THE EFFECTS OF DDE ON THE HEALTH OF THE
MOZAMBIQUE TILAPIA (OREOCHROMIS MOSSAMBICUS)

BY
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DISSERTATION

SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

AQUATIC HEALTH

IN THE

FACULTY OF SCIENCE

AT THE
UNIVERSITY OF JOHANNESBURG

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CO-SUPERVISOR: PROF V. WEPENER

2010
ACKNOWLEDGEMENTS

“The earth is the Lord’s, and everything in it; for he founded it upon the seas and established it upon the waters.” Psalm 24 vs 1-2.

It is with great appreciation and recognition that I wish to thank the following persons and institutions for the contributions that they have made to the completion of this study:

- Prof. J.H.J van Vuren for all his extreme patience, motivation, time and advice throughout the duration of the project.

- The Water Research Commission for the financial support.

- Dr. R. Greenfield, Moses and Sollie for all their help on fieldtrips and in the aquarium and with the help in the construction of the experimental system and its maintenance.

- L. Moolman for all her help and advice concerning the biomarkers

- Dr. M. Ferreira for his help with the statistical analysis

- Last but not least, my mom and my dad and my amazing friend Kerry Brink, for their endless love and continued support and encouragement throughout the study and without whom, the completion of this project would not have been possible. Thank you for never giving up on me.
The organochlorine insecticides were amongst the first pollutants shown to cause adverse population effects. The potential adverse effects of these pollutants on wildlife are a cause for great concern. Severities of their effects were sometimes surprising given the low levels of the compounds in environmental compartments such as surface waters and soils. High lipophilicity combined with chemical stability and very slow biodegradation are characteristic features of these toxic Persistent Organic Pollutants (POPs). Regional declines in fish, bird as well as invertebrate populations resulting from long term exposure to POPs such as 1,1-bis (4-chlorophenyl) -2,2,2-trichloroethane (DDT) and its stable metabolite 1,1-bis (4-chlorophenyl) -2,2-dichloroethene (DDE), could be related to some biochemical, endocrine and physiological effects in individuals. Some POPs have been suggested to have negative effects disrupting physiological processes and resulting in alterations of homeostasis, reproduction, development and behavior. Such adverse effects upon populations may be avoided if the potential of chemicals to cause them is recognized before problems arise. The aim of this study was to determine whether or not the ongoing spraying of DDT in the Limpopo Province is negatively affecting the health of aquatic species found in surface water of the area. Extensive research has shown that biomarkers have been very effective in the trace determination of a number of adverse effects caused by metals, and thus, are also being used for POPs. A battery of biomarkers (EROD, CAT and CEA) were used, both in the field and in a controlled laboratory environment, in order to try and determine the long term effects of exposure to low environmentally relevant levels of DDE in the selected area. DDT levels in the biota, water and sediment samples were also measured to determine the possible levels of exposure. Dose-response relationships were most successfully determined by the EROD and the CEA biomarkers in this study. In a controlled laboratory study, a definite effect was noted on the Mozambique Tilapia with increasing concentrations of DDE. In the natural environment, dose-response relationships to DDE exposure were more difficult to quantify as additional chemicals and natural environmental stressors also affect the results.
OPSOMMING

Die organochloor insektoders was van die eerste besoedelstowwe wat as oorsaak van nadelige gevolg vir die bevolking uitgewys is. Die potensiële nadelige gevolge van hierdie besoedelstowwe op wildlewe is 'n bron van groot kommer. Die erns van hul gevolge was soms verrassend, gegewe die lae vlakke van die verbindings in omgewings soos oppervlakwater en grond. 'n Hoe vlak van vetsug saam met chemiese stabiliteit en baie stadige afbreking is kenmerkende eienskappe van hierdie toksiiese organiese besoedelstowwe. Streeksgebonde afnames in vis-, voël- en werwedierbevolkings as gevolg van langdurige blootstelling aan SOBs soos DDT en sy stabiele metaboliet DDE, hou moontlik verband met sommige biochemiese, endokriene en fysiologiese versteurings by individue. Sommige organiese besoedelstowwe is uitgewys as moontlike oorsake van ontwrigte fysiologiese prosesse wat lei tot veranderings in homeostase, voortplanting, ontwikkeling en gedrag. Sulke negatiewe gevolge op bevolkings kan vermy word as die potentiaal van chemiese stowwe om versteurings te veroorsaak, raaksien en erken word voordat probleme ontstaan. Die doel van hierdie ondersoek was om vas te stel of die voortgesette bespuiting van DDT in die Limpopo Provinsie 'n negatiewe uitwerking op die gesondheid van waterorganismes het wat in hierdie omgewing voorkom. Uitgebreide navorsing het aangetoon dat biomerkers baie efektiw is in die vasstelling van tekens van verskeie negatiewe gevolge wat deur metale veroorsaak is. Dieselfde biomerkers kan daarom ook as indicators van organiese besoedelstowwe gebruik word. 'n Battery van biomerkers (EROD, CAT en CEA) is gebruik, beide in die veld sowel as in 'n gekontroleerde laboratoriumomgewing, met die doel om die langtermyngevolge van blootstelling aan lae omgewingsvriendelike vlakke van DDE in die gekose gebied te probeer vasstel. DDT-vlakke in die biota, water en sediment-monsters is ook bepaal om die moontlike vlakke van blootstelling te bereken. In 'n gekontroleerde laboratoriumomgewing, 'n effek was beslis opgemerk, veral met die EROD en die CEA biomerkers.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>CBR</td>
<td>Critical Body Residue</td>
</tr>
<tr>
<td>CEA</td>
<td>Cellular Energy Allocation</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>CYP1A</td>
<td>Cytochrome P4501A1</td>
</tr>
<tr>
<td>DDD</td>
<td>1,1-bis (4-chlorophenyl) -2,2-dichloroethane</td>
</tr>
<tr>
<td>DDE</td>
<td>1,1-bis (4-chlorophenyl) -2,2-dichloroethene</td>
</tr>
<tr>
<td>DDT</td>
<td>1,1-bis (4-chlorophenyl) -2,2,2-trichloroethane</td>
</tr>
<tr>
<td>dH₂O</td>
<td>Distilled water</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
</tr>
<tr>
<td>DOM</td>
<td>Dissolved Organic Matter</td>
</tr>
<tr>
<td>Eₐ</td>
<td>Total Energy Reserves</td>
</tr>
<tr>
<td>Eₑ</td>
<td>Energy Consumption</td>
</tr>
<tr>
<td>Eᵣ</td>
<td>Available Energy Reserves</td>
</tr>
<tr>
<td>EROD</td>
<td>7-Ethoxyresorufin-O-deethylase</td>
</tr>
<tr>
<td>ETS</td>
<td>Electron Transport Activity</td>
</tr>
<tr>
<td>GPx</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen Peroxide</td>
</tr>
<tr>
<td>INT</td>
<td>p-IodoNitroTetrazolium</td>
</tr>
<tr>
<td>KMnO₄</td>
<td>Potassium Permanganate</td>
</tr>
<tr>
<td>KNP</td>
<td>Kruger National Park</td>
</tr>
<tr>
<td>MFO</td>
<td>Mixed Function Oxygenase</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate (reduced form)</td>
</tr>
<tr>
<td>NTU</td>
<td>Turbidity</td>
</tr>
<tr>
<td>PAHs</td>
<td>Poly Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>PCA</td>
<td>Principle Component Analysis</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated Biphenyls</td>
</tr>
<tr>
<td>POPS</td>
<td>Persistent Organic Pollutants</td>
</tr>
<tr>
<td>RDA</td>
<td>Redundancy Analysis</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide Dismutase</td>
</tr>
<tr>
<td>SFG</td>
<td>Scope for Growth</td>
</tr>
<tr>
<td>TCI</td>
<td>Tissue Condition Index</td>
</tr>
<tr>
<td>TDS</td>
<td>Total Dissolved Solids</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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</tr>
<tr>
<td>TWQR</td>
<td>Target Water Quality Guidelines</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WRC</td>
<td>Water Research Commission</td>
</tr>
<tr>
<td>WWS</td>
<td>Warm Wet Season</td>
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1. INTRODUCTION

There are many different types of contaminants present in the environment but the potential adverse effects of Persistent Organic Pollutants (POPs) in the environment and on wildlife, in particular, are a cause for great concern. Toxic chemicals with long half lives in the environment were released on a large scale all over the world when there was still very little knowledge as to their potential effects on the environment (Vasseur and Cossu-Leguille, 2005). The organochlorine insecticides are just such chemicals and were some of the first pollutants shown to cause adverse population effects in various trophic levels. The severity of their effects was surprising given the low levels of the compounds in environmental compartments such as surface waters and soils. High lipophilicity combined with chemical stability and very slow biodegradation are characteristic features of these POPs, allowing them to become bioaccumulated as well as transported over very long distances (Vasseur and Cossu-Leguille, 2005; Vosloo and Bouwman, 2005).

1,1-bis (4-chlorophenyl) -2,2,2-trichloroethane (DDT) has been released into the environment since its discovery in the 1940s. Initially it was used to kill mosquitoes, thus combating the spread of malaria. In addition, it was also used as a form of pest control in crop protection. Following the discovery that DDT was responsible for declines in the populations of several predatory bird species, thus having had a distinct adverse effect on wildlife, DDT was restricted or totally banned in most countries during the 1970s and 1980s. It is still used in certain areas, however, particularly in developing countries like South Africa, for malaria vector control (Axmon and Rignell-Hydbom, 2006; Parvez and Raisuddin, 2005).

In nature the principal decomposition product of DDT is DDE (1,1-bis (4-chlorophenyl) -2,2-dichloroethene) (Solomons and Fryhle, 2000). According to Leanos-Castaneda et al. (2004), the two most common routes described for DDT metabolism in the environment are reductive dechlorination to DDD (1,1-bis (4-chlorophenyl) -2,2-
dichloroethane), and dehydrochlorination to DDE, with DDE being the main metabolite of the two. These metabolites are also highly persistent in the environment and have similar chemical and physical properties.

Adverse effects upon populations may be minimized and possibly even avoided if the full potential of these chemicals to cause them is recognized before problems arise. This approach needs to take into account the potential of the chemicals to have long term chronic effects. Because adverse effects are generally first at the sub-organismal level, sub-lethal or chronic effects are serious and far more common in the environment than acute effects (Joubert, 2000). Long term chronic effects of a pollutant in the environment and on its organisms may be due to compounds showing marked biological persistence, thus often leading to longer term biomagnification with movement along food chains, as in the case of POPs. There may also be the long term consequence of biochemical or physiological disturbances or even of population disturbances (Vasseur and Cossu-Leguille, 2005).

The need to be able to predict the effects of certain chemicals or pollutants on various populations in the environment from laboratory studies has become increasingly necessary. There is thus a need for physiological indicators and biochemical indicators of organism health and sub-lethal toxicant effects. Using biological indicators (biomarkers), it could be possible to identify the effects of environmental pollution before the health of the environment is seriously affected (Joubert, 2000). In past years, a large number of research projects have focused on the use of biomarkers in laboratory testing. It is, however, becoming increasingly important to apply established laboratory techniques in the evaluation of field conditions in order to ensure a healthy aquatic environment.

For the purposes of this study, it was hypothesized that the ongoing spraying of DDT in the Limpopo Province is negatively affecting the health and reproduction of aquatic species found in the area, with the main aim being to determine whether or not environmental levels of DDE, the metabolite of DDT that has historically been found in
the waters in the study area, are contributing to adverse health effects in aquatic species, more specifically, the Mozambique Tilapia (*Oreochromis mossambicus*).

With this hypothesis in mind, specific objectives were laid out:

- To determine the level of exposure of the Mozambique Tilapia to DDE by performing chemical analysis and biological testing for DDE concentrations in water, sediment and tissue samples.
- To determine the dose-response relationship induced when exposing the Mozambique Tilapia to varying degrees of environmentally relevant DDE concentrations in a controlled laboratory exposure using the specifically selected biomarkers.
- To determine whether or not DDE is having an effect on the health of the Mozambique Tilapia in the study area using specifically selected biomarkers.

Chapter 2 provides a detailed description of DDT in the aquatic environment and the metabolite DDE. Chapter 3 describes the study area and the location of each of the study sites chosen. The biomarkers used on the fish from the study area and the results of each are presented in Chapter 4. Chapter 5 presents the results and discussion of the laboratory exposure studies and toxicity testing of DDE on *O. mossambicus*. Lastly, the general conclusion and recommendations of the previous chapters are summarized in Chapter 6.
1.1 REFERENCES


2. DDT AND THE AQUATIC ENVIRONMENT: PRESENCE AND EFFECTS

2.1 DDT IN THE ENVIRONMENT

2.1.1 INTRODUCTION

The insecticidal properties of DDT were discovered in 1942. Since then, vast quantities of this chlorinated hydrocarbon, belonging to a group of chemicals known as POPs, has been sprayed over the surface of the earth in an effort to destroy insects (Solomons and Fryhle, 2000), with special mention of the Anopheles mosquito, a known vector of the malaria parasite. In the late 1950s and 1960s, however, it began to become clear that the extended and vigorous use of DDT has not been without some harmful and damaging side effects such as cancer and diabetes in humans and significant impacts on wildlife (particularly eggshell thinning in birds) (Solomons and Fryhle, 2000).

The U.S. Department of Agriculture, the federal agency with the responsibility of regulating pesticides before the formation of the U.S. Environmental Protection Agency (EPA) in 1970, began regulatory actions to prohibit many of DDT's uses because of mounting evidence of the pesticide's declining benefits and environmental and toxicological effects. In 1972, the EPA issued a cancellation order for DDT based on the adverse environmental effects of its use and since 1996, the EPA has been participating in international negotiations to control the use of DDT and other persistent organic pollutants used around the world. In 2001, several countries, including South Africa, joined together and negotiated a treaty, the Stockholm Convention on POPs, to enact global bans or restrictions on selected POPs, including DDT. South Africa has, and continues to play a significant role in the negotiations and implementation of the Stockholm Convention texts (Vosloo and Bouwman, 2005). A limited exemption for the use of DDT to control the spread of the Anopheles mosquitoes was, however, included. September 2006, saw the World Health Organization (WHO) declare its support for the indoor use of DDT in African countries where malaria remains a major health problem,
citing that the benefits of the pesticide outweigh the health and environmental risks. The full extent as to the effect of DDT on its surrounding environment is still not clear, however, and as such, it should still be treated with caution (Axmon and Rignell-Hydbom, 2006).

2.2 DDE, A METABOLITE OF DDT

The chlorohydrocarbon, DDT, is prepared from inexpensive starting materials, chlorobenzene and trichloroacetaldehyde. The reaction is catalyzed by acid. In nature the principal decomposition product of DDT is DDE (Solomons and Fryhle, 2000). DDT consists of six principle isomers: p,p′-DDT, o,p′-DDT, o,p′-DDD, p,p′-DDD, o,p′-DDE and p,p′-DDE.

According to Leanos-Castaneda et al. (2004), the two most common routes described for DDT metabolism are reductive dechlorination to DDD, and dehydrochlorination to DDE, with DDE being the main metabolite of the two (see Figure 1).

Figure 1: The chemical structures of DDT, DDD and DDE (National Cancer Institute, 1978).

It is thus quite obvious that an abundance of DDE among DDT metabolites will often point to the historical rather than the recent input of DDT to the environment, whereas failure of detecting DDE isomers in samples is likely to be an indication of relatively recent DDT exposure (Sapozhnikova et al., 2004).
2.2.1 PHYSICAL AND CHEMICAL PROPERTIES OF DDE

DDE (also a chlorinated hydrocarbon) is semi-volatile and exists in both the gaseous phase as well as bound to particles. It is also resistant to degradation and is only slowly destroyed by natural processes in the environment. As a result of this, DDE is very persistent in the environment (Riedel et al., 2002; Vosloo and Bouwman, 2005), with residues taking between three and sixteen years to be broken down fully depending on the various influencing environmental factors (United States Protection Agency, 2008). It is fat soluble and tends to accumulate in the fatty tissues of most animals, including humans, biomagnifying at each step in a particular food chain (Riedel et al., 2002). For example: the food chain that runs from plankton to small fish to larger fish to birds and to larger animals, will magnify the concentrations of DDE as well as other chloro-organic compounds at each step (Solomons and Fryhle, 2000). According to Vosloo and Bouwman (2005) and Axmon and Rignell-Hydbom (2006), DDE also has the potential for long range transport in the environment and as such, traces of the chemical can be found all over the globe. According to Walker et al. (2006), various means of transport include:

- **Oceanic transport:**
  DDE is insoluble in water, but can be dissolved in alcohol and taken up by fats and organic matter, thus allowing for DDE to adsorb onto particles and be taken up by biota or by settling out and forming part of the sediment component. Vertical transport (due to the effects of gravity) tends to result in the ocean sediment being the major final sink for POPs on a global scale.

- **Riverine transport:**
  Water solubilities are so low, that dissolved transport is generally not a significant vector for transport via rivers. Colloidal and suspended sediments are thus the main method of transport in riverine systems.
• **Air transport:**
  DDE does go into the gas phase and due to its resistance to chemical breakdown, can be transported over very long distances. In colder conditions, chemicals like DDE tend to condense out of the gas phase once again.

• **Migratory animals:**
  DDE bioaccumulates and bioconcentrates up a food chain, thus resulting in significantly increased concentrations of DDE in animals high up in the food chain and the migration of animals, with special mention of insects and birds, helps in the dispersal of DDT and its metabolites.

### 2.2.2 AQUEOUS ENVIRONMENTAL CHEMISTRY OF DDE

A major source of DDE contamination in an aqueous environment is through the use of the pesticide, DDT, for the control of disease carrying insects, where it is then washed into rivers and lakes from both sources of agricultural and urban runoff and during times of rainfall (Henry and Kishimba, 2006). DDE may also enter the aqueous environment by precipitating out of the atmosphere or through migration of animal species into those areas. DDE is not water soluble and as stated above, it adsorbs quickly onto organic particles and accumulates in sediments, moves out of the water into the gas phase, or is taken up by biota. According to Misumi *et al.* (2005) DDE is the most consistently found organochlorinated compound in aquatic biota and bed sediment in contaminated rivers and lakes, with the bottom sediments acting as major sinks for the pollutant (Zapata-Perez *et al.*, 2000; Vosloo and Bouwman, 2005).

DDE is highly toxic to many aquatic species (Sapozhnikova *et al.*, 2004) and due to the persistence, biomagnification and bioaccumulation, several populations have increased body burdens of this chemical (Axmon and Rignell-Hydbom, 2006). Some fish species, mainly bottom-dwellers, may represent appropriate targets to assess the biological effects of pollutants adsorbed to sediments (Zapata-Perez *et al.*, 2000) as the concentrations of chemicals such as DDE have been found to bio-magnify in the food web as a progressive ingestion of contaminated food. According to Vosloo and
Bouwman (2005), bioaccumulation in fish and mammals can be observed in adipose tissue, the liver, bone marrow as well as brain tissue and for most species; tissues will accumulate contaminants in proportion to their lipid contents.

2.2.3 KNOWN EFFECTS OF DDE

Exposure to chemical contaminants in the environment has been shown to cause a number of negative effects in the surrounding populations. Research has shown that DDE may induce a number of toxic effects in the environment. According to Walker et al. (2006), Misumi et al., (2005) and Riedel et al., (2002) these include:

- Non-specific toxicity (narcosis) where the dose of a compound in the organism reaches a threshold concentration called the critical body residue (CBR) (Walker et al., 2006; Misumi et al., 2005);
- Endocrine toxicology with interferences in the natural steroid hormones present in the body;
  - It disrupts a number of enzymatic and metabolic pathways and affects reproduction and behavioral patterns (Henry and Kishimba, 2006; Leanos-Castaneda et al., 2004);
- AhR-mediated toxic responses which include lethality, reproductive and developmental toxicity, immunotoxicity and cancer (Riedel et al., 2002);
  - Alterations in the function and development of immune systems, leads to an increase in disease susceptibility and, in turn, causes regional declines in the numbers of a variety of populations (Misumi et al., 2005).
- DDE prevents gene transcription and has also been shown to cause reduced body size in exposed populations (Bayley et al., 2002);
- Due to its stable nature, DDE is much more persistent in food chains than are either DDT or DDD and are thus more likely to bioaccumulate in tissues and bioconcentrate up a food chain;
• In fish, DDE exposure can lead to pericardial and yolk-sac oedema, subcutaneous haemorrhages, craniofacial malformations and arrested growth and development, culminating in death (Vosloo and Bouwman, 2005).

The full extent of the effect of DDE, however, is unknown and there are many gaps in this knowledge which remain to be filled. A number of recent studies have indicated that biomarkers may be a useful and accurate means of measuring the effect of DDE on organisms in the surrounding environment.

2.3 BIOMARKERS

Because of man’s increasing concern of the effect of pollution and other anthropogenic chemicals on the surrounding environment and its populations, there has been a rapid international growth of environmental monitoring in recent years. Biomarkers have been used widely as a means for assessing environmental hazards, environmental pollution or the effects this pollution may be having on organisms in the environment (De Coen, 1999). These biomarkers can be defined as xenobiotically induced variations in cellular or biochemical components or processes, structures, or functions that are measurable in a biological system or sample. They supply a measurable physiological change (De Coen, 1999; Jamil, 2001). In the environment, organisms are exposed to both complex and changing levels of mixtures of pollutants. Biomarkers may act as an ‘early warning system’ of pollution in the environment, where levels begin to deviate from ‘acceptable’ levels and may reveal both present or past exposure of an organism to pollution. They can be observed or measured at the molecular, biochemical, cellular, physiological or behavioral levels of an individual representing a larger group or population. Table 1 summarizes the various levels of biological organization which may be used as representative biomarkers (Jamil, 2001).
Table 1: Representative biomarkers which can be measured at various levels of biological organization (Adapted from Jamil, 2001).

<table>
<thead>
<tr>
<th>Biochemical</th>
<th>Physiological</th>
<th>Histopathological</th>
<th>Individual</th>
<th>Population</th>
<th>Communities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes</td>
<td>Creatinine and other enzymes</td>
<td>Necrosis</td>
<td>Growth</td>
<td>Abundance</td>
<td>Index of biotic integrity</td>
</tr>
<tr>
<td>Metabolites</td>
<td>Transaminase enzymes</td>
<td>Macrophage aggregate</td>
<td>Total body lipids</td>
<td>Size and age distribution</td>
<td>Intolerant/ resistant species</td>
</tr>
<tr>
<td>DNA integrity</td>
<td>Cortisol</td>
<td>Parasitic lesions</td>
<td>Organo indices genetic disorders</td>
<td>Sex ratio and susceptibility</td>
<td>Genotypes</td>
</tr>
<tr>
<td>Stress proteins</td>
<td>Tryglycerides, HSPs and Metallothioneins</td>
<td>Functional parenchyma tissues, etc.</td>
<td>Behavioural</td>
<td>Bioenergetic parameters</td>
<td>Locomotive parameters</td>
</tr>
<tr>
<td>Antioxidant enzymes</td>
<td>Steriod hormones</td>
<td>Carcinomas</td>
<td>Gross anomalies (lesions, etc)</td>
<td>Reproductive health</td>
<td>Stressed and weak types</td>
</tr>
</tbody>
</table>

Biomarkers offer a number of advantages compared to conventional ecotoxicity tests. According to De Coen (1999), biomarkers can generally be considered as measures of the first toxicological interactions between the chemical and the biological receptor site. This interaction induces a cascade of events starting at the sub-cellular level and eventually leads to adverse effects at higher levels of biological organization as can be seen in Figure 2.

Figure 2: Levels of biological complexity in the study of the affects of pollution and other chemicals. The extent of complexity increases as one progresses from left to right (Joubert, 2000).
The effects normally studied in conventional toxicity tests can be considered as the final result of accumulating damage at the sub-organismal level (De Coen, 1999). Biomarkers are thus extremely valuable indicators of toxic stress, with stress being defined by De Coen (1999) as “a state produced by an environmental or other factor which extends the adaptive responses of the organism beyond the normal range or which disturbs the normal functioning to such an extent that the chances of survival and/or reproduction are significantly reduced”.

According to De Coen (1999), biomarkers can be classified as either indicators of effects and/or exposure to a natural or a man-made stressor. Biomarkers of effects are those measurements which are indicative of the occurrence of a stress situation in the organism, without pinpointing the responsible agent. Biomarkers of exposure can be used to identify the specific classes of agents to which an organism has been exposed.

The biomarker response (Figure 3) is based on physiological alterations. The exposure of a healthy individual to a pollutant or chemical can result in the deterioration of its health and can eventually lead to an individual’s death. Early health problems are not as noticeable as a disease, but are associated with the initiation of compensatory responses, which can be seen on the physiological condition scale. When the ability of the individual to reach compensatory responses to the new environmental changes has been compromised, the survival potential of the organism may already have begun to decline. This takes place in the compensatory zone. Only if conditions improve sufficiently and quickly enough within the non-compensatory zone, will the organism possibly still be able to recover (Joubert, 2000). Different biomarkers can be used to assess the health status of an organism. It is desirable that these tests accurately reflect the differing status of the organism, thus providing a detailed picture of its health and also reflecting the status of the environment in which the organism lives at any given time (Connel et al., 1999). It is also apparent that instead of using single biomarkers it is more appropriate to make use of them as a “battery” and to take into account other influencing temporal and spatial variability in environmental factors in order to be able to accurately monitor the situation (Schiedek et al., 2006).
Figure 3: The health status curve (graph A) indicates a possible relationship between progression of a healthy individual to a diseased state and death. Graph B represents five hypothetical biomarker responses associated with changes in physiological condition (B1, B2 and B3 are responses as the non-compensatory phase is approached. B4 is a signal of the reversible non-compensatory stage) (Joubert, 2000).

Various advantages of making use of biomarkers in the monitoring of pollution have been identified (Joubert, 2000):

- A temporally and spatially integrated measure of bioavailable pollutants is provided.
• Depending on the biomarker, a very specific response is shown, and through this, they attribute exposure and risk to environmental pollutants.

• The application of different biomarkers to species from different habitats and different trophic habitats, helps to establish the importance of different routes of exposure.

• They can provide information on the relative toxicities of specific chemicals and effluents.

• They are applicable in both the laboratory as well as the field.

However, despite these advantages, there are limitations (Schiedek et al., 2006; Jamil, 2001; Joubert, 2000). These are listed below:

• Natural variables can influence the outcomes of both specific and general biomarkers and this must be considered when applying biomarkers in monitoring programmes.

• They have limited sensitivity because the responses are not always concentration specific and may not reflect ambient pollution.

• Dose-response responses are observed in the laboratory and in the field, but may also be absent, and this must be considered when using the biomarker as a biological measure of contaminant levels.

• A mechanistic understanding is necessary for the interpretation and application of biomarkers and their individual suitability to specific studies is evident.

• Technical expertise and equipment are required for assessment and some biomarkers require more technical expertise and equipment than others.

Biomarkers can detect effects over an entire continuum and they are thus good measures of early exposure to pollutants. Biomarkers should ideally be biologically relevant, sensitive and specific. They should also be easily accessible, inexpensive and technically feasible (Connel et al., 1999).
After a thorough literature review the following biomarkers were evaluated as being suitable for use as a battery of tests in this study: Ethoxyresorufin-O-deethylase (EROD), Catalase (CAT) and Cellular Energy Allocation (CEA). Both the CAT and the CEA biomarkers are considered to be indicators of general stressors in a specific environment, while EROD has been shown to be effective in the indication of some specific organic pollutants. Limited literature is available on biomarkers available specifically for the indication of the presence of organochlorine pollutants in isolation as well as the effectivity of the use of these biomarkers to determine any dose-response relationships. These biomarkers were selected to measure both the effectivity of the use of these specific biomarkers in the measurement of dose-response relationships with regard to DDE exposure and then with these results in mind, to use these biomarkers to measure the degree of the effect of DDE exposure in the field environment.

2.3.1 ETHOXYRESORUFIN-O-DEETHYLASE (EROD)

According to Schiedek et al. (2006), the Mixed Function Oxygenase (MFO) system plays an important role in the metabolism of many endogenous (e.g. Steroid hormones) and exogenous (e.g. Environmental pollutants) substrates in fish. Cytochrome P4501A1 (CYP1A1) is a terminal component of the MFO system. 7-ethoxy resorufin O-deethylase (EROD) activity is CYP1A1 dependent and is thus a useful biomarker of MFO induction. The use of EROD as a biomarker measuring the response of any given organism to the presence of pollutants in the aquatic environment is becoming increasingly popular with advantages such as: specificity, high sensitivity, feasibility and also the simplicity of its measurement (Riffat and Ahmad, 2006).

EROD activity has been shown to respond to a variety of organic pollutants such as polychlorobiphenyls, polycyclic aromatic hydrocarbons and various pesticides. Induction of EROD with respect to the control has been observed in a variety of systems (Riffat and Ahmad, 2006). According to Zapata-Perez et al. (2000), EROD activity is stimulated by the presence of o,p'-DDE, p,p'-DDE and p,p'DDD concentrations, however, EROD
induction by the pesticide may be inhibited to a degree by the presence of metals (Riffat and Ahmad, 2006).

2.3.2 CATALASE (CAT)

All aerobic organisms need molecular oxygen for their oxidative metabolic processes but as a consequence must also deal with the formation of dangerous Reactive Oxygen Species (ROS) including superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (.OH). ROS attack can chemically alter cellular macromolecules including proteins, lipids and DNA causing metabolic damage that is often severe (Bagnyukova et al., 2005) and may result in cumulative organ damage (Lushchack et al., 2005). All organisms therefore possess well-developed systems of antioxidant defence that include both low molecular weight antioxidants and antioxidant enzymes. The main antioxidant enzymes are Superoxide Dismutase (SOD), catalase, and Glutathione Peroxidase (GPx). Under normal physiological conditions there is a balance between ROS generation and their elimination by different antioxidant scavengers. If pro-oxidant processes are enhanced and/or the power of the antioxidant system is decreased, oxidative stress arises. Although all protective components are needed to cope with oxidative stress, each particular enzyme accomplishes specific functions that can only be partly replaced by the actions of other antioxidant defences (Bagnyukova et al., 2005).

Fish have been known to possess high antioxidant defenses, particularly in the liver and the kidney, which are both metabolically active tissues. These tissues are sites of xenobiotic detoxification and are thus considered to be powerful ROS generators (Bagnyukova et al., 2005).
2.3.3 **CELLULAR ENERGY ALLOCATION (CEA)**

Organisms exposed to suboptimal conditions face an *a priori* cost of combating stress in terms of metabolic resources. The energy available for maintenance, growth and reproduction, based on the biochemical analysis of the energy budget rather than on the direct measurement of those endpoints, thus provides a sensitive measure of stress in an organism. Under normal circumstances, fish will use available resources for maintenance, growth and reproduction. When facing an additional cost such as restoring damage from pollutant exposure, fish must re-allocate some of the acquired energy to repair and/or maintain their physiological integrity. If fish use more energy than can be generated, the energy budget may even turn negative. Thus, because changes in energy budget reflect how energy availability and accumulation evolves within an organism in time, it is often a sensitive and ecologically relevant sublethal indicator of stress in organisms (Smolders *et al.*, 2003).

According to Smolders *et al.* (2003), energy metabolism biomarkers have a high ecological relevance in ecotoxicology. Using CEA, available energy reserves and energy consumption are quantified on a cellular level and integrated into a general stress indicator. Energy reserves are quantified as glycogen, protein and lipid content of the test organism and energy consumption is estimated by measuring the Electron Transport Activity (ETS) at the mitochondrial level. The difference between energy reserves and energy consumption represents the net cellular energy budget of the test organism (Smolders *et al.*, 2003).

2.4 **BIOCONCENTRATION, BIOACCUMULATION AND BIOMAGNIFICATION**

The amount of a particular chemical substance in the body of an organism in the environment is rarely directly proportional to the concentration of that substance in the
surrounding water. This may be attributed to the fact that some chemical substances have the ability to be taken up into the tissues of an organism while others do not. Even though a particular organism may not have a specific mechanism for the uptake of some of these foreign substances, they are, more often than not, present in the food chain, or may mimic others chemically and are thus taken up incidentally (Davies and Day, 1998). The ecological fate and impact of many of these foreign substances in aquatic systems often involves both their trophic transfer to fish (i.e. the movement of metal through food webs) as well as the exposure and toxic effects on individuals and populations (Folt, 2004). If the rate of uptake is equalled by the rate of excretion, or the chemical is easily degraded, then the total body load will be constant and most likely not very large. If, however, a chemical is present that is not easily degraded, even in the slightest amounts, then it is likely that it will accumulate in the tissues of organisms present in the surrounding environment (Davies and Day, 1998).

The concepts of bioconcentration, bioaccumulation and biomagnification are very closely linked and it is necessary to have an understanding of each in order to have an understanding of the concept as a whole.

Bioconcentration is the intake of chemical contaminants through an organism's epithelial tissues or gills, and the subsequent concentration of that chemical substance within the organism's tissues to a level that exceeds ambient environmental concentrations (Hall, 2002). Bioaccumulation is the process by which chemical contamination increases in a single organism over time with the intake of food as well as toxic exposure and, as previously stated, it occurs as a result of the fact that the toxin is taken in at a rate faster than that at which it can be metabolised, if it can be removed at all (Hall, 2002). Biomagnification is the increase in the whole body concentration of a substance as it passes through two or more trophic levels (Folt, 2004).

Herbivores consuming large quantities of plant material will be exposed to a small amount of the substance in every plant they eat and will therefore accumulate more of the toxin in their tissues than did any of the individual plants that they consumed. The
process is repeated at each level of the food chain, with the toxin becoming more and more concentrated until the top carnivores may have accumulated even a lethal dose (Figure 4). It can thus be said that the phenomenae of bioconcentration and bioaccumulation will result in biomagnification.

Figure 4: A trophic pyramid. The increased shading from primary producers to tertiary consumers represents the increased concentrations of toxins that can be biomagnified or bioaccumulated ‘up the food chain’ (Davies and Day, 1998).

The process of biomagnification is a cause for concern because it means that even small concentrations of chemicals in the environment can find their way into organisms higher up in the food chain in high enough dosages so as to cause problems such as physiological disturbances (Mader, 2001). The cumulative build-up of toxins in aquatic food chains can have adverse effects on humans who consume fish from lakes and rivers where bioaccumulation occurs due to exposure to varying levels of the substance.
as well as other animals at the top of aquatic food chains, such as fish-eating wildlife, which are also highly susceptible to toxic contamination (Folt, 2004).

In order for biomagnification to occur, the pollutant must be:

- long-lived
- mobile
- soluble in fats
- biologically active (Mader, 2001)

If a pollutant is short-lived, it will be broken down before it can become dangerous. If it is not mobile, it will stay in one place and is unlikely to be taken up by organisms. If the pollutant is soluble in water it will be excreted by the organism. Pollutants that dissolve in fats, however, may be retained in the organism for a long time. It is traditional to measure the amount of pollutants in fatty tissues of organisms such as fish. In mammals, the milk produced by females is often tested, since the milk is rich in fat and because the very young are often more susceptible to damage from toxins (poisons). If a pollutant is not active biologically, it may biomagnify, but is not usually toxic to the organism (Mader, 2001).

A number of factors affect the potential uptake of a pollutant in fish. These include:

- The form of the pollutant (i.e. be it in colloidal form or in a suspension);
- The presence of other substances in the surrounding environment;
- The properties of the water system;
  - Temperature,
  - pH;
  - Dissolved oxygen;
  - Light;
  - Transparency;
  - Salinity;
  - Alkalinity;
Level of byproduction; 
Humic level; and 
Sediment trapping; and

- The properties of the biological system;
  - Age;
  - Size;
  - Food supply;
  - Activity;
  - System of protection; and 
  - Adoption to pollutants (Coetzee, 1996).

The toxicity of a pollutant is linked to the specific forms of the particular pollutant on the aquatic environment, which are influenced by interacting factors such as temperature, pH, etc. Bioaccumulation can often be influenced by factors relating to the organism itself such as species, physiological condition, growth, age, sex, etc.

2.5 DDT IN THE LIMPOPO PROVINCE

Spraying with DDT to control malaria has been an ongoing annual practice in the Limpopo Province, South Africa, since 1996 (Bornman et al., 2010). Although much research has been done, it is difficult to quantify the collective effects of this ongoing spraying of DDT in the surrounding environment. Additionally, the breakdown of DDT into its metabolites and the quantities of each currently in the environment over time are difficult to quantify. As such there is little knowledge as to the level of exposure and the effects of the runoff of DDT and its metabolites into the aquatic environment. Using methods for determining the level of bioaccumulation and bioconcentration in sediments and fish tissues, as well as determining the levels of DDT and its metabolites in the water, the level of exposure of aquatic organisms in the environment can be determined. Using the selected biomarkers discussed above, in both a laboratory and a field capacity, will aid in the determination of the extent to which environmental levels of DDE are affecting organisms in the aquatic environment.
2.6 REFERENCES


3. STUDY AREA

3.1 LOCATION AND DESCRIPTION OF THE STUDY AREA

The Luvuvhu Catchment forms part of the greater Limpopo system, which runs downstream into Mozambique. The Luvuvhu River, as well as some of its tributaries, forms the portion of the catchment on the southern side of the Soutpansberg Mountains east of Louis Trichardt. It continues to stretch another 200km, through the Kruger National Park (KNP) and confluences with the Limpopo River at Crook’s Corner on the Mozambican border, near Pafuri (State of the Rivers Report, 2001).
Figure 5: Location of the Luvuvhu River Catchment Study Area within Limpopo, South Africa.
3.1.1 PHYSICAL FEATURES

3.1.1.1 TOPOGRAPHY AND GEOMORPHOLOGY

The Limpopo Province consists of a number of diverse and contrasting biospheres including bushveld, sub-tropical forest and highveld grassland savannah (State of the Rivers Report, 2001).

The northern part of the Limpopo Province is the flattest, largest depositional basin in the world. The land changes to the west, where the Waterberg mountains rise in masses of syenite, a rock similar to granite (Brandl, 2007). This area features the fertile soil of an extinct volcano, allowing for an abundance of plant and animal species (Harmse, 2006).
Chapter 3

The incision of the upper river channel of the Luvuvhu River is due to the extreme susceptibility of this particular landscape area to erosion (Brandl, 2007; State of Rivers Report, 2001).

3.1.1.2 SOIL AND GEOLOGY

It is a well known fact that soil type is mainly determined by the underlying geology of an area as well as the climate. The Luvuvhu River Catchment area runs over the Waterberg Supergroup as well as the Soutpansberg, which are about 1 800 million years old (Brandl, 2007). The area consists of very porous, course grained sandstone, which absorbs a great deal of water. In many areas, soils of this rock type have formed deep soils that are used for agricultural purposes, but must be irrigated often due to the large infiltration rate. Very few nutrients are present in the soil, thus, a large quantity of fertilizers need to be added. However, in the Soutpansberg, there are some fertile valleys with crops found in this rock type because of the generally high rainfall of this area (Harmse, 2006).

3.1.1.3 HYDROLOGY

The river and all its tributaries rising in the Soutpansberg are perennial (Kabanda, 2003). The upper Luvuvhu consists of steep, narrow rivers dominated by cobble riffles and occasional pools, with a few bedrock rapids and waterfalls. The lower reaches of the Luvuvhu are diverse and varied in habitat with the occurrence of rapids, riffles, runs and pools. Dams along the Luvuvhu river include both the Albasini dam as well as the Nandoni dam, which was constructed in the middle section of the river. The Lotanyanda River contributes a constant flow to the Luvuvhu River downstream of the Albasini Dam (State of the Rivers Report, 2001).
3.1.1.4 VEGETATION

The floral diversity found within the Luvuvhu River Catchment area may be attributed to both the influence the Soutpansberg Mountains play on the wind and rainfall patterns of the area, as well as the several distinct floristic elements that act on it, which are:

- Tropical;
- Mozambique coastal;
- Lowveld;
- Afro-montane;
- Bushveld;
- Waterberg;
- Kalahari; and
- Limpopo valley (Hahn, 2002).

Riparian vegetation (Table 2) consists of dry acacia woodland species, with large areas having been removed to accommodate orchards leaving very narrow strips of riparian vegetation (State of the Rivers Report, 2001). Lower down in the catchment, the marginal vegetation is dominated by reeds with some areas becoming very dense, partly due to alien plant encroachment in the riparian zones (Fouche, 2003).
**Table 2: The aquatic and terrestrial vegetation of the Luvuvhu River catchment area (both alien and indigenous) (modified from State of the Rivers Report, 2001).**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alien plants</strong></td>
<td></td>
</tr>
<tr>
<td>Ageratum</td>
<td>Ageratum spp.</td>
</tr>
<tr>
<td>Bugweed</td>
<td>Solanum mauritianum</td>
</tr>
<tr>
<td>Castor-oil plant</td>
<td>Ricinus communis</td>
</tr>
<tr>
<td>Eucalyptus/ Gum trees</td>
<td>Eucalyptus spp.</td>
</tr>
<tr>
<td>Guava</td>
<td>Psidium guajava</td>
</tr>
<tr>
<td>Jacaranda</td>
<td>Jacaranda mimosifolia</td>
</tr>
<tr>
<td>Lantana</td>
<td>Lantana camara</td>
</tr>
<tr>
<td>Large cocklebur</td>
<td>Xanthium strumarium</td>
</tr>
<tr>
<td>Mauritius thorn</td>
<td>Caesalpinia decapetala</td>
</tr>
<tr>
<td>White mulberry</td>
<td>Morus alba</td>
</tr>
<tr>
<td>Paraffin bush/ Triffid weed</td>
<td>Chromolaena odorata</td>
</tr>
<tr>
<td>Peanut butter cassia</td>
<td>Senna didymobotrya</td>
</tr>
<tr>
<td>Pines</td>
<td>Pinus spp.</td>
</tr>
<tr>
<td>Poplars</td>
<td>Populus spp.</td>
</tr>
<tr>
<td>Red sesbania</td>
<td>Sesbania punicea</td>
</tr>
<tr>
<td>Syringa</td>
<td>Melia azedarach</td>
</tr>
<tr>
<td>Thistle (Spear/ Scotch thistle)</td>
<td>Cirsium vulgare</td>
</tr>
<tr>
<td>Wild tobacco</td>
<td>Nicotiana glauca</td>
</tr>
<tr>
<td><strong>Indigenous plants</strong></td>
<td></td>
</tr>
<tr>
<td>Common reed</td>
<td>Phragmites mauritianus</td>
</tr>
<tr>
<td>Cape willow</td>
<td>Salix mucronata</td>
</tr>
<tr>
<td>Jackal berry</td>
<td>Diospyros mespiliformis</td>
</tr>
<tr>
<td>Matumi/ Mingerhout</td>
<td>Breonadia salicina</td>
</tr>
<tr>
<td>Natal mahogany</td>
<td>Trichilia emetic</td>
</tr>
<tr>
<td>Sycamore fig</td>
<td>Ficus sycomorus</td>
</tr>
</tbody>
</table>
There are a number of factors posing a threat to the riparian vegetation. It is being disturbed and removed by farming and grazing activities in and around the riparian zone and terrestrial vegetation is encroaching into the riparian zone. In the area of Thohoyandou, the riparian vegetation has been largely destroyed for use as firewood and to provide grazing. This has in turn led to increased donga erosion.

### 3.1.1.5 Animal, Fish and Bird Species

The Luvuvhu River Catchment has a very diverse community of mammalian species, being particularly rich in bats, carnivores and some of the larger hoofed animals (Graigher and Stuart, 2003) and both hippos and crocodiles are abundant along the river (Angliss et al., 2001).

The riparian trees provide habitat for bird species such as the fish owl, bou bou, longtailed starling and white backed herons with the occurrence of at least 6 Red Data listed ‘vulnerable’ species and 11 ‘near-threatened’ species (Table 3) (Angliss et al., 2001).
Table 3: The Red Data-listed bird species occurring in the Luvuvhu River Catchment area (Angliss et al., 2001).

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vulnerable species</strong></td>
<td></td>
</tr>
<tr>
<td>White-backed Night Heron</td>
<td><em>Gorsachius leuconotus</em></td>
</tr>
<tr>
<td>Cape Vulture</td>
<td><em>Gyps coprotheres</em></td>
</tr>
<tr>
<td>Martial Eagle</td>
<td><em>Polemaetus bellicosus</em></td>
</tr>
<tr>
<td>African Finfoot</td>
<td><em>Podica senegalensis</em></td>
</tr>
<tr>
<td>African Grass Owl</td>
<td><em>Tyto capensis</em></td>
</tr>
<tr>
<td>Pel's Fishing Owl</td>
<td><em>Scotopelia peli</em></td>
</tr>
<tr>
<td><strong>Near-threatened</strong></td>
<td></td>
</tr>
<tr>
<td>Black stork</td>
<td><em>Ciconia nigra</em></td>
</tr>
<tr>
<td>Bat Hawk</td>
<td><em>Macheiramphus alcinus</em></td>
</tr>
<tr>
<td>Ayres' Hawk Eagle</td>
<td><em>Aquila ayresii</em></td>
</tr>
<tr>
<td>African Crowned Eagle</td>
<td><em>Stephanoaetus coronatus</em></td>
</tr>
<tr>
<td>Peregrine Falcon</td>
<td><em>Falco peregrinus minor</em></td>
</tr>
<tr>
<td>Lanner Falcon</td>
<td><em>Falco biarmicus</em></td>
</tr>
<tr>
<td>Half-collared Kingfisher</td>
<td><em>Alcedo semitorquata</em></td>
</tr>
<tr>
<td>African Broadbill</td>
<td><em>Smithornis capensis</em></td>
</tr>
<tr>
<td>Orange Ground Thrush</td>
<td><em>Zoothera gurneyi</em></td>
</tr>
<tr>
<td>Black-throated Wattle-eyed Flycatcher</td>
<td><em>Platysteira peltata</em></td>
</tr>
<tr>
<td>Pink-throated Twinspot</td>
<td><em>Hypargos margaritatus</em></td>
</tr>
</tbody>
</table>

Due to climatic diversity and abundance of the perennial streams in the Luvuvhu Catchment, the area presents a high diversity of aquatic biotopes for fish and invertebrates (Fouche and Graigher, 2003). However, this biodiversity is under threat due to the diminishing in-steam habitat. Impacts within the riparian zone are contributing towards erosion and deposition of sediments in the river. Flow-dependant fish species are suffering as a result of weirs, channels and water abstraction. In
addition, a number of highly invasive alien fish have been recorded in some reaches of the river (Table 4) (Angliss et al., 2001).

Table 4: Historically recorded fish species of the Luvuvhu River Catchment area (both alien and indigenous) (modified from Angliss et al., 2001).

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alien fish</strong></td>
<td></td>
</tr>
<tr>
<td>Black bass (Large mouth)</td>
<td><em>Micropterus salmoides</em></td>
</tr>
<tr>
<td>Common carp</td>
<td><em>Cyprinus carpio</em></td>
</tr>
<tr>
<td>Nile tilapia</td>
<td><em>Oreochromis niloticus</em></td>
</tr>
<tr>
<td>Rainbow trout</td>
<td><em>Oncorhynchus mykiss</em></td>
</tr>
<tr>
<td>Bluegill sunfish</td>
<td><em>Lepomis macrochirus</em></td>
</tr>
<tr>
<td><strong>Indigenous fish</strong></td>
<td></td>
</tr>
<tr>
<td>Line-spotted barb</td>
<td><em>Barbus lineomaculatus</em></td>
</tr>
<tr>
<td>Sawfin rock catlet</td>
<td><em>Chiloglanis paratus</em></td>
</tr>
<tr>
<td>Common mountain catfish</td>
<td><em>Amphilius uranoscopus</em></td>
</tr>
<tr>
<td>Southern barred minnow</td>
<td><em>Opsaridium peringueyi</em></td>
</tr>
<tr>
<td>Tigerfish</td>
<td><em>Hydrocynus vittatus</em></td>
</tr>
<tr>
<td>Mozambique tilapia</td>
<td><em>Oreochromis mossambicus</em></td>
</tr>
</tbody>
</table>

3.1.1.6 CLIMATE

The general climate in the Luvuvhu River Catchment area should be typed into two seasons only, a Warm Wet Season (WWS) and a Cool Dry Season (CDS) (Kabanda, 2003). During the WWS, temperatures are warm, ranging from about 16 °C to about 40 °C. In the CDS, temperatures range from about 12 °C to about 22 °C (Kabanda, 2003) and frost rarely occurs (Angliss et al., 2001).
The change in topography (altitude and relief) gives rise to varied climatic characteristics. The mountain zone has a rainfall of some 2000 mm and the dry lowveld in the KNP 400 mm/a (Angliss et al., 2001). More than 85 percent of the rain falls during the WWS, which usually occurs from about December to February. Evaporation increases gradually from 1 400 mm/a in the west to 1 900 mm/a in the east. About 60 percent of the evaporation occurs during the 6 months from October to March leading into the CDS occurring usually from about April to October (Kabanda, 2003).

The Soutpansberg mountains greatly influence both the wind and the rainfall patterns of the Luvuvhu River Catchment. They play a large role in creating the diverse microclimates found throughout the study area and have resulted in much diversity of the fauna and flora (Kabanda, 2003).

### 3.1.1.7 LAND USE

Forestry and agriculture are the two main land-use activities in and around the Luvuvhu River Catchment area. The upper catchment in the Soutpansberg Mountains is dominated by forestry plantations (pines, eucalyptus and mahoganies), while the lower catchment is dominated by a number of tea estates (Angliss et al., 2001). The Levubu agricultural area in the Levuvhu River Catchment produces citrus, mangos, bananas and macadamias, while further downstream in the Catchment, the area is dominated by rural community gardens, cattle and goats. Smallholdings that are developed intensively for orchards occur intermittently along the river. Large amounts of fertilizer and herbicides are being washed into the river, affecting the water quality, while urban and agricultural runoff has also begun to affect the turbidity of the river (Angliss et al., 2001).

Out of a population of 770 000 people living in the Luvuvhu Catchment, the majority live in rural villages, most of which are heavily concentrated along the river systems. Community activities with regards to the river include washing of clothes, bathing, fishing as well as washing of cars. Clay is removed from the riverbank for use in brick
manufacturing and pot making and these activities are damaging the channel banks as well as impacting negatively on the water quality of the river. The riparian vegetation is also over-utilized in many areas, mainly for firewood, fence construction, furniture, medicinal purposes and food (Angliss et al., 2001). Subsistence farming within the riparian zone is also commonly seen in these areas.

3.2 LOCATION OF SAMPLE SITES

Fieldwork was carried out along the Luvuvhu River in the Limpopo Province, South Africa, during both the low and high flow seasons of 2006 and 2007. Three sites were selected, namely the Albasini dam (to be used as a reference site), the Nandoni dam and then the Xikundu Fish Ladder. Each site was selected in order to sample in different areas affected by DDT.

3.2.1 SITE DESCRIPTION

3.2.1.1 SITE 1 (ALBASINI DAM)

The Albasini Dam (23° 06’ 26.3” S; 30° 06’ 16.5” E) is found in the Luvuvhu River headwaters, where the river rises as a steep mountain stream in the south-easterly slopes of the Soutpansberg Mountains. It is situated about 25 km from Makhado and about 45 km outside (west) of the DDT sprayed area. The Albasini Dam (situated outside of the DDT spraying area) was used as a control/unexposed reference site. The control site is necessary so that results from this site may be compared to those obtained from each of the sites within the DDT sprayed area. Any differences in the results and data observed will be correlated together in an effort to ascertain whether or not any changes are occurring as a result of the spraying of DDT.

The area around the Albasini Dam is a private conservancy. Riparian vegetation consists of dense stands of large trees, shrubs and reeds and land-use activities include
forestry (11 percent) and agriculture (20 percent) with subsistence farming constituting about a third of the total agricultural component.

Several introduced species such as the black bass and the common carp occur in the Albasini Dam. Additionally, fish populations are being heavily impacted by poaching in the area.

Figure 7: The Albasini Dam, Limpopo Province, South Africa.

3.2.1.2 SITE 2 (NANDONI DAM)

The Nandoni Dam (22° 59' 37.8" S; 30° 35' 36.7" E) is situated in the midst of a regularly sprayed DDT area, in the middle section of the Luvuvhu River 15 km east of the town of Thohoyandou. It was selected so as to compare the results from the upstream reference site Runoff from the town and the surrounding rural areas is likely to collect in this dam in the water and the sediments and is likely to also have affected and accumulated in the aquatic communities in this area.

The construction of the Nandoni Dam has impacted heavily on the area immediately down-stream. Access roads, in-stream coffer dams and diversions have damaged the
riparian vegetation and river bank, while the in-stream habitat has been degraded through excessive siltation.

![Image](image1.png)

**Figure 8: The Nandoni Dam, Limpopo Province, South Africa.**

### 3.2.1.3 SITE 3 (XIKUNDU FISH LADDER)

The Xikundu Fish Ladder (22° 48’ 29.4” S; 30° 47’ 55.3” E) is situated further east towards the Kruger National Park (KNP). This site was selected in order to assess the effect of spatial cumulative DDT spraying and whether or not the DDT concentrations become more diluted through environmental processes such as dilution, sediment deposition, etc or whether they become more concentrated in a downstream direction from added runoff. Such processes will affect the exposure of aquatic organisms to DDT and may thus affect the results obtained.

Large varieties of rare and sensitive species have been found in the area and a fishway was thus incorporated into the construction of the weir. Subsistence farming and other domestic activities impact heavily along the banks of this river. Additionally, poaching is rife which also impacts on fish abundance in this area.
Figure 9: The Xikundu Fish Ladder, Limpopo Province, South Africa.

### 3.3 **TILAPIA AS A TEST ORGANISM**

According to Viljoen (1999), in order for results to be relevant, meaningful, and ecologically significant, it is vital that one must select the correct test organism for the study at hand. A test species should possess the following:

- The species must be able to withstand a wide range of sensitivities.
- The test species should show a definite reaction to all toxicants tested.
- The test species must allow early detection of potential harm caused by toxicants.
- The test species should be available in practical size throughout the year.
- They should be indigenous, representative of the ecosystem to be studied.
- They should be of commercial, recreational, or ecological value.
- The test species should be amenable to routine laboratory maintenance and cultivation.
- Bioassay techniques for the test species should be well established and easy to perform.
- Sufficient knowledge on the ecology, physiology, genetics, and behavior of the test species should be available in order for data to be easily interpreted.
- Test organisms should be in good condition, free of parasites, and disease.

Fish are popular test organisms as their biology is generally well understood, they occupy a position in the food chain that leads to man, and they are perceived as a valuable organism in the environment (Buikema et al., 1982). Fish are generally used as sentinels of aquatic pollution, mainly due to the fact that they occur at higher levels of the food chain and are thus subjected to high levels of chemical pollutants through bioconcentration and biomagnification (Servos et al., 1989). Additionally, according to Grant and Briggs (2002), fish react to all synergistic and antagonistic effects of combined pollutants and stressors imposed on their environment and integrate their responses through time as opposed to analytical chemical analysis methods and in this way, fish can be considered convenient full-time monitors of the aquatic environment.

For the purposes of this study, the Mozambique tilapia (*Oreochromis mossambicus*) was chosen as the indicator species. It meets all the requirements for a test/experimental organism and is also endemic to the study area. It is a relatively large, deep-bodied, mouthbrooding cichlid, endemic to the east coastal rivers from the lower Zambezi system south to the Bushmans system in the Eastern Cape. It is also found south of the Phongolo system and is often naturally confined to closed estuaries and coastal reaches of rivers. The fish is also widely dispersed beyond this range to inland regions and to the south-west and west coastal rivers including the lower Orange and rivers of Namibia (Skelton, 2001). The Mozambique tilapia is common in the study area, the Luvuvhu river, in the Limpopo province.

The Mozambique tilapia occurs in all but fast-flowing waters, thriving in standing waters. It is quite a hardy fish being tolerant of fresh, brackish or marine waters and can survive temperature ranges from 15°C to 40°C. The fish feeds on algae, especially diatoms, and detritus (the organic matter in which DDT and its metabolites accumulate), while larger individuals might even take insects or other invertebrates (Skelton, 2001).
3.4 REFERENCES


Environmental Education Centre. African Arabian Wildlife Research Centre, South Africa.


4. BIOMARKERS IN FISH FROM THE STUDY AREA

4.1 INTRODUCTION

Aquatic ecosystems are being placed under increasingly large amounts of pressure from various anthropogenic pollutants originating from both point and non-point sources (Pruski and Dixon, 2002). As a result, aquatic organisms are being exposed to elevated levels and mixtures of pollutants, thus threatening the health of the organism (Luoma and Carter, 1991). Pollutant concentrations in aquatic systems vary to a large degree and levels are often below detection limit. The on-going control of water quality is thus indispensable and there is an increasing trend in using the behavior of pollutants (e.g. bioavailability and bioaccumulation) as well as pollution-induced biological effects on aquatic organisms, to evaluate or predict the impact of chemicals on aquatic ecosystems (van der Oost et al., 2003). To evaluate the presence of pollutants over time and to measure their toxic effect, biomonitors or bioindicators can be used and they play a prominent role in the monitoring of aquatic ecosystems (Mason, 1990; Ireland, 1991; Kramer and Botterweg, 1991; Salanki, et al., 2003). Aquatic organisms, along with physico-chemical monitoring, can provide useful information (spatially and temporally) on the water quality and the levels of various pollutants present in an aquatic ecosystem.

The continuous or periodic exposure to low levels of pollutants for extended time periods can cause chronic stress, starting at the cellular and sub-cellular levels of organisation. Chronic or sub-lethal effects are in general more subtle and qualitative, but it is difficult to monitor at population and community level (Joubert, 2000). The need to detect and assess the effects of pollutants at low concentrations and in complex mixtures has led to the development of a wide range of biomarkers of exposure and effect. The measurement of biochemical or cellular responses (biomarkers) to pollutants in organisms, allows the rapid detection of early biological effects of their exposure to anthropogenic pollutants. However, it is known that many organisms living in highly degraded and/or chronically polluted environments are able to cope with
increased pollution. These organisms demonstrate various compensatory mechanisms and some of them even using genetic adaptations to high levels of pollutants that may mask the expected biological responses of exposed organisms (Elder and Collins, 1991).

Nevertheless, changes in various biochemical and physiological biomarkers, at organism level, may be useful for identifying and predicting the impact of pollutants and variations in the biomarker responses to pollutant exposure have been demonstrated in several studies (Black et al., 1996; Lionette et al., 2003).

Chemical contamination can lead to increases in organisms over time with the intake of food as well as toxic exposure as previously stated through the process of bioaccumulation. It therefore occurs as a result of the uptake of the chemical at a rate faster than that at which it can be removed, if it can be removed at all (Hall, 2002). The amount of a particular chemical substance in the body of an organism in the environment is rarely directly proportional to the concentration of that substance in the surrounding environment (Coetzee, 1996). However, there are several reasons for measuring bioaccumulation of pollutants in organisms from the field environment and these include:

- to determine pollutant-specific bioavailability to develop realistic tissue-residue values;
- to assist in identifying possible causative agent(s) of toxicity; and
- to assess or predict effects of chronic, low-level exposures to pollutants (Chapman, 1992)

It should be noted that the bioaccumulation and toxicity of pollutants are situation dependant and it is thus difficult to compare results from any particular study to other situations where the biological species or environmental conditions are different (Elder and Collins, 1991). An important consideration in defining exposure in aquatic systems
is that total pollutant concentrations in solution and sediments, are not the total concentrations available to the organisms and various factors influence both pollutant bioavailability and toxicity to organisms. These include: geo-chemical factors, anthropogenic and physico-chemical factors that make the spatial and temporal distribution of pollutants complex (Luoma and Carter, 1991). Furthermore, various pollutants, present in an aquatic environment will interact with and alter the bioavailability of each other and thereby, the biological effects on aquatic organisms (Cossu et al., 2000).

The biomarkers, EROD, CAT activity and CEA were used as biomarkers of pollution for this part of the study, with the aim being to assess the effects of exposure to environmental stressors and pollutants on the health of *O. mossambicus* in the field environment. In order to do this, it was necessary to provide a measure of the exposure of the test organism to pollutants in the field environment by assessing both water quality and sediment quality. It is also necessary to assess the bioaccumulated pollutant levels present in the tissues of the organism for the determination of any relationships that may be present. The objective in this particular section of the study was to assess the various biomarker responses of the test organisms from each field locality along with the levels of bioaccumulation in the organisms and the environmental qualities of each site respectively.

### 4.2 MATERIALS AND METHODS

#### 4.2.1 DATA COLLECTION

**4.2.1.1 EQUIPMENT PREPARATION**

According to standard laboratory procedures, all glassware used for water and sediment collection was washed and placed in a soap bath containing a 2 percent Contrad TM (Merck Chemicals) solution for 24 hrs. Thereafter, it was rinsed with dH₂O and placed in a 1 M hydrochloric acid bath (Merck Chemicals) for another 24 hrs. It was then
rinsed again with dH$_2$O, allowed to dry and re-rinsed with chromatography grade ethanol.

4.2.1.2 SAMPLING PROTOCOL

One sampling survey was conducted during the high flow hydrological regime (10$^{th}$ – 11$^{th}$ March 2007) and two sampling surveys were conducted during the low flow hydrological regime (1$^{st}$ – 11$^{th}$ October 2006 and 22$^{nd}$ October – 2$^{nd}$ November 2007).

4.2.1.2.1 WATER AND SEDIMENT SAMPLE COLLECTION AND SYSTEM VARIABLE TESTS

Three triplicate sets of water and sediment samples were collected in labeled water bottles and honey jars respectively (all having gone through the washing process described above for glassware) at each site. Samples were frozen until further laboratory analysis. System variables were measured in situ. Temperature, oxygen percentage and oxygen content were measured with a Eutech CyberScan DO 310 meter, pH was taken with a Eutech CyberScan pH 310 meter, while total dissolved salts (TDS) and conductivity were measured with a Eutech CyberScan CON 400 meter.

4.2.1.2.2 FISH COLLECTION

Mozambique tilapia (O. mossambicus) were collected using gill nets and were sexed according to external features. 10 males were kept from each site. Each fish was weighed and measured and blood was drawn. The fish was then killed using cerebral severing and the ventrally open fish was then examined macroscopically for abnormalities.
4.2.1.2.3 BIOMARKER SAMPLE COLLECTION

Sections of liver, gills and muscle were collected, placed in sterile Eppendorf tubes, mechanically mixed with stabilizing Hendrikson buffer and stored in liquid nitrogen until being placed in -80 °C freezer so as to minimize any degradation of the samples and prevent contamination (Moolman, 2004).

4.2.2 ANALYSIS OF DATA
4.2.2.1 GLASSWARE PREPARATION

All glassware used for water and sediment collection was washed before use according to standard laboratory procedures. Glassware was washed and placed in a soap bath containing a 2 percent Contrad TM (Merck Chemicals) solution for 24 hrs. Thereafter, it was rinsed with dH$_2$O and placed in a 1 M hydrochloric acid bath (Merck Chemicals) for another 24 hrs. It was then rinsed again with dH$_2$O, allowed to dry and re-rinsed with chromatography grade ethanol.

Sterile Falcon tubes were used for the collection of all metal analysis tissue. Lightweight foil was used for the collection of tissue for DDT, DDE and EDC analysis (Bornman et al., 2010)

4.2.2.2 WATER QUALITY

Water samples were thawed for analysis. Each sample was analysed for the following chemical variables: turbidity (NTU), chloride (mg/l), orthophosphate (mg/l, sulphate (mg/l), ammonium (mg/l), nitrite (mg/l), COD (mg/l). Analyses were conducted using the Merck Spectroquant Spectrophotometer. Water samples were also sent for organochlorine content analyses and detailed metal analyses (Bornman et al., 2010).
4.2.2.3 SEDIMENT ANALYSIS

Sediment samples were thawed and analysis was done according to a method described in and adapted from Evans et al. (1990). An estimated wet-weight (± 100 g) of each sample was taken and dried at 60 °C for 72 hrs. The dry weight was then noted and each sample was shaken individually for grain size analysis using Endecott-mechanical sieves. 4000 µm, 2000 µm, 500 µm, 212 µm and 53 µm with a collecting pan at the bottom were used.

The percentage contribution of each size class was calculated as follows:

\[
\frac{\text{Mass of sediment in particular sieve}}{\text{Mass of original sample}} \times 100
\]

Dried sediment samples were partially digested and sent for organochlorine content analysis and detailed metal analysis.

Additional samples were weighed out with estimated wet-weights of ± 10 g, mixed with a mortar and pestle and dried for 72 hrs. The dry weight was noted and each sample was then incinerated for 48 hrs for the determination of the organic content percentage of each sample, which was calculated as follows:

\[
\frac{(\text{Mass [crucible + sediment]} - \text{Mass [crucible]}) \text{ before furnacing} \times 100}{(\text{Mass [crucible + sediment]} - \text{Mass [crucible]}) \text{ after furnacing}}
\]

4.2.2.4 TISSUE ANALYSES

4.2.2.4.1 EDC AND DDT ANALYSES

Muscle tissue for the metal analysis was collected and placed in sterile falcon tubes. Samples were then frozen until further laboratory analysis. EDC and DDT analyses
tissue (muscle and fat) was collected and wrapped in foil. Samples were then frozen until further laboratory analysis (Bornman et al., 2010)

4.2.2.4.2 BIOMARKER ANALYSES

Selected tissue samples were used for biomarker analysis. Tissues were thawed and homogenised on ice, in the appropriate homogenising buffer. The biomarkers assayed were: CAT activity, EROD and CEA. All samples were analysed in triplicate.

4.2.2.4.2.1 CAT ACTIVITY (CATALASE)

Catalase activity was determined by method of Cohen et al. (1970). Gill samples were homogenized with 0.01 M phosphate buffer (pH 7.0) on ice at a ratio of 1:2 and then centrifuged at 10 000 rpm for 10 min at 4 °C. To an aliquot of the supernatant was then added cold 6 mM H₂O₂ to start an enzyme catalyzed decomposition reaction of the H₂O₂. After an incubation period of 3 min, the reaction was stopped with the addition of 6 N H₂SO₄. The remaining H₂O₂ was measured by allowing it to react with a standard excess of 2 mM KMnO₄ and then measuring the residual KMnO₄ spectrophotometrically at a wavelength of 490 nm within 30 to 60 seconds of the addition of the KMnO₄. The absorbance was read using an Elx-800 universal microplate reader (Bio-tek instruments, USA). The protein content was determined using the method of Bradford (1976) and the CAT activity was expressed as μmol H₂O₂/mg protein.min.

4.2.2.4.2.2 EROD (ETHOXYRESORUFIN-O-DEETHYLASE)

EROD was determined by method of Burke and Mayer (1978), with some modifications. Liver samples were homogenized in 0.25 M sucrose – 0.05 M Tris (pH 7.4) on ice at a ratio of 1:3 and then centrifuged at 13 500 rpm for 20 min at 4 °C. To the supernatant of each sample was added a general reaction mixture of 0.1 M potassium phosphate buffer (pH 7.8) and 0.05 M Ethoxyresorufin in 1.25% Tween 80. A 0.05 M aliquot of NADPH was stirred into the mixture to start the reaction and the progressive increase in fluorescence, as
ethoxyresorufin was deethylated to resorufin. A baseline of fluorescence was recorded at an excitation wavelength of 544 nm and an emission wavelength of 590 nm with a fluorimeter and using resorufin as a standard. The excitation emissions were then recorded another six times at one minute intervals measuring the progressive increase in resorufin. Ethoxyresorufin and resorufin are both light sensitive compounds and the solutions of these compounds were thus prepared and stored in the dark and the metabolic reaction was performed in a room with dim infrared lighting.

4.2.2.4.2.3 CEA (CELLULAR ENERGY ALLOCATION)

The CEA was measured according to De Coen and Janssen (1997), with some modifications.

Sampled muscle tissue was homogenised in 3 parts of ice-cold homogenizing buffer before centrifuging at 3 000 rpm for 10 minutes at 4 °C separating the homogenate into 4 different fractions for energy reserve (lipids, carbohydrate and protein) quantification and energy consumption (ETS) quantification.

$E_r$ (Available energy reserves)

Lipids were separated, using one of the most common lipid extraction procedures derived by Bligh and Dye (1959). Thereafter, lipids were quantified by measuring absorbance at 405 nm, on an automated microplate reader and using cholesterol (tripalmitin) as a standard. The total carbohydrate concentration was determined using the glucose GOD-PAP colourmetric assay (Roche) at a 560 nm absorbance. Lastly, the protein fraction was quantified using Bradford's reagent (Bradford, 1976) and a bovine serum albumin standard at a wavelength of 630 nm. The lipid, carbohydrate and protein fractions were then transformed into energetic equivalents using their respective energy combustion values (39 500 mJ/mg lipid, 17 500 mJ/mg glycogen and 24 000 mJ/mg protein).
$E_c$ (Energy consumption)

The energy consumed was estimated by measuring the ETS. A 25 μl aliquot of the resultant supernatant was added to 75 μl buffered substrate solution and 25 μl NAD(P)H. The reaction was initiated with 50 μl 8 mM p-IodoNitroTetrazolium and the absorbance was read kinetically at 492 nm for 10 minutes. The cellular respiration rate was quantified using the theoretical stoichiometrical relationship that for each 2 μmol formazan formed, 1 μmol $O_2$ was consumed. The respiration rate was then transformed into energetic equivalents using an average oxyenthalpic equivalent of 484 KJ/mol $O_2$ (Gnaiger, 1983).

$CEA$

The difference between total energy reserves and energy consumed represents the net energy budget. The following equations illustrate how the $E_r$, $E_c$ and CEA values were calculated (Moolman, 2004):

$$E_r = E_{lipid} + E_{carbohydrate} + E_{protein}$$

$$E_c = E_{ETS}$$

$$CEA = E_r - E_c$$

4.2.2.5 STATISTICAL METHODS

Redundancy Analysis (RDA), a derivative of Principal Components Analysis (PCA) with one additional feature, was performed using Canoco version 4.5. The values entered into this analysis were not the original data but the “best-fit” values estimated and a second matrix of environmental data. Thus the PCA was constrained to optimise a fit to the environmental data so that this technique is the canonical version of PCA. Interpretation of RDA is through bi-plots which are maps of samples analysed on a two-dimensional basis, where the placements of the samples reflect the (dis)similarities between the samples (Ferreira et al., 2009).
Statistical analysis was also performed, using SPSS 14.0 and the homogeneity of data was assessed using Levene’s test. If the criterion of homogeneity was met, one-way analysis of variance (ANOVA) was used, to determine significant differences between the metal content and the biomarker responses of the test organisms and between the exposure periods. ANOVA was followed by Scheffé’s multiple comparison test as post-hoc criterion for homogeneity. If there was no homogeneity of variance, Dunnett’s-T3 test was applied to assign significant differences ($p<0.05$) between the variables (Mills, 2005). Significant differences are indicated as the letters a, b and c.

### 4.3 RESULTS

#### 4.3.1 WATER QUALITY

Table 5 shows the water quality variables taken at each of the sites at the time of each of the fieldtrips.

**Table 5: The physical water quality variables of the three selected sites at the time of each of the fieldtrips**

<table>
<thead>
<tr>
<th>Variable</th>
<th>October 2006</th>
<th>March 2007</th>
<th>October 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albasini</td>
<td>Nandoni</td>
<td>Albasini</td>
</tr>
<tr>
<td>Temp (T°)</td>
<td>21</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>5.5</td>
<td>5.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>103</td>
<td>113</td>
<td>112</td>
</tr>
<tr>
<td>pH</td>
<td>9.7</td>
<td>10.2</td>
<td>8.5</td>
</tr>
<tr>
<td>Conductivity (µS)</td>
<td>260</td>
<td>141</td>
<td>178</td>
</tr>
<tr>
<td>TDS</td>
<td>126</td>
<td>71</td>
<td>89</td>
</tr>
</tbody>
</table>
4.3.1.1 TEMPERATURE

Temperatures were generally lower at the Albasini Dam, but for the last fieldtrip this was not the case. The Albasini Dam was also considerably lower at the time of sampling for both the second and the last fieldstrips, which would have made the water more susceptible to higher water temperatures.

4.3.1.2 DISSOLVED OXYGEN

The dissolved oxygen content at Albasini in the last fieldtrip was found to be higher at this site than was found in previous fieldtrips, although oxygen levels at all three sites were generally higher than over the two previous fieldtrips. Generally, dissolved oxygen at Xikundu was found to be the highest for all three assessments.

4.3.1.3 PH, CONDUCTIVITY AND TDS

The pH of Xikundu is lower than the pH of the two dam sites. The temperature is significantly lower at Xikundu during the time of October 2007, as well as the dissolved oxygen content is higher at the same sight. pH is largely influenced by physico-chemical properties of the surrounding environment and there is any number of factors to which the differing pH values could be attributed.

The conductivity was generally higher in the two dams, with the exception of Xikundu at the time of the first fieldtrip. Albasini consistently showed higher amounts of TDS than the other two sites.

4.3.1.4 ORGANOCHLORINE CONCENTRATIONS

Table 6 shows the organochlorine concentrations of selected organochlorines in the water samples taken from the three selected sites. There is very little information
available as to the acceptable levels of these contaminants in the environment and, thus, these concentrations are to be used towards establishing a database of the environmentally relevant concentrations found in the natural environment in the Limpopo Province.

Table 6: The organochlorine concentrations present in the water samples taken from the three selected sites at the time of each of the fieldtrips (Bornman et al., 2010).

<table>
<thead>
<tr>
<th></th>
<th>Oct-06</th>
<th>Mar-07</th>
<th>Oct-07</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albasini</td>
<td>Nandoni</td>
<td>Xikundu</td>
</tr>
<tr>
<td>o,p'-DDE (ug/L)</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>p,p'-DDE (ug/L)</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>o,p'-DDD (ug/L)</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>p,p'-DDD (ug/L)</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>o,p'-DDT (ug/L)</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>p,p'-DDT (ug/L)</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>PCB 153</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
</tr>
</tbody>
</table>

4.3.2 SEDIMENT ANALYSIS

The particle size distribution of sediment (Table 7) collected from the three sites displayed clear spatial and temporal differences. Albasini Dam is characterised by course sediment composition whilst Xikundu is dominated by silts (>40%). The low flow periods resulted in generally higher siltation in the dams as seen from the increased fine silt content of the sediments. Xikundu sediments displayed the temporal variation the best being a riverine site, with high flows resulting in removal of fine sediments and medium sand predominating then during the low flow periods the fine silts increase again. During the high flow season in March 2007, Nandoni showed a significant increase in fine sediments as opposed to that observed in the two low flow periods. This
is likely due to increased runoff of fine sediments into the dam as a result of increased rainfall.

The concentrations of organochlorines in sediments from the three dams were all below analytical detection limits of <0.10 µg/kg and <0.02 µg/kg (Table 8).

Table 7: Particle size compositions of the sediment samples taken from the three selected sites at the time of each of the fieldtrips (expressed in grams (g)).

<table>
<thead>
<tr>
<th>Particle size (µm)</th>
<th>Sampling periods</th>
<th>Oct-06</th>
<th></th>
<th>Mar-07</th>
<th></th>
<th>Oct-07</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Albasini</td>
<td>Nandoni</td>
<td>Xikundu</td>
<td>Albasini</td>
<td>Nandoni</td>
</tr>
<tr>
<td>4000</td>
<td></td>
<td>41.47</td>
<td>5.89</td>
<td>0</td>
<td>–</td>
<td>0.54</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>16.30</td>
<td>7.98</td>
<td>0</td>
<td>–</td>
<td>2.43</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td>17.20</td>
<td>19.60</td>
<td>22.39</td>
<td>–</td>
<td>14.67</td>
</tr>
<tr>
<td>212</td>
<td></td>
<td>4.63</td>
<td>18.31</td>
<td>16.90</td>
<td>–</td>
<td>12.70</td>
</tr>
<tr>
<td>53</td>
<td></td>
<td>2.10</td>
<td>20.73</td>
<td>16.27</td>
<td>–</td>
<td>30.17</td>
</tr>
<tr>
<td>&lt;53</td>
<td></td>
<td>18.30</td>
<td>27.50</td>
<td>44.44</td>
<td></td>
<td>39.50</td>
</tr>
</tbody>
</table>

Table 8: The organochlorine concentrations present in the sediment samples taken from the three selected sites at the time of each of the fieldtrips (Bornman et al., 2010).

<table>
<thead>
<tr>
<th></th>
<th>Oct-06</th>
<th></th>
<th>Mar-07</th>
<th></th>
<th>Oct-07</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Albasini</td>
<td>Nandoni</td>
<td>Xikundu</td>
<td></td>
</tr>
<tr>
<td>o,p’-DDE (µg/kg)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>p,p’-DDE (µg/kg)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>o,p’-DDD (µg/kg)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>p,p’-DDD (µg/kg)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>o,p’-DDT (µg/kg)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>p,p’-DDT (µg/kg)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>PCB 153 (µg/kg)</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>
4.3.3 TISSUE ANALYSIS

4.3.3.1 BIOACCUMULATION

The concentrations of organochlorines in the fish tissues collected from the Albasini dam were all below analytical detection limits of <0.10 µg/km, while those collected from the Nandoni dam indicated increased levels of both the p,p'-DDE and the p,p'-DDD metabolites. The tissue samples collected from the fish at the Xikundu site, displayed very high levels of p,p'-DDE, p,p'-DDD and p,p'-DDT, with much lower levels of o,p'-DDD and o,p'-DDT (Table 9).

Table 9: The organochlorine concentrations present in the tissue samples taken from the three selected sites at the time of each of the fieldtrips (Bornman et al., 2010).

<table>
<thead>
<tr>
<th>Sampling periods</th>
<th>Oct-06</th>
<th>Mar-07</th>
<th>Oct-07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>o,p'-DDE (µg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>—</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Std Deviation (±)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE (µg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>—</td>
<td>80.50</td>
<td>1797.63</td>
</tr>
<tr>
<td>Std Deviation (±)</td>
<td>—</td>
<td>46.71</td>
<td>643.58</td>
</tr>
<tr>
<td>o,p'-DDD (µg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>—</td>
<td>&lt;0.01</td>
<td>18.14</td>
</tr>
<tr>
<td>Std Deviation (±)</td>
<td>—</td>
<td>—</td>
<td>12.39</td>
</tr>
<tr>
<td>p,p'-DDD (µg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>—</td>
<td>70.84</td>
<td>2233.71</td>
</tr>
<tr>
<td>Std Deviation (±)</td>
<td>—</td>
<td>29.42</td>
<td>921.19</td>
</tr>
<tr>
<td>o,p'-DDT (µg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>—</td>
<td>&lt;0.01</td>
<td>56.93</td>
</tr>
<tr>
<td>Std Deviation (±)</td>
<td>—</td>
<td>—</td>
<td>51.11</td>
</tr>
<tr>
<td>p,p'-DDT (µg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>—</td>
<td>&lt;0.02</td>
<td>2107.84</td>
</tr>
<tr>
<td>Std Deviation (±)</td>
<td>—</td>
<td>—</td>
<td>1305.49</td>
</tr>
<tr>
<td>PCB 153 (µg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>—</td>
<td>—</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
4.3.3.2 BIOMARKER ANALYSES

Figures 10, 11 and 12 show the biomarker concentrations derived from the selected tissue samples taken from the three selected sites at the time of sampling.

4.3.3.2.1 EROD

![Bar chart showing EROD activity](chart.png)

Figure 10: Average values (mean ± SE) of the biomarker levels and activities determined during each of the field assessments for EROD (EROD Activity (nM/min.mg protein)).

EROD activity was induced in samples obtained from the reference site, Albasini Dam. Additionally, the EROD inductions obtained at Albasini, were higher than those present in the samples obtained at Xikundu during the second low flow assessment. Much higher EROD inductions were noticed during the low flow periods, thus indicating seasonality to be a significant aspect to be considered in the use of biomarkers in the field environment.
4.3.3.2.2 CATALASE ACTIVITY

Figure 11: Average values (mean ± SE) for the biomarker levels and activities determined during each of the field assessments for catalase (µmol H₂O₂/mg protein.min).

Catalase activity concentrations obtained from Xikundu during the March 2007 high flow period showed significantly lower catalase inductions than were obtained at any of the other sites during any of the periods sampled. The catalase activity concentrations obtained from Xikundu and Albasini during the October 2007 low flow period differed significantly (P>0.05) from any results obtained in previous sampling periods with much higher catalase inductions. Results obtained in the low flow period of October 2006 show similar results for both Xikundu and Nandoni.

Results obtained in the high flow period of March 2007 show significantly higher catalase inductions at Albasini than those obtained at Xikundu. When comparing the high and low flow results obtained at Xikundu and Albasini, it is seen that catalase inductions were reduced at both sites during the high flow season. October 2007 was particularly dry with water levels significantly reduced and elevated TDS and conductivity readings.
4.3.3.2.3 CEA

Figure 12: Average values (mean ± SE) for the biomarker levels and activities determined during each of the field assessments for Cellular Energy Allocation (mJ/g).
The measurements of the available energy reserves and the energy consumption of *O. mossambicus* at each of the sites during each of the exposure periods can be seen in figure 12.

Significantly lower energy values (P>0.05) for the glucose content were observed at Xikundu and Nandoni during the sampling period of October 2006, while both sites also showed both higher lipid and protein measurements, than were obtained in the following two sampling trips. However, with repeated sampling, Xikundu, during the period of March 2007, appears to have undergone a continued impact from the October 2006 period, with lower levels of lipids as well as proteins measured in these samples.

The total energy reserves (Ea) gave an integrated overview of the temporal changes of the available energy reserves of *O. mossambicus*, obtained from each of the selected study sites (Figure 12). Xikundu and Nandoni during the period of October 2006 appeared to be the least affected of all the sampling periods with significantly higher overall energy reserves available than when compared to the following two fieldtrips. Albasini and Xikundu during the March 2007 sampling period show significantly lower energy reserves (P>0.05) than from the previous fieldtrip. While energy reserves from Albasini continued to deteriorate, it seems that fish from Xikundu recovered slightly by the time of sampling in October 2007. The cellular respiration rate (Ec) decreased very significantly from the October 2006 sampling period to the March 2007 sampling period, with respiration rates then increasing again at Albasini and Xikundu, but with Albasini showing more of an increase in the respiration rate at this period than was found at Xikundu.
4.3.4 PCA AND RDA PLOTS

The PCA plot below (Figure 13) shows the biomarker concentrations derived from the selected tissue samples in relation to the three selected sites and the time of sampling. The RDA bi-plots (Figures 14-17) derived from the PCA plot shows the biomarker concentrations and the (dis)similarities between various additional variables.

Figure 13: PCA plot showing (dis)similarity at the three selected sites during each of the sampling periods. The plot describes 97.5% of the variation in the data, where 60.0% is displayed on the first axis, while 30.5% is displayed in the second axis.
Figure 14: RDA bi-plot showing (dis)similarity at the three selected sites during each of the sampling periods, based on the biomarker results obtained, with water quality variables superimposed.
Figure 15: RDA bi-plot showing (dis)similarity at the three selected sites during each of the sampling periods, based on the biomarker results obtained, with DDT concentrations and particle sizes superimposed.
Figure 16: RDA bi-plot showing (dis)similarity at the three selected sites during each of the sampling periods, based on the biomarker results obtained, with DDT concentrations obtained in the tissue samples superimposed.
Figure 17: RDA bi-plot showing (dis)similarity at the three selected sites during each of the sampling periods, based on the biomarker results obtained, with the DDT bioaccumulation factor from tissue samples superimposed.

In the RDA bi-plots, each arrow points in the direction of the steepest increase of values for the corresponding variable. The angles between each arrow indicate the extent of the correlation between the variables, i.e. the approximated correlation is positive when the angle is acute and negative when the angle is >90°. The length of each arrow is a measure of fit for the variables. The distance between each sampling site in the bi-plot approximates their dissimilarity, measured by their Euclidean distance (Ferreira et al., 2009).
The PCA plot for both flow regimes indicated that there was a distinct difference in seasonal variation observed between high-flow and low-flow conditions. Similar reactions from both the Catalase and the EROD biomarker tests were observed during times of low flows when increased concentrations of salts and pollutants were likely.

Separate RDA bi-plots were also constructed. The bi-plots indicated that lower dissolved oxygen levels coincided significantly with both the Catalase and the EROD activity results obtained during the low flow seasons.

The bi-plots also indicated that the soils with the smallest particle sizes coincide with the Xikundu site. The Xikundu site is also the site with the highest DDT concentrations detected in the organic content of the soils. Additionally, the bi-plots indicate that the highest level of bioaccumulation occurred at the Xikundu site during October 2006. These results correlate with the Catalase and EROD activities observed at the time.

4.4 DISCUSSION

4.4.1 WATER QUALITY

The thermal characteristics of any given freshwater body, is dependent on various hydrological, climatic and structural features of the region, catchment area and river. Running waters in regions of seasonal climates exhibit daily and seasonal temperature patterns, in addition to longitudinal changes along a river course (Dallas and Day, 2004). Anthropogenic causes of temperature changes in these water bodies include those resulting from thermal pollution, stream regulation and changes in riparian vegetation and often, more silt-laden water adsorbs sun-rays and warms up more rapidly than does clear water (Mitchell and Stapp, 1994).

Temperature is the most important physical variable affecting dissolved oxygen, the rate of photosynthesis in water and the metabolic rate of any water life (Mitchell and Stapp, 1994). Temperature responses of fish can be divided into resistance, tolerance and
preference (DWAF, 1996) and it affects all aspects of fish biology. *O. mossambicus* prefers warm temperatures above 22 °C, but can tolerate temperatures of below about 15 °C in brackish or marine waters and can survive in temperatures of up to 42 °C (Skelton, 2001).

In South Africa, inland waters generally range from about 5 – 30 °C, with the thermal characteristics being dependant on the various features of the region and catchment area, which includes:

- the latitude and altitude of the river;
- hydrological factors such as the source of water, the relative contribution of ground water, and the rate of flow or discharge;
- climatic factors such as air temperature, cloud cover, wind speed, vapour pressure and precipitation events; and
- structural characteristics of the river and catchment area, including topographic features, vegetation cover, channel form, water volume, depth and turbidity (DWAF, 1996).

According to DWAF (1996), surface waters exhibit daily and annual periodicity patterns, in addition to longitudinal changes along a river course, and vertical stratification in deeper waters. Anthropogenic sources such as heated return flows of irrigation water, removal of riparian vegetation cover, inter-basin water transfers and discharge of water from impoundments may also cause changes in water temperatures (DWAF, 1996). In addition, very silt-laden water warms up more quickly than does clear water as more sun-rays are absorbed (Mitchell and Stapp, 1994).

Temperatures were generally lower at the Albasini Dam, but for the last fieldtrip this was not the case. However, this may be attributed to the fact that the area was experiencing cooler and more rainy weather during the sampling period at Xikundu, only clearing up well into the sampling at Nandoni. The Albasini Dam water level was also considerably
lower at the time of sampling for both the second and the last fieldstrips, which would have made the water more susceptible to higher water temperatures. This said, all the observed temperature changes over the course of this study may be attributed to natural seasonal and diurnal fluctuations and is therefore not considered to have had a negative influence on the water quality.

Waters with consistently high levels of dissolved oxygen are usually considered to be healthy and capable of supporting many different kinds of water organisms. Most often, dissolved oxygen in any given body of water is introduced from the atmosphere through rainfall, from tumbling water in fast moving streams and via water plants as a result of photosynthesis (Mitchell and Stapp, 1994). Often, large daily fluctuations of dissolved oxygen can occur in rivers and dams due to the effects of photosynthesis and plant and animal respiration. Effluent and agricultural runoff can enrich water, promoting the growth of algae and other water plants, while sewage effluent promotes large populations of bacteria which consume oxygen (Mitchell and Stapp, 1994; Dallas and Day, 2004).

The dissolved oxygen content at Albasini in the last fieldtrip was found to be higher at this site than was found in previous fieldtrips, although oxygen levels at all three sites were generally higher than over the two previous fieldtrips. Generally, dissolved oxygen at Xikundu was found to be the highest, but this is expected as Xikundu is a riverine environment, with more air to water contact and continuously flowing water, while Nandoni and Albasini are dammed environments with less surface area to water quantity and the absence of continuously flowing water. Additionally, it is important to note that the saturation of a particular gas in a liquid increases with temperature and varies under the different conditions such as salinity, biological oxygen demand, photosynthetic activity, atmospheric pressure, water movement and biological gas production (DWAF, 1996).

Reductions in the concentration of dissolved oxygen can be caused by several factors:
• The resuspension or precipitation of anoxic sediments due to disturbances in the river;
• Turnover or release of anoxic bottom water from a deep lake or reservoir;
• The presence of oxidizable organic matter, either of natural origin (detritus) or originating in waste discharges, can lead to reduction in the concentration of dissolved oxygen in surface waters. The potential for organic wastes to deplete oxygen is commonly measured as biochemical oxygen demand and chemical oxygen demand; and
• The amount of suspended material in the water affects the saturation concentration of the dissolved oxygen, either chemically, through the oxygen scavenging attributes of the suspended particles, or physically through reduction of the volume of water available for solution (DWAF, 1996).

Increased levels of dissolved oxygen obtained during the last fieldtrip may be due to rain in the area just before the time of sampling and also during the first half of the sampling trip, wind patterns, as well as increased water temperatures for Albasini Dam during the time of the October 2007 fieldtrip.

The pH of natural water is determined by geological and atmospheric influences, with atmospheric pollution such as nitrogen and sulphur dioxides from vehicles and thermal power stations producing acid rain and sewage and industrial effluent discharges can also affect the pH balance of rivers (Mitchell and Stapp, 1994). Some streams are naturally more acidic than others and their biotas are adapted to these conditions. Water Quality Guidelines require that TWQR for pH be stated in terms of the background site-specific pH regime. Guidelines are thus case- and site-specific and take diurnal and seasonal variation into account (Dallas and Day, 2004).

The pH of Xikundu is lower than the pH of the two dam sites. The temperature is significantly lower at Xikundu during the time of October 2007, as well as the dissolved oxygen content is higher at the same sight. As previously stated, pH is largely
influenced by physico-chemical properties of the surrounding environment and there is any number of factors to which the differing pH values could be attributed. The conductivity was generally higher in the two dams, with the exception of Xikundu at the time of the first fieldtrip. Albasini consistently showed higher amounts of TDS than the other two sites.

Table 5 shows the organochlorine concentrations of selected organochlorines in the water samples taken from the three selected sites. There is very little information available as to the acceptable levels of these contaminants in the environment and, thus, these concentrations are to be used towards establishing a database of the environmentally relevant concentrations found in the natural environment in the Limpopo Province.

4.4.2 SEDIMENT ANALYSIS

Many contaminants of concern to water quality are found to be associated with particulate matter in the environment. They often do not remain soluble in the water but are adsorbed and accumulated by bottom sediments, acting as a sink (Coetzee, 1996).

Natural water systems possess a variety of pathways for the deposition of pollutants to sediment. However, the two most relevant of these, as the case may be, are precipitation and adsorption. The relative balance between the two is dependent upon the degree of supersaturation and available solid surface area, as well as organic matter content, the presence of other pollutants or contaminants in the surrounding environment, the actual physical water quality parameters at a particular location and finally, the relevant external environmental influences (Coetzee, 1996).

The distribution of contaminants in the bottom sediments is affected by factors such as deposition, sorption, enrichment in organisms, organic material content and diagenetic redistribution of trace elements (Lacy et al., 1998). According to Evans et al. 1990), there exists a positive linear relationship between organochlorine concentrations in
sediment and the sediment’s organic matter content. It appears that size fractions also play a role in the localized distributions of these pollutants. Fine grained sediments rich in clay and organic materials tend to sorb organic pollutants, whereas course textured sediments poor in clay and organic materials sorb organic pollutants to a much lower degree. This was evident when looking at the RDA bi-plot (Figure 15), which indicated that the highest level of bioaccumulation took place at the Xikundu site, which constituted mostly of fine grained sediments. Organic matter content of aquatic sediments has been shown to be very important in terms of complexing hydrophobic toxic pollutants. Dissolved Organic Material (DOM) has been shown to complex specific pollutants, rendering them unavailable for uptake into aquatic organisms, whereas the degree of hydrophobicity of the pollutant affects the proportion of the compound able to bind to DOM or conversely the amount of DOM required to bind all of the contaminant present in the system. Evans et al. (1990) has shown that the partition of pollutants between water and sediment has been found to be linearly related to the organic matter content of both phases.

As was previously stated, DDT and its metabolites are extremely volatile and are able to traverse through many different watercourses in the environment, thus, explaining the exposure to DDT and its metabolites, even at the Albasini Dam reference site. Dam environments are generally still water bodies that collect all the runoff of water from the surrounding environments. With the Nandoni Dam being situated in the heartland of the DDT sprayed area and it would be logical to assume that there should be a greater runoff of DDT and its metabolites into this dam. This, however, is not evident in the organochlorine concentrations obtained in this particular field study, with all sediment and water concentrations found to be less than 0.02 µg/kg and 0.1 µg/L, respectively, at each of the sites selected for the study.
4.4.3 TISSUE ANALYSIS
4.4.3.1 BIOACCUMULATION

The RDA bi-plots (Figures 16 and 17) indicated that the highest level of bioaccumulation and bioconcentration took place at the Xikundu site. This correlates with the sediment data, which indicated that the Xikundu sediment samples contained the smallest particle size fractions in relation to the other two sites, thus making a higher organic content likely, which in turn is likely to contain higher levels of hydrophobic pollutants such as DDE. As was stated in Chapter 3, *O. mossambicus* are bottom feeders and feed on the organic matter present. Higher levels of DDT and its metabolites are thus likely in the tissues of fish caught in areas where the sediments have higher organic contents.

4.4.3.2 EROD

EROD activity has been proven to be induced specifically by exposure to certain planar organic compounds such as Poly Aromatic Hydrocarbons (PAHs), dioxins, and non-ortho-PCB (Schiedek *et al.*, 2005). It was, therefore, unexpected to find that there were EROD activity was induced in samples obtained from the reference site, Albasini Dam. It was even more unexpected to find that the EROD inductions obtained at Albasini, were higher than those present in the samples obtained at Xikundu during the second low flow assessment. This said, it should be noted that EROD activity has been shown to be influenced by a number of abiotic and biotic factors such as temperature, age as well as the reproductive stages of the organisms sampled (Schiedek *et al.*, 2005), all of which were shown, in this particular study, to vary to a large degree. As was stated in Chapter 2, Zapata-Perez *et al.* (2000), EROD activity correlates with o,p'-DDE, p,p'-DDE and p,p'DDD concentrations, however, EROD induction by the pesticides may be inhibited to a degree by the presence of metals (Riffat and Ahmad, 2006). The PCA plot (Figure 13) shows that much higher EROD inductions were noticed during the low flow periods, thus indicating seasonality to be a significant aspect to be considered in the use of biomarkers in the field environment.
The RDA bi-plot (Figure 14) indicates that EROD activity may be affected by the levels of oxygen that are present in the surrounding environment. This is not unusual, as EROD is a metabolic reaction the activity of which has been shown to be affected by the presence or absence of oxygen (Palace et al., 1996).

4.4.3.3 CATALASE ACTIVITY

Catalase activity concentrations obtained from Xikundu during the March 2007 high flow period showed significantly lower catalase inductions than were obtained at any of the other sites during any of the periods sampled. The catalase activity concentrations obtained from Xikundu and Albasini during the October 2007 low flow period differed significantly from any results obtained in previous sampling periods with much higher catalase inductions.

Results obtained in the low flow period of October 2006 show similar results for both Xikundu and Nandoni. This is not surprising as, although Nandoni dam is situated in the heartland of the DDT sprayed area; catalase is a very general biomarker and, as such, will indicate any number of impacts occurring in the area. In conjunction with direct DDT spraying, additional impacts to the Nandoni dam is construction, recreation as well as sideline fishing by the locals. Additionally, Xikundu is impacted by the presence of contaminants transported downstream from the DDT sprayed area as well as with livestock, agricultural, domestic and fishing activities.

Results obtained in the high flow period of March 2007 show significantly higher catalase inductions at Albasini than those obtained at Xikundu. This may, once again, be an indication of the general nature of the catalase biomarker and as such, indicates the presence of a variety of environmental stressors. Thus, even though Albasini dam was considered the control site, being situated upstream and outside of the DDT sprayed area, there are a number of other environmental impacts in the area including the presence of orchards and other agricultural activities, recreational activities and livestock. It is likely that any number of environmental contaminants such as fertilizers
and pesticides are being washed into the dam at any given time, thus explaining the higher inductions of catalase obtained at this site. The RDA bi-plot (Figure 14) indicates that catalase activity may be affected by the levels of oxygen that are present in the surrounding environment as catalase is an oxidative reaction (Cohen et al., 1970).

When comparing the high and low flow results obtained at Xikundu and Albasini, it is seen that catalase inductions were reduced at both sites during the high flow season, thus suggesting lower contaminant concentrations in the surrounding environment during this time and suggesting seasonality to be an influencing factor in the induction of catalase. This is supported by the results obtained in the PCA plot (Figure 13), which indicates that catalase activity was most pronounced in the low flow seasons. October 2007 was particularly dry with water levels significantly reduced and elevated TDS and conductivity readings. However, it is unlikely that this alone would explain the elevated catalase induction results from samples obtained at this time.

4.4.3.4 CEA

Significantly lower energy values for the glucose content were observed at Xikundu and Nandoni during the sampling period of October 2006, while both sites also showed both higher lipid and protein measurements, than were obtained in the following two sampling trips. The higher lipid content at these sites indicate that the effect of environmental disturbances and pollutants in these two areas were more recent than at Albasini, while the depleted lipid and protein reserves at Albasini indicate more of a constant historical impact than recent. However, with repeated sampling, Xikundu, during the period of March 2007, appears to have undergone a continued impact from the October 2006 period, with lower levels of lipids as well as proteins measured in these samples. The increased glucose levels indicate recent recovery in the fish.

The total energy reserves (Ea) gave an integrated overview of the temporal changes of the available energy reserves of O. mossambicus, obtained from each of the selected study sites (Figure 12). Xikundu and Nandoni during the period of October 2006
appeared to be the least affected of all the sampling periods with significantly higher overall energy reserves available than when compared to the following two fieldtrips. Xikundu during the March 2007 sampling period shows significantly lower energy reserves than from the previous fieldtrip. While energy reserves from Albasini deteriorated, it seems that fish from Xikundu recovered slightly by the time of sampling in October 2007. The cellular respiration rate (Ec) decreased very significantly from the October 2006 sampling period to the March 2007 sampling period, with respiration rates then increasing again at Albasini and Xikundu, but with Albasini showing more of an increase in the respiration rate at this period than was found at Xikundu.

It can be said with all surety that changes in the glucose, lipid and protein reserves contributed to the changes in the Ea and CEA. According to Muyssen and Janssen (2001) lipids are known as more efficient energy-storage products than either proteins or glucose and consequently, lipid reserves can be the first energy source to be consumed under conditions of toxic stress, this was observed in the results obtained during the second two sampling periods.

4.5 CONCLUSION

EROD inductions have been shown to be useful as a biomarker for a number of planar organic contaminants, however, a number of added influences and uncertainties introduced by various abiotic and biotic factors have influenced the results obtained in this study to a large degree. Additionally, the instability of the DDT metabolites in the field environment and the lack of a holistic understanding as to how these chemicals react in the environment makes interpretation of the results obtained difficult as the level of exposure of the organism to DDT and its metabolites, specifically, is not clear. CAT and CEA are both very general biomarkers and thus show the effects of all the pollutants in the selected study area. As an indication of induced stress experienced in a particular area to contaminated water and external stresses in general, both catalase
and Cellular Energy Allocation may be considered very useful. However, to indicate the stresses observed in the field environment due to exposure to planar organic compounds specifically, these biomarkers fall short.

Continued research with regard to the use of EROD inductions as a useful indicator of exposure to DDT and its metabolites in the field environment is considered necessary to build up a greater and more reliable data pool of results and to establish any trends in this data over time.
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5. TOXICITY TESTING OF DDE ON *OREOCHROMIS MOSSAMBICUS*

5.1 INTRODUCTION

Organisms under natural field conditions may respond quite differently to controlled pollutant exposures under laboratory conditions (Shugart, 1996). Laboratory studies generally lack ecological realism, because of many environmental factors that can influence organism responses at all levels of biological organization (Adams, 2001). Laboratory exposed organisms can exhibit an increased sensitivity towards environmental pollutants that are generally absent in many artificial culture media. Therefore, acclimation (tolerance) and adaptation (genetic) are factors related to organisms under field conditions that cannot be neglected when extrapolating laboratory data to field conditions (Muysse et al., 2002).

There is a growing need for more studies to demonstrate that biological responses to toxicants in the laboratory are comparable to field data. Exposures under laboratory conditions could help establish cause and effect relationships, as it is only in such an environment, that organisms can be exposed to pollutants under specific controlled conditions (Kruger, 2002). Under these controlled laboratory conditions it is possible to establish a dose-response relationship between concentrations of pollutants and between biomarker responses (Walker, 1998; Hyne and Maher, 2003), thus further contributing toward the validity of the use of biomarker responses in field monitoring. However, a number of biotic and abiotic factors can influence the extrapolation of individual biomarkers to the field monitoring of pollutant effects (Lagadic et al., 1994), thus making the comparison of field data and laboratory data difficult.

In addition to the field exposure study, the use of the Mozambique Tilapia (*Oreochromis mossambicus*) as a biomonitor was tested under laboratory conditions. A sub-lethal exposure study was undertaken. Predicting the potential effect of known amounts of a pollutant requires a task in which biomarkers might be employed. Biological responses
may link the bioavailability of compounds with their concentrations at target organs and intrinsic toxicity (van der Oost et al., 2003). Certain responses established for one species, however, are not necessarily valid for other species (van der Oost et al., 2003).

The determination of toxicity of a chemical, its by-products and its degradation products to aquatic organisms is important because any compound that is used and manufactured in considerable quantities is likely to become a contaminant of water sources (Nussey, 1994). To evaluate the effects that various concentrations of the chemical may have on aquatic organisms, toxicity tests are used. The information gained from various toxicity tests can be of use in the management of pollution for the purpose of either the prediction of environmental effects of a waste product, a comparison of toxicants and even test conditions (Nussey, 1994).

Available data for DDT and its metabolites indicate that acute toxicity to freshwater aquatic life may occur at concentrations as low as 11,600 μg/L and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of these chemicals to sensitive freshwater aquatic life (EPA, 1986).

Toxicity tests are designed to evaluate the relative toxicity of a test material or the level of toxicity of an agent to a selected group of aquatic organisms. They can be undertaken through injection of the test material into the organism or by inclusion in the food, however, most of these tests are carried out by exposing groups of organisms to several controlled environments in which different concentrations of the test material are mixed in water. The exposure is expected to produce a specific effect in the exposed test organisms over a specific period of time, for example, 24, 48 or 96 hours (Nussey, 1994).

Many factors can affect or modify the degree of sensitivity shown by an organism to different toxicants, for example the season of the year, specific diet and water quality variables such as hardness, pH and temperature. Since fish are ectothermic
(poikilothermic), temperature is the most important environmental factor controlling rates of biological processes, and thus, would be expected to influence tissue uptake of various substances as well as the toxicity tolerance in fish as changes in temperatures may cause increases and decreases in metabolism and oxygen uptake and consumption. An increase in water temperature will increase the rate of chemical reactions in fish, as predicted by the Arrhenius theory. This theory predicts that the toxicity of a toxicant also increases with a rise in temperature (Nussey, 1994). The calcium content (hardness) of water acts as a modifying factor with an increase in metal toxicity in water with a low calcium content. The toxicity of pollutants may be increased by a reduction in dissolved oxygen whilst the hydrogen ion concentration (pH) can affect the ionization and the solubility of the toxicant (Nussey, 1994).

This chapter reports on the effects observed on the biomarker values after exposure to sublethal concentrations of DDE. The objective was to conduct sublethal toxicity exposures using varying concentrations of DDE in a controlled environment with adult *Oreochromis mossambicus*, selected as indicator species as motivated in Chapter 2.

### 5.2 MATERIALS AND METHODS

Mature *Oreochromis mossambicus* males, all of comparable size and age, were obtained from Aquaculture Innovations (bred in captivity), Grahamstown, South Africa.

#### 5.2.1 GENERAL HOLDING SYSTEM AND LABORATORY CONDITIONS

Fish were exposed to five environmentally relevant DDE concentrations (Control, solvent control, 0.1µg/l, 0.4µg/l, 0.7µg/l of DDE) derived from levels observed in the Rietvlei Nature Reserve during a study funded by the WRC in 2007 (Bornman et al., 2007). Exposures took place at 24±1 °C for a period of 96 hours with a 12:12 hour day-night cycle. Eight healthy males were used per concentration per exposure and were
placed in a flow through system consisting of four exposure tanks and a stock tank (Figure 18).

![Figure 18: Schematic representation of test organisms exposed to DDE concentrations in a continuous flow through system.](image)

Fish were allowed to acclimatize for a period of two weeks and were fed commercial trout pellets (50% protein) every second day. The stock tank solution was made up using aged tap water and DDE in a 0.02 percent ethanol solution and pumped to exposure tanks 1-4.

### 5.2.2 WATER QUALITY

The physical water quality variables were monitored in each of the stock tanks at the following intervals: 1 and 96 hours. These included the pH, dissolved oxygen, temperature, conductivity and TDS. Temperature and dissolved oxygen content were measured with a Eutech CyberScan DO 310 meter, pH was taken with a Eutech CyberScan pH 310 meter, while total dissolved salts (TDS) and conductivity were measured with a Eutech CyberScan CON 400 meter.

### 5.2.3 SAMPLE PREPARATION

After the exposure, fish were removed from the tanks, each fish was weighed and measured and blood was drawn. The fish were then ventrally opened and were
examined macroscopically for abnormalities, the testes were removed, weighed and measured and were then stored in Bouin's solution for future testing. Sections of liver, gills and muscle were collected for biomarker analysis and stored at -80°C until further analysis.

5.2.4 BIOMARKERS

The biomarker responses of the test organisms were determined, after the 96 hour exposure period. Muscle, gills and liver tissue were used for each of the biomarker analyses. Tissues were thawed and homogenised on ice, in the appropriate homogenising buffer. The biomarkers assayed were: EROD, CAT activity and CEA (Table 10). All samples were analysed in triplicate.

All glassware used in the analyses was washed before use. Glassware was washed and placed in a soap bath containing a 2 percent Contrad TM (Merck Chemicals) solution for 24 hours. Thereafter, it was rinsed with double distilled H₂O (dH₂O) and placed in a 1 M hydrochloric acid bath (Merck Chemicals) for another 24 hours. It was then rinsed again with dH₂O and allowed to dry.

5.2.4.1 EROD (7-ETHOXY RESORUFIN O-DEETHYLASE)

EROD was determined by method of Burke and Mayer (1978), with some modifications. Liver samples were homogenized in 0.25 M sucrose – 0.05 M Tris (pH 7.4) on ice at a ratio of 1:3 and then centrifuged at 13 500 rpm for 20 minutes at 4 °C. To the supernatant of each sample was added a general reaction mixture of 0.1 M potassium phosphate buffer (pH 7.8) and 0.05 M Ethoxyresorufin in 1.25 percent Tween 80. A 0.05 M aliquot of NADPH was stirred into the mixture to start the reaction and the progressive increase in fluorescence, as ethoxyresorufin was deethylated to resorufin. A baseline of fluorescence was recorded at an excitation wavelength of 544 nm and an emission wavelength of 590 nm with a fluorimeter and using resorufin as a standard.
The excitation emissions were then recorded another six times at one minute intervals measuring the progressive increase in resorufin. Ethoxyresorufin and resorufin are both light sensitive compounds and the solutions of these compounds were thus prepared and stored in the dark and the metabolic reaction was then initiated in a room with dim infrared lighting.

5.2.4.2 CATALASE ACTIVITY

Catalase activity was determined by method of Cohen et al. (1970). Gill samples were homogenized with 0.01 M phosphate buffer (pH 7.0) on ice at a ratio of 1:2 and then centrifuged at 10 000 rpm for 10 minutes at 4 °C. To an aliquot of the supernatant was then added cold 6 mM H$_2$O$_2$ to start an enzyme catalyzed decomposition reaction of the H$_2$O$_2$. After an incubation period of 3 minutes, the reaction was stopped with the addition of 6 N H$_2$SO$_4$. The remaining H$_2$O$_2$ was measured by allowing it to react with a standard excess of 2 mM KMnO$_4$ and then measuring the residual KMnO$_4$ spectrophotometrically at a wavelength of 490 nm within 30 to 60 seconds of the addition of the KMnO$_4$. The absorbance was read using an Elx-800 universal microplate reader (Bio-tek instruments, USA). The protein content was determined using the method of Bradford (1976) and the CAT activity was expressed as μmol H$_2$O$_2$/mg protein.min.

5.2.4.3 CELLULAR ENERGY ALLOCATION (CEA)

Available energy reserves ($E_a$)
The Cellular Energy Allocations were determined my method of De Coen and Janssen (1997). The samples were homogenised on ice in 1 ml of ice-cold deionised water. The energy reserves determined were: the carbohydrate, the lipid and the protein content of the samples. Whole body carbohydrate content was determined using a glucose test kit (GOD-PAP 1 448 668, Roche) and glucose standard (C FAS 759 350, Roche) at a wavelength of 560 nm using an automated microplate reader. Total lipids were extracted following the method of Bligh and Dyer (1959), and the absorbance was
read at 340 nm using tripalmitin as a standard. The protein content was determined using Bradford’s reagent (Bradford, 1976). The absorbance was measured at 630 nm using bovine serum albumin (BSA) as a standard.

**Energy consumption (E\textsubscript{c})**

The energy consumption or cellular respiration rate was determined by measuring the electron transport activity (ETS). Each sample was homogenized in 1 ml homogenising buffer (0.1 M Tris-HCl pH 8.5, 15 percent (w/v) Poly Vinyl Pyrrolidone, 153 μM MgSO4 and 0.2 percent (w/v) Triton X-100). After centrifugation (at 3000 rpm for 10 minutes at 4 ºC), 25 μl supernatant was added to 75 μl buffered substrate solution (0.13 mM Tris-HCl and Triton X-100, pH 8.5) and 25 μl NAD(P)H solution (1.7 mM NADH and 250 μM NADPH). The reaction was started by adding 100 μl INT (p-IodoNitroTetrazolium) and the absorbance was measured kinetically at 490 nm for 10 minutes at 20 ºC. The amount of formazan formed was calculated using $e = 15900 / \text{M.cm}$. 

**CEA**

The different energy reserves (E\textsubscript{a}) were transformed into energetic equivalents, using the enthalpy of combustion values used by De Coen & Janssen (1997). The values are as follows: 17 500 mJ/mg glycogen, 39 500 mJ/mg lipid and 24 000 mJ/mg protein. The cellular respiration rate (E\textsubscript{c}) was determined, using the theoretical stoichiometrical relationship that for each 2 μmol of formazan formed, 1 μmol oxygen was consumed in the ETS system. The amount of oxygen was transformed into energetic equivalents using an average oxyenthalpic equivalent of 484 kJ/mol O\textsubscript{2}. The total energy budget was calculated using the following equation:

\[
\text{CEA} = E_a - E_c
\]

where: $E_a = E_{\text{carbohydrate}} + E_{\text{lipid}} + E_{\text{protein}}$

$E_c = E_{\text{ETS}}$
Table 10: A summary of the methods and apparatus used in the biomarker analysis

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Abbreviation</th>
<th>Apparatus</th>
<th>Absorbancy Wavelength (nm)</th>
<th>Method</th>
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<tbody>
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<td>Catalase</td>
<td>CAT</td>
<td>Elx800 Universal Microplate Reader</td>
<td>490</td>
<td>Cohen et al. (1970)</td>
</tr>
<tr>
<td>Cellular Energy Allocation</td>
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<td>Elx800 Universal Microplate Reader</td>
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<td>Bligh and Dyer (1959)</td>
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<td></td>
<td>Protein</td>
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<td>Bradford (1976)</td>
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<tr>
<td></td>
<td>Glucose</td>
<td></td>
<td>560</td>
<td>Glucose test kit</td>
</tr>
<tr>
<td></td>
<td>ETS</td>
<td></td>
<td>490</td>
<td>De Coen and Janssen (1997)</td>
</tr>
</tbody>
</table>

5.2.5 STATISTICAL ANALYSES

Statistical analyses were performed using the program, SPSS 14.0. The Levene’s test was used to test all data for homogeneity of variance. Differences among the biomarker responses following exposure to the five differing DDE concentrations were determined using one-way ANOVA. The Scheffé’s multiple comparison test (for homogenous data) as well as Dunnett’s-T3 test (for non-homogenous data) were applied as post-hoc criterion, when significant differences of P<0.05 were found amongst the variables.
5.3 RESULTS

5.3.1 WATER QUALITY

Table 11 shows the water quality variables measured for the water in each of the stock tanks at both the 1 and 96 hour intervals of the exposure periods. All the physical water quality parameters measured remained relatively constant throughout the exposure period.

Table 11: Selected water quality variables of the aged tap water used during the DDE toxicity tests.

<table>
<thead>
<tr>
<th>Water Quality Variables</th>
<th>Control, solvent control &amp; DDE Concentration (µg/L)</th>
<th>1 hr</th>
<th>96 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2nd Exposure</td>
<td>1st Exposure</td>
</tr>
<tr>
<td>pH</td>
<td>Control</td>
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<td></td>
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<tr>
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### Conductivity (mS m⁻¹)

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<td>0.1</td>
<td>275</td>
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<td>0.4</td>
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<tr>
<td>0.7</td>
<td>280</td>
<td>277</td>
<td>192</td>
<td>189</td>
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#### 5.3.1.1 pH

pH generally decreases with time since acid is produced during nitrification of ammonia to nitrate (DWAF, 1996). This correlates with the pH values for both exposure systems in the current study (Table 11), however, the pH values remained relatively constant throughout each of the exposures. In poorly buffered systems, the pH may decrease to 6 or less. Increased pH in recirculating systems is often indicative of poor biological filtration (DWAF, 1996), which was not the case here.

#### 5.3.1.2 DISSOLVED OXYGEN

Dissolved oxygen did not vary much over the life-time of each of the exposures. Oxygen levels were recorded as being relatively low, however, the accuracy of the
measurement apparatus is questionable as oxygen was constantly added to the exposure tanks by means of an air-stone for the duration of each exposure.

5.3.1.3 TEMPERATURE

The temperatures increased very slightly during both exposures respectively, but remained relatively constant throughout each for the extent of each of the exposures.

5.3.1.4 TDS AND CONDUCTIVITY

The conductivity and TDS values for both the first and the second exposure periods showed only very slight decreases in both the control and the solvent control. However, in both exposures, with the exception of the [0.1] of the first exposure, the values showed significant and similar decreases in the [0.1], [0.4] and [0.7] exposure concentrations, respectively.

All of the water quality values obtained were within the acceptable range with the South African Water Quality Guidelines for Aquaculture (DWAF, 1996).
5.3.2 BIOMARKERS

5.3.2.1 EROD

Figure 19: Mean values ± SE of the biomarker levels and activities determined during the first (A) and the second (B) aquarium exposures for EROD (EROD activity (nM/min.mg protein)).

The results in Figure 19 show that EROD enzyme activity was clearly affected, with clear dose-related inductions being noted at each of the exposure concentrations in both of the exposures. In the first exposure, the [0.1μg/l] concentration showed a significant induction when compared statistically with the control and a clear trend of
increasing EROD induction can be seen with increasing concentrations of DDE. In the second exposure, it is the [0.7μg/l] concentration that shows a significant EROD induction when compared statistically with both the control and the solvent control, thus further showing the effect of EROD induction in aquatic organisms on exposure to DDE despite slightly more erratic results obtained for EROD enzyme activity in the second exposure.

5.3.2.2 CATALASE

![Catalase Activity Graph](image)

Figure 20: Mean values ± SE for the biomarker levels and activities determined during both the first (A) and the second (B) aquarium exposure assessments for catalase (μmol H₂O₂/ mg protein.min).
Significant catalase activity (P>0.05) was noted in the [0.1], [0.4] and [0.7] exposure concentrations in the first exposure, with little induction being noted in both the control and the solvent control. However, there was no significant difference obtained between the different DDE exposure concentrations. Values obtained in the second exposure were erratic with no significant effects being noted between either the controls or the three DDE exposure concentrations (Figure 20).

5.3.2.3 CEA

The effects of the different DDE concentrations on the available energy reserves and the energy consumption of *Oreochromis mossambicus* in each of the exposures can be seen in figures 21 and 22.

Significant reductions in the energy values for the glucose content were observed between the control and the [0.4μg/l] and the [0.7μg/l] DDE concentrations respectively in the first exposure, while no significant differences (P< 0.05) were observed in the glucose energy reserves of fish exposed to DDE in the second exposure. However, when noting the results obtained for the available protein energy reserves in fish from each of the exposures, no significant differences (P< 0.05) were observed in the available protein energy reserves from the fish exposed to the different DDE concentrations in the first exposure, while significant reductions in the protein values for the glucose were observed between the control and the [0.4μg/l] and the [0.7μg/l] DDE concentrations, respectively, from fish in the second exposure. Significant reductions were observed in the available lipid energy reserves of the first exposure when comparing the values obtained for the controls and the [0.7μg/l] DDE concentration with a significant difference seen between the lipid energy reserves in the [0.7μg/l] DDE concentration and that obtained for the solvent control. A significant difference was also noted in the lipid energy reserves between fish in the control and the [0.7μg/l] DDE concentration in the second exposure.
The total energy reserves (Ea) gave an integrated overview of the temporal changes of the available energy reserves of *Oreochromis mossambicus*, after exposure to the different DDE concentrations during each of the exposures (Table 3.3). With increased DDE exposure in each of the exposures, the total energy reserves were significantly reduced, when compared to the control. The cellular respiration rate (Ec) decreased significantly during both of the exposures periods, when compared to the control. However, no significant differences in ETS activity were observed in either of the exposures, with comparison to the control. In the first exposure, a significant decrease in CEA was observed between the two controls and the [0.7] DDE concentration. However, although a decreasing trend in CEA can be seen in the second exposure, no significant differences were observed. Looking at the results as a whole and their individual contributions, it can be said that both the total energy reserves and the respiratory enzyme activity exhibited the same trend as the CEA values and clearly, contributed to the decrease in CEA in both exposures.
Figure 21: Average biomarker levels (Mean ± SE) and activities determined during the first aquarium exposure assessment for Cellular Energy Allocation (mJ/g).
Figure 22: Average biomarker levels (Mean ± SE) and activities determined during the second aquarium exposure assessment for Cellular Energy Allocation (mJ/g).
5.4 DISCUSSION

5.4.1 WATER QUALITY

pH is a measure of the acidic or basic character of a solution and its activity is the estimated hydronium ion concentration, commonly referred to as the hydrogen ion (H\(^+\)) (Dallas and Day, 2004). Water ionizes slightly, yielding both hydronium and hydroxyl ions (OH\(^-\)) and when the activities of the ions is equal (each at 10\(^{-7}\) mol/R) the solution is neutral. At 25 °C, neutrality is represented by a pH value of 7. The ionization constant of water is dependent on temperature; therefore, the exact point of neutrality varies with water temperature. Values of pH below neutrality indicate an increase in acidity or a predominance of hydrogen ions and those above neutrality signify increasing alkalinity or a predominance of hydroxyl ions (DWAF, 1996).

Measurement of pH is fundamental when assessing a potential water supply for fish, or when evaluating fish health conditions, as the effect of pH is often manifested through the likelihood or severity of the effects of water quality changes. Ionisation, solubility and the chemical species of many aquatic toxins found in a particular water sample are determined by the pH (Dallas and Day, 2004). Similarly, pH is largely influenced by physico-chemical properties of the surrounding environment. In closed recirculating systems, pH generally decreases with time since acid is produced during nitrification of ammonia to nitrate (DWAF, 1996). This correlates with the pH values for both exposure systems in the current study (Table 11), however, the pH values remained relatively constant throughout each of the exposures. In poorly buffered systems, the pH may decrease to 6 or less. Increased pH in recirculating systems is often indicative of poor biological filtration (DWAF, 1996), which was not the case here.

Many substances react differently in solution with changes in pH resulting in the precipitating out of solution of some dissolved compounds or increasing the volatility, thus affecting the bioavailability of dissolved compounds in the exposure solution.

Dissolved oxygen is vital for the endurance of life in water (Greenfield, 2001) and the maintenance of adequate dissolved oxygen concentrations is critical for unimpaired
survival and functioning. Dissolved oxygen fluctuates diurnally, depending on the relative rates of respiration of aquatic organisms present in a particular body of water and various other factors and may be expressed as either mg/l or percentage saturation (Dallas and Day, 2004). The degree of saturation of a particular gas determines its effects and availability and the sensitivity of aquatic species to changes in dissolved oxygen concentrations depends on the species, life stages and behavioral changes (Kruger, 2002). The saturation of a particular gas in a liquid increases with temperature and varies under different conditions such as salinity, biological oxygen demand, photosynthetic activity, atmospheric pressure, water movement and biological gas production (DWAF, 1996).

The extent of the impact of a depletion of dissolved oxygen on aquatic organisms depends on the frequency, timing and duration of such depletion. Continuous exposure to concentrations of less than 80 percent saturation is harmful and is likely to have acute effects, whilst repeated exposure to reduced concentrations may lead to physiological and behavioural stress effects. Additionally, the extent to which an aquatic organism is affected by a decrease in dissolved oxygen varies with the type of species, with the life-stages and with different life processes and size (Dallas and Day, 2004).

Dissolved oxygen did not vary much over the life-time of each of the exposures, as a result of oxygen being constantly added to the exposure tanks by means of an air-stone throughout. This eliminates the possibility of any additional stress being induced in the organism due to lack of oxygen during the exposure.

All organisms have a specific range of temperatures at which growth, reproduction and general fitness can occur optimally (Dallas and Day, 2004). In fish, temperature responses can be divided into resistance, tolerance and preference (DWAF, 1996). As fish are poikilotherms, their metabolisms are dependent on ambient water temperatures, with optimal temperature thus being that at which most biochemical reactions are at their most efficient (DWAF, 1996; Kruger, 2002). Additionally, increases in water temperature decreases oxygen solubility and may also increase
the toxicity of certain chemicals, both which result in increased stress in the associated organisms (Dallas and Day, 2004) and most often, resistance to disease and tolerance of toxins (metabolites and pollutants) is enhanced by maintaining an appropriate temperature regime (DWAF, 1996).

The temperatures increased very slightly during both exposures respectively, but remained relatively constant throughout each for the extent of each of the exposures and this variable is thus unlikely to have induced any stress-related reactions over the time of the exposure.

Total dissolved solids (TDS) in any given water body, provides a composite measure of the total amount of dissolved material present (DWAF, 1996). TDS during both of the exposures did not change and therefore no change in the concentration of dissolved compound can have taken place. Material dissolved in water is represented in three ways: as total dissolved solids (TDS), as salinity, or as conductivity. TDS and salinity are both measures of the mass of solutes in water; however, they differ in the components they measure. TDS represents the total quantity of dissolved material, organic and inorganic, ionized and un-ionized, in a water sample, whereas salinity measures only the dissolved inorganic content. Conductivity, on the other hand, is a measure of the ability of a sample to conduct an electrical current (Dallas and Day, 2004; DWAF, 1996). Often it is not the concentration of dissolved material in a water body that causes stress to an organism, but rather the rate of change of these concentrations (Dallas and Day, 2004).

The conductivity and TDS values for both the first and the second exposure periods showed only very slight decreases in both the control and the solvent control. However, in both exposures, with the exception of the [0.1] of the first exposure, the values showed significant and similar decreases in the [0.1], [0.4] and [0.7] exposure concentrations, respectively. As previously mentioned, DDE is a lipophylic substance and binds to organic particles in nature. The reduced conductivity and TDS values over time during the exposure periods suggest that the DDE in the exposure concentrations are binding to free ions and dissolved organic particles and
then either precipitating out of solution or being released into the atmosphere due to its semi-volatile nature.

All of the water quality values obtained were within the acceptable range with the South African Water Quality Guidelines for Aquaculture.

5.4.2 BIOMARKERS

5.4.2.1 EROD

In the first exposure, the [0.1μg/l] concentration showed a significant induction (P>0.05) when compared statistically with the control and a clear trend of increasing EROD induction can be seen with increasing concentrations of DDE. In the second exposure, it is the [0.7μg/l] concentration that shows a significant EROD induction when compared statistically with both the control and the solvent control, thus further showing the effect of EROD induction in aquatic organisms on exposure to DDE despite slightly more erratic results obtained for EROD enzyme activity in the second exposure.

It is well known that exposure to organic chemicals/xenobiotics causes the induction of EROD synthesis (Besselink et al., 1998). Both ethoxyresorufin and resorufin are intensely fluorescent. The excitation and emission maxima for both compounds lie in the visible spectrum. However, there is a distinct separation in the excitation maxima of the two respective compounds and this allows for easy differentiation between the two (Besselink et al., 1998). Increases in fluorescence at 590 nm with excitation set at 544 nm, suggests that the O-deethylation of ethoxyresorufin to resorufin was being observed. Inhibition of cytochrome p450 1A mediated catalytic activity occurs when fish are exposed to high levels of polychlorinated biphenyl congeners (Joubert, 2000). It is therefore not surprising that EROD enzyme activity was highly induced in the fish exposed to increasing concentrations of DDE. Evident EROD induction occurred at even the lowest DDE concentration in both exposures, thus indicating a high level of sensitivity with regard for use of this assay in measuring DDE exposure in Oreochromis mossambicus even at low environmentally
relevant concentrations (i.e. 0.1µg/l - 1µg/l). Additionally, according to Besselink et al. (1998) it must not be neglected the effectivity of EROD induction for indicating specific dose-related induction of the total cytochrome P450 content in hepatic microsomes. Thus, making EROD an effective measure of increased concentrations of organic pollutants not only in laboratory procedures, but also in field-related environments.

5.4.2.2 CATALASE ACTIVITY

Depending on the duration and intensity of the pollutant to which an organism is exposed, antioxidants may only be induced during the first phase of the response, while in other conditions organisms can exhibit no variations or transitory responses before adaptive mechanisms occur (Regoli et al., 2002). According to Zhang et al. (2005), there is ample evidence that suggests that if an exposure is prolonged, the induced response may decline, or even return to background levels. It is due to these adaptive mechanisms, that the exposure periods were kept at no longer than 96 hours each. Significant catalase activity was noted in the [0.1], [0.4] and [0.7] exposure concentrations in the first exposure, with little induction being noted in both the control and the solvent control. However, there was no significant difference obtained between the different DDE exposure concentrations. Values obtained in the second exposure were erratic with no significant effects being noted between either the controls or the three DDE exposure concentrations. Therefore, although several studies have found that an increase in CAT activity does occur in aquatic organisms after exposure to organic pollutants (Wenning et al., 1988; Nasci et al., 1999), the erratic nature of the results from the second exposure, make it impossible to provide an obvious explanation that relates the observed CAT activity to environmental conditions during the exposures in this study. However, it may be that due to its general nature as a bioindicator of stress, CAT activity may be less sensitive to specific exposure studies with pollutants such as p,p-DDE, as was the case in this study.
5.4.2.3 CELLULAR ENERGY ALLOCATION

Significant reductions (P > 0.05) in the energy values for the glucose content were observed between the control and the [0.4μg/l] and the [0.7μg/l] DDE concentrations respectively in the first exposure, while no significant differences (P < 0.05) were observed in the glucose energy reserves of fish exposed to DDE in the second exposure. However, when noting the results obtained for the available protein energy reserves in fish from each of the exposures, no significant differences (P < 0.05) were observed in the available protein energy reserves from the fish exposed to the different DDE concentrations in the first exposure, while significant reductions in the protein values for the glucose were observed between the control and the [0.4μg/l] and the [0.7μg/l] DDE concentrations, respectively, from fish in the second exposure. Significant reductions were observed in the available lipid energy reserves of the first exposure when comparing the values obtained for the controls and the [0.7μg/l] DDE concentration with a significant difference seen between the lipid energy reserves in the [0.7μg/l] DDE concentration and that obtained for the solvent control. A significant difference was also noted in the lipid energy reserves between fish in the control and the [0.7μg/l] DDE concentration in the second exposure.

The total energy reserves (Ea) gave an integrated overview of the temporal changes of the available energy reserves of Oreochromis mossambicus, after exposure to the different DDE concentrations during each of the exposures (Figures 21 and 22). With increased DDE exposure in each of the exposures, the total energy reserves were significantly reduced, when compared to the control. The cellular respiration rate (Ec) decreased significantly during both of the exposures periods, when compared to the control. However, no significant differences in ETS activity were observed in either of the exposures, with comparison to the control. In the first exposure, a significant decrease in CEA was observed between the two controls and the [0.7] DDE concentration. However, although a decreasing trend in CEA can be seen in the second exposure, no significant differences were observed. Looking at the results as a whole and their individual contributions, it can be said that both the total energy reserves and the respiratory enzyme activity exhibited the same trend.
as the CEA values and clearly, contributed to the decrease in CEA in both exposures.

The rationale for the use of energy-related measures in exposure studies is due to the costly requirements of the biological responses of organisms in terms of metabolic demands (Beyers et al., 1999). Additional costs such as, restoring cellular damage from pollutant exposures, requires the relocation of an organism’s energy expenditures, to repair and/or maintain their physiological integrity. Biological responses that are costly should increase the metabolic rate with increasing levels of toxicants until irreversible pathological effects impair metabolism itself (Koehn, 1989; Calow, 1991). Thus, the energy budget will reflect how energy availability and consumption changes within an organism under exposure conditions.

It can be said with all surety that changes in the glucose, lipid and protein reserves contributed to the changes in the Ea and CEA. The differential effect on the glucose, lipid and protein reserves for the two exposures is more difficult to explain. While the results observed for the first exposure appear to correlate with existing literature in that the toxicant stress preferentially causes a depletion of the more readily available glucose and lipid reserves instead of protein (Smolders et al., 2003), the protein energy reserves in the second exposure seemed to be more affected than the glucose reserves. This, however, is not entirely unusual, as protein is also considered a prominent source of energy in organisms such as fish (Verslycke et al., 2003) and as such, also has the potential to indicate metabolic disruptions caused by exposure to toxicants in various organisms. According to Muyssen & Janssen (2001) lipids are known as more efficient energy-storage products than either proteins or glucose and consequently, lipid reserves can be the first energy source to be consumed under conditions of toxic stress, this was observed in the results obtained in both exposures.

The observed reduction in Ea values is either a result of a depletion of the energy reserves caused by increased metabolic activity or a decrease in energy (food) uptake. According to De Coen (1999), when no increase in Ec is observed, as was the case in both the exposures in this study, then decreases in Ea must be a result
of reduced energy uptake. A lower respiration rate would result in a lower metabolic fuel demand, in turn, resulting in a lower CEA value.

5.5 CONCLUSION

During the controlled aquarium exposures, it was found that a response was induced in each of the biomarkers used (i.e. EROD, CAT and CEA).

EROD enzyme activity was highly induced in the fish exposed to increasing concentrations of DDE. Evident EROD induction occurred at even the lowest DDE concentration in both exposures, thus indicating a high level of sensitivity with regard for use of this assay in measuring DDE exposure in Oreochromis mossambicus even at low environmentally relevant concentrations. Thus making EROD an effective measure of increased concentrations of organic pollutants not only in laboratory procedures, but also in field-related environments. Additionally, EROD, appeared to be the most rapid and sensitive indicator of the physiological status of the organism.

The CEA biomarker also showed to be a relatively rapid and sensitive indicator as to the physiological functioning of the organisms in response to stressors in the surrounding environment that could affect its health. The CEA biomarker indicated the energetic costs required by the organism for combating environmental stresses. The altered physiological condition in the organism under the given exposure conditions, provided a link between the responses at cellular level, to higher levels of biological organisation.

CAT activity was found to be erratic and no obvious explanation can be given to relate the observed CAT activity to environmental conditions during the exposures in this study. However, it may be that due to its general nature as a bioindicator of stress that CAT activity may be less sensitive to exposure to specific pollutants such as p,p-DDE, as was the case in this study.
5.6 REFERENCES


6. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 INTRODUCTION

There is a growing awareness of the need for the rational management of aquatic resources in view of the expected increase in the development, urbanisation and socio-economic activities in South Africa. The sources and quantities of pollutants discharged to aquatic environments have to be identified and have led to the development of biomonitoring programmes, which use biomonitor or indicator organisms. The measurement of organochlorine concentrations in organisms is an acceptable approach for detecting bioavailable levels in aquatic environments if the chemistry and behaviour of the specific pollutant is well understood. The supplementary monitoring of biological responses for assessing the significance of pollutants, has become a reasonable measurement in biomonitoring too, although no biomarker has been validated as a unique tool to determine the effects of specific pollutants on organisms. Additionally, it is necessary to understand how bioaccumulation and biomarkers relate to each other on exposure to specific pollutants and how it varies between species, to interpret the effects of pollutant exposures in the field environment.

6.2 BIOMARKERS

6.2.1 BIOMARKERS IN THE FIELD ENVIRONMENT

The results indicate the need to understand the biochemical changes induced/inhibited in organisms, by factors that are independent of environmental concentrations of pollutants. These factors include the influence of environmental conditions, such as food and oxygen availability as well as factors such as metabolic adaptation capacity and the behaviour of organisms.
In many of the individuals found in a specific area, large differences were noted in the various activities of each of the biomarkers used in this study. However, clear dose-related responses were noted in each of the controlled aquarium exposures. There was an evident effect of the various external environmental influences such as temperature, pH, total dissolved salts and dissolved oxygen on the activities of the biomarkers used in the study. Capture-related stress may also be a contributing factor with the resultant crowding, coupled to temperature and oxygen differences in the holding and exposure tanks. Both the adaptation to sudden changes in temperature and the stress associated with the capture and holding of the fish, may have had a significant influence on the activities of the biomarkers used. The high flow period will have been more suitable as capture conditions were not as hot and water in the holding tanks was more monitored and controlled, with larger holding tanks as well as the pumping of additional air.

EROD inductions have been shown to be useful as a biomarker for a number of planar organic contaminants, however, the results obtained in Chapter 4 indicate that a number of added influences and uncertainties introduced by various abiotic and biotic factors influenced the results obtained in this study to a large degree. The instability of the DDT metabolites in the field environment and the lack of a holistic understanding as to how these chemicals react in the environment makes interpretation of the results obtained difficult as the level of exposure of the organism to DDT and its metabolites, specifically, is not clear.

CAT and CEA are both very general biomarkers and thus show the effects of all the pollutants in the selected study area. As an indication of induced stress experienced in a particular area to contaminated water and external stresses in general, both catalase and Cellular Energy Allocation may be considered very useful. However, to indicate the stresses observed in the field environment due to exposure to planar organic compounds specifically, these biomarkers fall short.

Continued research with regard to the use of EROD inductions as a useful indication of exposure to DDT and its metabolites in the field environment is considered necessary to build up a greater and more reliable data pool of results and to establish any trends in this data over time.
6.2.2 LABORATORY EXPOSURES

During the controlled aquarium exposures, it was found that a response was induced in each of the biomarkers used (i.e. EROD, CAT and CEA). The biomarker assay, EROD, appeared to be the most rapid and sensitive indicator of the physiological status of the organism. The CEA biomarker also showed to be a relatively rapid and sensitive indicator as to the health of the organism. The change in the CEA showed that the organisms compensated for the energetic costs required by the organism for combating environmental stresses. The altered physiological condition in the organism under the given exposure conditions, provided a link between the responses at cellular level, to higher levels of biological organisation.

EROD enzyme activity was highly induced in the fish exposed to increasing concentrations of DDE. Evident EROD induction occurred at even the lowest DDE concentration in both exposures, thus indicating a high level of sensitivity with regard for use of this assay in measuring DDE exposure in Oreochromis mossambicus even at low environmentally relevant concentrations. Thus making EROD an effective measure of increased concentrations of organic pollutants not only in laboratory procedures, but also in field-related environments. Additionally, EROD, appeared to be the most rapid and sensitive indicator of the physiological status of the organism.

The CEA biomarker also showed to be a relatively rapid and sensitive indicator as to the physiological functioning of the organisms in response to stressors in the surrounding environment that could affect its health. The CEA biomarker indicated the energetic costs required by the organism for combating environmental stresses. The altered physiological condition in the organism under the given exposure conditions, provided a link between the responses at cellular level, to higher levels of biological organisation.

CAT activity was found to be slightly erratic and no obvious explanation can be given.
The experimental work discussed in Chapter 5 shows that the selected biomarkers are in fact sensitive enough to show changes in specific physiological functions on exposure to environmentally relevant levels of DDE.

### 6.3 BIOACCUMULATION

The highest level of bioaccumulation took place in the area where the smallest particle sizes were noted in the sediment thus making them more likely to contain high organic content levels with a high level of adsorption from hydrophobic chemicals like DDT and its metabolites.

The highest level of bioaccumulation and bioconcentration took place at the Xikundu site. This correlates with the sediment data, which indicated that the Xikundu sediment samples contained the smallest particle size fractions in relation to the other two sites, thus making a higher organic content likely, which in turn is likely to contain higher levels of hydrophobic pollutants such as DDE. As was stated in Chapter 3, *O. mossambicus* are bottom feeders and feed on the organic matter present. Higher levels of DDT and its metabolites are thus likely in the tissues of fish caught in areas where the sediments have higher organic contents.

Other than some relation to the sediment particle sizes, the levels of DDT and its metabolites in the test species, *O. mossambicus*, did not reveal an obvious relationship between the organo-pollutant concentrations in the water and sediment. According to Batterman *et al.* (2009), DDT and its metabolites are not restricted to a specific form or phase in the environment and are considered semi-volatile when occurring in an inorganic phase of the environment. Residues present in the environment may fraction into vapour, particulate or solid matter, but are highly lipophillic and as such tend to accumulate in organic matter and tissues. This ability to fraction out into the various environmental compartments and phases may thus be an important factor influencing biomonitoring studies with regard to the chemical DDE and explains the ease with which the chemical traverses through the environment.
6.4 SHORTCOMINGS OF THE DATA

Repeatability, one of the most important functions testing reliability in any toxicity test, was a major challenge in this study. Different results were obtained for the same exposure concentrations in each exposure. Erratic results are attributed not to the biomarker assays themselves, as comparable results were obtained in each of the field locations chosen, but rather to the semi-volatile and lipophylic nature of the pollutant, DDE, itself. It is also possible that some of the pesticides were adsorbed onto the walls of the experimental glass containers, thus causing reductions in the exposure concentrations over time and also the likely cause of the more erratic results obtained in the second exposure. Looking at the results obtained it should be stressed that in any exposure studies making use of pollutants such as DDE, no experimental containers, such as the exposure tanks used, should ever simply be cleaned and reused for the next exposure. Rather, these should be discarded and use made of new containers to ensure that no residue remains on the container thus affecting further experiments and affecting repeatability of results.

With regard to the field studies investigated in chapter 4, the full quota of fish was not obtained on any of the field sampling trips and this makes seasonal comparisons of the data and results difficult as well as affecting the statistical reliability. In addition, there were not always available fat reserves and so it was necessary to test for the presence of organochlorines from the muscle tissues as well as the liver and this is not ideal as the different tissues are not comparable.

6.5 CONCLUSION

The test species, O. mossambicus, did not reveal an obvious relationship between the fat tissue organochlorine content and the environmental organochlorine concentrations, but did show to be a potential bioaccumulator of organochlorine contaminants. The effects of these pollutants in aquatic ecosystems are diverse, complex and often unpredictable. The biomarkers, CAT and CEA, are not specific to
a particular group of pollutants, but can represent an integrative response to the impact of multiple pollutants and/or environmental factors.

One of the main objectives for any biomonitoring programme is to delineate spatial trends in contamination. In this study, definite spatial trends in terms of organochlorine contamination were observed between the reference site (Albasini Dam) and the two exposure sites (Nandoni Dam and Xikundu Fish Dam). The lack of significant variation may be due to the low environmental levels of the organochlorine contaminants in both the sediment and the water at all three sites. However, it should not be ignored that fish are a highly mobile species and are thus not always relevant to long term bioaccumulation studies.

In this study, the level of exposure of the Mozambique Tilapia to DDE in its surrounding environment were successfully quantified through testing of the water, sediment as well as through bioaccumulation testing from tissues, however, further study as to the mechanisms and environmental paths of the various DDT metabolites in the environment is considered essential in order to better fully understand the level of exposure to organisms in the environment.

Dose-response relationships were most successfully determined by the EROD and the CEA biomarkers in this study. A definite effect was noted on the Mozambique Tilapia with increasing concentrations of DDE. In the natural environment, dose-response relationships to DDE exposure were more difficult to quantify as additional chemicals and natural environmental stressors also affect the results.

In conclusion, it was determined in this study that DDE does negatively affect the health of *O. mossambicus*. However, the extent to which *O. mossambicus* in the Limpopo Province are being affected by the ongoing spraying of DDT is difficult to quantify using only the selected battery of biomarkers. In the controlled laboratory experiments, in the absence of additional environmental stressors and other environmental contaminants such as fertilizers from agricultural runoff, a clear dose-response relationship was observed in both the EROD as well as the CEA biomarkers. Responses in the field environment, however, did not correlate with the
environmental levels of DDE observed in the water, sediment or bioaccumulation results. It is considered necessary to conduct further testing as to more pollutant specific biomarkers for use in the field environment.

6.6 RECOMMENDATIONS

Different organisms demonstrate different sensitivities for different pollutants/toxicants, therefore, the basic biology/physiology (e.g. basic biochemistry, growth rate) of the proposed biomonitoring organism must be known, so that the “normal” ranges of effects can be estimated and the sources of variation other than pollutant exposure can be taken into account.

The biomarkers applied in this study should be used in conjunction with complementary biomarkers. The antioxidant, CAT can be used in conjunction with SOD and GSH, which are induced concomitantly as a result of oxidative stress and could interpret the results of the single biomarker responses better. The physiological/organismal biomarkers, Scope for Growth (SFG) and Tissue Condition Index (TCI) can be used with the CEA technique to indicate the energetic and condition status of organisms effectively, following pollutant exposure. Biomarkers at the next level of biological organisation, such as reproduction and behavioural responses could be applied to help assess organism health and to extrapolate effects to population or community level.

Furthermore, in order to validate the use of O. mossambicus as an effective bioindicator, it should be used in several future biomonitoring studies.

The present study quantifies the total organochlorine concentrations in the water, sediment and fat tissue, however, this is by no means an absolute reflection as to what is happening at each of the three selected sites. That is, a number of other, more complex contaminants may be present. Thus, further studies should assess the effects of a wider range of contaminants on O. mossambicus in the field environment. Additionally, although organochlorines were assessed in all phases of
the environment (i.e. water, sediment and tissue) it is necessary that the wider range contaminants also be treated as such. In so doing, the identification of all possible sources of pollutants can be identified.

### 6.7 REFERENCES