APPLIED AQUATIC ECOTOXICOLOGY: SUB-LETHAL METHODS, WHOLE EFFLUENT TESTING AND COMMUNICATION

Report to the Water Research Commission

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EXECUTIVE SUMMARY

CHAPTER 1 INTRODUCTION

This report is the most recent in a series of WRC reports on the development of the capacity to undertake ecotoxicological research in South Africa. The development followed the following lines:

- Recognition, as a result of the Kruger National Park Rivers Research Programme, that there were virtually no data on the water quality requirements of South African macroinvertebrates.
- Development of the capacity to undertake experimental tolerance testing using riverine invertebrates in artificial stream systems.
- Investigation of the salt tolerances, and whole effluent toxicity responses, of both standard toxicity test taxa and South African macroinvertebrates.
- Development of both lethal and sub-lethal measures.
- Application of research results to the development of methods for water quality within ecological Reserve determinations, and the implementation of the National Water Act (NWA) (No 36. of 1998) and National Water Resource Strategy (NWRS).

The WRC is committed to funding research that underpins the implementation of the NWA and the NWRS. Over the past 12 years it became clear that there would not be a rapid uptake of ecotoxicology research results in South Africa, and that it was important to place ecotoxicology in the wider context of water quality. From this recognition, the concept of Environmental Water Quality evolved.

Environmental water quality (EWQ)

Environmental water quality includes:

- the physico-chemical composition of water (including surface water, ground water, and the water between sediment particles), and
- the responses of living organisms in ecosystems and ecosystem processes to the physico-chemical composition of the water;

There are three main approaches to the investigation of environmental water quality:

- *water physico-chemistry* – the measurement and monitoring of physical and chemical variables allows an understanding of the magnitude (often concentration), frequency and duration of specific abiotic conditions. This is a routine aspect of DWAF activities, although some ecologically important variables are not routinely monitored.

- *biomonitoring* - the measurement and monitoring of ecosystem health using a variety of indices allows an understanding of how living organisms and ecosystems respond to the physical and chemical environment. However, although biomonitoring can indicate condition (for example provide a "red flag" of poor condition), this approach does not identify the causes of the problem. This is a routine aspect of DWAF activities in those areas where the River Health Programme is active.

- *ecotoxicology* – the experimental linking of physical and chemical conditions to biological responses provides the crucial key for effective environmental water quality implementation. This approach provides an understanding of **why** ecological heath has changed. This is a relatively new technology and DWAF is in the process of evaluating and planning its use and application.

EWQ promotes the *integrated* use of water physico-chemistry, biomonitoring and ecotoxicology in water resource protection in order to:

- cost-effectively license and control industrial complex waste water discharges (Source Directed Controls - SDC); and
- set effective resource quality objectives (Resource Directed Measures - RDM).

The concept, components and methods for taking account of environmental water quality are therefore quite new. For research results to be applied now and in the future, water managers need to understand the concepts and methods and their potential for use in effective water resource management.

Current challenges and opportunities

Of the three approaches needed in the EWQ approach, ecotoxicology is the newest. Over the past decade, methods have been developed and research priorities identified, and it is clear from international experience that this is a core technology for water quality management.

At present DWAF have a policy document, a technical manual and a methods manual for how ecotoxicology can be used in licensing complex industrial wastes; and ecotoxicological data are central to the determination of water quality aspects of the ecological Reserve. Ecotoxicology has much to offer both RDM and SDC, and its integrated the use together with water physico-chemistry, and biomonitoring, even more so.

Project Aims

Therefore the aims of this project addressed both the technical development and communication of the EWQ approach:

- To develop methods to contributed to the use of lethal and sub-lethal ecotoxicity data in the development of ecologically protective water quality guidelines;
- To develop methods and to research sub-lethal responses to complex industrial waste water; and
- To communicate the EWQ concept widely and effectively within the South African water sector.

The third aim, together with the training of two MSc students, contributed to capacity building.

CHAPTER 2 WHOLE EFFLUENT CHRONIC TOXICITY TESTING USING THE FRESHWATER MOLLUSC *Burnupia stenochorias* (PULMONATA, ANCYLIDAE)

Dr. Heather Davies-Coleman graduated with her PhD in 2002 and her thesis addressed several of the aims of this project. The abstract is presented in this executive summary, and an extended summary is presented in Chapter 2. The entire thesis is available as a "pdf" file on the Internet: *"The growth and reproduction of the freshwater limpet Burnupia stenochorias (Pulmonata, Ancylidae), and an evaluation of its use as an ecotoxicology indicator in whole effluent testing."*

http://www.ru.ac.az/library/theses/collection.html#Zoology

For the protection of the ecological Reserve in South Africa, the proposed introduction of compulsory toxicity testing in the licensing of effluent discharges necessitates the development of whole effluent toxicity testing. The elucidation of the effects of effluent on the local indigenous populations of organisms is essential before hazard and risk assessment can be undertaken. The limpet *Burnupia stenochorias*, prevalent in the Eastern Cape of South Africa, was chosen to represent the freshwater molluscs as a potential toxicity indicator. Using potassium dichromate (as a reference toxicant) and a textile whole effluent, the suitability of *B. stenochorias* was assessed under both acute and chronic toxicity conditions in the laboratory. In support of the toxicity studies, aspects of the biology of *B. stenochorias* were investigated under both natural and laboratory conditions.

Using principal component and discriminant function analyses, the relative shell morphometrics of three feral populations of *B. stenochorias* were found to vary. Length was shown to adequately represent growth of the shell, although the inclusion of width measurements is more statistically preferable. Two of the feral populations, one in impacted water, were studied weekly for 52 weeks to assess natural population dynamics. Based on the Von Bertalanffy Growth Equation, estimates of growth and longevity were made for this species, with growth highly seasonal. Age is not easily discerned from shell size. Egg laying occurred all year round, with early summer (peak egg lay), mid summer (a second, smaller peak in egg lay), and winter (limited presence of eggs) phases. In toxicity testing, consideration is given to the choice of the test organism based on age and sexual development. Consequently, the sexual development of *B. stenochorias* relative to shell length was determined with the aid of histological examinations of transverse sections of limpets, of all sizes, collected over one year. Limpets less than 3mm shell length were found to be immature in the development of the oocytes and spermatozoa, and were later chosen for acute toxicity tests. A laboratory diet was developed for both culturing and maintaining limpets during toxicity tests; however, the diet requires optimisation. Under laboratory conditions, growth was linear, and individual fecundity highly variable.

Successful methods for the collection of limpets from naturally occurring populations, and their acclimation to the laboratory were developed. Three *B. stenochorias* populations, representing different hydrological and water quality conditions, were compared to a laboratory population (maintained for three years) in their responses to the textile whole effluent and potassium dichromate ($K_2Cr_2O_7$). Under acute conditions, variability of mortality between limpet populations and between seasons was consistent with acceptable international standards. However, seasonal differences between feral limpets were apparent, with early summer limpets significantly more susceptible to both potassium dichromate and textile effluent than winter limpets. Although mortality occurred within the effluent at all concentrations, no 96-hour LC_{50} values were obtained.

The chronic toxicity effects of the textile whole effluent were assessed over the entire life cycle of *B. stenochorias*, based on survival, growth and reproductive effects. Lower concentrations of effluent (10%) gave greater variability of responses and toxicity than higher concentrations, with a 43-day LC_{50} of 3.9% effluent. The No Observed Effect Concentrations (NOEC) for survival (over 43-days) were calculated in consecutive years as 0.1% and 1% effluent. Survival is considered a useful tool for determining toxicity endpoints using *B. stenochorias*. Limpet growth remained linear in effluent, with an apparent stimulation of growth at the 3-10% effluent concentration, confusing the toxicity and variability assessments. The possible addition of nutrients from the effluent points to either a potential inadequacy of the food quality provided in the chronic assessment, or the presence in the effluent of growth stimulants. Growth was also found to be too variable to allow adequate statistical conclusions about the toxicity of the effluent, although it is suggested that growth may be useful in the assessment of single compounds. Despite large individual variability in fecundity, statistical differences were discernible between effluent concentrations. The application of fecundity of *B. stenochorias* in hazard assessment therefore warrants further investigation. It was concluded that an assessment of textile whole effluent toxicity to *B. stenochorias* over an entire life cycle, and a F1 generation, is unnecessary. The development of the bucket/plastic bag method for both acute and chronic toxicity assessment of *B. stenochorias* was useful.

In the final assessment of the usefulness of *B. stenochorias* as a toxicity indicator, toxicity endpoints were compared with those of the standard laboratory organism *Daphnia pulex*. Both in acute and chronic toxicity, *B. stenochorias* was found to be more sensitive. *B. stenochorias* is therefore considered valuable as a South African freshwater molluscan ecotoxicological indicator, with a place in hazard assessment, although further development and research is necessary before the limpet can be effectively used.

CHAPTER 3 SALINITY IN AN ENVIRONMENTAL WATER QUALITY CONTEXT

Chapter 3 describes the role of salinization in a South African environmental water quality context. The background provides the motivation for the research described in Chapter 4. In this study, research in to the effect of increased salinization was extended in two separate but complimentary projects.

In the first study, the number of species for which their tolerance to NaCl and Na_2SO_4 is known was increased. Acute toxicity tests using species not previously tested substantially increased the database of indigenous invertebrate tolerances and will allow the development of species sensitivity distributions for indigenous species and contribute to the refinement of water quality guidelines for aquatic resources. This research is described in Chapter 4.1.

In a separate study, described in Chapter 4.2, techniques to extrapolate acute toxicity data to chronic toxicity levels in order to provide protective water quality guidelines, are described. The two extrapolation techniques are applied to acute toxicity data for indigenous invertebrates obtained from the UCEWQ database. A chronic toxicity test experiment, using an indigenous species, was undertaken and the NOEC derived from the experimental work was compared to the extrapolated results.

CHAPTER 4 METHOD DEVELOPMENT FOR SUB-LETHAL RESPONSE ASSESSMENT

The MSc theses of Samantha Browne and Andrew Slaughter contributed to different aspects of method development for the assessment of sub-lethal responses.

In the two studies, freshwater invertebrates were exposed to at least 2 salts (sodium chloride (NaCl) and sodium sulphate (Na_2SO_4)) for acute and chronic periods. In the first study, the emphasis was on gathering information for the widest possible range of taxa. These acute tolerance data, together with other similar data from the Unilever Centre for Environmental Water Quality (UCEWQ) database, were used to compare the responses of South African taxa with those from other regions, and to extrapolate chronic endpoints in the second study. In the second study, after acute:chronic extrapolation methods were applied, the modelled chronic results were compared with experimental chronic tolerance results generated as part of the study. In both studies, the results will be used to revise environmentally protective salinity guidelines.

4.1 A comparison of the salinity tolerances of selected South African freshwater invertebrates

Anthropogenically-induced salinization can negatively impact on freshwater systems. Previously water resource managers in South Africa's main tool in managing salinity was the South African Water Quality Guidelines based on a percentage deviation from the mean electrical conductivity

(DWAF, 1996a). These guidelines were formulated using mostly international data. This study proposed another approach in the derivation of water quality guidelines using tolerance data from acute 96-hour toxicity tests. Tests were conducted using wild-caught indigenous freshwater macroinvertebrates as test organisms (DWAF, 2000), collected from three lotic systems and one lentic system in the Eastern Cape Province. Two salts, NaCl and Na_2SO_4, were used as test toxicants. Artificial stream systems or channels were used for organisms collected from lotic environments according to the protocol for artificial stream systems (DWAF, 2000). The protocol was adapted and an experimental system designed for predacious organisms collected from a lentic system. LC_{50} data were generated using Probit and Trimmed Spearman-Kärber (TSK) statistical analyses. LC_{50} values for NaCl ranged from the most sensitive organisms 1807mg/L for *Baetis harrisoni* to 24410mg/L for Coenagrionidae, and from 280mg/L for *Demoreptus natalensis* to 29927mg/L for Coenagrionidae for Na_2SO_4. LC_{50} values were compared to LC_{50} values of similar taxa from other studies. Comparisons showed that invertebrates sharing taxonomic characteristics, regardless of their locality, behave similarly to acute osmotic stress caused by unnaturally, highly saline environments. A total of 35 acute toxicity data sets (18 for NaCl and 17 for Na_2SO_4) were generated for 14 different species, providing valuable data for future work on acute to chronic extrapolations. Most of the organisms proved to be suitable test organisms with the exception of Pleidae and Simuliidae larvae. Hence the study also provides valuable benchmarks for future toxicity testing using South African indigenous invertebrates. The approach aimed to generate data using as wide a range of species as possible, based on the Aldenberg and Slob data extrapolation method which determines concentrations of toxicants that should protect 95% of the species in an ecosystem (Aldenberg and Slob, 1993). Using this approach these data can best be applied in water resource management to refine water quality guidelines for salinity so that the guidelines are more representative of South African aquatic ecosystems and their species.

4.2 Generating chronic mortality data for sodium chloride and sodium sulphate using Two-Step Linear Regression Analysis (LRA) and Multi-Factor Probit Analysis (MPA) as extrapolation techniques

Salinization of freshwater resources is a major water quality problem in South Africa. The National Water Act (NWA) seeks to protect water resources so as to ensure sustainable, long-term resource use. Policies supporting the NWA specify the need for boundary values indicating a change of ecosystem health class between the Natural, Good, Fair and Poor classes. Indigenous chronic toxicity data for salts are needed to set protective guidelines and this research describes a method for defining the boundary between the Good and Natural river health classes. The study aimed to derive chronic mortality data for the salts NaCl and Na_2SO_4 for South African aquatic macro-invertebrates via a process of extrapolation using Linear Regression Analysis (LRA) and Multi–Factor Probit Analysis (MPA) on acute salinity toxicity data. An experimental chronic toxicity test using NaCl as a toxicant and *Caridina nilotica* as a test organism was run concurrently with the aim of validating the results of the extrapolations. Chronic mortality data were obtained by extrapolation, and extrapolated chronic mortality data for *C. nilotica* were compared to results obtained in the chronic toxicity experiment. Extrapolated low LC_x values ($LC_{0.01}$ – LC_{10}) for *C. nilotica* exposed to NaCl yielded values in the range of 550mg/L to 3500mg/L using LRA and 175mg/L to 1500mg/L using MPA. The chronic *C. nilotica* experiment yielded a survivorship No Observed Effect Concentration (NOEC) of 800mg/L. MPA yielded more accurate Predicted No Effect Concentration (PNOEC) when low LC_x values in the range of $LC_{0.1}$ to LC_{10} were considered. These values were also lower than values obtained from the LRA extrapolation. However, the LRA extrapolation technique allowed the deviation of $LC_{0.01}$ and $LC_{0.1}$ values, and the experimentally derived NOEC was found to be within that range. The study highlighted the value of acute (96-hour) toxicity tests, but also lethal, short-term chronic (10-day) tests. Validation of results from these extrapolation techniques is necessary.

CHAPTER 5 COMMUNICATION ABOUT ENVIRONMENTAL WATER QUALITY

In order to communicate widely with the water sector about the concept of Environmental Water Quality (EWQ), and particularly the role of ecotoxicology, an accessible handbook was developed and edited iteratively. Each stage was undertaken in co-operation with DWAF and selected stakeholders. The handbook outlines the concept of EWQ, and shows clearly how this concept is useful to water managers and users in the implementation of, and compliance with, the NWA. It is written in a simple style and aims specifically to assist water resource managers and users involved in any practice that affects water resource quality. The handbook has been presented to DWAF, edited in response to departmental requirements, and reflects DWAF policy as closely as possible.

The handbook is a product both of this project, and of WRC Project No: K5/1313: *The Development of an Environmental Water Quality Programme*. The first draft of the handbook was presented to DWAF personnel from the Eastern Cape. It was revised and then presented in Pretoria to 20 DWAF and industry participants. The presentation and document stimulated vigorous debate and 12 sets of comments were received. An illustrated draft was printed in September 2003 and presented at five "road-show" workshops.

CHAPTER 6 CAPACITY BUILDING REPORT

Capacity building is an integral part of the activities of the UCEWQ. This is variously achieved through training students (undergraduate lecturing and postgraduate supervision), public understanding of science (professional courses and roadshows) and technology transfer (teaching and courses).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AF	Assessment Factor
ANZECC	Australian and New Zealand Environment and Conservation Council
ASPT	Average Score per Taxon
BBM	Building Block Methodology
DO	Dissolved Oxygen
DRIFT	Downstream Response to Imposed Flow Transformations
DWAF	Department of Water Affairs and Forestry, South Africa
EC	Electrical Conductivity
ERA	Ecological Risk Assessment
ESD	Ecologically Sustainable Development
EWQ	Environmental Water Quality
FSR	Flow Stressor Response
IFR	Instream Flow Requirement
IWR	Institute for Water Research
IWRM	Integrated Water Quality Management
LC	Lethal Concentration (Dose)
LOEC	Lowest Observed Effect Concentration
LRA	Linear Regression Analysis
MPA	Multi-Factor Probit Analysis
NGO	Non-Governmental Organisation
NHEERLs	National Health and Environmental Research Laboratories
NOEC	No Observed Effect Concentration
NWA	National Water Act
NWRS	National Water Resource Strategy
OECD	Organization for Economic Co-Operation And Development
ORD	Office of Research And Development
PES	Present Ecological State
PNOEC	Predicted No Observed Effect Concentration
RDM	Resource Directed Measures
RHP	River Health Programme
RQO	Resource Quality Objective
SASS	South African Scoring System
SDC	Source Directed Control
STW	Sewage Treatment works
TDS	Total Dissolved Solids
TIMS	Toxicologically Important Major Salts
TIN	Total Inorganic Nitrogen
TP	Total Phosphate
TSK	Trimmed Spearman-Käber
TSS	Total Suspended Solids
UCEWQ	Unilever Centre for Environmental Water Quality
USEPA	United States Environmental Protection Agency
WMA	Water Management Area
WQG	Water Quality Guidelines
WQO	Water Quality Objectives
WRC	Water Research Commission
WUA	Water User Association

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CHAPTER 1

INTRODUCTION

This report is the most recent in a series of WRC reports on the development of the capacity to undertake ecotoxicological research in South Africa (Palmer *et al.*, 1996; Palmer and Scherman, 2000; DWAF, 2000; Zokufa *et al.*, 2001; Muller and Palmer, 2002; Scherman *et al.*, 2002). The development followed the following lines:

- Recognition, as a result of the Kruger National Park Rivers Research Programme, that there were virtually no data on the water quality requirements of South African macroinvertebrates.

- Development of the capacity to undertake experimental tolerance testing using riverine invertebrates in artificial stream systems.

- Investigation of the salt tolerances, and complex effluent toxicity responses, of both standard toxicity test taxa and South African macroinvertebrates.

- Development of both lethal and sub-lethal measures.

- Application of research results to the development of methods for water quality within ecological Reserve determinations, and the implementation of the National Water Act (NWA) (No 36. of 1998) and National Water Resource Strategy (NWRS) (DWAF, 2002).

The WRC is committed to funding research that underpins the implementation of the NWA and the NWRS. Over the past 12 years it became clear that there would not be a rapid uptake of ecotoxicology research results in South Africa, and that it was important to place ecotoxicology in the wider context of water quality. From this recognition, the concept of Environmental Water Quality evolved.

1.1 ENVIRONMENTAL WATER QUALITY

Environmental water quality includes:

- the physico-chemical composition of water (including surface water, ground water, and the water between sediment particles),
- the responses of living organisms in ecosystems and ecosystem processes to the physico-chemical composition of the water.

There are three main approaches to the investigation of environmental water quality:

- *water physico-chemistry* – the measurement and monitoring of physical and chemical variables allows an understanding of the magnitude (often concentration), frequency and duration of specific abiotic conditions. This is a routine aspect of DWAF activities, although some ecologically important variables are not routinely monitored.

- *biomonitoring* - the measurement and monitoring of ecosystem health using a variety of indices allows an understanding of how living organisms and ecosystems respond to the physical and chemical environment. However, although biomonitoring can indicate condition (for example provide a "red flag" of poor condition), this approach does not identify the causes of the problem. This is a routine aspect of DWAF activities in those areas where the River Health Programme is active.

- *ecotoxicology* – the experimental linking of physical and chemical conditions to biological responses provides the crucial key for effective environmental water quality implementation. This approach provides an understanding of **why** ecological heath has changed. This is a relatively new technology and DWAF is in the process of evaluating and planning its use and application.

EWQ promotes the *integrated* use of water physico-chemistry, biomonitoring and ecotoxicology in water resource protection in order to:

- cost-effectively license and control industrial complex waste water discharges (Source Directed Controls - SDC); and
- set effective resource quality objectives (Resource Directed Measures - RDM).

The concept, components and methods for taking account of environmental water quality are therefore quite new. For research results to be applied now and in the future, water managers need to understand the concepts and methods and their potential for use in effective water resource management.

1.2 CURRENT CHALLENGES AND OPPORTUNITIES

Of the three approaches needed in the EWQ approach, ecotoxicology is the newest. Over the past decade, methods have been developed and research priorities identified, and it is clear from international experience that this is a core technology for water quality management (Rand *et al.*, 1995; Chapman, 1995; Grothe *et al.*, 1996).

At present DWAF has a policy document, a technical manual and a methods manual for how ecotoxicology can be used in licensing complex industrial wastes (DWAF, 2003); and ecotoxicological data are central to the determination of water quality aspects of the ecological Reserve (Jooste and Rossouw, 2002; Palmer *et al.*, in press). Ecotoxicology has much to offer both RDM and SDC, and its integrated the use together with water physico-chemistry and biomonitoring, even more so.

1.3 PROJECT AIMS

Therefore the aims of this project addressed both technical development and the communication of the EWQ approach:

- to develop methods and to research sub-lethal responses to complex industrial waste water;
- to develop methods that contributed to the use of lethal and sub-lethal ecotoxicity data in the development of ecologically protective water quality guidelines; and
- to communicate the EWQ concept widely and effectively within the South African water sector.

The third aim, together with the training of two MSc students, contributed to capacity building.

CHAPTER 2

WHOLE EFFLUENT CHRONIC TOXICITY TESTING USING THE FRESHWATER MOLLUSC *Burnupia stenochorias* (PULMONATA, ANCYLIDAE)

Heather Davies-Coleman

Dr. Heather Davies-Coleman graduated with her PhD in 2002. Her thesis addressed several of the aims of this project and is available in full as a "pdf" file on the Internet: *"The growth and reproduction of the freshwater limpet Burnupia stenochorias (Pulmonata, Ancylidae), and an evaluation of its use as an ecotoxicology indicator in whole effluent testing."* http://www.ru.ac.za/libraty/theses/collection.html#Zoology

2.1 SUMMARY

The continual discharge of complex effluents into riverine systems suggests that the elucidation of the effects on local indigenous organisms is essential before hazard and risk assessments can be made. Future legislation in South Africa regarding licences for the discharge of chemically complex wastes will introduce compulsory toxicity testing. The overall aims of this research were to describe some of the toxicological responses of the freshwater limpet *Burnupia stenochorias* to a complex (textile) effluent, and to assess this species' suitability as an indigenous ecotoxicity indicator.

For the protection of the ecological Reserve in South Africa, the proposed introduction of compulsory toxicity testing in the licensing of effluent discharges necessitates the development of methods for whole effluent toxicity testing using indigenous species. The limpet *B. stenochorias*, prevalent in the Eastern Cape of South Africa, was chosen to represent the freshwater molluscs as a potential toxicity indicator. Using potassium dichromate ($K_2Cr_2O_7$) as a reference toxicant and a textile whole effluent, the suitability of *B. stenochorias* was assessed under both acute and chronic toxicity conditions in the laboratory. In support of the toxicity studies, aspects of the biology of *B. stenochorias* were investigated under both natural and laboratory conditions.

Using principal component and discriminant function analyses, the relative shell morphometrics of three feral populations of *B. stenochorias* were found to vary. Length was shown to adequately represent growth of the shell, although the inclusion of width measurements is more statistically preferable. Two of the feral populations, one in impacted water, were studied weekly for 52-weeks to assess natural population dynamics. Based on the Von Bertalanffy Growth Equation, estimates of growth and longevity were made for this species. Growth was estimated to be highly seasonal. Age is not easily discerned from shell size. Egg laying occurred all year round, with early summer (peak egg lay), mid summer (a second, smaller peak in egg lay), and winter (limited presence of eggs) phases. In toxicity testing, consideration is given to the choice of test organism based on age and sexual development. Consequently, the sexual development of *B. stenochorias* relative to shell length was determined with the aid of histological examinations of transverse sections of limpets, of all sizes, collected over one year. Limpets less than 3mm shell length were found to be immature in the development of the oocytes and spermatozoa, and were later chosen for acute toxicity tests. A laboratory diet was developed for both culturing and maintaining limpets during toxicity tests. However, the diet requires optimisation. Under laboratory conditions, growth was linear, and individual fecundity highly variable.

Successful methods for the collection of limpets from naturally occurring populations, and their acclimation to laboratory conditions were developed. Three *B. stenochorias* populations, representing different hydrological and water quality conditions, were compared to a laboratory population (maintained for three years) in their responses to the textile whole effluent and $K_2Cr_2O_7$. Under acute conditions, variability of mortality between limpet populations and between seasons was consistent with acceptable international standards. However, seasonal differences between feral limpets were apparent, with early summer limpets significantly more susceptible to both $K_2Cr_2O_7$ and textile effluent than winter limpets. Although mortality occurred within the effluent at all concentrations, no 96-hour LC_{50} values were obtained.

The chronic toxicity effects of the textile whole effluent were assessed over the entire life cycle of *B. stenochorias*, based on survival, growth and reproductive effects. Lower concentrations of effluent (10%) gave greater variability of responses and toxicity than higher concentrations, with a 43-day LC_{50} of 3.9% effluent. The no observed effect concentrations (NOEC) for survival (over 43-days) were calculated in consecutive years as 0.1% and 1% effluent. Survival is considered a useful tool for determining toxicity endpoints using *B. stenochorias*. Limpet growth remained linear in effluent, with an apparent stimulation of growth at the 3-10% effluent concentration, confusing the toxicity and variability assessments. The possible addition of nutrients from the effluent suggests either a potential inadequacy of the food quality provided in the chronic assessment, or the presence in the effluent of growth stimulants. Growth was also found to be too variable to allow adequate statistical conclusions about the toxicity of the effluent, although it is suggested that growth may be useful in the assessment of single compounds. Despite large individual variability in fecundity, statistical differences were discernible between effluent concentrations. The application of fecundity of *B. stenochorias* in hazard assessment therefore warrants further investigation. It was concluded that an assessment of textile whole effluent toxicity to *B. stenochorias* over an entire life cycle, and a F1 generation, is unnecessary. The development of the bucket/plastic bag method for both acute and chronic toxicity assessment of *B. stenochorias* was useful.

In the final assessment of the usefulness of *B. stenochorias* as a toxicity indicator, toxicity endpoints were compared with those of the standard laboratory organism *Daphnia pulex*. Both in acute and chronic toxicity, *B. stenochorias* was found to be more sensitive. *B. stenochorias* is therefore considered valuable as a South African freshwater molluscan ecotoxicological indicator, with a place in hazard assessment, although further development and research is necessary before the limpet can be effectively used.

2.2 DISCUSSION AND FUTURE RESEARCH

2.2.1 Biology and method development

Large individual variability was seen in growth and fecundity of *B. stenochorias*, in both natural and laboratory populations. However, although good toxicological practice requires the collection of consistent and comparable, legally defensible data, the possible use of *B. stenochorias* as an ecotoxicological indicator organism was pursued because of the necessity for the freshwater Mollusca to be represented in South African toxicity testing. As algal grazers on stones and rocks, limpets are an important component of the benthic freshwater community.

2.2.2 Toxicity tests

The difficulty in unravelling the toxic components of the textile effluent underlined the value of whole effluent, as opposed to single substance, toxicity testing. Responses of *B. stenochorias* to the textile effluent in terms of survival, growth and reproduction were variable. Consequently, the calculation of statistical endpoints was not always possible. Large innate variability in growth and reproductive parameters suggests that these experimental endpoints may not be appropriate as chronic toxicity test endpoints for *B. stenochorias*. However, this variability is characteristic of freshwater pulmonate molluscs, known to exhibit great inter-population and inter-specific variability in life cycle patterns and physiology (Dillon, 2000).

Since it is the magnitude of uncertainty that is important in the application of toxicity test data to hazard and risk assessment (Suter, 1995), responses of *B. stenochorias* were compared to those of the standard test taxon *Daphnia pulex*. The daphnids' clonal reproduction lends itself to genetically homogenous populations and, theoretically, to reduce variability of response endpoints. There is the question, however, regarding the suitability of such standard test organisms which inhabit lentic waters, and whether they reflect the responses of lotic dwelling, indigenous organisms. Laboratory cultures in general are also considered insufficient for hazard evaluation.

2.2.3 Acute Toxicity Tests

The use of the reference toxicant $K_2Cr_2O_7$ introduced a means of characterizing method variability between consecutive acute tests. Monotonicity of response to $K_2Cr_2O_7$ by both *B. stenochorias* and *D. pulex* was observed, suggesting consistency of response of both species towards the reference toxicant. A comparison of the LC_{50} values (1.41 and 11.46mg/L $K_2Cr_2O_7$ respectively) and coefficients of variation values (30% and 34.5% respectively) indicate both species gave consistent, and comparable, endpoints.

With textile effluent as the toxicant, *D. pulex* was less sensitive than *B. stenochorias* with infrequent, low mortality (5%) over 48-hours. Limpet response showed seasonality to toxicants, with significantly lower mortalities in winter to both effluent ($p < 0.001$) and $K_2Cr_2O_7$ ($p < 0.05$). Small, but not significant, population differences in mortalities also occurred between flowing and non-flowing sources of the populations sampled.

A 96-hour LC_{50} value for the textile effluent could not be calculated for *B. stenochorias*, necessitating chronic toxicity tests.

2.2.4 Chronic Toxicity Tests

Measuring growth for both *B. stenochorias* and *D. pulex* was both time consuming and statistically and practically unsatisfactory in revealing toxicological effects of the whole effluent. Stimulation in growth was generally seen with limpets maintained in effluent concentrations up to 20% effluent. This was possibly due to either the provision of extra nutrients or growth stimulants. However, growth stimulation may just reflect a shift in physiological response rather than a positive effect on the limpet life cycle; it needs to be carefully linked to reproductive efforts and longevity before a decision as to the advantage of this increase in growth is made. This is particularly so where the growth factors κ (rate at which λ is reached) and λ (longevity) in the Von Bertalanffy growth equation are known to be negatively correlated in indeterminate growers such as *B. stenochorias*. The uncertainty in the appropriateness of growth as a chronic toxicity indicator is also underlined by the dependency growth rates of *B. stenochorias* display on season and nutrition.

The fecundity of *B. stenochorias*, however, is a useful ecotoxicological measure, and has been used in biological monitoring with other pulmonate species. Despite the high innate variability in growth and reproduction of the limpets within each concentration of effluent, statistically significant differences in fecundity data became clear. Significantly increased fecundity was seen in the 3% effluent, compared to 20%, 10% and 1% effluent and the controls of dechlorinated tap water. This was possibly related to the increased growth seen when in 3% effluent, and the possibility of earlier sexual maturation. Egg lay was inhibited in half the 1% effluent replicates, where significantly increased age at first lay, and significantly fewer eggs per capsule and total number of capsules occurred. Possibilities for reproductive criteria include calculations of the number of eggs laid versus hatching, and egg development, both of which are easy to observe using a bi-focal microscope. The egg capsules adhere to the surface, and eggs and embryos within the capsule do not move around. Individual embryo development can therefore be monitored.

Although the investigation of fecundity was practically easier to monitor in *D. pulex*, its sensitivity to toxic effects was significantly lower than *B. stenochorias*. The NOEC was 12.5% effluent (*D. pulex*), compared to < 0.1% and <1% (*B. stenochorias*).

2.2.5 Application of *Burnupia stenochorias* toxicological data

With the vast range of textile dyes and processes used and applied in the textile industry, the importance of individual (factory) environmental risk assessments is emphasized.

As an immediate example of the application of the chronic whole effluent toxicity tests using *B. stenochorias*, the hazard-based guidelines for the disposal of textile whole effluent given by Zokufa (2001) can be examined. Post-irrigated effluent (the same source as used in the *B. stenochorias* tests) was not acutely toxic, defined in terms of an LC_{50} value, to mayflies, which complies with the results from this study of *B. stenochorias*. However, on the basis of both acute and 7-day chronic (survival) toxicity tests, using general (pre-irrigated) textile effluent from the same factory source, Zokufa calculated that a 3% effluent concentration would protect Class A (Natural) water resources i.e. those resources most natural in character. However, 43-day toxicity tests with *B. stenochorias* gave NOECs, also based on survival, of less than 0.1% (1998) and less than 1% (1999) post-irrigation textile whole effluent. Subtle toxic complexities, potentially cumulative to a toxic level, were therefore not found at the acute level tested by Zokufa. This clearly demonstrates the necessity, in the South African context, for chronic toxicity data with a variety of indigenous organisms to be tested to ensure adequate resource protection.

2.2.6 Conclusions

- The accumulation of biological data is generally a long term process before there is any confidence in suggestions of life cycle patterns.
- The standard laboratory organism *D. pulex* was not as protective as *B. stenochorias* in textile whole effluent toxicity tests.
- Extrapolation of acute toxicity data may not be as effective in predicting hazard for a Natural river health class as the completion of chronic toxicity tests.
- Large variabilities of response within a test organism to a toxicant should not exclude that organism from being useful in hazard and risk assessments.
- For chronic toxicity tests to be effective, detailed knowledge of dietary preferences and the effects of different water chemistry may be necessary.
- Understanding the mechanisms underlying the patterns of toxicity may contribute to more effective use of the test organism, and *B. stenochorias* in particular, as a toxicological indicator.

The chronic toxicity study undertaken here has pointed to worthwhile possibilities within the life cycle of *B. stenochorias* which warrant further investigation in the search for practical, reproducible chronic toxicity test methods to be introduced in site specific hazard and risk assessments. The keys to predictive utility of toxicity tests are interpretation and extrapolation to the receiving system in terms of estimated hazard of the toxicant; the sensitivity and variability of the responses of the test organisms used in the toxicity assessments; and the replicability by a wide variety of quality control personnel. Future research into the use of *B. stenochorias* should therefore be aimed with these points in mind, leading to the development of a practical, robust and unambiguous toxicity test method (or methods).

CHAPTER 3

SALINITY IN AN ENVIRONMENTAL WATER QUALITY CONTEXT

Samantha Browne

3.1 INTRODUCTION

Demands on aquatic ecosystems are numerous and include those made by industry, agriculture, and domestic users for basic human needs such as drinking water and sanitation. Aquatic ecosystems also provide important services such as the retention, supply and transport of water by means of rivers, estuaries, lakes, dams, wetlands and aquifers, all of which are part of the hydrological cycle. Other services include the dilution, removal and purification of wastes; commercial and subsistence supply products such as fish and plants; and sports and recreation facilities (Palmer, 1999). Supplies of freshwater are limited worldwide and demands on freshwater resources are increasing (Davies and Day, 1998) due to rising population and growing industrial and agricultural activity. Of the 72% of the earth's surface that is constituted by water (Barnes, 1980), only 2.53% is freshwater (Newson, 1994). Problems with supply are compounded by the naturally inequitable geographic distribution of water both spatially and temporally (Davies and Day, 1998, Gaylard, *et al.*, 2003).

Added to the challenges of water supply are those associated with water quality, which is also frequently impacted. The deterioration of both water quality and quantity are exacerbated by natural variability and changing climatic conditions, such as drought. A general lack of understanding of this variability has resulted in a paradigm of managing natural resources so to provide a reliable supply of good quality water. This in turn drove development and a focus on engineering solutions. The main approach to water resource management was to build dams to regulate supply. Dams have negative ecological consequences, disrupting aquatic ecosystems by severely modifying flow regimes of rivers which in turn modify natural structures of aquatic fauna and flora. However, the emerging field of holistic methodologies such as environmental flow assessment (Tharme, 2002), has led to a more comprehensive understanding of the negative impacts that impoundments have on aquatic ecosystems (O'Keeffe *et al.*, 1992) so as to improve manage dam releases, in an attempt to maximise aquatic ecosystem health. In the process of developing methods for managing aquatic ecosystems in South Africa, most of the method development has focused primarily on rivers, and since rivers are by far the most important freshwater systems in South Africa (Rabie and Day, 1992), they form the focus for this study.

Often the urgent need for rivers to provide more water-related services conflicts with the need to improve or maintain the ecology of the country's rivers (King and Louw, 1998). Water resource managers are under pressure to find equilibrium in managing and meeting the demands of the country's water while at the same time, adequately protecting the resource. This has prompted a change in paradigm to one that still appreciates growing demands of water supply, but also acknowledges that aquatic resources are limited and that ecosystem health is deteriorating. Aquatic ecosystems have been recognised, not as users of water in competition with other users, but rather as the base of the resource itself that needs to be actively cared for if development is to be sustainable (DWAF, 1997; King and Louw, 1998). Allanson *et al.* (1990, p.161) supports this paradigm shift: "the general conservation of rivers is complicated by their longitudinal nature, by their vulnerability to catchment changes, and by their importance as scarce water resources. Hence, the aim of river conservation cannot therefore be to preserve them in pristine condition, but rather to maintain them as renewable natural resources, to be exploited within limits for sustainable yields, and for multiple purposes". One of the greatest challenges riverine ecologists face today is adequately communicating their knowledge of spatial and temporal variability of rivers to managers in ways that enable managers to develop appropriate approaches to management (Rogers and O'Keeffe, 2003). An interdisciplinary understanding of river ecology is essential if this communication between scientists and managers is to be successfully achieved (Rogers and O'Keeffe, 2003).

As paradigms have changed, the tools used in water resource management have evolved. In apartheid South Africa, the Water Act (No. 54 of 1956) was based on the 'riparianity' principle, where the right to use water was based on land ownership (Palmer, 1999) and it focused primarily on inland running water (O'Keeffe *et al.*, 1992). Because land ownership was discriminatory, a privileged few had majority access to water. In 1994, with the beginning of democracy in the country, the principle of ripariarinty was challenged and a new water law was envisaged. To guide the process of developing new legislation, widespread public participation and consultation culminated in an outline of key principles, on which the new water law would be based. These principles provided the foundation for the 'White Paper on a National Water Policy for South Africa' (DWAF, 1997), which was based on two central principles: equity and sustainability (Palmer *et al.*, 2002). The White Paper formed the policy basis for the new legal framework for water resource management, the National Water Act, No. 36 of 1998 (Palmer *et al.*, 2002). Sustainability and equity remain as central guiding principles in the protection, use, development, conservation, management and control of water resources for the country (Chapter 1, National Water Act, No. 36 of 1998).

The new Act makes provision for both a basic human needs Reserve (that quantity and quality of water which provides for essential needs of individuals) and an ecological Reserve (that quantity and quality of water required to protect the ecosystems that comprise the water resource). The Reserve is viewed as one of the main policy and legal tools to ensure equity and sustainability (DWAF, 2000a). The National Water Act states that "the Minister, the Director-General, an organ of state and a water management institution, must give effect to the Reserve as determined in terms of this Part (the Reserve) when exercising any power or performing any duty in terms of the Act" (NWA, 1998).

With a conceptual basis for resource management in place (the White paper) and the establishment of a legal framework (the National Water Act), the need arose for an implementation strategy that would ensure action towards resource management. In the Integrated Manual for Resource Directed Measures (RDM) for Protection of Water Resources (DWAF, 1999), the Department of Water Affairs and Forestry identified three main components of policy implementation:

1. Protection to ensure sufficient water quantity and water quality (especially in relation to human health) to meet basic human needs.

2. Protection of ecosystem structure and function, in order to ensure that utilisation of water resources can be sustained on a long-term basis.

3. Meeting of water quality requirements for other water users (agriculture, industry, recreation) as far as possible, within the constraints of requirements for protection of basic human needs and the protection of water resources.

These components further highlight the need for water resource managers to adopt an integrated and adaptive approach to management. The aim of this approach is to ensure sustainable, equitable and efficient use of the country's aquatic resources. This is set out in the proposed first edition National Water Resource Strategy (NWRS) (DWAF, 2002). Essentially, the strategy acts as the implementation framework for the National Water Act (No. 36 of 1998) and outlines the goals and objectives of water resource management for the country. It also provides plans, guidelines and strategies on how to achieve these goals.

In order for these new holistic ideas to be practically implementable, the Department of Water Affairs has identified four regulatory activities (DWAF, 1999):

- Firstly, **resource directed measures (RDM):** this involves co-operatively defining the appropriate level of protection for a water resource, and on that basis, setting clear numerical or descriptive goals for the resource quality of the resource i.e. Resource Quality Objectives (RQOs).
- Secondly, **source directed controls (SDC)**; this requires controlling impacts on the water resource through the use of regulatory measures such as registration, permits, directives, prosecution, and economic incentives such as levies and fees, in order to ensure that the RQOs are met.
- Thirdly, **managing water resource demands:** this is in order to keep utilisation within the limits required for protection.
- Lastly, **continual monitoring:** this involves monitoring the status of the country's water resources on a continual basis, in order to ensure that the RQOs are being met and to enable DWAF to modify programmes for resource management and impact control as and when necessary.

The need for water resource protection is plain in light of the goods and services aquatic ecosystems provide, coupled with threats of overuse. Water resource managers have needed quantity-led goals for water resource protection. This has prompted the development of a classification system which identifies different levels of protection according to kinds of use. The Australian and New Zealand Guidelines for Fresh and Marine Water Quality advocate ecologically sustainable development (ESD) principles, which imply acceptance of a degree of environmental protection, as long as the integrity of ecosystems is not threatened (ANZG, 2000). Guideline values are derived to protect 95% of aquatic life species but with varying levels of certainty that ecosystems will be protected. The required level of certainty decreases according to the degree of accepted modification of the natural system. Similarly, the Canadian Water Quality guidelines recommend benchmark values for the protection of 100% of species, 100% of the time but site-specific water quality objectives are adopted as local conditions dictate (CCREM, 1987).

In South Africa the National Water Act focuses on water resource protection to ensure efficient **use** and how much use is permissible for a particular river or section of river is governed by a classification system. The system firstly classifies the current level of ecosystem health into one of five ecological Reserve categories (**Figure 3.1**). Secondly, one of four water resource classifications is then selected defining the state towards which the water resource needs to be managed and an appropriate management class is assigned to the resource. The management objective, stipulated by the management class, is set according to the desired level of ecosystem health, and the associated level of resource protection. For example, a management objective exhibiting a high level of ecosystem health can receive an ecological Reserve category of 'A' and be assigned an 'A' management class associated with high levels of protection and restricted use of the resource. Only three management classes exist, as a 'poor' management class is not considered a management option.

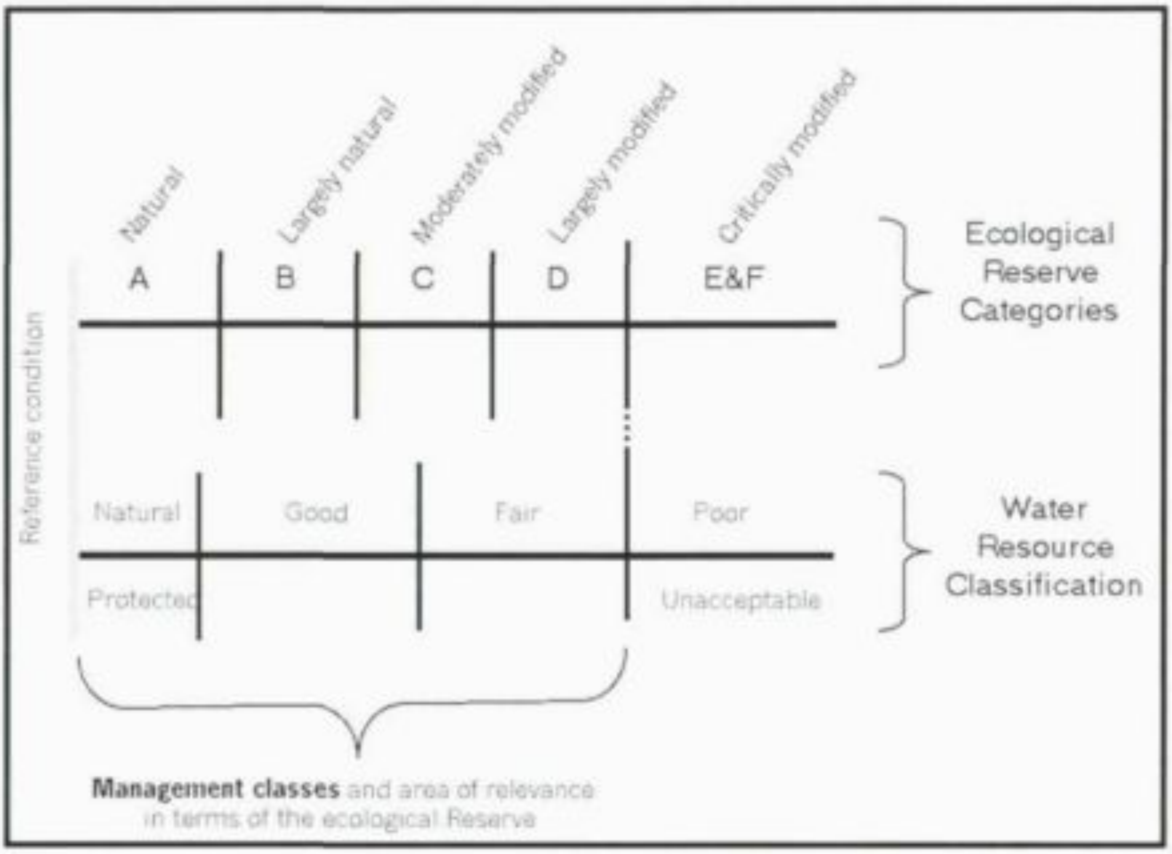


Figure 3.1 South African ecosystem health and management classification system

Once a particular resource is classified, RQOs are determined. These are targets or objectives set for each water resource in terms of the level of protection the water resource requires. The setting of RQOs is part of the Reserve determination process and serves to meet the requirements of the Reserve, i.e. both the basic human needs Reserve and the ecological Reserve. RQOs are derived using primarily Instream Flow Requirement (IFR) Methods. The IFR can be defined as "that which describes, in space and time, the minimum amount of water that is felt will facilitate maintenance of the river at some pre-defined state" (King and Louw, 1998). Methods to derive IFRs include the Building Block Methodology (BBM) (King and Louw, 1998), Downstream Response to Imposed Flow Transformations (DRIFT) and the Flow Stressor-Response (FSR) method. The BBM rapidly provides scientific guidance on required flows for a river in cases where biological data and understanding of the functioning of the river are limited (King and Louw, 1998). The initial data-collection steps of the BBM closely approximate those of DRIFT and like BBM, DRIFT uses a holistic, scenario-based approach addressing all bio-physical aspects of a river in question. A database is created which can be queried to describe bio-physical consequences of any number of potential future flow regimes (scenarios). It is also designed to detail and quantify links between changing river condition and the social and economic impacts for the riparian people who rely on the river for subsistence. Where the BBM 'builds up' a recommended flow regime, DRIFT takes the present day flow regime as a starting point. It describes the consequences for all aspects of the river when reducing or increasing flow regimes in different ways and it also addresses socio-economic links (Brown and King, 2000).

The Flow Stressor-Response method was designed for usage within the BBM and DRIFT methodologies as a tool to guide the evaluation of the ecological consequences of modified low flow regimes, based on principles of Ecological Risk Analysis (Suter, 1993, cited in O'Keeffe and Hughes, in press). It aims to consistently capture specialist knowledge on the relationship between flow, hydraulic habitat and the responses of instream biota (O'Keeffe *et al.*, 2002). These relationships are then translated directly into stress profiles for any flow regime in terms of magnitude, frequency and duration. The method concentrates on water quantity requirements and is independent of the level of biological knowledge available although this will affect the level of confidence associated with the recommended flows (O'Keeffe and Hughes, in press).

The concept of setting an IFR for any particular river complements the water resource objectives stipulated in the National Water Act of 1998. These include seeking out a balance between the need to protect and sustain water resources on the one hand, and the need to develop and use resources on the other. IFR methods link sound data to relevant management issues to ensure successful implementation. However, the IFR methods are limiting in that they deal only with flows and don't incorporate a water quality component. Malan and Day (2002) outline how characteristic flows of rivers are intimately linked with water quality, because if flow is altered, water quality frequently changes. A recent approach has been outlined as part of a Reserve determination, termed the environmental water quality (EWQ) approach and involves understanding how chemical, microbiological, radiological and physical characteristics of water link to the responses of living organisms and ecosystem processes (Palmer *et al.*, in press). In 1999 the Olifants River ecological Reserve assessment was the first to include an assessment of water quality (DWAF, 2000a). Since then methods for including water quality in reserve assessments have been developed and refined, the most recent records are outlined in Palmer *et al.* (in press).

Guidelines for managing water quality provide the structure that managers need to manage water quality and can support management decisions that are socially, economically and ecologically sustainable. They have been an important component of water resource management over the years (Hart *et al.*, 1999). In South Africa the South African Water Quality Guidelines for Aquatic Ecosystems were formulated in 1996 (DWAF, 1996a) and offered decision support for water resource managers (mainly the Department of Water Affairs and Forestry) in the management and protection of aquatic ecosystems (Dallas *et al.*, 1998). Furthermore, the guidelines served as the primary source of information for determining the water quality requirements of different water uses (DWAF, 1996a). However these were drafted prior to the establishment of a classification system aimed at protecting resources in an excellent or near Natural condition. Additional guidelines are in the process of being drafted which ideally will provide boundary or trigger values (discussion to follow) which are the numerical values or concentrations of a toxicant which allow managers to distinguish between the different Ecological Health Classes.

One particular water quality constituent that has come under increasing surveillance over the last 15 years, and which is the main focus for this study, is salinity. Furthermore, salinization has been identified as one of the major contributors to decreased water quality in South Africa (O'Keeffe *et al.*, 1992) and warrants careful research and investigation.

3.2 SALINITY AND SALINIZATION

One of the water quality parameters described in the South African Water Quality Guidelines for Aquatic Ecosystems is salinity or total dissolved salts/solids (TDS). TDS concentration is a measure of the quantity of all dissolved compounds in water that carry an electrical charge (DWAF, 1996a). Salinity can be defined as the mass measurement of dissolved salts in a solution of given mass. Salinity is not exactly equivalent to total dissolved solids (TDS), however the two are closely related (US Dept. of the Interior, 1999) (TDS concentration is directly proportional to the electrical conductivity or EC). A common approximation for TDS concentrations from EC for South African inland waters is (DWAF, 1996a):

- TDS (mg/L) = EC (mS/m at 25°C) x 6.5

The factor of 6.5 is approximate and can be influenced by the actual ionic make-up of the dissolved solids. It can be as high as 9.0 or as low as 4.0, however the 6.5 is fairly accurate for the average mixed ionic make-up of natural water (Urban-Econ, 2000b).

TDS can vary in natural waters according to geological formations and physical processes (e.g. evaporation), hence TDS are governed by constituent inorganic salts (DWAF, 1996a). Most commonly, the relative concentrations of major ions tends to be:

- Cations: $Na^+ > Ca^{2+}/Mg^{2+} > K^+$
- Anions: $Cl^- > HCO_3^- > SO_4^{2-} > CO_3^{2-}$

Salinization is the process which refers to an increased concentration in water, or in soil, of naturally occurring mineral ions, particularly those of sodium (Na^+), chloride (Cl^-) and sulphate (SO_4^{2-}) (Davies and Day, 1998). Salinization is a useful measure of monitoring water quality because for the most part, the usefulness of water for most purposes diminishes with an increasing salt content (Du Plessis and van Veelen, 1991). The process of salinization can be driven by both natural and anthropogenic activities.

3.2.1 Natural drivers of salinization

3.2.1.1 Climate

The rate that salinization occurs is affected by various environmental components, such as annual rainfall, the ratio of precipitation to evaporation, groundwater hydrology and surface run-off rates. Seasonal and inter-annual variations in climate are also major drivers of solute concentrations in rivers (Interlandi and Crockett, 2003). The association of arid and semi-arid areas with high rates of salinization is a common phenomenon (Roos and Pieterse, 1995; Kotb *et al.*, 2000; Young, 2001a; Jorenush and Sepaskhah, 2003; Farber *et al.*, 2004; Oren *et al.*, 2004) especially where these regions are associated with shallow, saline water tables (Jorenush and Sepaskhah, 2003). South Africa is typically characterised by large areas of semi-arid areas. The average rainfall for the country is approximately 450mm per year, well below the world average of about 860mm per year (DWAF, 2002). Its climate and landscape exacerbates the process of salinization due to high evaporation:precipation ratios and low run-off:rainfall ratios. For South Africa, the runoff coefficient is only 10%, meaning that 90% of rainfall is lost through evapotranspiration. Evapotranspiration is defined as the combined water loss by evaporation from open water surfaces and from soil and groundwater through transpiration by plants (Rowntree, 2000). South Africa's rivers, large dams, canals and farm dams suffer salinity problems due to evaporative losses from surface waters. Many of the country's salinity problems have also occurred during, or as a result of, extreme climatic events.

3.2.1.2 Geology

Salinization is often affected by the nature of geological formations of a particular area (Davies and Day, 1998). The mass and type of mineral dissolved in solution depend on geochemical characteristics of the soil and the surrounding environment. Limestone bedrock and other calcareous sedimentary deposits can contribute to increasing solutes (Interlandi and Crockett, 2003). Decomposed shales release high concentrations of sodium, chloride and sulphate, while decomposed dolomites contribute principally calcium, magnesium and carbonate ions (Loewenthal, 1995). The mixing of leachates with formation water varies depending on hydrological conditions (Farber *et al.*, 2004). In the Jordan Valley (Jordan), sulphate-rich groundwater is derived from leaching of the Pleistocene and Neogene sediments (Pleistocene sediments induce dissolution of saline marls and gypsum) (Farber *et al.*, 2004). In South Africa, many water bodies are naturally high in dissolved salts, especially where rivers flow over old marine sediments such as the Karoo series (O'Keeffe *et al.*, 1992). Dominance of specific ions has shown to correlate with geographical patterns (Day and King, 1995). For example, ground waters of much of the country's coastal belt, and all of the Karoo, were categorised as highly mineralized chloride-sulphate waters with TDS values >1000mg/L (Day and King, 1995).

3.2.2 Anthropogenic drivers of salinization

The process of salinization can be exacerbated by anthropogenic activity, leading to unnaturally high levels of salinity in the natural environment. This is sometimes referred to as secondary salinization (Hart *et al.*, 1991). The three main causes for increased salinization due to anthropogenic activity are: urban activity; industrial and mining operations; and agricultural activity and practices. In South Africa, urbanisation, industrialisation and irrigation have caused increases in salinity which greatly threaten the potential usefulness of the country's rivers (O'Keeffe *et al.*, 1992).

3.2.2.1 Urban activity

Worldwide urban sprawl has resulted in an increase in dissolved solutes in rivers. In urban areas with high populations, high rates of freshwater abstraction exacerbate salinization. This is especially so when water abstraction occurs during low flow periods, increasing salt concentrations in surface water (Young and Hillman, 2001). A United States example in Southeastern Pennsylvania region reveals a directly proportional relationship between increasing solute levels over the past several decades with quantity of developed land area in suburban portions of the Schuylkill catchment (Interlandi and Crockett, 2003). One of the main drivers in Northern Hemisphere examples of salinization is the use of road salts for de-icing (Interlandi and Crockett, 2003). Similar relationships between developed areas and solute levels have been observed in South Africa, for example in the Vaal River (Urban-Econ, 2000b). Middle reaches of the Buffalo River have ceased to be a resource due to salinization and pollution, that the river has become a potential health hazard (O'Keeffe *et al.*, 1992). With increased economic pressure, more industrialisation is likely to take place, with possible effects on salt levels in rivers throughout the country (Urban-Econ, 2000b).

3.2.2.2 Industrial and mining operations in relation to salinity

The mining sector within South Africa is diverse and water usage patterns and the impacts of increased salinity vary significantly throughout (Urban Econ, 2000a). Saline pollution by mineral salts, particularly those derived from irrigation seepages, mining and industrial effluents, and storm runoff from mining areas, has been documented as creating serious problems from as early as the 1970's in the Commission of Enquiry into Water Matters, Report of 1970 (DWAF, 1986a), contributing significantly to the country's salinity problems. One of the problematic factors of mining effluent is the contribution of acid mine drainage and sulphate pollution. In the process of coal mining in South Africa, coal deposits contain pyretic formations which, under certain conditions, are oxidised to sulphuric acid and iron sulphate (Thompson, 1980). Resultant acid mine drainage from these by-products is extremely acidic and can be treated with hydrated lime ($CaCO_4$) before discharge into the environment. The resultant effluent is saline (gypsiferous) water, mainly due to Ca^{2+} and SO_4^{2-} in solution (Jovanovic *et al.*, 1998). Another potential contributor to sulphate-enriched effluent is in the process of heavy mineral extraction from dune sand. The chemical impacts relating to smelting processes are of environmental concern. Effluent resulting from the smelter complex are most likely to cause raised salinity levels in the receiving aquatic ecosystems, particularly due to the contribution of sulphate ions (Palmer and Wade, 1997).

Examples of South African rivers that have been subject to intense pressure from mining activities include the Olifants and upper Vaal River catchments (Van der Merwe and Grobler, 1990). The Olifants River catchment formed the basis of one of the first comprehensive Reserve determinations carried out in the country. The assessment revealed that various segments of the river are highly impacted by numerous coal mining and power generation activities and discharges from slime dams; not excluding the contribution of irrigation return flows and poor land use practices to increased salt loads in the system (DWAF, 2000).

3.2.2.3 *Agriculture*

Agricultural activity can contribute to salinization on a large geographical scale (El-Ashray *et al.*, 1985). Salt loading refers to where salts are added to water. The main contributing factor to salt loading is irrigation, especially when saline groundwater is the significant or sole source of water (Oren *et al.*, 2004). This results in the recycling of salts (mainly chloride, sulphate, sodium and calcium) dissolved in irrigation water. Salt concentrations are increased when water containing salts in solution is lost by evaporation and transportation, leaving behind salts that precipitate. Major salts are not taken up substantially by plants and return to rivers and groundwater from water runoff from fields and by percolating through soil respectively. The problem intensifies as repeated irrigation results in increasing salt accumulation (El-Ashray *et al.*, 1985). High rates of evapotranspiration and the lack of flushing by rain of near-surface and soil root zones in arid and semi-arid areas only exacerbate the process of increasing salt concentrations. Infiltrating water from agricultural fields can cause water table levels to rise, increasing chances of evaporation (Kotb *et al.*, 2000). In a study conducted in the Arava Valley in Israel, it was found that the main source of chloride came from irrigation water after evapotranspiration. Nitrate concentrations of recharging water are also easily affected as nitrate salts are highly soluble and easily flushed towards the water table (Oren *et al.*, 2004).

Various factors have resulted in agriculturally-induced salinization becoming a major problem worldwide. These include reduced annual flows, over-irrigation, insufficient drainage systems, over-use of salt-generating agrochemicals, dumping of diverted saline springs or wastewater, intrusion of seawater into freshwater systems, and the acclimation of surface runoffs in low-lying areas (Kotb *et al.*, 2000). Most of these factors are linked either to poor planning, in the case of poorly designed irrigated systems, or are due to poor catchment management, in the case of poor land-use practices (e.g. land clearing) and overuse of natural resources within a catchment. Various countries have experienced problems in managing saline water bodies either caused by man-related activities or due to rising saline groundwater tables and expanding saline lakes (Kefford, 1999). Such environmental concerns are not only a product of man's activities over recent years. The San Joaquin Valley of California, for example, has been subject to intensive development for over 70 years (50 years stated by Orlob and Ghorbanzadeh in 1981), and its major rivers regulated for power production, water supply, flood control and irrigation (Orlob and Ghorbanzadeh, 1981). It is even thought that irrigated agriculture caused the decline of certain ancient civilizations (El-Ashry, 1985).

In summary, contributing factors to salinization in South Africa, as a result of agricultural activity, include runoff from dryland agriculture and irrigation return flows, including seepage losses from irrigation water storage and distribution systems; and flow reduction in rivers due either to upstream diversion of dilution waters or drought conditions (SA Water Bulletin, 1990).

As previously discussed, South Africa has large semi-arid areas, which implies that it shares some of the above-mentioned problems of salinization associated with agricultural activity. However, the potentially negative affects of salinization on South Africa's natural environmental is not well-documented and it was only in 1986 that the Department of Water Affairs and Forestry first clearly identified salinization as one of the main water quality problems experienced in the country, alongside with eutrophication (DWAF, 1986b).

3.2.3 Effects of salinity on aquatic ecosystems

The focus of the salinity problem in South Africa has centred mainly on mining and agricultural activities, and early emphases were placed on researching de-salinization processes to help meet supply demands of industry, agriculture and urban centres (DWAF, 1986a). Limited focus has been given to the salinity problem in terms of management of the country's aquatic resources. In small quantities, salt is essential for life; however, when it reaches greater concentrations in the natural environment it has the capacity to adversely affect many plant and animal species (Beresford *et al.*, 2001). Some isolated references in the literature do examine the effects of salinity on aquatic ecosystems. However it is only recently that salts have been formally classified as toxicants (Kefford *et al.*, 2002). The big question that arises in the whole salinity debate is: what impact, positive or negative, does an increase of salinity (either naturally- or anthropogenically-induced) have on aquatic ecosystems?

Some fauna and flora are able to cope with varying degrees of salinity. Usually these coping mechanisms have a threshold beyond which extremely variable salinity conditions can directly and indirectly cause changes to the physical and biological aquatic environments. The main concern for aquatic organisms associated with salinity is osmotic stress, when cells of the organisms have either a lack of water or an excess of ions (or both) that can result in a range of toxic effects (Hart *et al.*, 1991). Most aquatic organisms are stenohaline, hence salinity changes might affect aquatic organisms in two ways (ANZG, 2000):

i) direct toxicity through physiological changes (particularly osmoregulation) – both increases and decreases in salinity can have adverse effects;
ii) indirectly by modifying the species composition of the ecosystem and affecting species that provide food or refuge.

Hart *et al.* (1991) provides a comprehensive review on the salt sensitivity of Australian freshwater biota which is useful in understanding how unnatural levels of salinity can affect biota. On a fundamental level, salinity increases can affect microbe communities at three levels:

- effects and adaptations of individual types;
- effects on community structure; and
- effects on microbial processes, such as metabolism and nutrient recycling.

Algae can be indirectly affected when turbidity levels lower due to a change in levels of TDS in the water. This results in an increase of underwater light levels as colloids precipitate out (Urban-Econ, 2000b).

In terms of flora, riparian vegetation species that are most vulnerable to salinity increases are non-halophytes, which achieve best growth conditions in non-saline conditions and whose growth is reduced as salinity increases. Such species compose the majority of riparian flora of streams and associated wetlands (Hart *et al.*, 1991). Ion uptake reaches toxic levels, or the plant diverts it's metabolites to maintenance respiration and results in reduced growth. A further consideration for plants in salt-affected land is prolonged or occasional waterlogging. Recent studies suggest waterlogging and increasing salinity can act synergistically. Salinity appears to interfere with the plant's adaptations to waterlogging and vice versa. It also appears that tolerance to combined effects of salinity and waterlogging are greater in waterlog-tolerant species (Hart *et al.*, 1991).

Freshwater invertebrates can also be adversely affected by salinity as they are hyper-osmotic regulators. Hence, they are incapable of maintaining body fluid solute concentrations below that of the water they live in. If the salinity of their environment is increased above a threshold level below which they can maintain a constant ionic concentration, they take up increasingly more ions. They then loose more water from their cells, until they no longer function properly and the organism dies. For invertebrates, factors such as degree of acclimation, life stage and temperature can influence their sensitivity to salinity increases. An organism's condition such as reproductive state, stage within a moult cycle, sex and body size and nutritional state can affect an invertebrate's ability to regulate cell solute concentrations in varying external salinities (Hart *et al.*, 1991). Varying salinities can further modify community structures of macroinvertebrate communities (Kefford, 1998).

Fish fauna are known to have coping mechanisms to deal with varying salinities in their surrounding environment, i.e. they use either hyper-osmotic (transport ions across gill surfaces) or hypo-osmotic regulation (loose water through gills) (Hart *et al.*, 1991). A large proportion of fish species have marine origins and as a result have some inherent tolerance to salinity. The ability of a certain fish species to tolerate salinity can be related to how recently it has evolved from a species of marine origin. Furthermore, in conditions where chemical conditions are variable, freshwater species may be more likely to retain characteristics of salinity tolerance. Life stages can also affect tolerance capabilities as larval fish do not have adult osmoregulatory abilities, placing them at higher risk in varying salinity conditions (Hart *et al.*, 1991. Further discussion pertaining to salt sensitivities of amphibians, mammals and birds can be found in literature (Hart *et al.*, 1991; US Dept. of the Interior, 1999).

On a more widespread scale, salinization can threaten biodiversity hotspots in areas associated with high species diversity and with rare species of fauna and flora. The South-West region of Australia, has a pending salinity crisis, threatening the natural environment with potential large scale loss of species diversity (Beresford *et al.*, 2001).

In concentrated areas worldwide, including South Africa, anthropogenically-induced salinization is currently threatening the health of aquatic ecosystems. In order for these particular ecosystems to be managed accordingly to prevent further degradation and unsustainable use of the resource, the impact of specific levels in the aquatic environment needs to be quantified. Toxicology and ecotoxicology can be used as valuable tools to quantify the impact of salinity increases on aquatic ecosystems.

3.3 ECOTOXICOLOGY

Toxicology in the broader sense has been defined as that which is concerned with the deleterious effects of chemical and physical agents on all living systems (Plaa, 1998). More specifically, environmental toxicology is a field of study that deals with the potentially deleterious impact of chemicals, present as pollutants of the environment, to living organisms (Plaa, 1998). Incorporated within the field of environmental toxicology is aquatic toxicology. This has been defined as the study of the effects of manufactured chemicals and other anthropogenic and natural materials and activities (collectively termed toxic agents or substances) on aquatic organisms at various levels of organization, from subcellular through individual organisms to communities and ecosystems (Rand *et al.*, 1995). Ecotoxicology is an area of study that has evolved as an extension of environmental toxicology as it is also 'concerned with the toxic effects of chemical and physical agents on living organisms', but especially 'in populations and communities within defined ecosystems' and is known to include the study of 'the transfer pathways of those agents and their interactions with the environment' (Plaa, 1998).

Ecotoxicology can facilitate the implementation of ecosystem protection by adopting a risk-based approach. This approach to water resource management is in response to the unpredictability, uncertainty and complexity of aquatic ecosystems. For example, it can be used to quantify the impact of increasing salinity to biota. One way in which this can be done is by conducting acute toxicity tests using a salt as the toxicant and to generate tolerance data of different types of aquatic biota at various concentrations of the salinity. Single-species acute toxicity tests are preferred over multi-species bioassays and chronic toxicity testing. This is because single-species tests are relatively simple, easy to standardise and are reproducible and rapid, whereas multi-species bioassays are expensive, time-consuming and particularly *in-situ* bioassays are characterised by high variability (ANZG, 2000). However, the shortcoming of single-species tests is that they exhibit low levels of biological and environmental realism, excluding significant inter-species and ecosystem-level interactions. Despite this, such data continue to dominate toxicity data sets and are mostly used in the derivation of water quality guidelines (Schudoma, 1994). In some cases acute to chronic extrapolations can also be used, but ideally acute toxicity data should be combined with data from comprehensive field data where possible when setting management objectives. These methods and also the philosophies surrounding the use of toxicity data are variable and have developed over the last 20 years.

3.3.1 Use of toxicity data in the development of water quality guidelines

Ideally, water quality guidelines should allow for the sustainable functioning of healthy and balanced aquatic ecosystems. The setting of water quality guidelines or the use of water quality criteria has proven to be an important tool in supporting resource management decisions. Various methods have been applied in using toxicity data to set numerical data for specific substances that indicate levels at which protection of sensitive components of aquatic ecosystems can be ensured (Roux *et al.*, 1996).

One of the earliest approaches to guideline development was the use of the assessment factor (AF) approach. In this approach, the lowest reported toxicity value is divided by a constant that is variously called an assessment, uncertainty, application or safety factor. The magnitude of the AF is governed by the perceived 'quality' of the toxicity data (Warne, 1998). Assessment factor methods have been used in the United States by the United Stated Environmental Protection Agency (USEPA, 1984, 1994) and Australia by the Australian and New Zealand Environment and Conservation Council (ANZECC, 1992). They've also been used in South Africa based largely on the methodology developed by Stephan *et al.* (1985). However, more recent approaches have shifted towards the use of extrapolation methods that are statistically based (Warne, 1998). The stimulus for extrapolation methods has been due firstly to the need for a risk-based approach, secondly to a lack of existing multiple species experiments and thirdly, to the lack of scientific basis for assessment factors.

Extrapolation methods use toxicity data obtained from tests on individual species and fit a statistical distribution to the data to derive a concentration that should protect 95% of the species in the environment (Warne, 1998). Extrapolation methods have been adopted in the Netherlands, Denmark as well as the United States and are best explained according to three main types of extrapolation techniques, grouped according to the authors that have described them:

1. Stephan *et al.*, 1985.
2. Wagner and Løkke, 1991.
3. Aldenburg and Slob, 1993 (developed from the methodology first proposed by Kooijman, 1987; Van Straalen and Denneman, 1989).

The technique proposed by Stephan *et al.* (1985) has been rejected for several reasons (Warne, 1998):

- It assumes a threshold toxicity value below which no detrimental effects will occur. This is not supported by scientific literature and risk assessment theory.
- It assumes ecosystems can tolerate high concentrations for short periods of time.
- It has extensive data requirements.

As proposed by Warne (1998) the Aldenburg and Slob method (1993) is the more favourable technique over the Wagner and Løkke (1991) method. This is because it is recommended by the Organisation for Economic Co-operation and Development (OECD); is adopted in the Netherlands; and has received more validation work than the Wagner and Løkke (1991) method.

3.3.2 Toxicological databases

Various methods have been developed and are in the process of being developed to use toxicity data in deriving water quality guidelines. One of the most practical ways that toxicity data can be accessed is through toxicological databases. One of the most prominent international toxicity databases currently in existence, is the USEPA ECOTOX database. This database was created and is currently maintained by the United States Environmental Protection Agency (USEPA), the Office of Research and Development (ORD) and the National Health and Environmental Effects Research Laboratory's (NHEERLs) Mid-Continent Ecology Division (USEPA, 2002). The database provides single chemical toxicity data for aquatic life and terrestrial plants as well as wildlife. The original intended use for this database was for accessing toxicity information for use in characterizing, diagnosing and predicting effects associated with chemical stressors and to support the development of ecocriteria for natural resource use (USEPA, 2002). This database has been used extensively in the development of Water Quality Guidelines for South Africa because indigenous organism response data specific to South Africa are limited (DWAF, 1996a; Roux *et al.*, 1996) and no standard, official database of toxicity data currently exists in the country.

In an effort to reduce this dominance of internationally based data, the Unilever Centre for Environmental Water Quality (UCEWQ) as part of the Institute for Water Research (IWR) (Rhodes University, Grahamstown) has a toxicological database of South African aquatic macroinvertebrate tolerances. Data generated from this study will contribute directly to this database. Ideally this database will expand in the future, incorporating data from a wider variety of toxicants, across a broader range of taxonomic groups, so as to increase the accuracy and representivity of toxicity data in the ongoing development of South African Water Quality Guidelines.

3.4 SALINITY RESEARCH UNDERTAKEN IN THIS STUDY

In this study, research in to the effect of increased salinization was extended in two separate but complimentary projects. In the first, the number of species for which their tolerance to NaCl and Na_2SO_4 is known was increased. Acute toxicity tests using species not previously tested substantially increased the database of indigenous invertebrate tolerances and will allow the development of species sensitivity distributions for indigenous species and contribute to the refinement of water quality guidelines for aquatic resources. This research is described in Chapter 4.1.

In a separate study, described in Chapter 4.2, techniques to extrapolate acute toxicity data to chronic toxicity levels in order to provide protective water quality guidelines, are described. The two extrapolation techniques are applied to acute toxicity data for indigenous invertebrates obtained from the UCEWQ database. A chronic toxicity test method, using an indigenous species, was undertaken and the NOEC derived from the experimental work was compared to the extrapolated results.

CHAPTER 4

METHOD DEVELOPMENT FOR SUB-LETHAL RESPONSE ASSESSMENT

Samantha Browne and Andrew R Slaughter

The MSc theses of Samantha Browne and Andrew Slaughter contributed to different aspects of method development for the assessment of sub-lethal responses.

In the two studies, freshwater invertebrates were exposed to at least 2 salts (sodium chloride and sodium sulphate) for 1) acute and 2) chronic exposure periods. In the first study, the emphasis was on gathering information for the widest possible range of taxa. These acute, lethal, tolerance data, together with other similar data from the Unilever Centre for Environmental Water Quality (UCEWQ) database were used to compare the responses of South African taxa with those from other regions, and to extrapolate chronic endpoints in the second study. In the second study, after acute:chronic extrapolation methods were developed, the modelled chronic results were compared with experimental chronic tolerance results generated as part of the study. In both studies the results will be used to revise environmentally protective salinity guidelines.

4.1 A COMPARISON OF THE ACUTE SALINITY TOLERANCES OF SELECTED SOUTH AFRICAN FRESHWATER INVERTEBRATES

Samantha Browne

4.1.1 Introduction

The negative impacts that anthropogenically-induced salinization can have on freshwater systems is well documented (El-Ashray, 1985; Davies and Day, 1998; Williams, 1999; Beresford *et al.*, 2001; Kefford *et al.*, 2003). Previously water resource managers in South Africa's main tool in managing salinity was the South African Water Quality Guidelines based on a percentage deviation from the mean electrical conductivity (DWAF, 1996a). However, another approach is proposed in the derivation of water quality guidelines, particularly with respect to guidelines for salinity. The approach uses tolerance data derived from toxicity tests using salts as toxicants and aims to generate data using as wide a range of species as possible. This approach is based on the Aldenberg and Slob data extrapolation method, which determines concentrations of toxicants that should protect 95% of the species in an ecosystem (Aldenberg and Slob, 1993).

Standard acute toxicity test protocols used in South Africa are based on internationally accepted protocols, which are dominated by non-indigenous test organisms such as daphnids (e.g. *Daphnia magna*), guppies (*Poecilla reticulata*) and fathead minnows (*Pimephales promelas*) (Cooney, 1995). As a result, few protocols exist that use indigenous South African species in routine testing and water quality guidelines are heavily weighted by international toxicological response data. This study focuses on testing wild caught populations of indigenous macroinvertebrates so as to generate response data that would be representative of South African aquatic ecosystems and its species and that could therefore contribute to refining South Africa's water quality guidelines. Due to the fact that chronic toxicity tests are lengthy, complex and time consuming, only 96-hour tests were conducted. The aim was to expand the South African tolerance database and to cover as wide a range of taxa of invertebrates as possible that would facilitate more accurate extrapolations concentrations that would protect 95% of species existing in a natural ecosystem.

It is unrealistic to conduct tests for all salts, hence this study focused on acute toxicity tests using sodium chloride and sodium sulphate.

Na^+ and Cl^- are two of the most common ions and anions in South Africa' s surface waters (Day and King, 1995) and their concentrations are commonly exacerbated through agricultural activities. Sodium sulphate as the SO_4^{2-} ion is known to exacerbate ion toxicity and it is a common ion associated with mining (Thompson, 1980; Jovanovic *et al.*, 1998). The resultant acute toxicity data is then placed in the context of international data and where possible, comparisons drawn between salinity responses of South African organisms, mostly invertebrates, and other non-indigenous organisms and recommendations made for the application of this data in refining South Africa's water quality guidelines for salinity.

Hence, this research aims to follow international trends by adopting the Aldenburg and Slob (1993) method in the process of developing water-quality criteria, by using Species Sensitivity Distributions (SSD), in contrast to the original assessment-factor approached proposed by Roux *et al.* (1996).

Although this study focuses on the toxicity of whole salts, some research has focused on the differential toxicity of different salts and their ionic compositions once in solution. Mount *et al.* (1997) acknowledges the need for a broader understanding of major ion toxicity and that salinity toxicity is dependant on ionic composition. In the South African context, Jooste and Rossouw (2002) consider the varying toxicity of ionic compositions when proposing benchmark or boundary values for the boundary between the Natural/Good and Fair/Poor ecological health classes. Jooste and Rossouw (2002) developed a model for Toxicologically Important Major Salts (TIMS), which takes into consideration ionic ratios and concentrations, and derives both a lethality benchmark and a sub-lethality benchmark. Acute and chronic endpoints from the USEPA ECOTOX (USEPA, 2002) database are extrapolated to 336-hours (time at which toxicity reaches a steady state) to derive the lethality and sub-lethality benchmarks respectively. This study adopts the idea of using acute toxicity data in deriving the salinity boundary values for the Fair/Poor ecological health classes but uses a SSD approach instead of a direct data extrapolation approach. It also focuses on two main salts, sodium chloride and sodium sulphate, rather than ionic complexes.

An important component in deriving SSDs is having large sets of data with which to work with. The greater the amount of data used in SSD derivation and the broader the taxonomic representation of that data, the more accurately the SSD is likely to represent 95% of the species in the ecosystem. Thus, toxicological databases serve as important tools in accurately deriving water quality guidelines.

4.1.1.1 Motivations and Aims

The above discussion has served to highlight several motivations for the aims of this study.

These motivations are:

- South Africa's current Water Quality Guidelines were formulated using mainly international data. It is important to include South African species information in the development of guidelines, specifically for salts, as salt combinations and exposures are geographically distinct.

- Unnatural increases in salinity are evident in certain regions of South Africa's rivers. These high levels of unnaturally occurring salinity levels can potentially have adverse affects on aquatic ecosystems and their biota.

- Ecotoxicology is a useful tool to develop risk-based water quality guidelines (WQGs) and water quality objectives (WQOs), which can then be applied by water resource managers, with a relevant level of confidence, in the process of decision making.

- Macroinvertebrates are recommended test organisms in toxicity tests, however, test protocols using species indigenous to South Africa are lacking.

- Current South African toxicological databases for response data for indigenous organisms to salinity, needs expansion.

Hence, the aims of this study:

1. Generate acute toxicity data for salinity so as to expand the acute salinity toxicity database and thereafter use a wide range of freshwater invertebrate taxa to assess the relevance of Species Sensitivity Distribution-based salinity guidelines.

2. Evaluate the role of acute toxicity data in the development of salinity guidelines and make recommendations to water quality managers and those responsible for deriving Water Quality Guidelines.

4.1.2 Methods

4.1.2.1 Collecting test organisms

Wild-caught indigenous macroinvertebrates were selected as test organisms (DWAF, 2000). Fourteen different species were collected from four study sites in the Eastern Cape Province. Study sites were relatively unimpacted to ensure that organisms were not adapted to deteriorating water quality conditions (DWAF, 2000). The first site characterized by lotic riffles, situated in the Kat River is in close proximity to Amhurst Village (32°38'S, 26°41'E). The Kat River has its source in the Hogsback Mountains, flows through the small town of Fort Beaufort and eventually joins the Great Fish River. Organisms collected from this site included:

- mayflies *Afronurus barnardi* (Heptageniidae),
- *Euthraulus elegans* (Leptophlebiidae),
- *Tricorythus discolour* (Tricorythidae), and
- the freshwater limpet *Burnupia stemochorias* (Ancylidae).

The second site, a lotic riffle situated in the Balfour River (32°30'S, 26°40'E), is a tributary to the Kat River. Organisms collected here included:

- mayflies, *Baetis harrisoni* (Baetidae),
- *Demoreptus natalensis* (Baetidae);and
- *Oligoneuropsis lawrencei* (Oligoneuridae).

The third collection site, also a lotic riffle, was situated above Maden Dam (33°18'S 26°40'E) in the Buffalo River. Organisms collected from this site included:

- the mayfly *Caenid sp.1* (Caenidae),
- the black fly larvae *Simulium medusaeforme and Simulium sp.*, and
- snipe fly larvae *Suragina sp.* (Athericidae).

Mayfly nymphs from the Kat, Balfour and Buffalo rivers were collected according to methods recommended by DWAF (2000) except for Oligoneuridae nymphs from the Balfour River which were collected by rubbing a hand gently over an area of bedrock in fast flowing water and holding a hand-held net close to catch any dislodged nymphs. Nymphs were transferred to aerated cooler boxes containing river water and were aerated with battery-operated air pumps to maintain high oxygen levels during transportation (DWAF, 2000). Limpets were collected and transported according to methods described by Davies-Coleman (2002). Athericid larvae were collected using a modification of the SASS collection method for stones in current (Davies and Day, 1998) (collection time was not limited). Debris collected in the SASS net was transferred to a SASS tray and athericids sorted into 5-litre plastic jugs before being transferred to an aerated cooler box containing river water for transportation. Simuliidae were collected from riffle rocks by gently removing larvae using paintbrushes. Between five and ten larvae were placed in plastic petri dishes lined with moistened filter paper, placed on a layer of crushed ice (De Moor, 2002) in a large plastic container, for transportation.

Organisms were collected from a fourth study site, Dräger's Dam, situated on a private farm 15km outside of Grahamstown. The collection site, a lentic system characterised by marginal vegetation, is one of three catchment dams. The first of the dams fills up with surface runoff from the surrounding catchment during rainfall events which overflows into the second dam, which in turn overflows into the third dam. The second dam was chosen as a collection site due to easy accessibility and an abundance of marginal vegetation. Organisms collected included:

- the mayfly *Cloeon virgilae* (Baetidae),
- the bug *Plea pullula* (Pleidae),
- the damselfly nymphs *Coenagrionid sp.* (Coenagrionidae) and
- cased caddisflies *Leptoceridae sp.* (Leptoceridae).

All organisms were transferred to plastic bags containing river water and marginal vegetation, supported in a large bucket for transportation. No aeration was used, due to a short transportation time of 20 minutes.

Electric conductivity (EC), total dissolved solids (TDS), pH, temperature and dissolved oxygen (DO) readings were recorded at all field sites.

4.1.2.2 Identification of test organisms

Species identification was confirmed by the curators of the National Freshwater Invertebrate collection at the Albany Museum (De Moor and James, pers. comm.) with the exception of two species. *Burnupia stenochorias* was identified by Heather Davies-Coleman of the UCEWQ-IWR, Grahamstown. Athericid larvae were identified as a *Suragina sp.* by Dr. Brian Stuckenberg of the Natal Museum. The current collection of Caenidae at the Albany Museum are described according to 10 different species. The Buffalo River Caenidae nymphs were described as *Caenid sp.1* (James, pers. comm.). Two species of Simuliidae larvae were identified although identification of simuliidae was not confirmed to species level as this data was excluded from final results due to unsuitable test results with this organism.

Coenagrionidae nymphs were reared to adult stage to facilitate species identification. Coenagrionidae nymphs were reared by adapting a method described by Samways and Wilmot (2003). Modified honey jars were used as rearing vessels. Each honey jar had two squares cut from either side of it and this was replaced with a plastic mesh secured on with silicon. A hole was cut in the top of each lid. Each honey jar had two holes cut on either side near the top and threaded through with string so that the jars could be secured onto the sides of the glass tank. Six jars were then partly submerged into the glass tank filled with dechlorinated tap water. The tank was aerated with two aerators. Water quality parameters were measured (EC, TDS, °C and DO). A strip of plastic gauze was placed in each jar onto which the emerging adult could climb. One nymph of approximately 2cm in length was placed in each jar. The nymphs were fed with mosquito larvae (Samways and Wilmot, 2003) and small baetid nymphs. Five coenagrionid nymphs were reared successfully between days 13 and 34 after commencement of rearing.

Reared specimens were sent to the Albany Museum, Grahamstown for identification. Two species were identified: *Ischnura senegalensis* and *Enallagma sp*.

Further investigation was conducted using nymphs from Experiment 2 to determine diagnostic nymphal characteristics that could allow for the experimental sample to be separated out into the two sets of tolerance data for two different species. The diagnostic traits between the two species were the number of premental setae, but because early instar life stages and the identification guides were formulated for identification at late instar stages (Samways and Wilmot, 2003), the test individuals could not separated (premental setae could not be accurately counted as these were still developing at the instar stages). Hence, the Coenagrionidae nymphs were treated as a family complex comprising *Ischnura senegalensis* and *Enallagma sp.* and the number of organisms per experimental vessel was increased so as to minimise the chance of one species dominating.

4.1.2.3 Experimental systems

Salt exposure experiments were set up prior to field collection of organisms. Artificial stream systems or channels were used for organisms collected from lotic environments (Balfour, Kat and Balfour rivers), according to the protocol for artificial stream systems (DWAF, 2000). This protocol was adapted for the freshwater limpet *Burnupia stenochorias* (Ancylidae). Limpets were placed on plastic Petri-dishes and then submerged in the channel to allow the limpets an immediate and easy foothold on a substrate (**Figure 4.1**). Athericidae are known to be predacious and were therefore used in a lentic experimental system (50ml plastic vials) to keep larvae separate[1].

Figure 4.1 The UCEWQ-IWR wet laboratory – recirculating channels

Using the underlying principles of toxicity testing described in DWAF (2000), an experimental system was designed to accommodate organisms collected from a lentic aquatic system, i.e. Dräger's dam. The system was designed for predacious damselflies (Coenagrionidae) which had to be accommodated individually to avoid cannibalism (Samways and Wilmot, 2003). The

[1] no lotic experimental system, at the time of the experiment was conducted, had been developed to keep predacious organisms separate from each other – the experimental setup proved reliable as suitable tolerance data were generated for the athericids.

bottoms of 50ml plastic vials were sawed off, and a large hole was drilled in the lid. The containers were then turned upside down and mesh gauze was secured in the bottom with the lid (**Figure 4.2**). Two further holes were drilled in to allow several containers to be suspended on a single dowel stick, which was then suspended across the rim of a glass tank allowing three quarters of the container to be submerged in solution (**Figure 4.3**). Each tank was vigorously aerated and oxygen levels maintained in each vial. Plastic tubs were replaced with glass tanks to increase capacity of vials (from 24 to +40) to accommodate the variability introduced by two coenagrionid species (**Figure 4.5**). Vials were also used to conduct experiments using the *Cloeon virgilae* (Baetidae) collected from Dräger's Dam with approximately five baetids allocated to one pill container during a toxicity test.

Similarly, glass tanks were used with larger 500ml plastic vials to accommodate Leptoceridae larvae and Pleidae with an area for swimming. Vials had two squares cut from both sides and a plastic mesh secured in their place, using silicon. The plastic mesh allowed for circulation. Two holes were drilled in the top of each vial. String was thread through these holes and, using the string, the jars were secured to a single dowel stick with three vials jars per dowel stick.

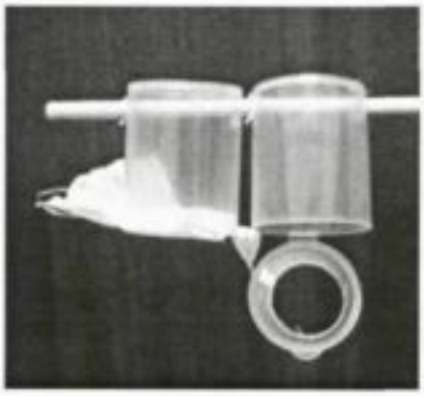


Figure 4.2 Two single 50ml plastic vials on left illustrating how gauze is secured into container

Figure 4.3 Lentic experimental system – glass tanks with 50ml plastic vials

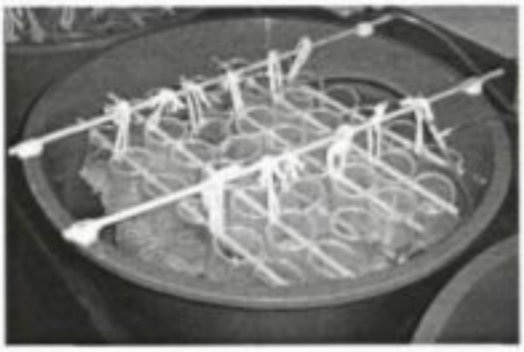


Figure 4.4 Lentic experimental system – plastic tubs with 50ml plastic vials

4.1.2.4 96-hour acute toxicity tests

De-chlorinated tap water was used as the diluent medium (prepared by passing tap water through a carbon filter) (DWAF, 2000). Experimental vessels were filled with de-chlorinated tap water to allow water to cool to laboratory temperature to ensure constant temperature of diluent medium. After transportation, organisms were transferred into experimental vessels excluding damaged organisms and those with wingbuds (indicating last instar before emergence) and allowed 36-hour acclimation time (DWAF, 2000). Prior to toxicants being added, experimental vessels were examined and acclimation deaths were removed, recorded and preserved in 75% ethanol to confirm identification. Vessels exhibiting >10% acclimation mortality were rejected from the experiment (DWAF, 2000). After acclimation, toxicants (NaCl or Na_2SO_4) were added to experimental vessels at selected concentrations using de-chlorinated tap water as the experimental medium.

Daily measurements of the following were taken in each experimental vessel: EC and TDS using Amel 160 and CyberScan 200 conductivity meters; pH using CyberScan 10 and Beckman 10 pH meters; DO using WTW OXI92 dissolved oxygen meter and temperature using a thermometer (0-50°C). Immobility was used at the test endpoint. Immobility was recorded at 12-hour intervals for a period of 96-hours, beginning at Day 0, PM i.e. 12-hours after toxicants were added. Dead or immobile organisms were collected at each of these readings, labelled and preserved in 75% ethanol. Surviving organisms at the end of the 96-hour period were also collected, labelled and preserved in 75% ethanol. Water samples were collected from each experimental vessel at the end of the experimental period for analysis of macro-elements, nutrients, phosphate, ammonium, dissolved oxygen and metals. Water quality analysis was undertaken by Resource Quality Services (RQS) DWAF. Laboratory equipment was washed and cleaned in detergent and 2% hydrochloric acid.

4.1.2.5 Data analysis

LC_{50} values were calculated using the parametric, Probit method where a parametric normalised distribution of the percentage of organisms responding to a chemical concentration is derived and a LC_{50} is estimated with 95% confidence limits (Cooney, 1995). Where the Probit model yielded a LC_{50} value, but without confidence limits a warning was given (that the data were not suited to this model), the Trimmed Spearman-Kärber (TSK) method was used to calculate LC_{50} values. The use of this non-parametric method was first recommended by Hamilton *et al.* (1977, In: Cooney, 1995) which uses interpolation to calculate the LC_{50}. A 20% trim of data was objectively decided upon as the upper acceptable limit of percentage trim. Where the Probit method was used, the calculated and tabulated Chi-squared values are reported. Where the TSK method was used the percentage trim is reported.

4.1.3 Results

A total of 35 acute toxicity data sets (18 for NaCl and 17 for Na_2SO_4) were generated for 14 different species. A summary of test species, organism source, experimental system and toxicant (salt) is listed in **Table 4.1**. Organisms that exhibited >10%mortality in the controls during tests are summarized in **Table 4.2**.

Table 4.1 Summary of 96-hour acute toxicity tests

Experiment No.	Date	Experiment type	Salt	Site	Species
1	06/3/03-10/03/03	Channels	NaCl	Kat	*Euthraulus elegans*
				Balfour	*Oligoneuropsis lawrencei*
				Balfour	*Demoreptus natalensis*
				Balfour	*Baetis harrisoni*
			Na_2SO_4	Kat	*Euthraulus elegans*
				Kat	*Afronurus barnardi*
				Kat	*Tricorythus discolor*
				Kat	*Burnupia stenochorias*
2	05/04/03-09/04/03	Glass tanks	NaCl	Dräger Dam	Coenagrionid sp. (RF)
3	17/04/03-21/04/03	Glass tanks	NaCl	Dräger Dam	*Cloeon virgilae*
				Dräger Dam	*Plea pullula* (RF)
			Na_2SO_4	Dräger Dam	*Cloeon virgilae*
				Dräger Dam	*Plea pullula* (RF)
				Dräger Dam	Coenagrionid sp. (RF)
4	08/05/03-12/05/03	Plastic tubs	NaCl	Dräger Dam	Coenagrionid sp.
			Na_2SO_4	Dräger Dam	Coenagrionid sp.
5	11/09/03-15/09/03	Glass tanks	NaCl	Dräger Dam	Leptoceridae (RF)
				Dräger Dam	*Plea pullula*
			Na_2SO_4	Dräger Dam	Leptoceridae (RF)
				Dräger Dam	*Plea pullula*
6	25/09/03-29/09/03	Plastic tubs	NaCl	Dräger Dam	Leptoceridae
			Na_2SO_4	Dräger Dam	Leptoceridae
7	16/10/03-20/10/03	Glass tanks	NaCl	Dräger Dam	*Plea pullula*
			Na_2SO_4	Dräger Dam	*Plea pullula*
8	30/10/03-03/11/03	Plastic tubs	NaCl	Dräger Dam	Coenagrionidae
			Na2SO4	Dräger Dam	Coenagrionidae
9	13/11/03-17/11/03	Channels	NaCl	Balfour	*Baetis harrisoni*
				Balfour	*Demoreptus natalensis*
				Kat	*Burnupia stenochorias*
			Na_2SO_4	Balfour	*Baetis harrisoni*
				Kat	*Euthraulus elegans*
				Kat	*Burnupia stenochorias*
10	29/01/04-02/02/04	Channels	Na_2SO_4	Balfour	*Oligoneuropsis lawrencei*
				Balfour	*Demoreptus natalensis*
11	01/03/04-05/03/04	Glass tanks	NaCl	Buffalo	Athericidae
		Channels	NaCl	Buffalo	Simulidae
				Buffalo	*Caenid sp.1*

The mayfly *Demoreptus natalensis* exhibited >10% control mortality for every experiment the organism was used in (experiments 1, 9 and 10), whereas *Baetis harrisoni* exhibited >10% control only in one experiment (experiment 1). Simuliidae larvae exhibited both high acclimation and control mortalities, 39.29% (±13.02%) and 80% respectively.

Table 4.2 Organisms exhibiting >10% control mortalities
*This data set was not excluded as control mortality between 10-20% was still considered acceptable

Experiment	Salt	Organism	Numbers responding	Numbers exposed	% control mortality
1	NaCl	*Baetis harrisoni*	12	25	48.0
		Demoreptus natalensis	6	8	75.0
7	NaCl	*Plea pullula*	22	39	56.4
	Na_2SO_4	*Plea pullula*	17	44	38.6
9	NaCl	*Demoreptus natalensis*	2	17	11.8*
10	Na_2SO_4	*Demoreptus natalensis*	9	15	60.0
11	NaCl	Simulidae	12	15	80.0

LC_{50} values for all organisms for NaCl and Na_2SO_4 are reported in **Tables 4.3** and **4.4** respectively. LC_{50} values for NaCl ranged from the most sensitive organisms 1807mg/L for *Baetis harrisoni* to 24410mg/L for Coenagrionidae and from 280mg/L for *Demoreptus natalensis* to 29927mg/L for Coenagrionidae for Na_2SO_4. LC_{50} values could not be calculated for experiment 3 for *Plea pullula* as this was a range finding experiment. This suggests the concentration ranges selected were unsuitable for the organisms to exhibit a typical sigmoidal concentration-response curve (Rand *et al.*, 1995).

Table 4.3	Summary of LC 50 data for NaCl *Mimimum required trim was too large (81.2%), therefore LC50 not be calculated **Data excluded where control mortality >20%							
Organism	**Exp. No.**	**LC50**	**Upper and Lower Confidence Limits (UCL/LCL)**		**Method of LC50 derivation**	**χ^2 calculated**	**χ^2 tabulated**	**% Trim**
Baetis harrisoni	9	1807.09	1243.00	LCL				
			2627.15	UCL	TSK			16.67
Baetis harrisoni	1	1950.00	1350.00	LCL				
			2800.00	UCL	TSK			0.00
Burnupia stenochorias	9	3899.00	3572.00	LCL				
			4299.00	UCL	Probit	9.29	12.59	
Demoreptus natalensis	9	3800.00	3320.00	LCL				
			4340.00	UCL	TSK			0.83
Demoreptus natalensis	1	4370.00	3490.00	LCL				
			5470.00	UCL	TSK			0.00
Cloeon virgilae	3	4682.19	4282.01	LCL				
			5119.78	UCL	TSK			1.96
Caenid sp.1	9	4800.00	3130.00	LCL				
			7350.00	UCL	TSK			6.25
Oligoneuropsis lawrencei	1	4858.67	4286.10	LCL				
			5314.81	UCL	Probit	1.67	14.10	
Leptoceridae	5	5620.00	4430.00	LCL				
			7140.00	UCL	TSK			0.00
Euthraulus elegans	1	6674.82	5177.52	LCL				
			8605.13	UCL	TSK			6.45
Plea pullula	5	6740.00	5220.00	LCL				
			8710.00	UCL	TSK			0.00
Leptoceridae	6	8290.00	7530.00	LCL				
			9120.00	UCL	TSK			0.00
Plea pullula	3			LCL				
				UCL	*			
Plea pullula	7	10190.00	9300.00	LCL				
			11160.00	UCL	TSK			0.00
Suragina sp.	11	18606.00	14074.00	LCL				
			25952.00	UCL	Probit	4.88	11.07	
Coenagrionidae	8	20056.00	18945.00	LCL				
			21046.00	UCL	Probit	1.43	12.59	
Coenagrionidae	4	21397.26	20300.01	LCL				
			22553.81	UCL	TSK			0.00
Coenagrionidae	2	24410.00	2188.00	LCL				
			2722.00	UCL	TSK			0.00

Table 4.4	Summary of LC 50 data for Na_2SO_4 *Mimimum required trim was too large (90.3%), therefore LC50 not be calculated **Data excluded where control mortality >20%							
Organism	**Exp. No.**	**LC50**	**Upper and Lower Confidence Limits (UCL/LCL)**		**Method of LC50 derivation**	**χ^2 calculated**	**χ^2 tabulated**	**% Trim**
Demoreptus natalensis	10	280.02	78.96	LCL				
			381.01	UCL	Probit	6.46	11.10	
Oligoneuropsis lawrencei	10	704.31	482.74	LCL				
			1075.26	UCL	Probit	1.68	11.07	
Cloeon virgilae	3	3368.59		LCL				
				UCL	TSK			1.52
Baetis harrisoni	9	3647.38	3091.39	LCL				
			4303.37	UCL	TSK			15.79
Burnupia stenochorias	1	4580.17	3786.45	LCL				
			5540.28	UCL	TSK			0.00
Burnupia stenochorias	9	5272.00	4836.00	LCL				
			5801.00	UCL	Probit	9.28	12.60	
Afronurus barnardi	1	5923.67	4839.18	LCL				
			7128.60	UCL	Probit	4.78	14.07	
Euthraulus elegans	1	7907.97	7271.97	LCL				
			8599.60	UCL	TSK			17.86
Euthraulus elegans	9	9264.77	8759.17	LCL				
			9799.56	UCL	TSK			6.85
Plea pullula	3			LCL				
				UCL	*			
Plea pullula	5	9355.00	7117.00	LCL				
			11487.00	UCL	Probit	3.76	11.07	
Tricorythus discolor	1	9402.13	8234.00	LCL				
			12187.46	UCL	Probit	7.13	14.07	
Leptoceridae	5	9720.00	7910.00	LCL				
			11950.00	UCL	TSK			4.00
Plea pullula	7	10000.00	7630.00	LCL				
			13100.00	UCL	TSK			6.88
Leptoceridae	6	11345.00	10555.00	LCL				
			12343.00	UCL	Probit	5.82	14.07	
Coenagrionidae	8	23609.00	22633.00	LCL				
			24505.00	UCL	Probit	3.57	12.59	
Coenagrionidae	4	26224.00	25026.00	LCL				
			27365.00	UCL	Probit	6.87	14.07	
Coenagrionidae	3	29 926.71	24 223.67	LCL				
			36 972.42	UCL	TSK			14.29

4.1.4 Discussion

4.1.4.1 The use of South African indigenous macroinvertebrates in acute toxicity tests

Certain organisms did not prove to be suitable test organisms. These included the baetid *Demoreptus natalensis*, the bug *Plea pullula* and the simulid larvae. According to DWAF's protocol for using riverine invertebrates in acute toxicity tests, baetids should only be used if other test organisms are not available, as they often occur in species complexes (DWAF, 2000). The high control mortalities observed for the baetids can be attributed to handling stress and the lack of suitable numbers of organisms per experimental vessel (at least 40 organisms per experimental vessel is recommended, DWAF, 2000). Although *Baetis harrisoni* exhibited >10% mortality for one experiment in this study, *Baetis harrisoni* collected from lotic aquatic systems have successfully been used in previous toxicity tests (Binder, 1999; Williams, 1996). Because baetids require extra care and minimal handling, it is suggested that collecting trips are not allocated for various different organisms for one trip but that collecting be allocated to baetids only (to minimise collecting time and handling stress).

LC_{50} values could be calculated for *Plea pullula* (excluding the range finding experiment no.3), although the concentration response curves continued to display inconsistencies in following a typical sigmoidal curve (Rand *et al.*, 1995). This may be due to the fact that they are air-breathing organisms (Gerber and Gabriel, 2002) and could have been displaying behavioural avoidance to the toxicant. Some Pleids also became buoyant as a result of handling, inhibiting swimming. If pleids are to be considered for future acute toxicity tests, handling methods would need to be refined so as to minimize inconsistencies in results. If simuliidae are to be considered in future tests, the method of collection and the nature of the experimental vessel would need to be reviewed. The method of larval transportation as described by De Moor (2002) is for rearing purposes and might not be suitable for toxicity purposes. Furthermore, Simuliidae are found in very fast flowing reaches (Davies and Day, 1998) of a river and the recirculating channels might not provide the most suitable exposure-habitat for the larvae.

Most of the test organisms in this study proved to be suitable test organisms with the exception of Pleidae and simuliidae larvae, and in some instances baetid larvae. Repeated toxicity tests on individual species would help assess the variability in responses introduced from field-collected organisms.

This study also provides a valuable platform for further development of experimental methods using invertebrates collected from lentic aquatic environments.

4.1.4.2 South African acute toxicity data for salinity

Three main sources of literature exists that might allow for some comparison of salinity tolerance data. These include, Hart *et al.* (1991), Kefford *et al.* (2003) and the AQUIRE database of toxicology data (USEPA, 2002). Hart *et al.* (1991) reviews the salt sensitivity of Australian freshwater biota including invertebrates and reports the range of field salinities that invertebrates are found in and where possible, their relative abundances. Kefford *et al.* (2003) conducted 72-hour acute toxicity tests using Ocean Nature salt which has a similar ionic composition to sea water. LC_{50} values are reported as EC (mS/cm). The AQUIRE database reports 48-hour and 96-hour LC_{50} values for both NaCl and Na_2SO_4 as well as other salts. LC_{50} values are reported as TDS (mg/L). Problems arise when trying to compare South African salinity tolerance data with international data as there is no international standard for reporting salinity. Salinity can be reported in electrical conductivity (EC) (mS/m, µS/m, mS/cm) or in total dissolved solids (TDS) (ppm, mg/L, g/L). Conversions from EC to TDS exist TDS (mg/L) = EC (mS/m at 25°C) x 6.5 (DWAF, 1996a), however errors could be incurred in converting values where the ionic make-up of the dissolved solid is not known as the factor of 6.5 is influenced by the ionic make-up of dissolved solids and can range from 4.0 to 9.0. Some work has been done to derive conversions for individual salts using regression equations (Palmer *et al.*, 2004). Despite these contributing factors, some comparisons are shown in **Table 4.5**, comparing LC_{50} data for taxa which are common to both the AQUIRE database, Kefford *et al.* (2003) and this study. These taxa include baetids (Ephemeroptera), other Ephemeroptera, Trichoptera, Hemiptera and Odonata. LC_{50} values reported in mg/L from the AQUIRE database and this study were converted to mS/cm using the conversion:

EC (mS/m) = TDS (mg/L) ÷ 6.5 (DWAF, 1996a)

Table 4.5 LC_{50} values for taxa common to three salinity tolerance studies (all values reported in mS/m)

Taxa	Kefford *et al.*, 2003 – 72hr LC50 with Ocean nature salt	This study		Other studies (from Kefford, *et al.*, 2003)	AQUIRE database
		NaCl	Na_2SO_4		
Baetidae	550-620	278-739 (x=580, n=4)	43-561 (x=374, n=3)	100-1400	
Ephemeroptera	>1260-1400	748-1027 (x=887, n=2)	108-1447 (x=1022, n=5)	1400-2000	385 (n=1)
Trichoptera	6400->25 600	865-1275 (x=1070, n=2)	1495-1745 (x=1620, n=2)	930-3400	35-869 (x=208, n=3)
Hemiptera	1500-25 600	1037 (n=1)	1439 (n=1)	1800	
Odonata	>12 600-6000	3085-3755 (x=3378, n=3)	3632-4604 (x=580, n=3)	2000-2100	3538-3692 (x=3615, n=2)

Between taxa, salinity responses from this study followed similar trends to the other two studies. The range of LC_{50} values for the five taxa for the three studies are between 550 and 6000mS/m (Kefford *et al.*, 2003), 278 and 4604mS/m (this study) and between 385-3692mS/m (AQUIRE database). Baetids proved to be the most salt sensitive taxa with the lowest range of LC_{50} values (**Table 4.5**), followed by Ephemeroptera, Trichoptera and Hemiptera. Odonata exhibited the highest tolerance to salinity in all three studies, this study exhibiting the highest LC_{50} of 4604mS/m. Hart *et al.* (1991) report finding Odonata present in field salinities of up to 3230mS/m.

These studies show that invertebrates that share taxonomic characteristics, behave similarly to acute osmotic stress caused by unnaturally highly saline environments. However, the ionic make-up of different salts is known to affect living organisms differently (Mount *et al.*, 1997) and individual salts are known to vary in toxicity (Jooste and Rossouw, 2002; Palmer *et al.*, 2004). This has highlighted the need for water resource managers to establish ionic compositions of water bodies to facilitate a more accurate approach in water resource management. Unfortunately, such an approach is time consuming and data are limited. Methods have been developed for South Africa which propose how EC can be used in water quality management as part of Reserve assessment, taking into account individual ion toxicity and conversion factors from EC to TDS and vice versa (Palmer *et al.*, 2004). Furthermore, the chronic effects of long-term exposure of lower concentrations of salts on invertebrates and other aquatic organisms are not accurately quantified in literature and should be highlighted for further research possibilities. Because chronic toxicity tests are expensive, time consuming and complex to conduct, acute response data is often used to extrapolate chronic response. Hence, this study makes a valuable contribution towards those considering undertaking acute to chronic extrapolations. The need to understand chronic effects of salinity on aquatic ecosystems is important as these could have much broader impacts on aquatic ecosystems as a whole. These impacts could include a decrease in biodiversity, lowering in productivity and changes in the natural characteristics of aquatic ecosystems (Williams, 1999), as well as a breakdown of food-web structure, loss of riverine habitat and overall environmental degradation (Bailey and James, 2000).

4.1.5 Conclusion

Most of the test organisms in this study proved to be suitable test organisms with the exception of Pleidae and the Simuliidae larvae. Further toxicity tests would have to be conducted on each organism to assess the variability in responses introduced from field-collected organisms. Further tests would also assess the validity of using the lentic experimental system to develop a protocol for organisms collected from lentic systems. However, some of these data represent concentration-response data for South African indigenous invertebrates that have not been used before. Hence, they provide valuable benchmarks for future testing.

This study has shown that invertebrates are salt sensitive and can exhibit a wide range of tolerances to salinity. South African invertebrate taxa follow similar trends in salinity responses compared to international taxa. It is recommended that the tolerance data generated from this study can best be applied in water resource management by adopting the Aldenburg and Slob (1993) approach and using these data to derive SSDs to derive water quality criteria (Warne, 1998). Furthermore, there is a need for an international standard in reporting salinity tolerance data so as to facilitate comparisons between similar studies. The process of developing accurate water quality guidelines in South Africa for salinity is a complex one, but should remain under review as more data is gathered using indigenous organisms for toxicity tests and as methods for applying this data in water resource management are refined.

4.2 GENERATING CHRONIC MORTALITY DATA FOR SODIUM CHLORIDE AND SODIUM SULPHATE USING TWO-STEP LINEAR REGRESSION ANALYSIS (LRA) AND MULTI-FACTOR PROBIT ANALYSIS (MPA) AS EXTRAPOLATION TECHNIQUES

Andrew R Slaughter

4.2.1 Introduction

South Africa's freshwater resources are utilized by the industrial, agricultural and domestic sectors of South Africa and are subject to intensive use. There is therefore the need for legislation to ensure freshwater resources in South Africa are used in a sustainable manner. The South African National Water Act (No. 36 of 1998) (NWA) stipulates the need for an ecological Reserve. The NWA defines the ecological Reserve as the water required in terms of both quantity and quality, to sustain the health of aquatic ecosystems while allowing sustainable use of water resources.

Water quality is an important factor affecting the health of aquatic ecosystems (Jooste and Rossouw, 2002). Water quality guidelines are numerical concentration units or narrative statements for substances, recommended to support and maintain a designated water use or ecosystem condition (ANZECC and ARMCANZ, 2000). Environmental water quality guidelines are generally derived with the intention of providing some confidence that there will be no significant impact on aquatic ecosystem health if they are achieved (ANZECC and ARMCANZ, 2000).

Various countries have compiled water quality guidelines including Australia (ANZECC and ARMCANZ, 2000) and South Africa (DWAF, 1996a-g). The South African Water Quality

Guidelines provide water quality guidelines for the protection of South African freshwater resources (DWAF, 1996a). In addition, Jooste and Rossouw (2002) have proposed generic benchmarks for various system variables nutrients and toxicants for South African freshwater resources, taking account of different levels of ecosystem health.

The salinization of South Africa's fresh water resources has become an increasingly serious water quality problem (Van der Merwe and Grobler, 1990). The term salinization refers to the process where water in fresh water resources becomes salty, but the term 'salt' encompasses a large number of chemical compounds. A study by Day (1993) showed that less saline waters of southern Africa are dominated by salt inputs from the catchment substratum, while waters subject to evaporation or agricultural input are dominated by the salt sodium chloride (NaCl). Generally sodium sulphate (Na_2SO_4) is the salt associated with industrial or mining input (DWAF, 2000). A Water Research Commission (WRC) workshop held in 1989 (Anon, 1990) recognized that there is a strong trend of increasing salinity in South African water resources and identified the following main causes:

- flow reduction in rivers
- evaporative losses in surface waters
- irrigation return flow
- runoff from dry-land agriculture
- saline industrial effluents
- urban development contribution point and non point sources
- atmospheric deposition.

Salinization has been shown to have a negative effect on fresh water biota in numerous studies. Kefford (1999) showed that there was a decrease in species richness and abundance in the macro-invertebrate community at sites in the Barwon River in Australia associated with saline water disposal while Kefford and Doeg (1999) showed a decline in macro-invertebrate faunal abundance and community species richness associated with an increase in salinity in two irrigation channels in Victoria, Australia. A reduction in the variability in salinity can have an affect on euryhaline fish species and general high salinities may affect stenohaline fish species (Bluhdorn and Arthington, 1995). Changes in salinity may also allow invasive aquatic species to become dominant (Bluhdorn and Arthington,1995).

Since salinization of fresh water resources seems to reduce biodiversity and change community structure, salinization may well have negative effects on freshwater ecosystem integrity. Therefore salinization is a problem that should be addressed with urgency if fresh water resource use in South Africa is to be sustainable.

Salinity water quality guidelines for South African water resources do exist. The South African Water Quality Guidelines (DWAF, 1996a) give ion specific guidelines based predominantly on data obtained from international databases and literature, and recommend that TDS should not vary by more than 15% from natural. There has been an increasing awareness of salts as toxicants (Goetsch and Palmer, 1997; Kefford, 2003). Jooste and Rossouw (2002) compiled inorganic salt specific benchmarks based on USEPA toxicity data, using a model to account for ion recombination in salt mixtures, and based on 95% protection of species biodiversity. Very limited South African indigenous species tolerance data was used to compile the benchmarks and the guidelines (Jooste and Rossouw, 2002; DWAF, 1996a).

Aquatic toxicity data are generally the basis for the derivation of toxicant guidelines (ANZECC and ARMCANZ, 2000; DWAF, 1996a). The endpoints used for guideline development are often levels of a toxicant that are deemed 'safe' for the test organism used and are the results of acute and chronic toxicity tests. Acute toxicity tests are typically run over a short time period which may only encompass a fraction of the test organism's life cycle and tend to measure mortality as a test end point (Rand *et al.*, 1995). Chronic toxicity tests measure long term effects of a toxicant on sub-lethal endpoints such as mortality, growth and reproduction and should be run over the test organism's entire life cycle or at least a major part of the life cycle (Rand *et al.*, 1995). Chronic toxicity test data can therefore be used to generate more relevant water quality guidelines, which are more protective of the aquatic ecosystem.

Besides the compilation of water quality guidelines, toxicity data are also used in ecological Reserve assessments and resource classification (Jooste and Rossouw, 2002). It has been suggested that river ecosystem health in South Africa should be classified according to the following classes (Palmer *et al.*, 2002):

- Natural
- Good
- Fair
- Poor.

Benchmark values define the boundaries between these classes. Jooste and Rossouw (2002) used the 5^{th} percentiles of all chronic data available to define the boundary between "Natural" and "Good" health classes for a river, while acute toxicity data are used to define the upper boundary for the "Poor" class.

Although both acute and chronic response data are used to set the resource quality objectives associated with ecosystem health classes, there are considerably more data available on acute salt tolerance responses, both nationally and internationally, than on chronic salt tolerances. Many more acute toxicity data are available because of the expense and difficulty of running chronic toxicity tests.

Ion specific salinity guidelines given by the South African Water Quality Guidelines (DWAF, 1996) and inorganic salt specific benchmark boundary values proposed by Jooste and Rossouw (2002) need to be validated, and amended if necessary, by the results of chronic salinity toxicity data for aquatic biota indigenous to South Africa. Therefore there is a need for both chronic experimental research and reliable extrapolations of acute response data to chronic responses.
Various acute to chronic mortality data extrapolation methods have been proposed (Lee *et al.*, 1995; Mayer *et al.*, 1994; Sun et al, 1995; Warne, 1998). Although extrapolated chronic data are relatively simple to generate, the resultant guideline values need to be validated with the results of experimental chronic toxicity tests to assess their accuracy.

In this study, chronic mortality extrapolations were generated from acute mortality data using the Two – Step Linear Regression Analysis (LRA) (Mayer *et al.*, 1994) and the Multi-Factor Probit Analysis (Lee *et al.*, 1995) techniques, for the salts NaCl and Na_2SO_4. Acute toxicity data were obtained from the MSc research of Samantha Browne and the Unilever Centre for Environmental Water Quality (UCEWQ) database, which contains results of toxicity tests undertaken using mainly indigenous invertebrates. In conjunction with these extrapolated results, chronic sub-lethal toxicity data were obtained by running a three-month chronic growth and survival toxicity test on the indigenous fresh water shrimp, *Caridina nilotica*, using NaCl as the toxicant.

The aims of this study were to produce chronic salinity data via a process of acute to chronic extrapolation and validation through chronic experimental data.

4.2.2 Methods

4.2.2.1 Acute data

Acute toxicity data were obtained from the Institute for Water Research (IWR) - Unilever Centre for Environmental Water Quality (UCEWQ) toxicity database. The database contains acute data for a range of indigenous invertebrates exposed to a range of toxicants(Scherman *et al.*,2003). The toxicity data on the database has been obtained using organisms from various areas of South Africa (**Figure 4.5**):

- Eastern Cape: Bushman's River, Kat River, Balfour River, Palmiet River
- KwaZulu Natal: Mooi River
- Western Cape: Breede River, Keurbooms River
- Gauteng: Vaal River
- Northern Province: Sabie River, Olifants River

The toxicological data therefore represents the salinity tolerance of indigenous macro-invertebrates on a national level.

Figure 4.5 Map of South Africa showing rivers from which macro-invertebrates were collected for acute toxicity tests contained in the UCEWQ-IWR toxicity database where the toxicants used were sodium chloride and sodium sulphate. The map also displays provincial boundaries and major cities/towns near the rivers.

4.2.2.2 *LRA acute – chronic extrapolations*

Two – Step Linear Regression Analysis (LRA) is an extrapolation method that considers concentration of toxicant, response to toxicant and time-course of effect simultaneously. LRA involves two regression processes run sequentially. As outlined in Mayer *et al.* (1994) the first regression process is the determination of LC_x values at each time period of observation within relevant toxicity tests. For example using the data of one acute toxicity test, an LC_1 value can be generated for response data taken at 12-hours, another at 24-hours etc. The regression model chosen to obtain the LC_x value is the model that best fits the data. The second regression involves plotting the LC_x values as the dependant variable versus the reciprocal of time as the independent variable. The Y intercept of this regression gives an indication of the LC value over an infinite time period or chronic exposure period.

Toxicological data were grouped by toxicant, and organism, for analysis. Measured salt concentrations and response data for each concentration, at all measured time periods during the acute tests, were recorded. Records were grouped by experiment identification number, replicate and time for analysis. Acute tests (<96-hours exposure) were rejected where there were greater than 10% mortality in the control and short-term chronic tests (>96 hours and up to 10-days exposure) were rejected where there were greater than 20% mortality in the control (Rand *et al.*, 1995). Even when toxicity tests had unacceptably high control mortalities at the time the test ended, survival observations would still have been used at observation times during the test when control mortalities were still acceptable.

The Probit test (Probit Program Version 1.5 developed by the Ecological Monitoring Research Division of the USEPA), or least–square linear regression equations with data transformations other than those used in the Probit test, were generated for each time period in each test. Probit data were rejected where the data did not fit the model (as indicated by a chi-square value) or where the slope of the regression generated by Probit was not significant. Least–square regression was performed using Statistica Version 6 when the Probit model did not fit the data, with the following being regressed:

Regression equation 1:

% mortality = Y intercept + slope * toxicant concentration

OR

Regression equation 2:

log(% mortality + 1) = Y intercept + slope * log(toxicant concentration + 1).

The LC_x value from the regression giving the highest R^2 value was selected from
regression one and two. $LC_{0.01}$, $LC_{0.1}$, LC_1, LC_5 and LC_{10} values were generated from the valid Probit equations or from regression one or two. Low LC_x values ($LC_{0.01} - LC_{10}$) were selected as they are low toxicity endpoints and may represent chronic toxicity endpoints (Lee *et al.*, 1995; Mayer *et al.*, 1994). The LC_x values grouped, by toxicant and organism, were plotted over time to assess whether to use or reject data. Data that did not show increasing toxicity with time were rejected. Values for 1/time, 1/log(time) and $\log(LC_x)$ were generated.

In Statistica, R^2 values for the following regressions were established:

i. $LC_x = y + m/t$
ii. $\log_{10}LC_x = y + m/t$
iii. $\log_{10}LC_x = y + m/\log_{10}t$

where:

- LC_x represents a toxicity value ranging from $LC_{0.01} - LC_{10}$ generated from mortality observations taken at time t,
- y represents the y-intercept of the regression,
- m represents the slope of the regression line, and
- t represents the observation time.

The regression that gave the highest R^2 value was chosen and the regression line plotted in Statistica. The y – intercept of each regression line estimantes the magnitude of an indefinite LC_x value.

4.2.2.3 MPA acute – chronic extrapolations.

Multifactor probit analysis is an acute to chronic toxicity data extrapolation method that simultaneously evaluates concentration, time and effect and was first proposed by Lee *et al.* (1995). The method uses two basic models of which there are three variations of each, depending on the data transformations used (Lee *et al.*, 1995). As stated by Lee *et al.* (1995), the first model assumes that the derivative of Probit P (probability of death) with respect to C_x (concentration of a toxicant causing a certain effect) is constant at each acute exposure time. This is termed 'parallelism'.

Equation 1:

$$\text{Probit}\,(P) = \alpha + \beta C_x + \delta T_x$$

The second model given by Lee *et al.* (1995) is the model for non-parallelism and allows for a change in slope as exposure time varies.

Equation 2:

$$\text{Probit}\,(P) = \alpha + \beta C_x + \delta T_x + \gamma\, CT_x$$

where:

- P is probability of death,
- C_x may be concentration or log concentration,
- T_x is exposure time or the reciprocal of exposure time or the reciprocal of log exposure time.

Other parameters, namely α, β, δ and γ are estimated via maximum likelihood techniques.

Lee *et al.* (1995) gave six variant models based on the two main models stated in Equation 1 and 2.

Pair **A** based on log dose and log time:

Ai: $\text{Probit } P = \alpha + \beta \log(C) + \delta \log(T)$

where: $C = 10^{(\text{Probit } P - \alpha - \delta \log(T))/\beta}$

Aii: $\text{Probit } P = \alpha + \beta \log(C) + \delta \log(T) + \gamma(\log(C)\log(T))$

where: $C = 10^{(\text{Probit } P - \alpha - \delta \log(T))/(\beta + \gamma \log(T))}$

Pair **B** based on log dose and reciprocal of time:

Bi: $\text{Probit } P = \alpha + \beta \log(C) + \delta/T$

$$\text{where: } C = 10^{(\text{Probit } P - \alpha - \delta/T)/\beta}$$

Bii: $\text{Probit } P = \alpha + \beta \log(C) + \delta/T + \gamma(\log(C))/T$

$$\text{where: } C = 10^{(\text{Probit } P - \alpha - \delta/T)/(\beta + \gamma/T)}$$

Pair **C** based on log dose and reciprocal of log time:

Ci: $\text{Probit } P = \alpha + \beta \log(C) + \delta/\log(T)$

$$\text{where: } C = 10^{(\text{Probit } P - \alpha - \delta/\log(T))/\beta}$$

Cii: $\text{Probit } P = \alpha + \beta \log(C) + \delta/\log(T) + \gamma(\log(C)/\log(T))$

$$\text{where: } C = 10^{(\text{Probit } P - \alpha - \delta/\log(T))/(\beta + \gamma/\log(T))}$$

Associated with each model is a 'goodness of fit' statistic that reflects the degree of discrepancy between the actual responses in the data and the responses estimated from the model. The results from the model with the best 'goodness of fit' statistic is chosen. Making 'C' (concentration of toxicant) the subject of the formula will give the concentration of a toxicant that will cause a particular effect. The degree of effect is specified by Probit P and T (time of exposure) and must be entered at the modeller's discrepancy. Some idea of the life cycle of the test organism or the time taken for mortality to stabilise (threshold time) in a toxicity test (Jooste and Rossouw, 2002) is needed to enter a meaningful value for T in the models.

Concentrations causing a small degree of effect can be estimated such as 0,01% - 10 % effect (Lee *et al.*, 1995).

The models for Multifactor Probit Analysis were run in Microsoft Excel. Only acute toxicological data where there were at least 5 concentrations and 4 observation times were used (recommended by Lee *et al.*, 1995). The format of the spreadsheet follows the format recommended by Caux and Moore (1996) for running models in Microsoft Excel. The spreadsheet has a data entry section, a summary section and a section for each of the specified six models. A goodness of fit statistic is calculated as:

$$\Sigma_{(i=1-n)} (O_i - E_i)^2/E_i \text{ (Rand } et\ al.\text{, 1995)}$$

where:

- O_i is the observed effect,
- E_i is the expected effect calculated from the model, and
- n is the number of data entries for the acute toxicological data available.

The parameters α, β, δ and γ for the models are estimated via maximum likelihood techniques using SOLVER in Excel. The threshold exposure time to be inputted into the model is estimated by plotting low LC_x data for a particular organism exposed to a particular toxicant against time. The time taken for the LC_x values to stabilise is taken as the threshold time. This analysis is run in STATISTICA Version 6. Jooste and Rossouw (2002) found that LC50 values generally stabilized after 10 days. Therefore, where there were insufficient LC_x data available to estimate a threshold time, a threshold time of 240-hours was assumed.

4.2.2.4 *Caridina nilotica chronic toxicity experiment*

Laboratory-reared *Caridina nilotica* neonates, aged less than 14-days, were used in an experiment where they were exposed to low concentrations of NaCl in three replicated concentration ranges. After 15-days mortality stabilized in all replicates, and lethality data were analyzed at the end of the three month experimental period.

Free swimming individuals were kept in glass tanks of approximately 12-litres volume dechlorinated tap water. Tank temperature was controlled by thermostats 24°C (± 1°C), with air conditioning set at 22 °C (± 2°C). The light/dark regime chosen was 12/12 hours. The test animals had a 48-hour acclimation period in the experimental tanks before exposure to salt. The concentration range for NaCl was 0, 200, 400, 600, 800, 1000, 1500 and 2000mg/L.

The shrimp were fed Tetramin® flakes enriched with algae. Tanks were also seeded with green algae attached to a ceramic tile. A piece of square netting was suspended in each tank to provide an attachment surface for young neonates. Faecal pellets were left in the tanks as the algae or bacteria growing on the pellets served as an additional source of food.

Test solutions were renewed once a week so as to prevent a build up of toxic excretory products.

Tanks were aerated at all times using air stones, and covered with plastic cling wrap to prevent excessive evaporation. Laboratory and individual tank temperatures were recorded daily. Electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), nutrients, light and pH were measured weekly, immediately prior to and after the water change.

Mortality data for all three replicates were grouped and analysed for the estimation of a no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). The mean accumulated mortality and standard deviation per concentration were plotted using whisker plots. Responses were taken to be significantly different where whiskers did not overlap. In addition mortality responses were analysed using ANOVA or a non-parametric alternative using Statistica Version 6.

These results were compared to the extrapolated LRA and MPA results to evaluate the validity of such extrapolations.

4.2.3 Results

4.2.3.1 *Caridina nilotica chronic experiment*

The last replicate in the *C. nilotica* experiment suffered complete mortality in 4 of the tanks during the acclimation period. Therefore a reduced concentration range was used, namely control, 200mg/L, 400mg/L, 600mg/L 800mg/L and 1000mg/L NaCl. The replicates were analysed together for the estimation of a NOEC but only within the concentration range of the last replicate.

The results of a Bartlett's Test using the mortality data showed that variance in all treatments was equal so an ANOVA was undertaken. The ANOVA indicated that the ratio of among treatment variance to within treatment variance was not significant at α = 0,05 due to high within treatment variance. The NOEC was therefore estimated by from the whisker plot of mean mortality plotted against concentration (**Figure 4.6**). The NOEC was estimated to be 800mg/L.

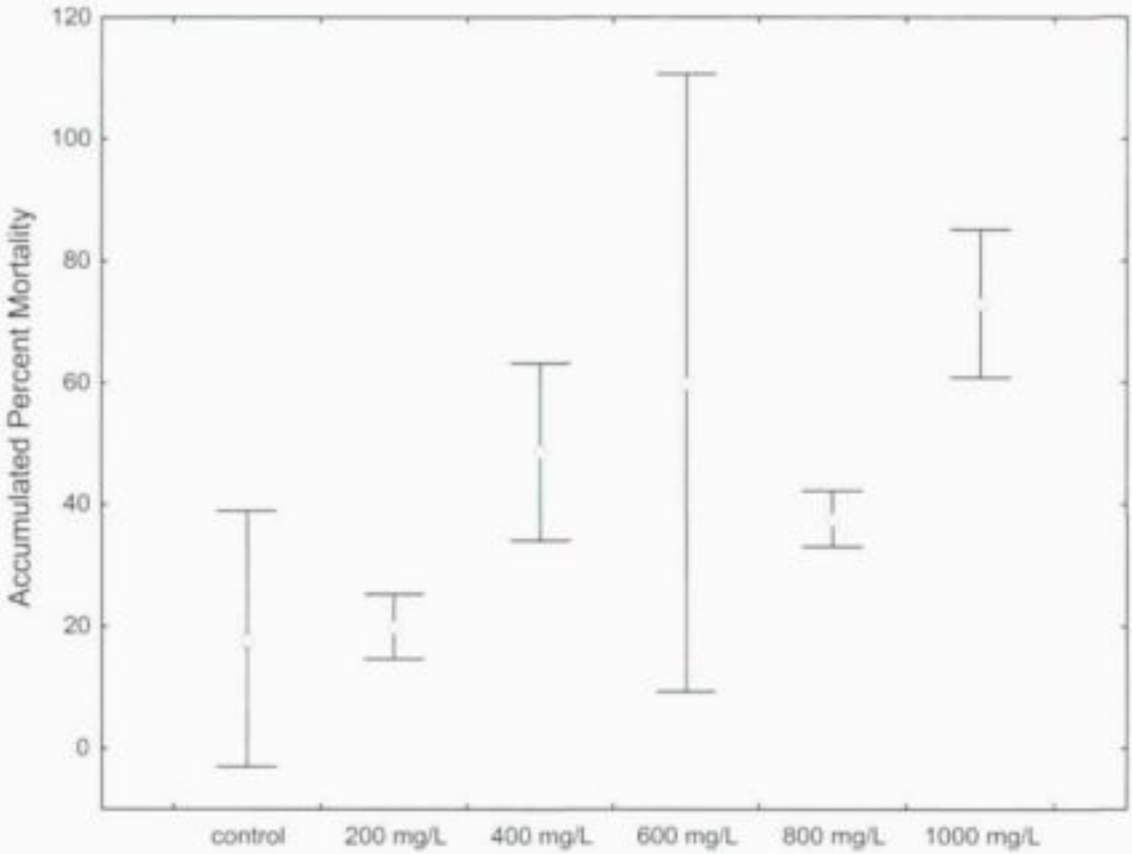


Figure 4.6 The means and standard deviations of mortality results for the three replicates grouped from the chronic toxicity experiment. The standard deviations of the highest concentration do not overlap with the standard deviations of the control treatment indicating that this treatment is a possible LOEC.

4.2.3.2 *Acute:chronic extrapolations*

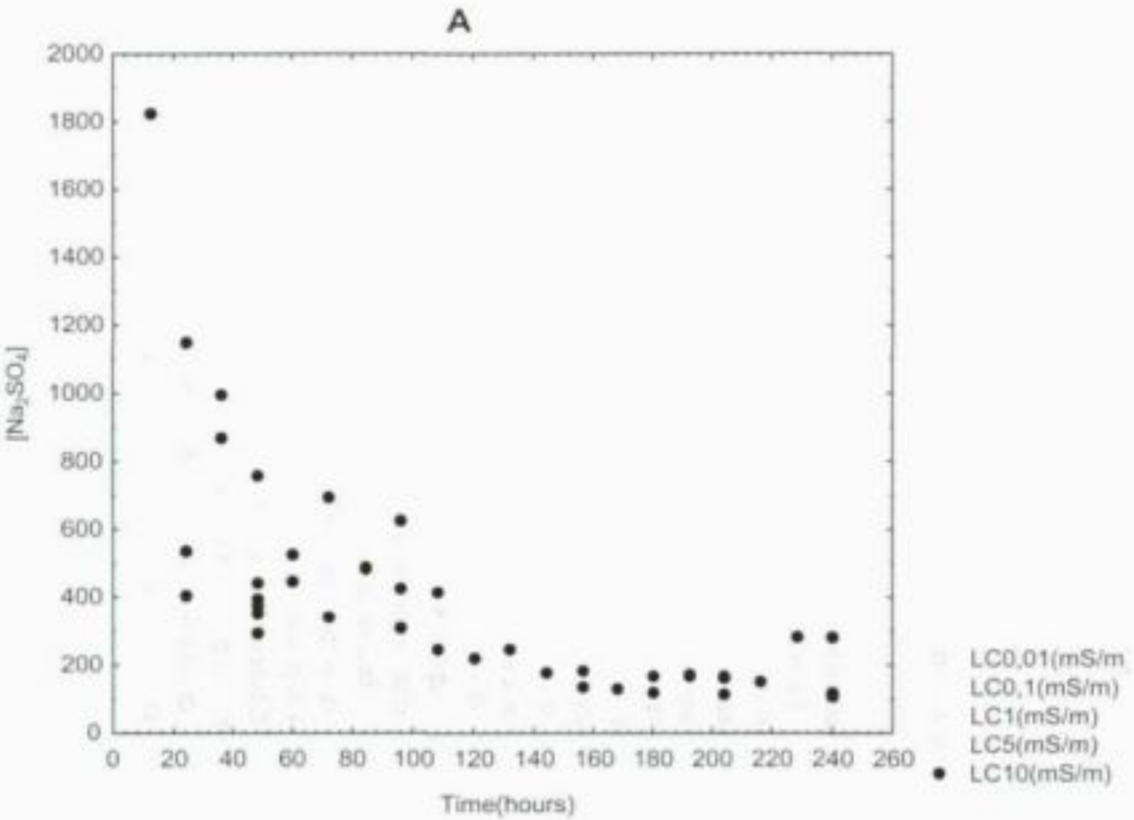
The results for acute to chronic extrapolation techniques using suitable acute toxicity data from the IWR/UCEWQ database are shown in **Table 4.6** and **Table 4.7**. In some cases, obtaining results for the full range of threshold LC_x values from 0,01 percent effect to 10 percent effect was not possible due to the unsuitability of the data. Threshold times for use in MPA were estimated by plotting low LC_x values against time (**Figures 4.7A-F and 4.8A-B**). A threshold time of 360-hours was used for *C. nilotica* exposed to NaCl since mortalities were found to stabilise after 15-days in the chronic NaCl experiment. A threshold time of 240-hours was assumed for *Adenophlebia sylvatica* exposed to NaCl since the available LC_x data was not suitable for estimating a threshold time.

Table 4.6 A summary of the LCx values obtained using LRA associated with R^2 values.
* Species used in chronic toxicity test

Species	Salt	$LC_{0,01}$		$LC_{0,1}$		LC_1		LC_5		LC_{10}	
		mg/L	R^2	mg/L	R^2	mg/L	R^2	mg/L	R^2	mg/L	R^2
Adenophlebia auriculata	NaCl	1966,6	0,89	1973,27	0,89	2026,6	0,89	2146,6	0,9	2540,6	0,92
Afronurus barnardi	NaCl	515,27	0,2					101,27	0,15	223,93	0,26
*Caridina nilotica**	NaCl	547,93	0,61	939,93	0,7	1593,27	0,75	2658,6	0,77	3511,27	0,76
Cloeon virgiliae	NaCl	86,6	0,7	729,93	0,38	1529,9	0,11	1791,27	0,23	1434,07	0,56
Euthraulus elegans	NaCl	63,9	0,55	233	0,54	560,2	0,52	1088,6	0,53	1543,27	0,57
Tricorythus discolor	NaCl									353	0,65
Adenophlebia auriculata	Na_2SO_4	1171	0,09	199	0,12	446,6	0,16	501,1	0,21	560,12	0,25
Afronurus barnardi	Na_2SO_4									363	0,95
Afroptilum sudafricanum	Na_2SO_4	167	0,5	458,2	0,48	907,2	0,47	1468,6	0,46	1875,8	0,4
Euthraulus elegans	Na_2SO_4							1331	0,98	731	0,99

Table 4.7 A summary of the LC_x values obtained using MPA associated with goodness of fit values.

Species	Salt	Time to Threshold	Model	λ^2 goodness of fit	Tabulated λ^2	$EC_{0,1}$ (mg/L)	EC_1 (mg/L)	EC_5 (mg/L)	EC_{10} (mg/L)
Adenophlebia sylvatica	NaCl	180	B2	104	162		220	1386,67	2646,67
Caridina nilotica	NaCl	360	A2	180,8	254	175,33	571,33	1115,33	1509,33
Cloeon virgiliae	NaCl	240	A2	21	62				61,2
Euthraulus elegans	NaCl	240	C2	1061	1163		180	1893,33	4166,67
Adenophlebia auriculata	Na_2SO_4	240	C2	215,6	259	20	940	2460	3716
Afronurus barnardi	Na_2SO_4	100	A2	22	34			92,7	1097
Caridina nilotica	Na_2SO_4	240	A2	274	761			470	840
Cloeon virgiliae	Na_2SO_4	100	C2	15,01	49		271,8	866	1292
Euthraulus elegans	Na_2SO_4	240	A2	17,67	34		579	1480	2158
Plea pullula	Na_2SO_4	240	A2	48,8	79				341,2



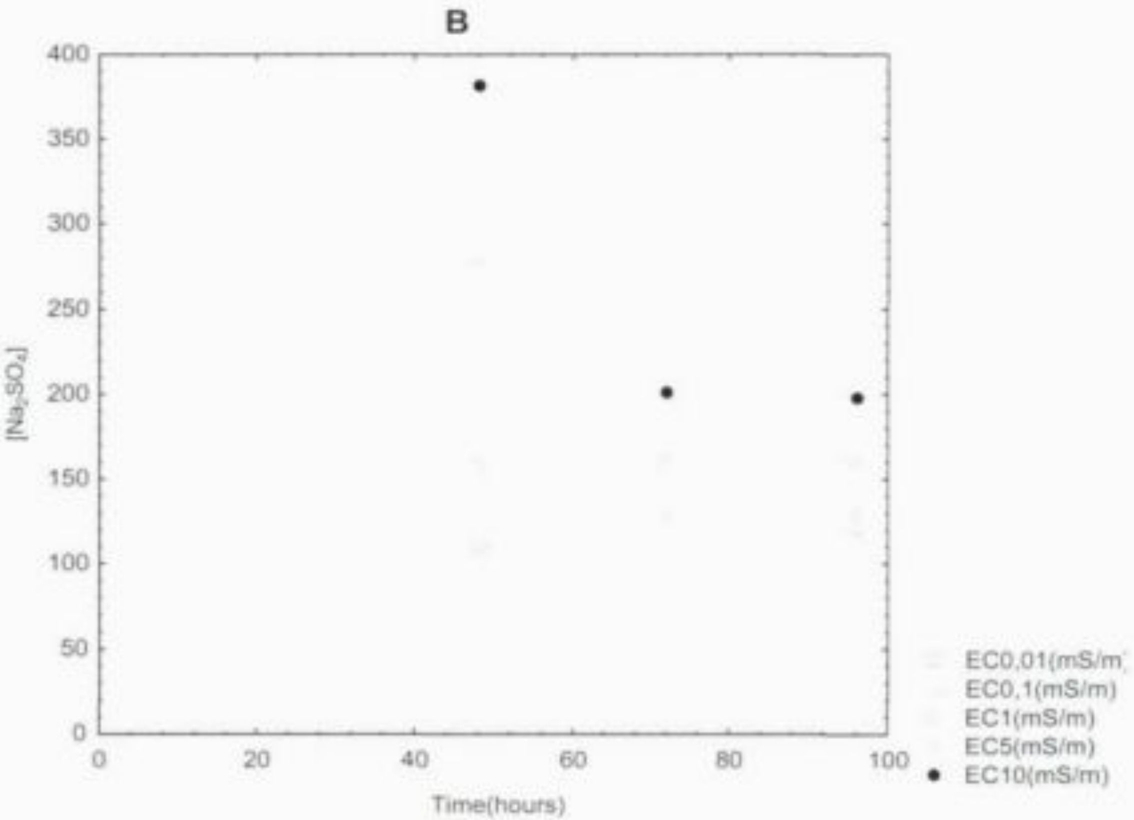
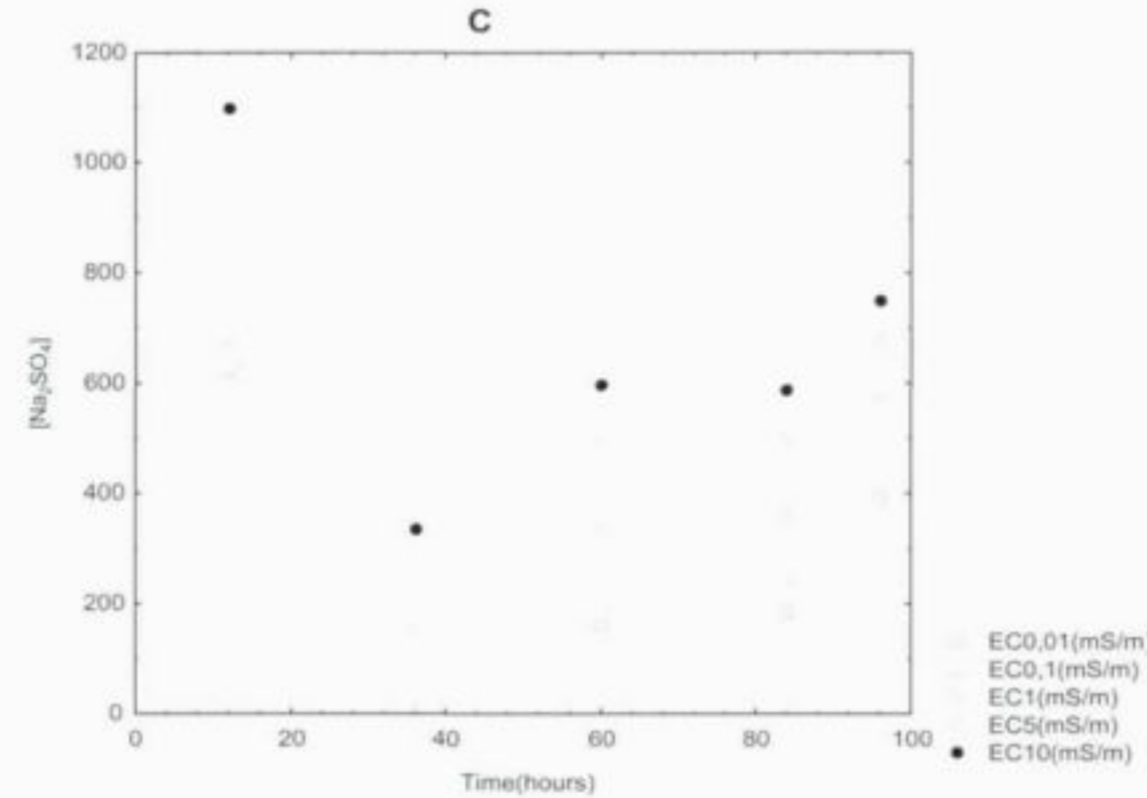


Figure 4.7 To estimate threshold time for use in MPA, low LC_x values were plotted against time for Na_2SO_4. Threshold time was taken to be the time where LC_x values appear to stabilise. (**A**: *Caradina nilotica* and **B**: *Cloeon virgillae*).



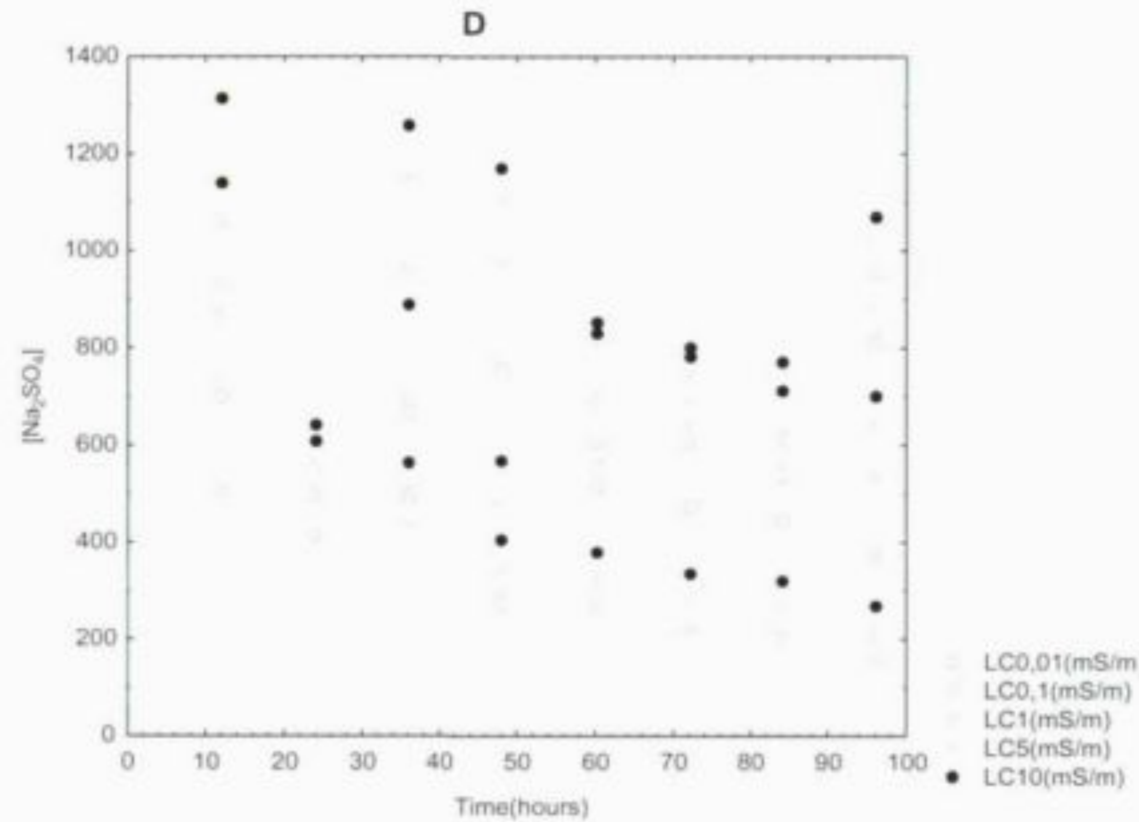
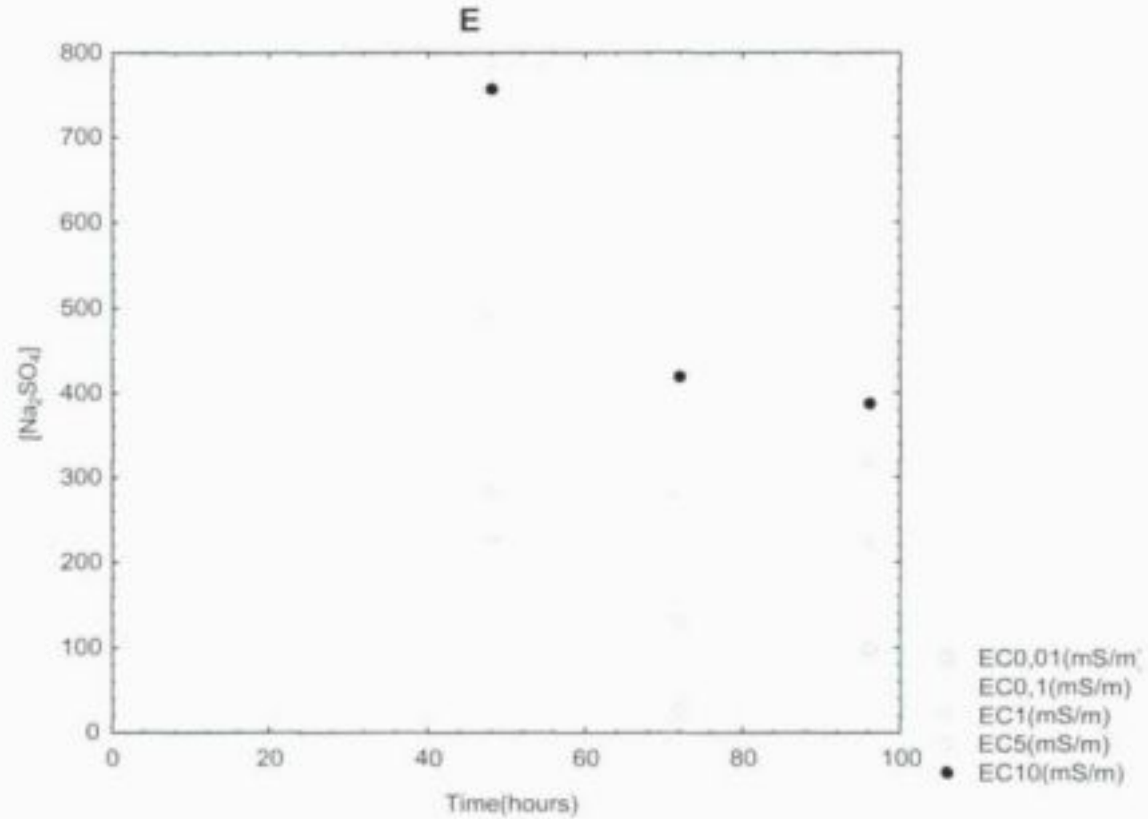


Figure 4.7 **Continues:**
To estimate threshold time for use in MPA, low LC_x values were plotted against time for Na_2SO_4. Threshold time was taken to be the time where LC_x values appear to stabilise. (**C**: *Plea pullula* and **D**: *Adenophlebia auriculata*).



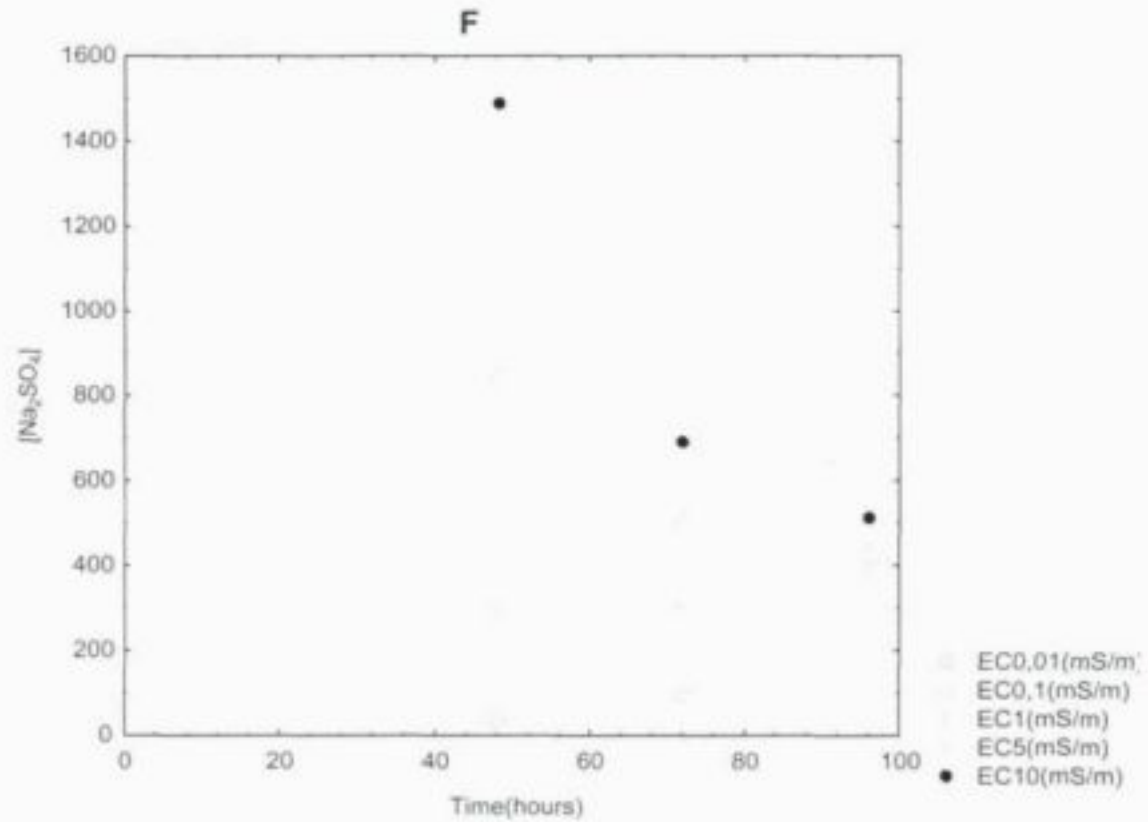


Figure 4.7 **Continues:**
To estimate threshold time for use in MPA, low LC_x values were plotted against time for Na_2SO_4. Threshold time was taken to be the time where LC_x values appear to stabilise. (**E**: *Afronurus barnardi* and **F**: *Euthraulus elegans*).

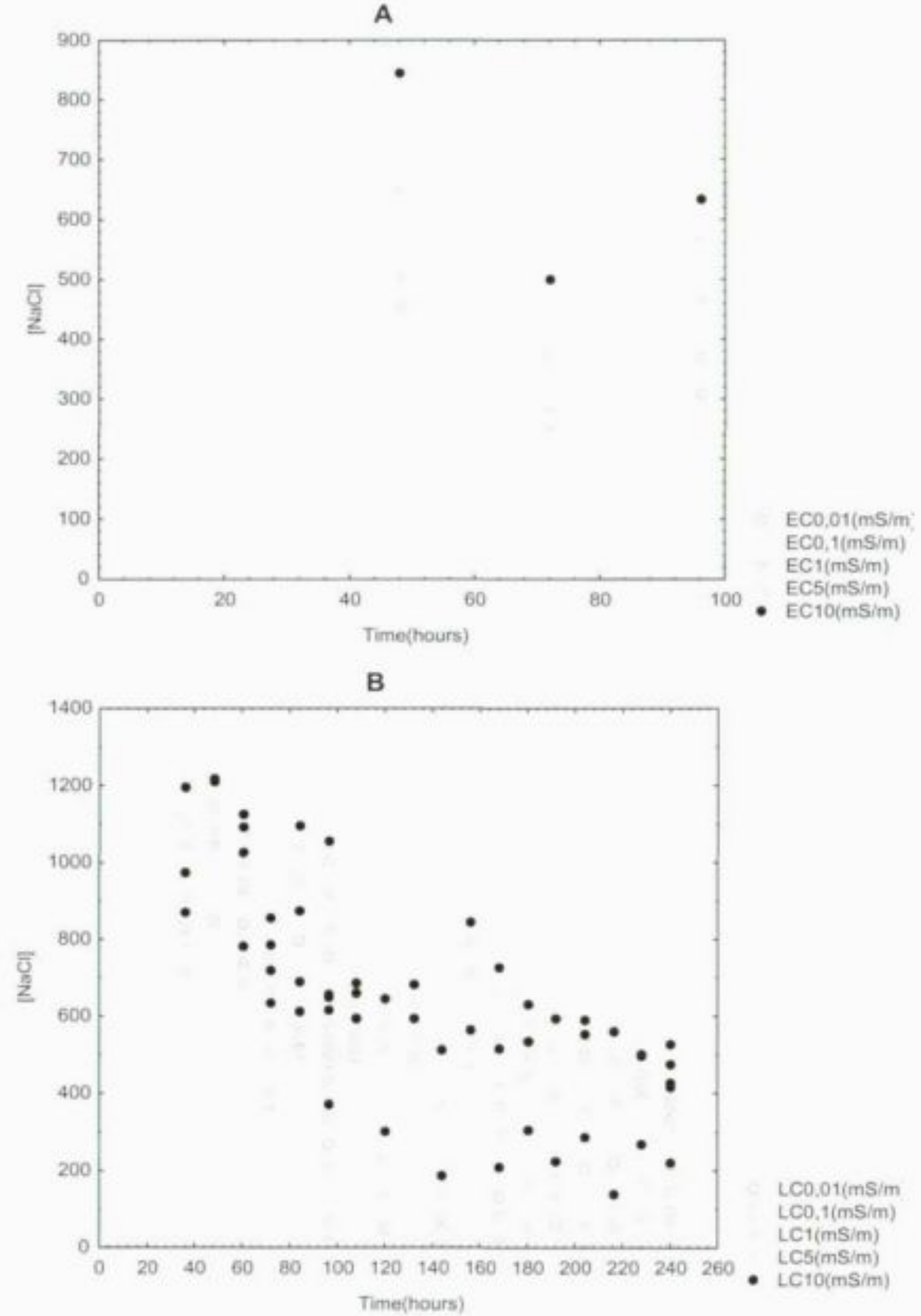


Figure 4.8 To estimate threshold time for use in MPA, low LC_x values were plotted against time for NaCl. Threshold time was taken to be the time where LC_x values appear to stabilise. (**A**: *Cloeon virgillae* and **B**: *Euthraulus elegans*).

4.2.4 Discussion

Low LC_x ($LC_{0.01}$ – LC_{10}) obtained from LRA and MPA are equated with a possible predicted no observable effect concentration (PNOEC) (Lee *et al.*, 1995; Mayer *et al.*, 1994). Extrapolated low LC_x values ($LC_{0.01}$ – LC_{10}) for *C. nilotica* exposed to NaCl yielded values in the range of 550mg/L - 3500mg/L using LRA and 175mg/L – 1500mg/L using MPA. Although both extrapolation techniques give reasonable estimates of the experimental NOEC, MPA yielded a more accurate PNOEC. Values generated by MPA also appear to be more conservative then LRA (based on comparisons of the LC_x and EC_x values) and are therefore potentially more protective.

The experimentally obtained NOEC of 800mg/L NaCl for *C. nilotica* falls between the $LC_{0.01}$ and $LC_{0.1}$ values obtained by the LRA extrapolation method (548 and 940mg/L NaCl respectively) and the EC_1 and EC_5 values obtained by the MPA extrapolation method (571 and 1115mg/L NaCl respectively). This seems to suggest that these extrapolation methods, using acute toxicity test results with mortality as an endpoint, may be appropriate for developing environmental water quality criteria. It appears that the lower LC_x values ($_{0.01}$ and $_{0.1}$) derived using LRA extrapolation, may better approximate NOECs. However more experimental validation of the results given by LRA and MPA is needed before results of these extrapolations can be confidently used.

The high degree of variability of mortality responses among and within the treatments of the *C. nilotica* chronic toxicity test suggests that the experimental methodology used is not sufficiently refined. High mortalities may have contributed to the low LC_x values yielded by the experiment although the control mortalities were acceptable for two of the replicates.

A limitation of these two extrapolation techniques is that often the only acute toxicity data available are those from mature test organisms. An experimental chronic test would normally be run over a test organism's entire life cycle including more sensitive stages. Ideally, acute data run on very young test animals should be used for acute to chronic mortality extrapolations.

Acute toxicity tests lasting longer then 96-hours are typically more accurate for use in acute to chronic mortality data extrapolations. It is recommended to extend acute toxicity tests until mortality appears to stabilize, i.e. undertake time-independent acute toxicity tests. In most cases mortality appears to stabilise within 10-days. The amount of additional work that needs to be done to extend a 96-hour acute test to a 240-hour acute test is eclipsed by the wealth of additional information that can be obtained.

CHAPTER 5

COMMUNICATION ABOUT ENVIRONMENTAL WATER QUALITY

A hand book entitled: *Environmental Water Quality For Water Resource Managers* by Tally Palmer, Robert Berold and Nikite Muller is available as a stand-alone WRC TT report in an A5 format.

In order to communicate widely with the water sector about the concept of Environmental Water Quality (EWQ), and particularly the role of ecotoxicology, an accessible handbook was developed and edited iteratively. Each stage was undertaken in co-operation with DWAF and selected stakeholders. The handbook outlines the concept of EWQ, and shows clearly how this concept is useful to water managers and users in the implementation of, and compliance with, the NWA. It is written in a simple style and aims specifically to assist water resource managers and users involved in any practice that affects water resource quality. The handbook has been presented to DWAF, edited in response to departmental requirements, and reflects DWAF policy as closely as possible.

The handbook is a product both of this project, and of WRC Project No: K5/1313: *The Development of an Environmental Water Quality Programme*. The first draft of the handbook was presented to DWAF personnel from the Eastern Cape. It was revised and then presented in Pretoria to 20 DWAF and industry participants (see Chapter 6 for more details). The presentation and document stimulated vigorous debate and 12 sets of comments were received. An illustrated draft was printed in September 2003 and presented at a series of "road-show" workshops:

Date (2003)	Location	Number Attending	Host And Representation
25 June	Grahamstown	8	UCEWQ-IWR: DWAF Eastern Cape Province, academics
10 July	Pretoria	30	DWAF: DWAF Gauteng and Head Office, industry, mining
22 October	Richards Bay	28	Richards Bay Minerals: DWAF KwaZulu-Natal, mining, local government
23 October	Durban	56	Durban Chamber of Commerce: DWAF KwaZulu-Natal, industry, local government
30 October	Cape Town	22	Water Institute of Southern Africa AGM: DWAF Western Cape, industry, academics, local government
11 November	Witbank	19	AMCOAL: Mining, industry, local government, consultants
12 November	Sasolburg	36	SASOL: Industry, mining, consultants

All comments were collated and the handbook is available as a WRC TT report.

CHAPTER 6

CAPACITY BUILDING REPORT

6.1 UNDERGRADUATE AND POSTGRADUATE CAPACITY BUILDING

Capacity building, communication and technology transfer are an integral part of UCEWQ activities. In this project, this was achieved through undergraduate lecturing and postgraduate supervision, technology transfer workshops and presentations and a professional course.

Postgraduate Research:

Prof. Palmer and Dr. Muller supervise postgraduate research. This project funded the MSc Bursary of Samantha Browne and her research contributes substantially to this report (chapters 3 and 4.1). Andrew Slaughter's MSc thesis makes a contribution to the project (Chapter 4.2) but is funded by another WRC project.

Teaching:

Prof. Palmer and Dr. Muller are actively involved in both undergraduate and postgraduate teaching. Both teach aspects of the ECL301 Applied Freshwater Studies course, offered to 3rd year Environmental Science, Geography, Zoology and Entomology students. At honours level, a course in Environmental Water Quality is offered to Zoology, Entomology, Environmental Science and Geography students.

Details of the technology transfer workshops and presentations and the professional course are presented in Chapters 6.2 and 6.3.

6.2 EWQ PROGRAMME CAPACITY BUILDING AND COMMUNICATION

Communication about environmental water quality was a core component of this project. A handbook was produced (see Chapter 5) and a series of workshops and presentations organised. A summary of these are found in Chapter 5. This section tabulates workshop and presentation participants and shows the range of potential stakeholders who may have an interest in, or need to know about, environmental water quality.

GRAHAMSTOWN, 25 JUNE 2003: Attendees At The Presentation	
Andrew Lucas	DWAF, East London
Vin Kooverji	DWAF, East London
Mzuvukile Tonjeni	DWAF, East London
Pieter Retief	DWAF, Port Elizabeth
Nikite Muller	UCEWQ-IWR, Rhodes University
Heather Davies-Coleman	UCEWQ-IWR, Rhodes University
Andrew Gordon	UCEWQ-IWR, Rhodes University
Jay O'Keeffe	UCEWQ-IWR, Rhodes University

PRETORIA, 10 JULY 2003: ATTENDEES AT THE PRESENTATION	
Dirk Roux	CSIR
Sakkie Van Der Westhuizen	DWAF, Pretoria
Magda Ligthelm	DWAF, Pretoria
Themba Duma	DWAF, Pretoria
Bonani Madikizela	DWAF, Pretoria
Thandiwe Zokufa	DWAF, Pretoria
Sarah Letshwene	DWAF, Pretoria
Bill Rowlston	DWAF, Pretoria
Philip Kempster	DWAF, Pretoria
Paul Herbst	DWAF, Pretoria
Sebastian Jooste	DWAF, Pretoria
Suzan Oelofse	DWAF, Pretoria
Marius Keet	DWAF, Pretoria
Jurgo van Wyk	DWAF, Pretoria
Harrison Pienaar	DWAF, Pretoria
Fortunate Makhubu	DWAF, Pretoria
Priya Moodley	DWAF, Pretoria

RICHARDS BAY, 22 OCTOBER 2003: ATTENDEES AT THE PRESENTATION	
Johnson Dayal	Richards Bay Minerals
Roy Jones	Richards Bay Minerals
Sbu Ndwandwe	Dept Environmental Affairs
Malcolm Moses	Dept Environmental Affairs
Pat Jennings	Dept Environmental Affairs
Jim Dowe	Sasol
Tania Dunne	Foskor
Muhammad Ali	Foskor
Ushanta Naicker	Foskor
JH Boonzaaier	Impala Water Users Assoc
F Cronje	Impala Water Users Assoc
Sean Jaganath	Huletts Sugar Association
Jan de Beer	Bayside Aluminium
Herman Swanepoel	Bayside Aluminium
Anton Prinsloo	Bayside Aluminium
Toby Myburgh	Bayside Aluminium
Clive Winder	Bayside Aluminium
Sipho Mosai	Mhlathuze Water
Kevin Coetzee	Mhlathuze Water
Jan Thompson	Mhlathuze Water
Nico Serfontein	Mhlathuze Water
Bruce Kelber	University of Zululand
Mark Hains	BKS
Samuel du Rand	BKS
Horst Weyermuller	AAFC
Hannes Coetzee	uMlalazi Municipality
Hendrik Louw	Hillside Aluminium
A.N.Other	Hillside Aluminium

DURBAN, 23 OCTOBER, 2003: ATTENDEES AT THE PRESENTATION	
Ian Naidoo	
Alison Haycock	FFS Refiners
Joey Singh	UOS
Armar Sooklal	Durban Chamber of Commerce
Terri Clapperton	Island View Storage Ltd
Rob McInerney	Nintex
Geoff Purnell	SBA Durban
Raj Pillay	DWAF KwaZulu-Natal
Renelle Karen Pillay	DWAF KwaZulu-Natal
Kelello Ntoampe	Waste Services
Judy Pitts	Huntsman
Bill Pfaff	DMWS
Liz Anderson	MJVN
Greg Matthews	WSP Walmsley Environmental Consultants
Mark de Souza	FFS Refiners
Rory Lynsky	Environ Liaison Manager, SA Sugar Assoc
Tobile Bokwe	Umgeni Water
Sandile Sithole	Umgeni Water
Smanga Shange	Umgeni Water
Don Hodgkinson	Umgeni Water
Poppy Dlamini	Umgeni Water
Manu Pillay	Umgeni Water
Asha Ramtajan	Umgeni Water
Thubendran Naidu	Umgeni Water
Mark Graham	Umgeni Water
Peter Thompson	Umgeni Water
Simon Mashigo	Umgeni Water
Pamela Ngwenya	Umgeni Water
Bongani Dumesa	WSA Manager (uMgungundlovu DM)
Lin Gravelet-Blondin	DWAF KwaZulu-Natal

CAPE TOWN, 30 OCTOBER, 2003
PRESENTATION GIVEN AT THE WATER INSTITUTE OF SOUTHERN AFRICA ANNUAL GENERAL MEETING. A REGISTER WAS NOT TAKEN.

WITBANK, 11 NOVEMBER, 2003: ATTENDEES AT THE PRESENTATION	
H. Lodewjks	Anglo Coal
J. Froneman	Anglo Coal
S. Gioimi	Xstrata Coal SA
J. Adam	Exigent
M. Mohring	Greenside Coll
N. Dooge	Xstrata Coal SA
J. Kuntonene-van't Riet	BANK
P. Meulenbeld	Goedehoop – AC

F. Cessford	SRK consulting
A. James	METAGO
S. Dill	METAGO
W. Lubbe	BANK
G. Trusler	Digby Wells and Associates
D.B. Venter	Anglo Coal – Klein Kopje
R.E. Gardner	Anglo Coal
J. van der Walt	Anglo Coal – Kriel
B. Wolmarans	WSSA
L. van der Merwe	Water, Sewage & Effluent magazine
V. Rall	Ecosun

SASOLBURG, 12 NOVEMBER, 2003
Representatives of the local catchment forums and industry including SASOL. A register was not taken.

6.3 PROFESSIONAL COURSE

UCEWQ staff organised and lectured a professional course titled "Introduction to Applied Aquatic Ecotoxicology: Theory, Policy and Practical" in 2001 and 2003 (in 2003 the practical course was run collaboratively with EcoSun, an environmental consulting company based in Johannesburg). The courses were attended by delegates form Government (DWAF and Nature Conservation), industry (water service providers and manufacturing industries), tertiary institutes and consultants.

CHAPTER 7

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