study Sites

Table 1: Site description

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The farm has a settling pond along its sewer drain, and the drain spans approximately a kilometre before entering the lake. Along the way, the drain also receives effluent from fish ponds just before it enters the lake.</td>
</tr>
<tr>
<td>2</td>
<td>The farm has no settling pond, and drains directly into the lake without any treatment of the effluent.</td>
</tr>
<tr>
<td>3</td>
<td>The area is relatively unperturbed as it lies within the Parks and Wildlife area, but is along the same bay as the study sites</td>
</tr>
</tbody>
</table>

3.2 Data Collection

The research project focused on the diversity of benthic macroinvertebrates in areas receiving effluent discharge from crocodile farming. Physico-chemical water parameters and benthic fauna were investigated in one summer 2010/2011. The summer season has the highest loading due to crocodile feeding habits, feed ingestion tend to maximize in warm weather. Continual discharge of effluent also makes it possible to collect samples any season and possibly get similar results each season. Samples were collected monthly from December, 2010 to March 2011. The BMI sampling technique coupled two methods so as to collect a greater diverse macroinvertebrates and was simultaneously done with the water sampling. The researcher participated in the field collection and laboratory analyses that provided all the data utilized in the present study.
3.2.1 Physicochemical parameters

Water quality at the two study areas and the control site was assessed by applying standard techniques and protocols (R.I.C., 2000). One farm drained directly into the lake as it had no settling pond; the effluent was collected at the drain outlet/lake entry point and randomly within 50 m on either side of the outlet. The three samples were poured into a bucket and a one-litre sub sample extracted and used for the analysis. At the other farm, which had a settling pond along its waterway, the effluent was collected before treatment in the settling pond and after treatment just before it discharged into the lake and at the actual discharge point at the lake. Three random samples per point were again collected, pooled and a one-litre sub sample extracted for analysis. At the reference point, three water samples were collected randomly within the 100 m stretch that would be sampled for macroinvertebrates, and after pooling, a sub sample was extracted for analysis. Water samples, for chemical analysis, were taken in 1000 ml plastic bottles and stored in the dark at 4 ºC until analysis that was performed within 24 hours from sampling. Samples were immediately placed in cooler boxes with ice in the field for transportation back to the laboratory for analysis. Samples collected were analysed at the University of Zimbabwe Lake Kariba Research Station (UZLKRS) wet chemistry laboratory.

Temperature, dissolved oxygen, pH, and electrical conductivity were measured in situ. Temperature and dissolved oxygen were measured with a portable Oxymeter YSI, Professional Series model Pro20. pH was measured with a portable ionmeter EcoScan.
Ion 6 (Eutech Instruments). Electrical conductivity was measured with a portable conductivimeter WTW model LF91 (SEMAT, UK).

In the laboratory, nitrate (NO\textsuperscript{3-}) was analysed using the Cadmium Reduction Method; Ammonium (NH\textsubscript{4}\textsuperscript{+}), was analysed using the Indophenol method; Orthophosphate (H\textsubscript{2}PO\textsubscript{4}) was analysed by the Molybdenum blue method; Total phosphorus was analysed by the Molybdenum blue method after digestion with 8% potassium persulphate and concentrations were spectrophotometrically determined using a Hitachi UV-visible spectrophotometer, model 100-40 (Elenbaas, 1994).

Biochemical oxygen demand (BOD\textsubscript{5}) was determined using the oxygen electrode method (Subramanian, 2006). Two bottles were filled with the water sample and the D.O. of the first bottle immediately determined by the oxygen electrode method. The second bottle was incubated under 20\textdegree C for 5 days, which are meant to be ideal to promote microbial activity, and the D.O. was once again measured. The difference between the two D.O. values is the amount of oxygen that is consumed by microorganisms during the 5 days and is reported as BOD\textsubscript{5}. Turbidity was measured in the laboratory using a Turbidimeter Model 16800 (Hach Chemical Company, USA).

3.2.2 **Benthic macroinvertebrates**

A square-shaped (25 by 25 cm) scoop net with a 500-\(\mu\)m mesh was used to sample 50m on each side of the point of discharge, giving a stretch of a 100 metres. The scoops were
1m long, along the stretch to a depth of 0.5m along the shoreline, paying attention to cover all microhabitats present. Washing the epiphytes in the scoop net while in the water collected epiphytic macroinvertebrates.

The snail scoop method (Rosenberg et al, 1998) was used over the same 100m stretch of the shoreline at each site. The snail scoop is a shovel-like implement with a rigid metal sieve base and a long handle like a scoop net. The sieve base is 15cm X 15cm. Scooping was done along the water edges, taking one scoop per metre up to a depth of 0.5m along the stretch. All macroinvertebrates collected in the sampling were fixed with 70% ethanol for preservation and transportation back to the laboratory for identification using a dissection microscope and dichotomous keys. The identification of taxa was done using keys presented in Day et al, (2002a, b) and de Moor et al, (2003a, b). All macroinvertebrates were identified to at least family level.

3.2.3 WET tests

The test organisms were collected from the lake, and were let to acclimatize to the laboratory conditions for two weeks in an aquarium 40cm long X 40cm wide X 30cm high. The test organisms were fed daily on powdered fish food. On the day of the test, effluent and lake water from the relatively undisturbed site were collected and a dilution series, with each concentration replicated three times made. The test organisms were added and mortality noted hourly over a 24-hour period. The test organisms were not fed during the experiment.
The completely randomized experimental design was achieved by labelling the eighteen beakers from 1-18, then forming a grid of 3 X 6 spaces by using 18 white A4 size paper on the experiment desk. Each paper was labeled alphabetically from A to R. A list of random numbers 1-18 was generated using a table of random numbers and the beakers were allocated space on the grid so formed on the experimental desk according to the list of random numbers.

3.3 Task Safety Requirements

The area in which the project was carried out is a wildlife area with high numbers of wild game, which include elephants, buffaloes, zebras, lions, crocodiles and hippopotamuses. As a safety measure, the inclusion of a game guard in the team during sampling was imperative. The waters also have schistosomiasis/bilharzia vector snails. To minimize infection by bilharzias causing schistosomes, the sampler wore waders, and as a precaution, bilharzia medication was taken every three months.

3.4 Data Analysis

Field data analysis

The physico-chemical parameters measured at each site were pooled and the mean and standard deviation calculated. One way analysis of variance (ANOVA) was used to determine whether physico-chemical parameters differed among sites and when significant differences were noted, Tukey post hoc test was used to determine which sites
differed. The test for homogeneity of variances was carried out using the Bartlett Chi-square and the parameters that complied were subjected to one-way ANOVA with ln(x+1) transformed data. The Kruskal-Wallis test was further carried out on the data that did not comply with the ANOVA. This was performed using the Paleontological Statistical package (PAST) version 2.08b (Hammer et al, 2001).

Principal components analysis was used to explore for differences in water physicochemical variables among the sites. This was performed using the Paleontological Statistical package, PAST. PCA indirectly assesses relationships and accurately portrays environmental gradients (Zimmer et al, 2000). The data were log(x+1) transformed to reduce clustering at the centre of the ordination plot thereby allowing for the examination of qualitative differences in the physicochemical characteristics of the sites.

In evaluating environmental conditions in a water body knowledge of the relative abundance of taxa, is much more useful than the knowledge of their presence or absence (Phiri 2000). The number of different taxa in each sample was determined, counts from samples of each site pooled and the percent relative abundance of each taxa, calculated. The Shannon-Wiener and Pielou’s evenness indices were calculated for the macroinvertebrate taxa collected at the three sites. The community indices were calculated using the statistical package OpenStat (Miller 2009). One way analysis of variance (ANOVA) was used to determine whether indices differed among sites and when significant differences were noted, Tukey post hoc test was used to determine which sites differed. The statistical analyses were done using the PAST package.
The average linkage cluster analysis and non-metric multidimensional scaling (nMDS) were used to explore for similarities and differences in benthic macroinvertebrate assemblages among the sites using the PRIMER statistical package (Clarke and Gorley 2006). In order to reduce the contribution of taxa represented by many individuals to patterns, averaged macroinvertebrate abundance data with all taxa included, were log(x + 1) transformed before analyses. Hierarchical classification groups similar items indicating relationships among groups (Gauch, 1982 in Phiri, 2000).

The study also assessed whether passing the effluent through a settling pond at Site 1 had an effect on the quality of effluent disposed into Lake Kariba. Thus, samples of the effluent before and after its passage through the settling pond were collected and analysed. The physico-chemical parameters were subjected to two-sample t-test to determine any significant differences using the PAST package.

Bioassay data analysis

The experiments were conducted in April 2011. Crocodile effluent concentrations by volume used were 0.00% (control), 12.5, 25, 50, 75 and 100%. All crustaceans died within 1 h in this preliminary, range finding bioassay (Browne, 2005) with crocodile effluent concentrations >12.5% and so lower concentrations were used for the experiment. The crocodile effluent concentrations by volume used are in appendix D. Behavioural changes were recorded and mortality recorded every hour for 24 hours. The lethal concentration (LC₅₀) for fresh water shrimps was calculated using Probit Analysis. In this method, a parametric normalised distribution of the percentage of organisms
responding to a chemical concentration is derived, and a LC_{50} is estimated with 95% confidence limits (Cooney, 1995 in Browne, 2005). The calculated and tabulated Chi squared values for heterogeneity were reported. The EPA Probit Analysis Program (Version 1.5) was used for calculating LC_{50} values (Appendix E).

3.5 Limitations of the study

The time allocated for the project was limited; hence the study could not touch on seasonal variations that could have shaded more light on macroinvertebrates community structures over a longer period. They are also once off which means they fail to provide indication on variability or non-variability across seasons. They are of short term nature in that they do not provide an indication of effect of time. This way a trend analysis is not possible. The results obtained in this study are site specific, and may not be generalized for other sites on Lake Kariba.
CHAPTER 4

4 RESULTS

4.1 Physicochemical parameters

Table 2: Summary of the physico-chemical variables (mean ± standard deviation) measured during this study. (n=4)

<table>
<thead>
<tr>
<th>Physical and chemical water variables</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>29.7 ± 0.8</td>
<td>29.2 ± 0.6</td>
<td>29.9 ± 0.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.5</td>
<td>7.1 ± 0.4</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹)</td>
<td>188.5 ± 36.1</td>
<td>168.6 ± 44.6</td>
<td>95.7 ± 6.2</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>47.8 ± 21.7</td>
<td>47.8 ± 19.3</td>
<td>20.8 ± 5.6</td>
</tr>
<tr>
<td>Dissolved O₂ (mg l⁻¹)</td>
<td>2.6 ± 0.5</td>
<td>3.1 ± 1.5</td>
<td>5.2 ± 0.8</td>
</tr>
<tr>
<td>BOD₅ (mg l⁻¹)</td>
<td>23.8 ± 20.1</td>
<td>34.4 ± 14.9</td>
<td>14.5 ± 14.6</td>
</tr>
<tr>
<td>Nitrate-N (NO₃⁻) (mg l⁻¹)</td>
<td>0.59 ± 0.41</td>
<td>0.38 ± 0.25</td>
<td>0.07 ± 0.1</td>
</tr>
<tr>
<td>Ammonium-N (mg l⁻¹)</td>
<td>3.142 ± 1.85</td>
<td>3.166 ± 2.46</td>
<td>0.07 ± 0.05</td>
</tr>
<tr>
<td>Phosphates (PO₃⁻₄) (mg l⁻¹)</td>
<td>0.19 ± 0.08</td>
<td>1.35 ± 0.88</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Total phosphorous (mg l⁻¹)</td>
<td>0.58 ± 0.10</td>
<td>3.57 ± 2.21</td>
<td>0.07 ± 0.04</td>
</tr>
</tbody>
</table>

Temperature, pH, turbidity and BOD₅ did not show marked variability between sites ($P > 0.05$) in this study (Table 2). Conductivity was significantly higher (ANOVA, $F = 13.15$,
\( P < 0.05 \) at Sites 1 and 2, compared to Site 3. Nitrates (ANOVA, \( F = 8.255, P < 0.05 \)), Ammonium (ANOVA, \( F = 13.49, P = 0.02 \)), Phosphates (ANOVA, \( F = 14.58, P < 0.05 \)) and Total phosphorous (ANOVA, \( F = 36.71, P < 0.05 \)) were significantly different at Sites 1 and 2 compared to Site 3. Pair wise post hoc analyses for the physicochemical variables with significant differences are given in Appendix A.

Principal component analysis (PCA) showed that the first and second axes accounted for 46.32 % and 16.96 % of the variation in water physico-chemical aspects of the study sites, respectively. The first axis was largely negatively correlated with ammonium, turbidity, orthophosphates, total phosphorous and conductivity, while the variation in the second axis was largely due to orthophosphates, total phosphorous, temperature, conductivity and dissolved oxygen (Table 3). These were the key components that separated the sites in respect to physico-chemical parameters. The PCA scatter plot (Fig 2) shows complete separation of the Site 3 from the perturbed Sites 1 and 2. The two perturbed sites show some degree of overlap in their physico-chemical parameters.
Figure 2: PCA scatter plot of water physicochemical variables from the three sampling sites
Table 3: Loadings for environmental variables

<table>
<thead>
<tr>
<th></th>
<th>Axis 1</th>
<th>Axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>eigenvalue</td>
<td>4.63198</td>
<td>1.6956</td>
</tr>
<tr>
<td>% variance</td>
<td>46.32</td>
<td>16.956</td>
</tr>
<tr>
<td>Temp</td>
<td>0.08586</td>
<td>-0.4166</td>
</tr>
<tr>
<td>DO</td>
<td>0.2572</td>
<td>0.3259</td>
</tr>
<tr>
<td>Cond</td>
<td>-0.3314</td>
<td>-0.4121</td>
</tr>
<tr>
<td>pH</td>
<td>0.1896</td>
<td>-0.05691</td>
</tr>
<tr>
<td>Tur</td>
<td>-0.41</td>
<td>-0.09358</td>
</tr>
<tr>
<td>BOD$_5$</td>
<td>-0.3182</td>
<td>0.2099</td>
</tr>
<tr>
<td>Nit</td>
<td>-0.2631</td>
<td>-0.3205</td>
</tr>
<tr>
<td>TP</td>
<td>-0.3523</td>
<td>0.422</td>
</tr>
<tr>
<td>PO$_{3-4}$</td>
<td>-0.3614</td>
<td>0.4293</td>
</tr>
<tr>
<td>NH</td>
<td>-0.4329</td>
<td>-0.1711</td>
</tr>
</tbody>
</table>

4.2 Benthic macroinvertebrates

The percent relative abundance of the macroinvertebrates and total number of identified families collected from each sampling station are shown in Table 4. Overall, 23 taxa were recorded across all sites sampled. The gastropod *Melanoides* was the most ubiquitous, occurring in large numbers in the perturbed sites. A sign of great reproductive proliferation in this taxon was evidenced by the numbers of snails of all sizes at the perturbed sites.
Table 4: Percent relative abundances of taxa collected during the study

<table>
<thead>
<tr>
<th>FAMILY</th>
<th>GENUS</th>
<th>SITE 1</th>
<th>SITE 2</th>
<th>SITE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viviparidae</td>
<td>Bellamya</td>
<td>9.67</td>
<td>6.08</td>
<td></td>
</tr>
<tr>
<td>Planorbidae</td>
<td>Biomphalaria</td>
<td>0.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiaridae</td>
<td>Cleopatra</td>
<td>7.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiaridae</td>
<td>Melanoides</td>
<td>78.56</td>
<td>77.31</td>
<td>22.34</td>
</tr>
<tr>
<td>Physidae</td>
<td>Physa</td>
<td>0.14</td>
<td>4.10</td>
<td></td>
</tr>
<tr>
<td>Corbiculidae</td>
<td></td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actyidae</td>
<td>Caridina</td>
<td></td>
<td>16.57</td>
<td></td>
</tr>
<tr>
<td>Aeshnidae</td>
<td></td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomphidae</td>
<td></td>
<td>0.14</td>
<td>0.30</td>
<td>0.61</td>
</tr>
<tr>
<td>Libellulidae</td>
<td></td>
<td>0.22</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Coenagrionida</td>
<td></td>
<td>0.65</td>
<td>0.45</td>
<td>7.45</td>
</tr>
<tr>
<td>Glossiphoniida</td>
<td></td>
<td>2.96</td>
<td>1.41</td>
<td>1.52</td>
</tr>
<tr>
<td>Baetidae</td>
<td></td>
<td>3.18</td>
<td>10.33</td>
<td></td>
</tr>
<tr>
<td>Hydrophilidae</td>
<td></td>
<td>0.22</td>
<td>0.15</td>
<td>0.61</td>
</tr>
<tr>
<td>Gyrinidae</td>
<td></td>
<td>0.07</td>
<td>0.07</td>
<td>0.76</td>
</tr>
<tr>
<td>Gerridae</td>
<td></td>
<td></td>
<td></td>
<td>1.67</td>
</tr>
<tr>
<td>Corixidae</td>
<td></td>
<td>0.58</td>
<td>0.07</td>
<td>0.30</td>
</tr>
<tr>
<td>Notonectidae</td>
<td></td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>Belostomatida</td>
<td></td>
<td>3.68</td>
<td>0.30</td>
<td>0.76</td>
</tr>
<tr>
<td>Chironomidae</td>
<td></td>
<td>9.10</td>
<td>9.23</td>
<td>4.10</td>
</tr>
<tr>
<td>Syrphidae</td>
<td></td>
<td>0.51</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Hydrachnellae</td>
<td></td>
<td></td>
<td>6.53</td>
<td></td>
</tr>
<tr>
<td>Cyclosteriida</td>
<td></td>
<td></td>
<td>7.14</td>
<td></td>
</tr>
<tr>
<td>Total families</td>
<td></td>
<td>13</td>
<td>13</td>
<td>20</td>
</tr>
</tbody>
</table>

There were more macroinvertebrate families at Site 3 than at the two perturbed sites. Thus the highest number of taxa or richness was recorded at Site 3 (Figure 3a). The control site had a total of 20 families. The number of taxa was significantly different between the sites (ANOVA, $F = 29.84$, $P < 0.05$). Total abundance (Figure 3b) was higher at Site 1 because of the snail numbers, and this emphasized the dominance by this...
mollusc. There was no significant difference between sites in relation to total abundance (ANOVA, F = 2.55, P > 0.05) (Table 5). Community evenness (Figure 3c) was significantly different between the perturbed sites and the control (ANOVA, F = 10.80, P < 0.05). Shannon Wiener index (Figure 3d) was also significantly greater at Site 3 as compared to the two perturbed sites (ANOVA, F = 19.67, P < 0.05) (Table 5). Tukey’s pair wise post test results are in Appendix C.

**Table 5**: One-way ANOVA results for community indices (n = 4). All the indices were analysed at 0.05 level of significance.

<table>
<thead>
<tr>
<th>Community Index</th>
<th>F (df = 2,9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richness (S)</td>
<td>29.84</td>
<td>0</td>
</tr>
<tr>
<td>Total Abundance</td>
<td>2.55</td>
<td>0.13</td>
</tr>
<tr>
<td>Evenness</td>
<td>10.80</td>
<td>0</td>
</tr>
<tr>
<td>Shannon Wiener</td>
<td>19.67</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 3: Macroinvertebrate community indices of sites. Error bars represent standard deviation of mean.
Figure 4: nMDS plot showing possible links between discharge sites

Non-metric Multidimensional Scaling (nMDS) Ordination showed strong differences in the macroinvertebrate assemblages at discharge Sites 1 and 2, and the control site. The perturbed sites showed little differentiation between them. The differences between the assemblages can hypothetically be grouped in the nMDS ordination diagram in Figure 4. The assemblages of the perturbed site can also be shown to be similarly linked by the dotted circle in the same figure.
Hierarchical clustering also showed strong differences in the macroinvertebrate communities at the control site and the perturbed sites. Cluster analysis gives similarities for the control site at more than 70%. Similarities between the perturbed sites is given at 60%. There was a complete separation of the control site from the perturbed sites. This was separated on the Bray-Curtis similarity axis at a similarity level of 40% (Figure 5).

4.3 WET tests

Symptoms of toxicity in fresh water shrimps.

The symptoms of distress in the fresh water shrimps were easily recognizable. At low effluent concentrations, the shrimps remained active throughout the observation period
and some even moulted. At higher concentration, the crustaceans exhibited degrees of restlessness, random motion, surfacing movements, increased swimming and chelepede scrapping. At even higher concentrations, overturning (loss of balance), sluggishness and bunching effect was noticed, with a reduction in all activities up to maximum letharginess. With time progression, shrimps at higher concentrations of effluent exhibited inhibited movement and bottom settling, with decreased chelepede scrapping and responded feebly to gentle prodding. There was colour change to a chalky white and a gradual turning to pink as rigor mortis set in the afflicted organisms. The pink colour was retained even after death in the shrimps.

Lethal concentration.
The LC50 of crocodile effluent for *Caridina nilotica* was 3.11% effluent concentration, with 1.98-5.96 confidence limits at 95% level of significance. There were no deaths in the control (0% effluent concentration). Chi-square for heterogeneity calculated was 6.8, and was less than chi-square for heterogeneity tabular value of 7.8 at 0.05 level of significance. The effluent proved to be toxic to *C. nilotica*. In the Probit analysis carried out on the results of the acute toxicity test, the tabular chi-square value, $X^2_{(crit)}$ was lower than the calculated chi-square value, $X^2_{(calc)}$ for heterogeneity. This supports that the Probit model was appropriate for this data, and the results may be accepted as valid for interpretation. Appendix E shows the full result of probit for 24 hours exposure time. Crocodile effluent was highly toxic to freshwater shrimps at very low concentrations.
4.4 Settling pond

The physico-chemical data collected at the farm with a settling pond were analysed to aid in assessing its effectiveness in pre-treatment of the farm effluent. There were no significant differences in any of the measured physicochemical variables between effluent before and after passage through the settling pond (Table 6).

**Table 6: T-test results (n\textsubscript{1} = 4; n\textsubscript{2} = 4) of the settling pond entrance and exit points**

<table>
<thead>
<tr>
<th>physico-chemical variables</th>
<th>Ent mean</th>
<th>Exit mean</th>
<th>Ent std dev</th>
<th>Exit std dev</th>
<th>t-Test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp</td>
<td>31.2</td>
<td>29.9</td>
<td>1.2</td>
<td>1.3</td>
<td>1.529</td>
<td>0.177</td>
</tr>
<tr>
<td>DO</td>
<td>1.97</td>
<td>2.00</td>
<td>2.43</td>
<td>2.49</td>
<td>-0.019</td>
<td>0.986</td>
</tr>
<tr>
<td>Cond</td>
<td>601.8</td>
<td>609.8</td>
<td>135.2</td>
<td>121.5</td>
<td>-0.088</td>
<td>0.933</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
<td>7.3</td>
<td>0.29</td>
<td>0.40</td>
<td>-0.711</td>
<td>0.504</td>
</tr>
<tr>
<td>Turb</td>
<td>68</td>
<td>58.25</td>
<td>7.35</td>
<td>24.81</td>
<td>0.754</td>
<td>0.498</td>
</tr>
<tr>
<td>BODs</td>
<td>37.5</td>
<td>32.8</td>
<td>2.658</td>
<td>14.997</td>
<td>0.624</td>
<td>0.556</td>
</tr>
<tr>
<td>Nit</td>
<td>418.3</td>
<td>416.1</td>
<td>286.9</td>
<td>203.4</td>
<td>0.012</td>
<td>0.991</td>
</tr>
<tr>
<td>TP</td>
<td>5888.4</td>
<td>5386.6</td>
<td>4691.48</td>
<td>878.20</td>
<td>0.210</td>
<td>0.840</td>
</tr>
<tr>
<td>PO\textsubscript{3-4}</td>
<td>3119.2</td>
<td>3660.6</td>
<td>1874.06</td>
<td>2272.53</td>
<td>-0.368</td>
<td>0.726</td>
</tr>
<tr>
<td>NH4</td>
<td>28967</td>
<td>25364</td>
<td>20242.78</td>
<td>13222.33</td>
<td>0.298</td>
<td>0.776</td>
</tr>
</tbody>
</table>

Note: Ent mean = entrance mean
Ent std dev = entrance standard deviation
Exit std dev = exit standard deviation
CHAPTER 5

5 DISCUSSION

5.1 Preamble

This study assessed the effect of crocodile effluent on macroinvertebrate community structure on Lake Kariba. It was hypothesized that the macroinvertebrates community at impacted sites would be significantly different from the one at a control site. The results, as expected, showed that the macroinvertebrates communities were significantly impacted by the effluent from crocodile farming. Species richness and general diversity (Shannon index) were significantly lower at the impacted sites than at the control site. Multidimensional scaling ordination and hierarchical cluster analyses completely separated the macroinvertebrate community at the control site from those obtained at the two impacted sites. The calculated taxa richness, Shannon's diversity and evenness indices revealed the decimating effects of the crocodile effluent on communities of benthic macroinvertebrates. Reduced values of these indices and an increase in dominance index at Sites 2 and 3 are similar to the typical response of benthic communities to organic pollutants (Kirkagac et al, 2004; Couceiro et al, 2006). The taxa that were encountered at these sites tallied with taxa reported to be relatively tolerant to very tolerant to organic pollution from findings of other studies (Woodecock and Huryn, 2007; Yucel-Gier et al, 2007). The settling pond meant for pre-treatment of effluent at Site 1 was not effective. The physico-chemical parameters measured indicated high organic nutrients loading.
5.2 Physico-chemical parameters

The two sites at which crocodile farm effluent was discharged greatly differed from the control site with respect to the physico-chemical variables. The water quality at the impacted sites was much lower than at the control site. However, between the two impacted sites, Site 1 was less impacted than Site 2. Dissolved oxygen is one of the most important abiotic factors relating to the survival of most aquatic organisms, which depend on oxygen dissolved in the water. The maintenance of adequate dissolved oxygen concentrations is thus critical for the survival and function of aquatic biota. Aerobic decomposition of organic matter by microorganisms affects the concentration of dissolved oxygen in the water. Depletion of DO plays a major role in driving biological changes. The oxygen requirements of aquatic organisms vary with type of species, with life stages, i.e. eggs, larvae, nymphs, adults, and with different life processes, i.e. feeding, growth, reproduction and size. Juvenile life stages of many aquatic organisms are more sensitive to physiological stress arising from oxygen depletion than adults, and in particular to secondary effects, such as increased vulnerability to predation and disease. If possible, many species will avoid anoxic or oxygen depleted zones (Dallas and Day, 2004). Insects like ephemeropterans (mayflies), plecopterans (stoneflies) and trichopterans (caddisflies) respire through gills or by direct cuticular exchange, and are thus when exposed to hypoxia for prolonged times may result in tissue damage, increase in size of organs and other stresses. In some insects like the caddisfly Distronia magnifica, low dissolved oxygen could cause reduced moulting success and delays in adult emergence (Dallas and Day, 2004).
Impairment of oxygen concentration at Sites 1 and 2 may be attributed to the high content of organic matter. The BOD₅ obtained at Sites 1 and 2 shows the degraded state of water at these sites, which can be ascribed to the crocodile effluent impact. The values obtained were higher than those at the control site, but not significantly different (p = 0.29). BOD₅ values indicate the extent of organic pollution in aquatic ecosystems, which adversely affects water quality (Jonna lagadda and Mhere, 2001). There is a close relationship between the BOD₅ values obtained in this study and those reported for polluted Lake Chivero in Zimbabwe (Nhapi, 2009). BOD₅ summarizes the quality of all organics in a single value. It thus makes it an extremely useful measure of organic enrichment. When organic matter exceeds the capacity of a system to assimilate it, a degradation cycle begins. The enhanced level of oxygen consumption by aerobic decomposers exceeds the rate of re-aeration and dissolved oxygen concentrations begin to fall. If DO decline continues, aerobic decomposers cease to function and anaerobic organisms populate the water and sediment. The high organic loading as evidenced in this study may be contributing to oxygen depletion shown by the low readings of dissolved oxygen at the perturbed sites. This could lead to a vicious cycle as low levels of DO are reported to facilitate the release of nutrients from sediments, further enriching the waters (R.I.C. 2000).
5.2.1 Nutrient enrichment

The major nutrients that contribute to eutrophication, phosphorous as phosphate ions ($\text{PO}_4^{3-}$), nitrogen as nitrate ($\text{NO}_3^-$) and ammonium ($\text{NH}_4^+$) ions were in high quantities at study Sites 1 and 2. Orthophosphate was higher at Site 1 and even higher at Site 2 than at the control site. The difference between Site 1 and Site 2 at the point of discharge may partly be attributed to the effect of the settling pond at Site 1 and the length of the drainage channel which spans approximately a kilometre to the lake. The measured parameters on the effluent on entering the settling pond showed some reduction in readings on exit from the settling pond. However, the differences were not statistically significant (Table 6). Thus, the length of the sewer drain could be contributing to the pre-treatment of the effluent whose readings were lower at the point of disposal to the lake. The drainage channel is not lined with concrete, and thus some of the orthophosphate could have been lost to sediments. Another possible explanation could be that floating mats of $E. \text{crassipes}$ that occupy part of the channel within the farm perimeter but after the settling pond, may have contributed to the reduction of the nutrient. This form of phosphorous is the most readily available for plant uptake during photosynthesis (R.I.C., 2000). Despite the differences for entrance and exit readings not being statistically significant, the difference may be sufficient to have implications on other biological processes.

Total phosphorous (TP) was high at Site 1 and Site 2 in comparison to Site 3. TP is an essential plant nutrient and is often the limiting nutrient to plant growth in fresh water
and is rarely found in significant concentrations in surface waters. Most lakes not affected by anthropogenic inputs have concentrations of TP generally less than 0.01mg/l (R.I.C. 2000). Most recent recordings for Lake Kariba on pelagic areas (1-5m depth) of the lake’s Charara basin had mean nutrient concentrations ranging between: 0.007-0.024mg/l for total phosphates, 0.003-0.004mg/l for phosphate phosphorus (Lindmark, 1997). These recordings are far below the recordings in this study. Since phosphorous is generally the most limiting nutrient, its input to fresh water systems can cause extreme proliferation of algal growth, thus its introduction by the effluent is the prime contributing factor to eutrophication in the effluent disposal points. Macroinvertebrates do not directly respond to phosphorus enrichment, but instead respond to changes in dissolved oxygen concentration and habitat alterations that accompany high TP levels (Roberts, 2005). Knowledge of the role of processes and mechanisms that control the supply of bioavailable phosphate, that is orthophosphate, is essential for the management of lakes to avoid eutrophication (Webster et al, 2001).

Nitrates being the end products of aerobic stabilization of organic nitrogen are seldom abundant in natural surface waters with concentrations less than 0.10mg/l as photosynthetic action is constantly converting them to organic nitrogen in plant cells (R.I.C., 2000). This nutrient is toxic at high concentrations and readings obtained in the present study of 0.59mg/l and 0.38mg/l at Site 1 and Site 2, respectively, are relatively high and may possible contribute to the fewer taxa of macroinvertebrates observed at the perturbed sites.
Ammonia occurs as free unionized form NH$_3$ or as ammonium ions NH$_4^+$ in effluent, and its toxicity is directly related to the concentration of the un-ionized form since the ammonium ion has little or no toxicity (Williams et al, 1986 in Dallas and Day, 2004) though it contributes to eutrophication. The readings of ammonia obtained at the study sites are relatively very high as compared to typical concentrations of ammonia and ammonium compounds of less than 100µg$l^{-1}$ (R.I.C., 2000; Dallas and Day, 2004). At low to medium pH values, the ammonium ion dominates, but as pH increases ammonia is formed. Ammonia is highly toxic to aquatic organisms (Magadza, 1997). However, a high concentration of ammonia ions in water at low pH is not toxic, but if the pH is raised toxicity will develop. Ammonia toxicity can be exacerbated by concentrations of DO, carbon dioxide and presence of other toxicants, like metal ions (Dallas and Day, 2004). Most recent recordings for lake Kariba on pelagic areas (1-5m depth) of the lake’s Charara basin had mean nutrient concentrations ranging between 2-20µg$l^{-1}$ for nitrate nitrogen (NO$_3$-N) and 5-17µg$l^{-1}$ for ammonium nitrogen (Lindmark, 1997). The ammonia readings in the present study were high, but the pH recorded for the sites was relatively not high, leaving the observed BMI assemblages to other confounding factors, or to synergistic mechanisms of toxicity if ammonia is playing a part. In a separate study, Schofield et al, (1990) in Dallas and Day, (2004) found significant deleterious effects on macroinvertebrate communities in a stream subjected to 3,0mg$l^{-1}$ – 5,0mg$l^{-1}$ (baseline) and 20,0mg$l^{-1}$ (peak) un-ionized ammonia from an intensive dairy farm. Thus, the ammonia in the present study may be contributing to benthic macroinvertebrate losses. Water pollution represented by all forms of nitrogen and total phosphorous affect macroinvertebrate fauna by causing reduction in taxa richness, simplifying the
macroinvertebrate community composition without changing abundances (Couceiro et al, 2006). This was also evident in the present study.

Eutrophication might be welcomed as a process, as it increases productivity of the water and thus, for instance potential fish yields. It should be realized, however, that increased productivity is not always translated in increased yields of interesting products. Ecologically, there are important negative side effects which include the reduction of biodiversity and the loss of species that are dependent upon presence of aquatic vegetation and clear water. Highly valued species such as *Hydrocynus vittatus* and *Limnothrissa miodon* that require clear water may disappear, and may be replaced by less valued species like *Clarias gariepinus* and *Sinodontis zambezensis*. Highly trophic and shallow lakes may experience winterkills of fish caused by overturn as experienced at Lake Chivero in 1996 (Moyo, 1997).

5.3 Benthic macroinvertebrates

The benthic composition determined in the present study was partially consistent with other studies of organic pollution in perturbed areas that have recorded dominance of insects and molluscs in the benthic samples (Bredenhand, 2005; Sarang and Sharma, 2006). BMI have been used successfully in other studies to determine the ecological conditions of water bodies receiving aquaculture effluents and organisms like *chironomids* (Woodcock and Huryn, 2007), polychaeta and molluscs (Yucel Gier et al, 2007) have been identified as possible bioindicators of perturbed areas. Most of the work
on the use of BMI has largely concentrated on fish farms in lotic systems. This study recorded high numbers of the gastropod *Melanoides* alongside non biting midge larvae *chironomids* at the two perturbed sites of this lentic system. The genus *Cleopatria* was not as prevalent in the perturbed sites though it also belongs to the Thiaridae family as the *Melanoides*. However, the genus *Bellamya* was also more prevalent at the polluted sites than at the control site indicating some tolerance to organic enrichment. From observations of the present study, the gastropod *Melanoides* may serve as a potential indicator species of nutrient enriched sites. The decapoda *Caridina nilotica* was abundant at the control site but absent at the perturbed sites. Also found at the control site but absent at the perturbed sites were members of the families Cyclosteriidae, Hydrachnellae and Notonectidae. The present study has shown that BMI may be used as tools to assess the impact of aquaculture effluent on shallow inshore of a lentic system like Lake Kariba. It is evident from the study that water quality deteriorated at the discharge point of aquaculture effluent. Consequently, this has resulted in environmental degradation, which means that only tolerant species can survive while sensitive species are doomed to local extinction. Furthermore, tolerant species may be increasing in population numbers due to the decline of competition with more sensitive species.

### 5.4 WET tests

The Actyidae, *Caridina nilotica*, showed great sensitivity to crocodile farm effluent as depicted in the WET tests carried out over 24 hours. The lethal concentration of crocodile effluent is so small, indicating high toxicity of whole effluent on this test organism. This
supports the observations in the field where the species was notably absent from all the perturbed sites but prevalent in relatively unperturbed area.

Symptoms observed during the toxicity test were quite comparable to observations on other crustaceans (Lodhi et al, 2006). Though behavioural responses to toxicants have been shown to indicate sensitivity, there are no standardized behavioural responses established for early detection of toxicity in crustaceans. These would facilitate early detection of pollution before lethality sets in when perturbation occurs. *Caridina nilotica* response to crocodile effluent in this study forms the baseline data for comparison with LC$_{50}$ of other known pollution sensitive macroinvertebrates. This will enable the compilation of possible indicator species for aquaculture effluent on the lake. It is also imperative that the life history of the *C. nilotica* be documented to enable chronic toxicity tests to be carried out, as these are more reliable in production of effluent standards than acute toxicity tests (Dallas and Day, 2004).

### 5.5 Settling pond

The difference in means of the physico-chemical parameters to determine the impact of the settling pond showed no significant differences according to t-test, despite the readings physically showing some reductions in relation to entering effluent and exiting effluent. Thus, it can be assumed that the settling pond has little if any effect in pre-treating the effluent before it is disposed of in the lake. This implies that the lake is receiving untreated effluent, and is thus exposed to the subsequent effects of reduction in macroinvertebrates diversity. The design of the settling pond or the lack of it could be
contributing to the ineffective screening of the effluent, because the pond is just a dug out hole within the sewer drain, and basically receives no attention at all as to the removal of sediment. A number of escapee crocodiles from the pens inhabit it and churn the sediments in their movements, thus not allowing the sediment to settle and somehow pre-treat the effluent.

5.6 Mechanisms of toxicity

The main causes of reduced macroinvertebrate numbers reported in other studies of fish effluents include increased substrate embeddedness and organic matter blanketing the stream bed and the animals themselves (Roberts, 2005).

Roberts, (2005) produced clear evidence that organic growth and particles on the invertebrates were the most likely causes of toxicity. Solid fecal and food particles from the fish collected between the legs and upon the external gills of the organisms in the effluent samples. This is important to organisms with external gills, such as most Ephemeroptera, Plecoptera, and Trichoptera taxa, because respiration can be hindered. High readings of turbidity in this study, in comparison to the control site may infer that there exist high suspended solids in the crocodile effluent, such as fecal and food particles. In studies on man-made lakes in South Africa, it appeared that 1mg l⁻¹ is roughly equivalent to 1NTU (Walmsley and Butty, 1980 in Dallas and Day, 2004). Thus, it could be that most gilled taxa within the perturbed areas are directly being affected by the effect of suspended sediment concentrations at the effluent disposal points. This could also be compounded by the absence of effective settling ponds and the reduction in sewer
drain flow speed as the effluent enters the relatively still waters of the lake which accelerates the deposition of the suspended particles within the confines of the area of disposal.

Observed in the field during this study was the apparent absence of submerged or floating aquatic vegetation at the perturbed sites, save for sparse floating mats of *E. crassipes* at the effluent disposal points. The absence of submerged or floating vegetation in the perturbed sites could be depriving an important habitat for many aquatic macroinvertebrates that feed, shelter or emerge among plants. This absence of refugia for macroinvertebrates reduces habitat heterogeneity, and may contribute to the low diversity of macroinvertebrates. In similar studies, macroinvertebrates have shown strong preference for submerged vegetation, possibly because they are complex habitats that provide refuge from predation and food for a variety of herbivores and detritivores, attachment for filter feeding taxa and exit points for emerging insects (Chakona *et al*, 2008). The vegetation mainly associated with presence of the fresh water shrimps was composed mainly of *Vallisneria aethiopica* though the control site was characterized by the presence of this submerged macrophyte and other aquatics like *Lagarosiphon sp*, *Polygonum sp* and the oxygen weed, *Myriophylum aquaticum*, in the shallow marginal areas studied. In vegetated aquatic systems, habitat structure is often provided by macrophytes, and their importance as a habitat is demonstrated by a far greater abundance of macroinvertebrates than in unvegetated area. The abundance and richness of the macroinvertebrates community appears to be proportional to the density or biomass of freshwater macrophytes (Warfe and Barmuta, 2006). The absence of shrimps may not
necessarily be attributed wholly to mortality due to the effluent at the disposal site as the absence of typical vegetation in shrimp areas was observed at the study sites. The vegetation could explain to some degree the absence of these macroinvertebrates at impacted sites.

Aquatic resources need to be sustainably managed in order not to compromise the needs of the future generations. From a management perspective, these results have implications for the conservation of water reservoirs in Zimbabwe. Some lakes in Zimbabwe suffer from severe pollution due to organic enrichment as is the case with Lake Chivero (Magadza 1997; Moyo, 1997; Nhapi, 2009). Since macroinvertebrates are an important component of both aquatic and terrestrial food webs, reductions in macroinvertebrates production and biodiversity have ramifications for the management of native fisheries and insectivorous birds (Chakona et al, 2008). Evidence exists that support the notion that changes in invertebrate production can affect successful recruitment in fish and water birds (Jenkins and Boulton, 2007 in Chakona et al, 2008). In order to enhance sustainable management of crocodile ranching, various measures could be undertaken and some of these form the basis of the next chapter on conclusions and recommendations.
CHAPTER 6

6 CONCLUSION AND RECOMMENDATIONS

It can be concluded from the findings of the present study that untreated crocodile effluent is contributing high levels of nutrients to the lake in the form of ammonia and phosphorous, thus causing eutrophication at the points of discharge of the effluent. The study showed that water quality deteriorated around the crocodile farm effluent discharge points. Macroinvertebrate diversity, evenness and richness were low at the impacted sites. Benthic macroinvertebrates may be used as tools to assess the impact of aquaculture effluent on shallow inshores of a lentic system like Lake Kariba. The gastropod *Melanoides tuberculata* and decapoda *Caridina nilotica* are hereby proposed for consideration as potential bioindicators of organic enrichment in Lake Kariba.

It is imperative that the aquaculture establishments install or upgrade the system of wastewater purification before disposing of it back into the lake. This will reduce the nutrient loading at the points of disposal. The impacts of crocodile effluent on Lake Kariba must be monitored to avoid aquaculture-related biodiversity loss, which is already declining in polluted areas as this study has highlighted. The impact discussed here may be considered preliminary, owing to limitations on the scope and depth of parameters used as criteria. Further detailed studies with more survey sites at impacted areas are necessary to fully document changes in water quality and benthic macroinvertebrate community structure and the extent and duration of such changes so as to understand
better pollution processes in this lake that might call for improved regulation and policy development.


de Moor, I.J., Day, J.A. and de Moor, F.C. (2003b) Guides to the freshwater invertebrates of Southern Africa, vol 8: Insecta II, Hemiptera, Megaloptera, Trichoptera and


Miller, W.G. (2009). OpenStat. OPENSTAT@MSN.com


Sarang, N. and Sharma, L.L. (2006). Macrobenthic fauna as bioindicator of water quality in Kishore Sagar Lake, Kota (Rajasthan) India. Department of Aquaculture,


APPENDIX A.

PHYSICO-CHEMICAL INDICES ANOVA POST TEST
TUKEY’S PAIRWISE COMPARISONS Q>p (same)

<table>
<thead>
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<th>site 1</th>
<th>site 2</th>
<th>site 3</th>
</tr>
</thead>
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APPENDIX B

COMMUNITY INDICES VALUES

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<thead>
<tr>
<th>Sample</th>
<th>S</th>
<th>N</th>
<th>d</th>
<th>J'</th>
<th>H'(loge)</th>
<th>1-Lambda'</th>
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<td>Site 1 (D)</td>
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<td>0.5606</td>
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<tr>
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</tr>
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<td>0.2892</td>
<td>0.6013</td>
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<td>2.282</td>
<td>0.834</td>
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</table>

S= No. of taxa  
N= Abundance  
d= Margalef  
J'= Evenness  
H'= Shannon  
1-Lambda= Simpson
### APPENDIX C

#### COMMUNITY INDICES ANOVA POST TEST

**TUKEY’S PAIRWISE COMPARISONS Q\(p\) (same)**

<table>
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<th>SITE 1</th>
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<th>SITE 3</th>
</tr>
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<tbody>
<tr>
<td><strong>a) RICHNESS (S)</strong></td>
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<td></td>
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<tr>
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</tr>
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<td>SITE 3</td>
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<td><strong>b) ABUNDANCE (N)</strong></td>
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<td></td>
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<td>SITE 1</td>
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</tr>
<tr>
<td>SITE 3</td>
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</tr>
<tr>
<td><strong>c) EVENNESS (J')</strong></td>
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<td></td>
<td></td>
</tr>
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</tr>
<tr>
<td><strong>d) SHANNON WIENER (H')</strong></td>
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<td></td>
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</table>
APPENDIX D

DATA ON MORTALITY RATE OF *CARIDINA NILOTICA* EXPOSED TO DIFFERENT CONCENTRATIONS OF WHOLE EFFLUENT FROM THE CROCODILE FARM.

<table>
<thead>
<tr>
<th>Conc.</th>
<th>log conc.</th>
<th>No. tested</th>
<th>% mortality at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>0.20</td>
<td>-2.7</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>0.50</td>
<td>-2.3</td>
<td>15</td>
<td>0</td>
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<td>1.00</td>
<td>-2</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>2.00</td>
<td>-1.7</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>10.00</td>
<td>-1</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>
APPENDIX E

EPA PROBIT ANALYSIS PROGRAM (Version 1.5)
USED FOR CALCULATING LC/EC VALUES

Crocodile farm effluent

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Number Exposed</th>
<th>Number Resp.</th>
<th>Observed Proportion Responding</th>
<th>Proportion Responding Adjusted for Controls</th>
<th>Predicted Proportion Responding</th>
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<td>0.2000</td>
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<td>1</td>
<td>0.0667</td>
<td>0.0667</td>
<td>0.0116</td>
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<tr>
<td>0.5000</td>
<td>15</td>
<td>1</td>
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<td>0.0667</td>
<td>0.0652</td>
</tr>
<tr>
<td>1.0000</td>
<td>15</td>
<td>2</td>
<td>0.1333</td>
<td>0.1333</td>
<td>0.1739</td>
</tr>
<tr>
<td>2.0000</td>
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<td>3</td>
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<td>0.2000</td>
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<tr>
<td>10.0000</td>
<td>15</td>
<td>14</td>
<td>0.9333</td>
<td>0.9333</td>
<td>0.8335</td>
</tr>
</tbody>
</table>

Chi - Square for Heterogeneity (calculated) = 6.804
Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Mu = 0.492252
Sigma = 0.524383

Parameter                  Estimate    Std. Err.         95% Confidence Limits
------------------------------------------------------------------------
Intercept                  4.061274    0.215657    (3.638587, 4.483961)  
Slope                      1.907002    0.391284    (1.140086, 2.673918)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

<table>
<thead>
<tr>
<th>Point</th>
<th>Exposure Conc.</th>
<th>95% Confidence Limits</th>
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</thead>
<tbody>
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<td>0.036</td>
</tr>
<tr>
<td>LC/EC 5.00</td>
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<td>LC/EC 10.00</td>
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<td>LC/EC 15.00</td>
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<td>0.424</td>
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