MACROINVERTEBRATE COMMUNITY AND SPECIES RESPONSES TO CHLORINATED SEWAGE EFFLUENT IN THE UMSUNDUZE AND UMBILO RIVERS, KWA ZULU-NATAL, SOUTH AFRICA

THESIS

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ABSTRACT

Chlorine has a wide variety of applications in water treatment. Because of its disinfectant efficacy, it is used world wide for the treatment of potable water, sewage, swimming pools and for the control of nuisance organisms in cooling towers. A problem arises when such chlorinated water enters the natural environment, as chlorine's greatest advantage, i.e. its germicidal capacity, becomes its greatest disadvantage. In particular, the discharge of heated, chlorinated water from cooling towers and chlorinated, treated sewage into rivers have severe consequences for the riverine flora and fauna. This study focused on the effects of chlorinated, treated sewage effluent on the community structure of benthic macroinvertebrates in two rivers in KwaZulu-Natal viz. the Umsunduze River in the Pietermaritzburg area, and the Umbilo River in the Durban area.

The study was conducted in three phases. The first two phases comprised a *toxicological* investigation of the effects of chlorine on a selected riverine macroinvertebrate, and the third phase comprised an *ecotoxicological* investigation of the effects of chlorinated treated sewage on benthic macroinvertebrate community structure.

The first phase of the study involved the development of an artificial stream system which would be suitable for determining the response of a selected macroinvertebrate species to chlorine. Chlorine is both reactive and volatile, so this necessitated the development of a specialised flow-through system with apparatus which would allow continuous dosing of a sodium hypochlorite solution. The system was set up at the Process Evaluation Facility at Wiggins Waterworks, Durban, where raw water from Inanda Dam was used.

The second phase involved the use of this artificial stream system to conduct acute 96 h toxicity tests. Baetid mayfly nymphs (*Baetis harrisoni* Barnard) were selected as the test organisms after a preliminary investigation found them to be suitable for survival under laboratory conditions. For comparative purposes, tests were run first on *B. harrisoni* from a relatively uncontaminated stream in a residential area of Westville, then on specimens from the severely impacted Umbilo River. The LC_{50} of chlorine for organisms from both

sources was found to be in the region of 0.004 mg/ℓ (free chlorine). This value was well below the general effluent standard of 0.1 mg/ℓ in effect at the time. The recommended acute environmental guideline is 0.001 mg/ℓ.

The third phase of the study involved field validation of the toxicity test results. It was hypothesised that since the LC_{50} for free chlorine was 0.004 mg/ℓ, *B. harrisoni* would not be found downstream from a point source of chlorinated effluent where the concentration of free chlorine ranged from 0.06 to 0.2 mg/ℓ, and that the macroinvertebrate community structure would also be altered. In order to test these hypotheses, benthic macroinvertebrate community structure was investigated at several sites up- and downstream from the outlets of the Darvill Wastewater Works in the Umsunduze River and the Umbilo Sewage Purification Works in the Umbilo River. In addition, in order to differentiate between the effects of chlorinated and unchlorinated treated sewage, a section of the Umbilo River (upstream from the chlorinated discharge) was exposed to unchlorinated, treated sewage. In this way, a limited "before and after" sewage and an "upstream and downstream" from sewage investigation could be carried out.

Organisms were collected from riffles (and from pools in the Umbilo River) and the samples were then sorted and organisms were identified to species level, where possible, otherwise to genus or family. Changes in community composition were shown graphically as pie charts of relative proportions of organisms found at each site, graphs of the average number of taxa at each site; and graphs of the average number of individuals at each site; Data from the Umbilo River were also analysed using TWINSPAN (Two-way indicator species analysis).

In both the Umsunduze and the Umbilo rivers, the deleterious effects of the chlorinated effluent were clearly evident. At Umsunduze Site 3 and Umbilo Site 5 (both immediately downstream from the chlorinated effluent) both the number of taxa and number of individuals were substantially reduced, sometimes to zero. Where organisms were found at the next sites downstream (Sites 4 and 6 respectively), the samples were dominated by

Chironomus. In contrast, the unchlorinated effluent in the Umbilo River caused very little difference in community structure.

As predicted, *B. harrisoni* was not found in downstream samples in which chlorine was present, yet appeared to be relatively unaffected by the unchlorinated effluent, suggesting that chlorine, rather than the effluent was responsible for its absence at downstream sites.

In conclusion, it would appear that while treated sewage effluent certainly causes *changes* in macroinvertebrate community structure, chlorination of this effluent leads to large scale *destruction* of the riverine community. This in turn delays the recovery process of the river, rendering a longer stretch unfit for use. The consequences of this delayed recovery are that the failure to meet the water quality requirements of the natural environment results in those of the other water users (agriculture, industry, domestic and recreation) not being met.

This reduces the natural capacity of the riverine community to process organic waste and recover from the discharge of sewage effluent. Chlorination increases the distance of impaired water quality and environmental integrity which result from organically enriched treated sewage effluent.

The results of the study indicated that the draft water quality guidelines for aquatic ecosystems, derived from inadequate data, and calculated with a safety factor, were the correct order of magnitude. The approach followed in the study will be useful in the development and refinement of water quality guidelines for aquatic ecosystems.

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CHAPTER 1

INTRODUCTION

INTRODUCTION

South Africa is a semi-arid country with limited water resources (DWAF, 1994). Meeting the growing water needs of the country in terms of both quantity and quality requires sound management practices, part of which involves the setting of water quality guidelines which will be used to ensure that what little water there is, is maintained in a state fit for use. In order to supplement water quantity, effluents, including treated sewage, have to be re-used. Usually, when treated sewage effluent is released into the natural environment (e.g. into rivers) it is further processed by aquatic organisms. For human consumption, it can then be processed further at a waterworks. However, if the sewage load is too large or is chlorinated, the organisms which would normally process this effluent are eradicated, and the cleansing process is retarded (Davies & Day, 1986). This aims of this study were to investigate some of the effects of chlorinated sewage effluent on riverine macroinvertebrates, and communities. Both toxicological (experimental chlorine tolerance testing) and ecotoxicological (relating tolerances to community structure in the field) approaches were used.

WATER AVAILABILITY IN SOUTH AFRICA

South Africa is an old land and a dry one (Davies & Day, 1986). Most parts of the country do not have high rainfall and droughts are an ever-present threat in all regions. The average annual rainfall of 500 mm is about 60% of the world average, and 65% of the country receives less than this annually. Twenty-one percent of the country receives less than 200 mm (DWAF, 1994). The country's aridity results from the fact that the south-easterly trade winds drop much of their rain in the south eastern part of the country, leaving the interior of the country in a "rain shadow", and because dry, stable, high-pressure air masses may persist for long periods over the warmer parts of the country and do not produce rain (Davies & Day, 1986).

In addition to the low rainfall, evaporation rates are high. Over most of the country the average annual potential evaporation is well in excess of the annual rainfall. Evaporation rates range from 1 100 mm in the east to more than 3 000 mm in the west, which reduces the water available from surface runoff. It is estimated that owing to the variability of rainfall and the high evaporation losses from dams, only about 62% (33 000 million m^3) of the average annual runoff of 53 500 million m^3 can be used cost effectively with present technology (DWAF, 1994).

In many parts of the country, the local water resources are fully utilised or overdrawn, and the question of the equity of the present water allocations demands attention (DWAF, 1994). The past political situation, together with the geographical factors, have resulted in a situation of gross inequity in the distribution of water. At present more than 12 million people do not have access to an adequate supply of potable water and nearly 21 million do not have basic sanitation. A small amount of water is needed for physical survival and a limited amount for personal hygiene (DWAF, 1994), and a lack of clean water for these basic needs has community health implications as many diseases are water-born. A combination of safe drinking water, sanitation facilities and personal hygiene would do much to reduce the incidence of disease in under-developed communities.

According to the White Paper on Water Supply and Sanitation Policy of 1994:
The goal of Government is to ensure that all South Africans have access to essential basic water supply and sanitation services at a cost which is affordable to both the household and the country as a whole.

In 1990 municipal and domestic use was estimated to account for 12% of the water used and this is expected to increase to 17.3% by the year 2010. It is, of course, not only households which require water: agriculture accounted for 52.4% in 1990 but this is expected to decrease to 47.3% by 2010. The main water users of South Africa are depicted in the following pie chart:

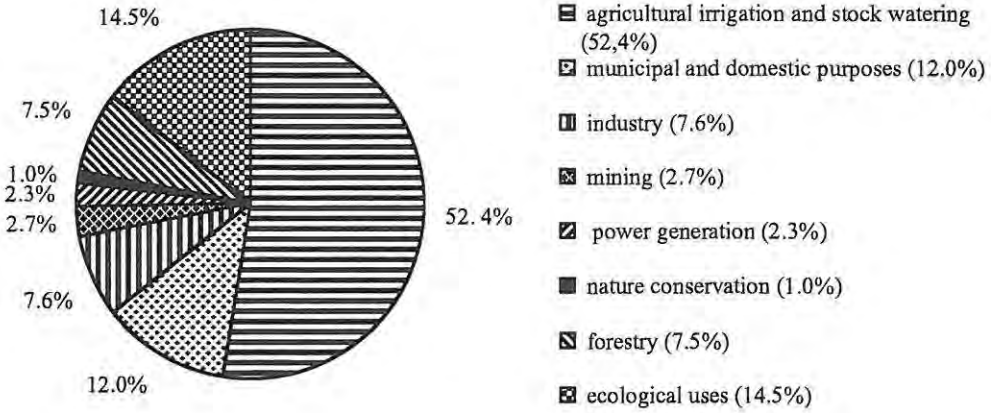


Fig. 1.1 Water Users in South Africa

(information from South African DWAF, 1986, *adapted by* Davies *et al*, 1993)

The current annual water demand is 25 888m kℓ and it would probably increase to 49 000m kℓ by the year 2010 as South Africa's population (31 million in 1991) is growing at an annual rate of between 2,3 and 3,6% (Davies *et al.*, 1993; Brown, 1995).

The question arises as to where all this water which is required will come from. In South Africa, there is a paucity of true inland lakes (Allanson *et al.*, 1990), so almost all water comes from rivers. Although the subcontinent appears to be well endowed with rivers, (especially the eastern part, which receives a large proportion of the rainfall), many rivers in the rest of the country are seasonal, flowing for only part of the year (Davies & Day, 1986). The importance of ground water is growing rapidly throughout the country because of dwindling surface water resources and the need to develop local resources optimally. However, most of South Africa is underlain by hard rock formations, so only about 5 400 million m^3 of water per annum may be obtainable from underground sources. In addition, over large areas of the country, the ground water is saline (DWAF, 1994), so is of limited use. Without desalination or recycling, we are likely to deplete our available water resources within the next fifteen years, even with careful use and management of water supplies and the addition of exploitable groundwater resources. This means that at present, South Africa is facing a severe water crisis (Davies *et al.*, 1993).

It is not only the *quantity* of water which is of concern in South Africa, but also the *quality*. The two are obviously linked as having a large *volume* of water is usually of little value if it is not in a state which is *fit for use*. One of the most important water users is the natural environment as it is the source for all other water users. Failure to meet the needs of the environment in terms of both quantity and quality would mean that the needs of most other users would not be met. Sanitation services and economic activities which can pollute water and render it unfit for use must therefore be controlled (DWAF, 1994).

The limited water resources of South Africa are a national asset which must be properly managed if they are to bring maximum benefit to the country as a whole (DWAF, 1994). This is the role of the Department of Water Affairs and Forestry (DWAF).

WATER QUALITY MANAGEMENT IN SOUTH AFRICA

In April 1991, the DWAF produced a document entitled "Water quality management policies in the RSA" in which various aspects of water quality management (WQM) in South Africa at the time were outlined. These included historical perspectives of WQM, the status of WQM at the time, strategies that affected WQM, and the evolution of policy related to WQM. A brief overview of selected aspects of this document is presented below.

HISTORICAL PERSPECTIVE

Water quality management in South Africa began with the promulgation of the Public Health Act of the Union of South Africa, 1919 (Act 36 of 1919). The Chief Health Officer of the Public Health Department was responsible for controlling pollution by ensuring that "the best known or the only or the most practical methods" for sewage disposal were being used. The Chief Health Officer could prevent effluent from sewage treatment works from being discharged into water courses, so, sewage or sewage effluent had to be disposed of on land.

After 1950, the South African economy changed from being agriculturally based to one in which mining and industry played the major role. At about the same time, the Department of Irrigation changed to the Department of Water Affairs and Forestry. The Water Act of 1956 (Act 54 of 1956) vested in the Minister of Water Affairs and Forestry the necessary powers to exercise control over the management of South Africa's water resources. Its aim was to control the use of water by industry and the treatment and disposal of effluent. It soon became apparent that reconciling water supply and demand was becoming increasingly difficult and that effluent would have to be re-used to supplement the country's scarce water resources. The requirements of the 1919 Act were subsequently changed. The new Act required that all effluent be returned to the body of water from which it was originally abstracted.

The Water Amendment Act, 1984 (Act 96 of 1984) broadened water quality management by making industrial effluent and sources other than effluent, e.g. water which arises as a by-product from industrial and mining activities and seepage or stormwater runoff from a site, subject to pollution control regulations.

THE PRESENT STATUS OF WATER QUALITY MANAGEMENT

Definition of water quality management

Water quality management embraces decisions and actions which lead to the development, implementation and execution of strategies to achieve the stated mission and objectives.

These activities are:

- Planning
- Comprehensive studies, research and monitoring
- Co-ordination and communication
- Education and training (for skilled manpower)
- Organisation
- Decision-making
- Regulation, operation and control (of water pollution)
- Monitoring and evaluating success
- Information.

The overall goal of water quality management

The two alternative goals of water quality management could be either-

- to maintain water quality in some original pristine state; or
- to maintain water quality in such a state that it remains fit for recognised uses.

According to section 23 of the Water Act (Act 54 of 1956) it is an offence to pollute water by rendering it less fit for-

- the purposes to which it could ordinarily be put to use by other persons;
- the propagation of fish or other aquatic life; or
- recreation or other legitimate purposes.

In common with water pollution control legislation in most developed countries in the world, the Water Act does not require Water Affairs to ensure that the quality of South Africa's marine and freshwater resources remain in some original or pristine state unless this can be justified in terms of maintaining such quality for the purpose of meeting the requirements of one of the recognised water uses.

The Water Act neither requires or implies the concept of zero pollution. As South Africa is a water scarce region, re-used effluents are considered vitally important supplements to freshwater resources. Purified effluents, with their pollutants, are required to be returned to natural bodies for re-use.

The concept of 'fitness for use' implies an evaluation of water quality in terms of the requirements of a particular user and is measured in relation to criteria or norms representing ideal quality for a particular use. The 'fitness for use' concept should be viewed as one where the overall risk to the consumer or environment is minimised and the risk or level of protection is defined.

There are various degrees of fitness for use, ranging from one hundred percent fit to completely unfit for use. Between these extremes is a wide transition zone in which water quality may be fit for a particular use.

The fitness for use concept, therefore, does not demand that water quality requirements be stated in absolute terms but rather according to the particular use or category of uses or acceptable level of risk.

Factors which affect water quality management

Some of the factors which affect water quality management in South Africa are outlined below:

- It is a semi-arid country so effluents should be re-used to reconcile water supply and demand.

- Economically South Africa has a mixture of undeveloped, developing and developed components which places constraints on the options available for protection of the water resources.

- The uneven distribution of water resources means that whereas in arid parts of the country the re-use of effluent may be crucial for matching water supply and demand, in the wetter parts this may not be as pressing.

- There are serious shortages of skilled manpower, especially in the public sector, and this places constraints on decision-making systems and levels of information required to support the decision-making process.

- Fundamental political, social, economic and constitutional changes taking place at present may drastically alter the present dispensation regarding water quality management. The question of environmental management is at present under review.

Fundamental approaches to water pollution control

Control over sources of water pollution is central to water quality management. Each country has its own approaches to water pollution control but generally they are variations and/or combinations of a few fundamental approaches. These are the Uniform Effluent Standards Approach, the Receiving Water Quality Objectives (van der Merwe & Grobler, 1990) and the Pollution Prevention Approach.

1. Uniform effluent standards

This approach aims to control the imput of pollutants to the water environment by requiring that effluents comply with uniform standards. The underlying philosophy is that minimum pollution (from point sources) is the desirable ultimate goal.

The standards should be set so as to achieve levels of effluent pollution concentration that would result from applying "best available technology not entailing excessive cost"(BATNEEC). In the past, aspects of this approach have been adopted by the European Community and have formed the basis of pollution control by DWAF.

Several drawbacks are evident in this approach:

- It focuses on effluent and effluent treatment technology, largely ignoring the impact on the quality of the receiving waters.

- This approach may fail to protect the quality of water resources where there are multiple point sources or high background levels of pollution arising from diffuse sources.

- Since it requires all effluent to comply with the same standards, irrespective of variations in the assimilative capacity of the receiving waters and regardless of the costs involved, it is not necessarily cost-effective.

- There is no incentive for industry to locate at the most environmentally advantageous location.

- As it provides no framework for control of non-point sources it cannot guarantee the meeting of receiving water quality objectives.

The advantages are, however, that:

- It is simple, understandable and straightforward to enforce.
- Pollution from point sources should be minimised as standards are updated to incorporate the latest and best pollution abatement technology.

2. Receiving water quality objectives

This approach involves specification of the desired quality of the receiving water environment and the control of sources. It takes into account non-point and point sources of pollution to the degree necessary to maintain the desired quality. It recognises the capacity of the receiving water to assimilate pollution to a certain extent without serious detriment to quality requirements of recognised users. Point source pollution can be controlled effectively by setting site-specific effluent standards that take into account the contribution of diffuse sources of pollution.

There are three main advantages of this approach:

- Both point and non-point sources of pollution have to be taken into account as the focus is on the quality of the receiving waters and minimum interference with legitimate uses of the environment.

- It is cost effective as it optimises the level of control required by considering the capacity of the receiving water environment to assimilate particular pollutants.

- It offers an incentive for industry to locate where the receiving environment is least sensitive to pollution.

From a regulatory point of view its drawbacks are:

- Thorough understanding of the fate of pollutants and their impacts on the water environment is needed so its application is technologically more demanding.

- Since site-specific effluent standards have to be specified, considerably more detailed investigation is required than would be necessary with the application of the Uniform Effluent Standards approach.

3. Pollution prevention approach

Toxicity, persistence and capacity for bioaccumulation present major threats to the environment. The pollution prevention approach is aimed specifically at control of the handling and disposal of hazardous substances. The Receiving Water Quality

Objectives approach would be inappropriate in this context because of the difficulty of setting safe receiving water quality standards for these pollutants.

Various strategies have been recommended:

- In the United Kingdom, industries which discharge significant amounts of hazardous substances are required to comply with effluent standards based on BATNEEC and must "carry out all other functions ... in accordance with best practice and in a manner that renders any emissions that do occur harmless and inoffensive to people and the environment as a whole".

- In the United States of America concepts of pollution prevention and waste minimisation focus on source reduction and recycling and can involve reduction of the quantity and toxicity of the waste.

- In some countries, the Pollution Prevention approach has been extended and embraces all pollutants, not only hazardous ones.

South Africa's approach to water pollution control

The *Uniform Effluent Standards* approach was applied by Water Affairs for almost three decades, and still forms the basis of most permits. Although it retarded deterioration in water quality, focused public attention on pollution and promoted the development of improved wastewater treatment technology and management techniques, the quality of South Africa's water resources has deteriorated. Water Affairs has therefore adopted the *Receiving Water Quality Objectives* (RWQO) approach for non-hazardous substances and the *Pollution Prevention* approach for hazardous substances (van der Merwe & Grobler, 1990).

The RWQO approach as applied at present in South Africa involves:

- the compilation of water quality guidelines based on the requirements of the recognised water users;

- the formulation of water quality management objectives which recognise the water quality requirements of water users as well as economic, social, political and

technological considerations (the management objectives therefore do not necessarily conform to the water quality guidelines); and

- the imposition of site-specific effluent standards or other measures to ensure that the water quality management objectives determined for the particular water body will be met.

In effect, effluent producers have to comply with minimum effluent standards, namely the uniform General and Special Effluent Standards. Exemptions to the Standards may be granted if motivated satisfactorily on technical and/or economic grounds and justified by the Receiving Water Quality Objectives approach. Site-specific effluent standards are then substituted. Site-specific standards may also be stricter than the General and Special Effluent Standards.

Fundamental to the Receiving Water Quality Objectives is the determination of the quantity and quality requirements of the different user sectors (DWAF, 1991).

SOUTH AFRICAN WATER QUALITY GUIDELINES

The Department of Water Affairs and Forestry considers itself to be the custodian of South Africa's water resources, part of its goal being to protect the aquatic ecosystems so that they remain in a healthy and viable state, in order that they be fit for a variety of water uses on a sustained basis. The DWAF recognises that water use for purposes such as recreation, irrigation and domestic use will result in some impact on and modification of aquatic ecosystems, and accepts that its responsibility is to determine the limits or thresholds of the systems (above which they could not recover or maintain their integrity) and to ensure that these limits are not exceeded.

South African Water Quality Guidelines for domestic, recreational, industrial and agricultural use were published in 1993 (DWAF, 1993), but not so for the natural environment (Palmer *et al.*, in press), for which only a draft guideline has been published (DWAF, 1995).

DEFINITIONS

The following definitions referred to in the guidelines for aquatic ecosystems (DWAF, 1995) are useful:

Water quality is defined as "the physical, chemical, biological and aesthetic properties of water which determine its fitness for a variety of water uses and protection of the health and integrity of aquatic ecosystems".

Guidelines are defined as "a set of information provided for a specific constituent. It consists of the water quality criteria, including the Target Water Quality Range (TWQR) for that constituent and other supporting information, e.g. the occurrence of the constituent in the aquatic environment, the norms used to assess its affects on aquatic ecosystems, how these effects may be mitigated and the rationale for case-, site- and region-specific modifications".

Criteria are defined as "scientific and technical information provided for a particular water quality constituent, in the form of numerical and/or narrative descriptions of its potential effects on the health and integrity of aquatic ecosystems, and the fitness of water for other uses. The criteria used in these guidelines were derived on the basis that they have to provide long term protection assuming continuous exposure to water of a given quality".

THE APPROACH TO THE DERIVATION OF WATER QUALITY GUIDELINES

The approach to the derivation of water quality guidelines for aquatic ecosystems differs in several significant ways from the approach taken for agricultural, domestic, recreational and industrial water use because the environment should not be regarded as a "user" of water in competition with other users, but as a base from which the resource is derived and without which no development is sustainable.

With regard to effluent disposal, DWAF policy is to require all effluent to be treated and returned to natural water courses to augment the quantity of water, with the recognition that this affects the water quality. An important purpose of developing

water quality guidelines for aquatic ecosystems is, therefore, to provide information that can be used to determine the degree to which water quality could be altered through the return of effluent, while still maintaining healthy aquatic ecosystems.

A precautionary approach is required to reach the goal of protecting the health of aquatic ecosystems because of the vulnerability of aquatic ecosystems to changes in water quality, **the uncertainty of tolerance limits**, and the fact that there are very few options for mitigating the effects of impaired water quality (DWAF, 1995).

THE DEVELOPMENT OF WATER QUALITY GUIDELINES

In order to set water quality guidelines, information can be drawn from

1. historical patterns of chemical concentrations,
2. water quality guidelines set for other countries,
3. the relation between regional patterns of water quality and the natural distribution patterns of freshwater biota, and
4. ecotoxicological studies which can provide experimental results about the tolerance limits of specific taxa to specific chemicals or conditions (Palmer *et al.*, in press.

1. Historical patterns of chemical concentrations

The DWAF has an extensive water chemistry database which includes about 1000 sampling stations, some of which have been sampled since the 1960's. The variables reported include conductivity (sometimes TDS), pH, major ions (including alkalinity) various nutrients, silicate and fluoride (Dallas *et al.*, 1994). Analysis of previously recorded determinands and comparison with current ones could provide some insight into the changes occurring in water quality, although, as pointed out by Dallas *et al.*, (1994), the accuracy of some of the values reported is sometimes questionable, the sampling frequency varies from site to site, few data are available for seasonal and ephemeral rivers, and sampling programmes were initiated at different times for different stations.

2. Water quality guidelines set for other countries

Overseas conditions are seldom the same as those in South Africa, so that the extrapolation from overseas data to the local situation is not an adequate substitute for

local research (Dallas & Day, 1993). However, in the absence of data pertaining to the South African situation, use has had to be made of data published in international literature, for example, American, Canadian and Australian water quality guidelines (DWAF, 1995; Roux *et al.*, in press).

The aim of developing *South African* water quality guidelines is to develop a single set of guidelines and criteria that is appropriate for prevailing ecological conditions in South Africa, based on consensus amongst experts and water quality managers. In this way, it is hoped that the confusion that often arises from the use of different criteria and guidelines will be eliminated and that a sound basis for the establishment of water quality requirements for aquatic ecosystems and for each water use will be provided. In the past, certain guidelines or criteria have differed by a factor of 100 or more because different approaches and methodologies were used in their development (DWAF, 1995).

3. Regional patterns of water quality

Rivers are naturally divided into distinct zones according to their physical, chemical and biological characteristics (Day & King, 1995). Besides zonal differences, there are regional differences, for example KwaZulu-Natal rivers are very different from those in the south-western Cape. These regional differences result from differences in climate (temperature, mean annual precipitation and evaporation), geomorphology (gradient and erosion), geology and biota, and need to be considered when establishing guidelines for the protection of riverine ecosystems. Community composition in each region or zone is determined by the water quality, the type of habitat, characteristic hydraulic conditions, temporal variability and historical distribution of species. Water quality variables which could affect riverine ecosystems may be physical (turbidity, suspensoids, temperature) or chemical (pH, TDS, salinity, conductivity, nutrients, organic enrichment and dissolved oxygen (all non toxic), and the toxic biocides and trace metals. Each variable affects aquatic organisms to some extent - the overall effect in the case of more than one variable depending on whether they act synergistically or antagonistically. The effect of each variable on individual organisms is also influenced by the tolerance limits of the organism (Dallas & Day, 1993).

4. Toxicity tests and ecotoxicological studies

Toxicity tests are desirable in water pollution evaluation because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota (APHA, 1992). Recent developments in aquatic toxicity testing have resulted in the standardisation of methodologies through which test organisms respond in a clear and relatively consistent manner, not only interpretable by toxicologists, but useful to regulators and managers. However, few people in South Africa are currently competent to carry out toxicity tests, and only two or three laboratories can conduct tests with a representative range of freshwater organisms on a relatively routine basis (Roux, 1994).

The information gathered from various toxicity tests can be used in the management of pollution for the purposes of *prediction* of environmental effects of a waste, *comparison* of toxicants or animals or test conditions, or *regulation* of discharge (Buikema *et al.*, 1982) so would be useful in the setting of water quality guidelines.

While *toxicology* is concerned with effects of pollutants on single organisms, *ecotoxicology* is concerned with their effects on ecosystems and Moriarty (1983) makes a clear distinction between these two concepts. While the immediate effects of pollutants are on individual organisms (either by direct toxicity or by altering the environment), the ecological significance, or lack of it, depends on the indirect impact on the populations of species. A pollutant which kills half the individuals in a species population may have little or no ecological significance, whereas a pollutant which kills no organisms but retards their development may have a considerable ecological impact. Some pollutants have no effects on individual organisms but still have considerable ecological consequences (Moriarty, 1983), which makes the interpretation of toxicity test data difficult.

Although the new guidelines for aquatic ecosystems were developed to be representative of South African conditions, much of the information has come from data published in international literature and in international toxicological databases because very few data are available with which to assess the water quality requirements

of local species (DWAF, 1995). The information gained from both toxicity tests and ecotoxicological studies can therefore be of use in effluent regulation and control and in the setting of environmental water quality guidelines.

THE SCOPE OF THE PRESENT STUDY

This study involved both toxicity testing and ecotoxicology.

The toxicity aspect of the study involved the design and construction of a laboratory-based artificial stream system which would be suitable for testing chlorine toxicity to a selected species of riverine macroinvertebrate. The organism which was selected for this test series was the nymphal stage of the mayfly *Baetis harrisoni* Barnard (Order Ephemeroptera, Family Baetidae), which was found abundantly upstream from chlorinated sewage outlets, but was absent from the outfall, for a considerable distance downstream.

The ecotoxicological aspect involved an investigation of the changes in macroinvertebrate community structure in response to chlorinated, treated sewage effluent in the Umsunduze and Umbilo Rivers. This was done by collecting samples with a modified Surber-sampler at each site and examining the types and numbers of organisms which were present in riffle and pool samples at sites upstream from the effluent and comparing them to those collected at a number of sites downstream from the effluent.

It is not only the *chlorine* in sewage effluent which has toxic effects on the biota. The effluent itself is toxic to certain riverine organisms (Odum, 1971; Davies & Day, 1986; Dallas & Day, 1993). A secondary study was therefore undertaken in the Umbilo River in which the change in the community structure at a particular site after exposure to *unchlorinated*, treated sewage effluent was investigated.

Lamberti & Steinman (1993) suggest that one of the most useful applications of artificial stream research is the generation of testable hypotheses which can then be validated in natural stream ecosystems. This has been the approach followed in this study, and the aims of the study were therefore:

1. To develop an appropriate artificial stream system, and use it to establish experimentally the acute toxicity of chlorine to an organism tolerant of sewage effluent.

2. To use the results to hypothesise in-stream effects of chlorine both in respect of the test organism and the macroinvertebrate community.

3. To investigate the effects of chlorinated sewage effluent on a riverine macroinvertebrate community in the field, and to attempt to distinguish the effects of chlorine from those of sewage effluent.

4. To use the tolerance data and the field study to assess the newly developed environmental water quality guideline for chlorine (DWAF, 1995).

SUMMARY OF THESIS STRUCTURE

CHAPTER 1. *INTRODUCTION*

This chapter describes the context in which this study was carried out. It refers to the aridity of the country and the need for adequate water quality management if the water needs of the country are to be met. It mentions the setting of new water quality guidelines for the aquatic environment as appropriate to South Africa, and the lack of toxicity data relevant to indigenous species. The need for studies such as the present one is noted.

CHAPTER 2. *CHLORINE AND THE DEVELOPMENT OF AN ARTIFICIAL STREAM SYSTEM*

The use of chlorine in the treatment of sewage is discussed in this chapter. Mention is made of its chemistry (as it is a very versatile and reactive element) and its efficient disinfectant properties. The deleterious effect of its disinfectant properties in the context of riverine ecosystems is discussed, and the development of an artificial stream system suitable for chlorine toxicity testing is described.

CHAPTER 3. ***THE STUDY AREAS***

This chapter describes the Umsunduze and Umbilo rivers with regard to the geological and geographical features of the catchments and the nature of the pollutants which are found in the rivers. The sites selected for the ecotoxicolgical studies are described. A brief description of the Westville stream (from which mayflies were collected for toxicity tests) is also given.

CHAPTER 4. ***ACUTE CHLORINE TOLERANCE OF BAETIS HARRISONI FROM THE UMBILO RIVER AND A WESTVILLE STREAM***

This chapter deals with the issues of chlorine toxicity to benthic macroinvertebrates and the use of acute toxicity tests to determine lethal levels of chlorine. It describes the use of an artificial stream system to carry out these toxicity tests with the mayfly *Baetis harrisoni.*

CHAPTER 5. ***MACROINVERTEBRATE COMMUNITY AND SPECIES RESPONSES TO CHLORINATED, TREATED SEWAGE EFFLUENT IN THE UMSUNDUZE AND UMBILO RIVERS***

This chapter deals with the effects of chlorinated sewage effluent on riffle-dwelling macroinvertebrate communities in these two severely impacted rivers in KwaZulu-Natal. The methods of collection, sorting and identification of macroinvertebrates are described and an analysis of the data is presented.

CHAPTER 6. ***CONCLUSION***

This is the concluding chapter in which the results of the ecotoxicolgical study and the toxicity tests are discussed in the light of the Water Quality Guidelines.

CHAPTER 2

CHLORINE AND THE DEVELOPMENT OF AN ARTIFICIAL STREAM SYSTEM

The addition of chlorine to treated sewage prior to its release into the natural environment is deemed necessary in terms of reducing the risk to human health. However, when this treated, chlorinated sewage is discharged into aquatic environments it has serious consequences in terms of ecosystem integrity. In this study, an artificial stream system has been designed in order to conduct acute 96 h toxicity tests to determine the LC_{50} of chlorine for riffle-dwelling macroinvertebrates. In this chapter, the use of chlorine in the treatment of wastewater is discussed and the development of an artificial stream system which can be used for testing chlorine toxicity is described.

2.1 CHLORINE

INTRODUCTION

Water supplies and polluted waters are chlorinated mainly to destroy or deactivate disease-producing organisms (viruses, bacteria, spores, cysts and eggs). The inactivation mechanism of viruses by chlorine and other oxidants has not been resolved, but it is thought that chlorine kills living organisms by penetration of the cell wall or membrane and destruction of part of the enzyme system, thus preventing growth (White, 1992).

A secondary benefit of chlorination, especially in the treatment of drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulphide and some organic substances (APHA, 1992).

Chlorination can, however, also produce adverse effects. Taste and odour characteristics of phenols and other organic compounds present in a water supply may be intensified (APHA, 1992), and potentially carcinogenic chloro-organic compounds (e.g. chloroform and other trihalomethanes) may be formed (Shuval *et al.*, 1994). In addition, combined chlorine formed during chlorination of ammonia- or amine-bearing waters adversely affects aquatic life (APHA, 1992). During the late 1970's and early 1980's, considerable effort went into the collection of data concerning the acute effects of chlorine in fresh and salt water systems (Roberts, 1990).

In order to understand some of the behaviour and applications of chlorine, a brief account of its chemistry and manufacture are included in this chapter.

CHLORINE CHEMISTRY

Chlorine is an element in the halogen family, but is never found uncombined in nature. It is estimated to account for 0.15% of the earth's crust in the form of soluble chlorides e.g. common salt (NaCl), carnallite ($KMgCl_3 \cdot 6H_2O$), and sylvite (KCl). In nature, therefore, it exists only as the negative chloride ion with a valence of –1 (Cl^-). Chlorine is a most unusual and versatile chemical, since its properties differ so widely in the gaseous, liquid, and aqueous states (White, 1992).

When chlorine gas is dissolved in water, it hydrolyses rapidly to form hypochlorous acid according to the following equation:

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

Hypochlorous acid is a "weak" acid which means that it tends to undergo partial dissociation thereby producing a hydrogen ion (H^+) and a hypochlorite (OCl^-) ion as follows:

$$HOCl \leftrightarrow H^+ + OCl^-$$

In waters of pH between 6.5 and 8.5 the reaction is incomplete and both species are present to some degree. Hypochlorous acid (HOCl) is the most germicidal of all chlorine compounds with the possible exception of chlorine dioxide (ClO_2). The hypochlorite ion, on the other hand, is a relatively poor disinfectant because of its inability to diffuse through the cell wall of microorganisms (White, 1992).

Hypochlorous acid is always the active ingredient in any **hypochlorite** solution. It is produced when sodium hypochlorite or calcium hypochlorite dissociate according to the following equations:

$$NaOCl + H_2O \rightarrow HOCl + Na^+ + OH^-$$

$$Ca(OCl)_2 + 2\ H_2O \rightarrow 2\ HOCl + Ca^{++} + 2OH^-$$

Hypochlorous acid is known officially in the industry as **free available chlorine residual** (White, 1992).

MANUFACTURE OF CHLORINE

These days (1990's) most chlorine is manufactured by three types of electrolytic cells: diaphragm, mercury and membrane. Other methods of production are designed according to the available raw material containing the chloride ion. These methods include the electrolysis of hydrochloric acid, the electrolysis of brine (as carried out by 'Polifin' in South Africa (Polifin, 1995)) and the HCl oxidation process. Chlorine is also often produced as a by-product of heavy metal recovery e.g. the tungsten sponge process or the extraction of magnesium from magnesium chloride ore.

Commercially, chlorine is packaged as a liquefied gas under pressure in steel containers. This liquid vaporises easily at normal atmospheric temperature and pressure, producing an unmistakable irritating, penetrating and pungent odour (White, 1992). In those instances where facilities do not exist for handling this toxic gas, chlorine may be added to water in the form of a sodium hypochlorite solution (NaOCl) e.g. 'Jik' used as household bleach, or as calcium hypochlorite granules ($Ca(OCl)_2$) e.g. 'HTH' used in swimming pools.

The preparation of sodium hypochlorite is relatively simple and involves the reaction of chlorine with caustic soda (NaOH) according to the following equation :

$$2\ NaOH + Cl_2 \rightarrow NaOCl + NaCl + H_2O + Heat$$

sodium hydroxide chlorine sodium hypochlorite sodium chloride

The strength of a soda bleach (NaOCl) solution is commonly expressed in terms of its available chlorine content as *trade percent* or *percent by volume*. (Household bleach e.g.*Jik* states on the bottle "3.5% m/v when packed") i.e. 3.5g sodium hypochlorite/100g water.

During storage, sodium hypochlorite solutions can lose a significant amount of available chlorine in a few days and so, where specific chlorine concentrations are required, the user should monitor the decay rate of available chlorine of the stock solution. Also, it does not lose its strength at a constant rate per day, but at a decreasing rate as it loses its strength. **Fig 2.1** illustrates the chlorine strength decay rate over a period of 160 days for three different sources of hypochlorite under controlled conditions. These data reflect a 'best case' situation (White, 1992). In general, the more concentrated the solution, the more rapid is its initial decay.

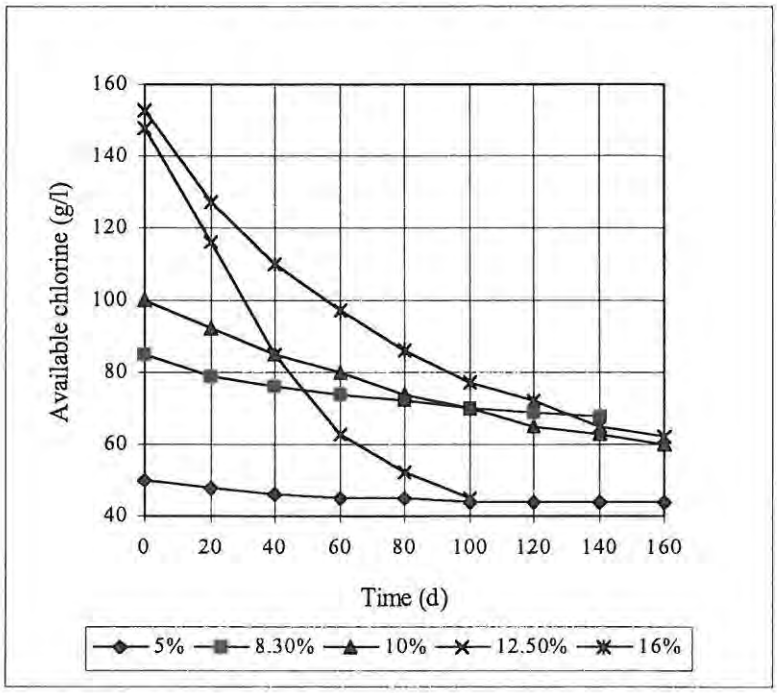


Fig. 2.1. Decay rate of various sodium hypochlorite solutions (after White, 1992). The concentrations of chlorine from various sources were measured over periods of up to 160 d to determine the rates of decay (chlorine loss) of the solutions.

Heat, light, pH and the presence of heavy metal cations (iron, copper, nickel and cobalt) affect the stability of hypochlorite solutions. Solutions should be stored in the dark below 21°C as it is estimated that the rate of decomposition of 10 and 15% solutions almost doubles with every 10°C rise in temperature (White, 1992).

THE USE OF CHLORINE AND HYPOCHLORITE IN WASTEWATER TREATMENT

Chlorine has a number of uses in wastewater treatment e.g. it is used for the control of odour in wastewater and foul air, and for disinfection. These days, chlorine use is often associated with disease control in humans, but until the late nineteenth century it was believed that disease was spread by odours (Baxter, 1994), e.g. the name *malaria* is derived from the words *mal aria* meaning *bad air* (Fripp, 1983). It was therefore believed that the control of odours should control the spread of disease. Chlorine was therefore used as a deodorant long before its value as a disinfectant was recognised. The earliest recorded large-scale sewage chlorination was in 1854 when the Royal Sewage Commission used chloride of lime ($Ca(OCl)_2$) to deodorise London sewage.

Bacteria were discovered around 1680 but only in about 1880 did investigations reveal that certain bacteria (now described as pathogens) caused specific diseases. Many of these pathogenic bacteria were found to be present in sewage. Chlorine was probably first used for sewage disinfection in 1879 when William Soper of England used chlorinated lime ($Ca(OCl)_2$) to treat the faeces of typhoid patients before disposal into a sewer. Chlorine was first used for sewage disinfection on a plant scale at Hamburg, Germany in 1893 to stem a disastrous typhoid epidemic (White, 1992).

Chlorine has been in continuous use for water disinfection since 1904 and as a result, water borne diseases such as typhoid, cholera, dysentery, amoebiasis, salmonellosis, shigellosis and hepatitis A have decreased greatly in the United States of America in the last 80 years (Johnson & Jolley, 1990).

Chlorine is used extensively in sewage treatment in South Africa. Human pathogens, which include viruses, bacteria, cysts of Protozoa (*Entamoeba, Cryptosporidium* and *Giardia*) and helminth eggs (*Ascaris, Trichuris, Taenia* and *Schistosoma*) are found in high numbers in sewage (Fripp, 1983) and conventional sewage treatment processes reduce but do not eliminate them. From the point of view of community health it is important to inactivate these pathogens before treated sewage enters rivers as, for

example, in KwaZulu-Natal, a large proportion of the population lives in informal settlements along the river banks and uses raw water for domestic purposes. Exposure to such pathogenic organisms would increase the incidence of disease in communities already affected by disease and poverty. Durban has a long history of amoebiasis among people living in informal settlements with no access to treated water or sanitation, so much so that for many years Durban has been the world centre for research in this field. (Mr T. Jackson, Medical Research Council (MRC), Durban. *pers. comm.*) The MRC (the former *Research Institute for Diseases in the Tropical Environment*) is probably the only place in the world which maintains stock cultures of the causative protozoan, *Entamoeba histolytica*. Failure to inactivate these pathogens in sewage would increase the morbidity and mortality of this sector of the population.

In addition, a recent survey carried out among children from rural schools around Durban has shown that 95% of them were infected with worms, many harbouring more than one species. (Mr I. Bailey, Umgeni Water, *pers. comm.*) This obviously has consequences in already malnourished children, as a high worm load increases lethargy, decreases mental alertness and retards both mental and physical development. As most worm infections are spread via the faecal-oral route, the incidence of these infections is high in communities who do not have access to sanitation facilities or clean water and so do not practise basic hygiene (i.e. washing hands after visiting the toilet or before preparing or eating food, or washing vegetables fertilised with night soil). The provision of sanitation to this sector of the community would decrease the incidence of helminth infections.

Quite apart from the point-source effluent discharges, many rivers in KwaZulu-Natal are highly polluted with raw sewage from informal settlements. The contaminated state of the Umsunduze and Umgeni rivers is the focus of much media attention in KwaZulu-Natal in January each year when participants in the annual 'Duzi Canoe Marathon take to the river for three days. In the past, a large number of the canoeists has suffered from diarrhoea (the well known *'Duzi guts*) following accidental consumption of the polluted water.

Chlorination of wastewater is standard practice in many other countries as well as South Africa. In more developed countries, other methods are also in use and these will be discussed further in **Chapter 6**.

In summary, chlorine is used in wastewater treatment for the following:

- disinfection (killing of pathogenic organisms);
- odour control and prevention of septicity (odours are the result of putrefaction of the solids in wastewaters);
- improvement of grease and scum removal;
- prevention of filter ponding;
- fly control;
- control of activated sludge bulking;
- odour control in the sludge-thickening process;
- control of waste-activated sludge disposal;
- control of foaming;
- destruction of cyanides;
- destruction of phenols;
- foul air scrubbing;

The trade-off of modern-day water chlorination is to make the best use of chlorine's excellent disinfection efficacy while reducing its environmental impacts and by-product toxicity. Chemists, biologists and engineers need to work together to identify, quantify and make the most effective use of the disinfectant forms of chlorine for effective disinfection; at the same time, they also need to identify, quantify and reduce the toxic forms of by-products formed by chlorine's reactions with organic compounds found in water (Johnson & Jolley, 1990).

While the biocidal properties of chlorine are undoubtedly of great benefit in water treatment, from an ecological point of view its killing efficiency can have serious implications, particularly in the context of sewage treatment where the treated effluent is released into rivers.

Under normal circumstances, aquatic organisms are able to process treated sewage effluent which enters rivers, and return the river to its original condition, provided that the river remains uncanalised and that other human intervention is not too severe (Davies & Day, 1986). If the effluent is chlorinated, however, the very organisms which would normally *process* this effluent are eradicated, which is detrimental to the health of the river as it would retard the recovery process. In this study, the effect of chlorine on some of these aquatic organisms is investigated. As chlorine exists in different chemical forms in wastewater, a brief overview of the chemistry of chlorine in wastewater is given below.

CHEMISTRY OF CHLORINE AND WASTEWATER

Chlorine dosed to water in its molecular or hypochlorite form initially undergoes hydrolysis to form **free chlorine** consisting of aqueous molecular chlorine, hypochlorous acid (HOCl) and hypochlorite ion (OCl^-), the relative proportions of which are pH and temperature dependent. Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form **combined chlorine**. Chlorine reacts with ammonia to form the chloramines: monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and nitrogen trichloride (NCl_3)(trichloramine). These compounds have very low, if any, gemicidal effect, and this aspect will be discussed further in **Chapter 4.** The nitrogenous compounds "consume" free chlorine rendering it unfit for disinfection purposes, hence the term "chlorine consumption". The term "chlorine demand" is used as an indication of the concentration of these nitrogenous compounds in the effluent: the higher the amounts of these compounds in the water being treated, the higher the chlorine demand i.e. more clorine would have to be dosed to achieve the desired level of disinfection. The presence and concentrations of these combined forms of chlorine are determined by pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand and reaction time.

Both free and combined chlorine may be present simultaneously. Combined chlorine in water supplies may be formed in the treatment of raw waters which contain ammonia or to which ammonia or ammonium salts have been added. Chlorinated wastewater effluents usually contain only combined chlorine (APHA, 1992).

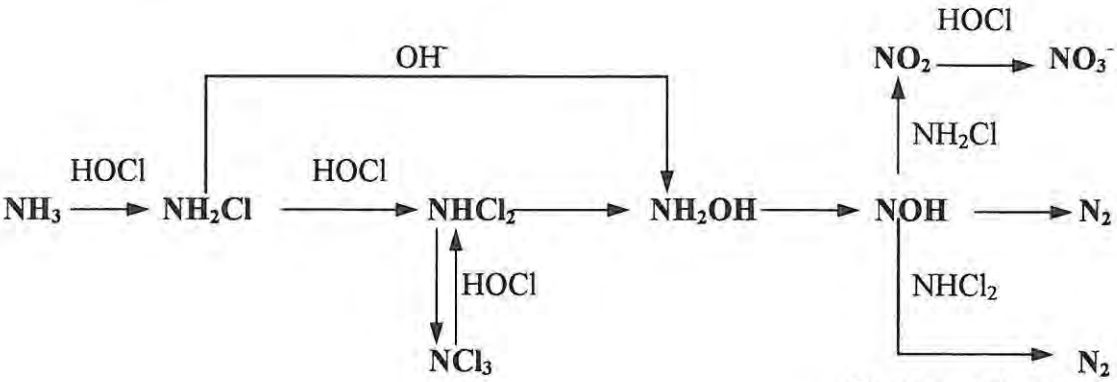
The chemistry of the chlorination of potable water, wastewater, and industrial waste is fundamentally the same. The reactions differ only because of the differences in chemical species and amounts of interfering organic and inorganic chemicals which either contribute to excessive chlorine consumption or impair the bacteriocidal efficiency of the chlorine residual.

The most important of these interfering substances in raw sewage are:
ammonia nitrogen, organic nitrogen, tannins, cystine, uric acid, and humic acid, Researchers have isolated and studied each of these to determine their effect on chlorine application, and the following are reported in White (1992):

i) Ammonia nitrogen is usually present in all wastewater effluents and reacts with chlorine according to the following equation at usual wastewater pH values (when the chlorine to ammonia nitrogen ratio is less than 5:1)

$$HOCl + NH_4^+ \leftrightarrow NH_2Cl + H_2O + H^+$$

A schematic reaction pathway for the chlorine/ammonia reactions is shown below:



(Gordon *et al.*, 1990)

If the pH is less than 7, dichloramine will begin to form. The rate of the ammonia-chlorine reactions is pH dependent, occurring fastest at pH 8.3 and slowest at pH 2. Chlorination processes usually operate in the neutral pH range and the formation of monochloramine from the chlorine-ammonia reaction is almost instantaneous. These reactions seem to take precedence over the penetration of chlorine into bacteria so may be a significant factor in the kinetics of wastewater disinfection.

ii) Organic nitrogen compounds are present in all wastewaters containing domestic sewage but their reactions with chlorine are relatively obscure. Studies of some of these compounds (e.g. proteinacious matter, amino acids and various nitrogenous compounds of urine) have shown that they are stable over a long period of time, even in the presence of free chlorine. Chlorinated organic nitrogen compounds (N-chloro compounds or organic chloramines) appear in the dichloramine fraction in the procedures for chlorine residual determination.

iii) Tannins

These compounds of organic nitrogen exert an extremely high chlorine demand and should be treated at the source before entry into the collection system.

The application of hypochlorite and chlorine gas in potable water and waste water treatment both achieve the same results, i.e. both result in the formation of hypochlorous acid. The only difference between the reactions of the hypochlorites and chlorine gas is the side reaction of the end products: the reaction with the hypochlorites increases the hydroxyl ions by the formation of sodium hydroxide whereas the reaction with chlorine gas and water increases the proton concentration by the formation of hydrochloric acid.

Sodium hypochlorite (often referred to as liquid bleach or soda bleach liquor) is the most widely used of the hypochlorites for potable water and wastewater treatment. It requires much more storage space than calcium hypochlorite does, and long distance transport is more expensive, but it is easier to handle and causes the least maintenance problems with pumping and metering equipment (White, 1992).

MEASUREMENT OF CHLORINE CONCENTRATIONS

Several methods are available for the measurement of chlorine concentrations. Because of the complexities of the reactions of chlorine with other substances in water, methods have been developed to distinguish between free chlorine, chloramines, chlorine dioxide, chlorite (ClO_2^-) and chlorate (ClO_3^-). Methods for most chlorine species (except chlorate (Freese & Knoble, 1994)) are described in *Standard Methods*

for the Examination of Water and Wastewater (APHA, 1992). Historically, the principal analytical problem has been to distinguish between free and combined forms of chlorine.

The choice of method depends on a number of factors e.g. the degree of accuracy required, the range of concentrations encountered, where the measurements are to be done (in a laboratory or in the field), the availability of appropriate equipment and the level of expertise of the operators. In addition, the choice of method depends on whether the water is natural / treated water or waste water.

1) Natural and treated waters

Iodometric methods are suitable for measuring total chlorine concentrations greater than 1 mg/ℓ but for greater sensitivity the amperometric end-point of these methods should be used (APHA, 1992). The iodometric titration method is used by most laboratories as a reference method, rather than being used routinely (Gordon *et al.*, 1990).

The amperometric titration method is a standard of comparison for the determination of free or combined chlorine. A low-level amperometric titration procedure is necessary to determine total chlorine at levels below 0.2 mg/ℓ (APHA, 1992). According to Aieta *et al.* (1984), in drinking water the detection limits using amperometric or potentiometric titrations are believed to be approximately 0.05 mg/ℓ for chlorine dioxide, 0.02 mg/ℓ for chlorine and chlorite, and 0.25 mg/ℓ for chlorate.

The DPD colorimetric methods (described later) are operationally simpler for determining free chlorine than the amperometric titration (APHA, 1992), and have become the most widely used for the measurement of chlorine (Gordon *et al.*, 1990), but high concentrations of monochloramine can interfere with the free chlorine determination unless this reaction is stopped with thioacetamide (Steadifac) (Palin, 1986 ; Freese, 1992) or arsenite (APHA, 1992).

The **F**ree (**A**vailable) **C**hlorine **T**est, **S**yringaldazine (FACTS) was developed specifically for free chlorine over the range of 0.1 to 10 mg/ℓ and is unaffected by significant concentrations of monochloramine, dichloramine, nitrate, nitrite and oxidised forms of manganese. In this method, a saturated solution of syringaldazine (3,5-dimethoxy-4-hydroxybenzaldazine) in 2-propanol is used (APHA, 1992), and while this is comparable to the DPD test, one drawback is the difficulty of dissolving the syringaldazine in 2-propanol or in water (Gordon *et al.*, 1990).

2) Wastewaters

Water samples which contain organic matter present special problems in the determination of total chlorine. Residual chlorine exists in a combined state because of the presence of ammonia, amines and organic compounds, especially organic nitrogen. Although a considerable chlorine residual may exist there may be an appreciable unsatisfied chlorine demand. The addition of reagents in the determination may change these relationships and result in the loss of residual chlorine during analysis. Only the DPD method for total chlorine is performed under neutral pH conditions. The differentiation between free and combined chlorine is usually not made in wastewater as the dosage of chlorine to wastewater is seldom great enough to produce free chlorine.

For free chlorine determinations, the amperometric method is the method of choice because it is not subject to interference from colour, turbidity, iron, manganese or nitrite nitrogen. While the DPD method is subject to interference from high concentrations of monochloramine, this can be avoided by the addition of thioacetamide immediately after the addition of the DPD reagent. As with natural or treated waters, the FACTS method is unaffected by concentrations of monochloramine, dichloramine, nitrite, iron, manganese and other interfering compounds normally found in domestic wastewaters.

For total chlorine in samples with significant amounts of organic matter, the DPD methods, amperometric or iodometric back titration methods can be used (APHA, 1992). The DPD method using the various reagents in tablet form is the most widely accepted method in England (White, 1992).

In addition to the methods mentioned above, Gordon *et al.* (1990) evaluated a number of other methods for determination of free and combined chlorine, chlorite, chlorate and chlorine dioxide. These included:

- ultraviolet methods: generally not considered useful in routine monitoring of chlorine residuals because the molar absorptivities are quite low for chlorine and chloramine species. However, absorption spectrophotometric analysis can be used for the determination of relatively high concentrations of the species in relatively pure water.

- chemiluminescence: limited data are available but this method shows promise as an analytical method.

- fluorescence: shows potential but needs more research.

- membrane electrode methods: may play prominent roles in chlorine residual measurements in the future. The potentiometric electrode is suitable for continuous measurements and seemed to give results that were acceptable when compared with the amperometric titrator.

For field measurements, the DPD method is most convenient (and is the method used by Umgeni Water in KwaZulu-Natal). Because chlorine in aqueous solution is not stable and the chlorine content of samples or solutions, particularly weak solutions, decreases rapidly, chlorine determinations should be started immediately after sampling, avoiding excessive light and agitation. Exposure to sunlight or other strong light or agitation accelerates the reduction of chlorine and samples to be analysed for chlorine should not be stored (APHA, 1992). In this project, the DPD method using the Lovibond® Comparator was chosen for the determination of free and combined chlorine as it is one of the few methods which can be used in the field and in the laboratory.

The DPD Method

The Palin method for determination of chlorine, chlorine dioxide and chloramines uses the reagent diethyl p-phenylene diamine (DPD). A red colour results from the reaction of this reagent with either chlorine, chlorine dioxide or iodine at pH values between 6.0 and 7.0. In the presence of a small amount of potassium iodide, monochloramine reacts with the reagent, whilst if more potassium iodide is present, dichloramine will also react (Adams *et al.* 1966).

The DPD test reagents are available in tablet form and are used in conjunction with a Lovibond® Comparator in which the colour which develops in the sample in one glass cuvette is matched with a blank in a second glass cuvette, behind which is a 'chlorine disc'. This disc has nine different pink coloured glass inserts each corresponding to a particular chlorine concentration. The disc is rotated behind the cuvette containing the blank until a colour match is obtained. This method is used by field staff of Umgeni Water as a reliable and accurate means of measuring free and total chlorine. The principle tablets of the DPD system are numbered 1 to 4 as follows:

Tablet No.	Active ingredient	Chlorine species
DPD No. 1	diethyl p-phenaline diamine, with some ethyl diamine tetra acetic acid (EDTA) to complex any interfering metal ions and a buffer	free chlorine
DPD No. 2	stabilised potassium iodide	monochloramine
DPD No. 3	potassium iodide	dichloramine
DPD No. 4	all reagents in a single tablet	total chlorine

Table 2.1 The contents and uses of DPD Tablets 1 to 4 (after Palin, 1986)

In this study, only the DPD No. 1 and No. 3 tablet were used for free and total chlorine respectively.

Three comparator discs were used :

3/40 A for range 0.1 to 1.0 mg/ℓ available chlorine (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1.0) *(Note: there was no match for 0.9 on this disc so this had to be estimated between 0.8 and 1.0)* Confidence limit: ±0.1 mg/ℓ

3/40 B for range 0.2 to 4.0 mg/ℓ available chlorine (0.2, 0.4, 0.6, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0) *(Note: this was suitable for "range-finding" as the intervals above 1.0 were quite large)* Confidence limit: ±0.2, 0.5 or 1.0 mg/ℓ depending on the concentration.

The discs above were used with the 13.5 mm/10 mℓ glass cells in which one of each DPD tablet was used.

3/40 E for range 0.02 to 0.30 mg/ℓ available chlorine (0.02, 0.04, 0.06, 0.08, 0.1, 0.15, 0.2, 0.25.) *(Note: this disc was used where chlorine concentrations below 0.1* mg/ℓ *were present.)* Confidence limit: ±0.02 mg/ℓ for concentrations below 0.1 mg/ℓ

The disc above was used with 40 mm/20 mℓ glass cells, and two of each DPD tablet were used. (Lower concentrations of chlorine can be detected with this cell because twice the volume of sample is used and the glass cell is placed sideways into the comparator so that the viewer looks through a greater depth of sample, which therefore appears more intense than it would in the 10 mℓ cell. For this reason a separate disc, calibrated differently, is necessary.)

The Lovibond® Comparator DPD technique is, however, not without pitfalls and discrepancies. Care must be taken when preparing samples for analysis e.g.

1. The DPD No.1 tablet should under no circumstances be touched as this affects the reading. If dropped, it should be discarded (Freese, 1992).

2. The sample cuvettes should be rinsed well between determinations, particularly after total chlorine analysis, as contamination with potassium iodide affects the free chlorine reading. Trace quantities of iodide cause monochloramines to react during the free chlorine determination and give rise to positive errors. Separate glass cells should therefore be used for free and combined chlorine measurements. (APHA, 1992; Freese, 1992.)

   In this study, 3 of each of the glass cells were purchased: one for the free chlorine determination, one for the total chlorine and one for the blank. They were labelled as such and were never interchanged.

3. As mentioned previously, a significant breakthrough of combined chlorine into the free chlorine DPD determination can occur when combined chlorine (e.g. chloramine) concentrations are high (i.e. > 0.5 mg/ℓ). By adding 2 drops of thioacetamide solution to the free chlorine solution immediately after mixing the DPD No. 1 tablet, reaction between the chloramines and the DPD tablet can be prevented.

4. Discrepancies can result in concentrations which lie between the upper limits of the "low concentration" disc and the lower limits of the "high concentration" disc. It has been recommended that the high range disc be used only for concentrations greater than 1 mg/ℓ (Freese, 1992).

5. The colour matching could be subject to human error as it is sometimes difficult to decide which coloured glass is the "best match" for the sample in the cuvette, and where there is no glass for a particular concentration, an educated guess must suffice.

6. The limit of detection (using the 40 mm cell) is 0.02 mg/ℓ. With the "Nessler Attachment", the limit is 0.01 mg/ℓ. The purchase of a Nessler Attachment was considered at one stage, but it was decided that the small increase in the number of concentrations which could be measured did not justify the considerable extra expense, especially since the concentrations which were important to this study were less than 0.01 mg/ℓ. The main benefit of the Nessler Attachment is that the concentrations indicated on the disc (code NDPB) increase by increments of 0.01 in the 0.01 to 0.1 mg/ℓ range, whereas the increments for the same range on the 3/40 E disc are 0.02 mg/ℓ. The disadvantages (apart from the initial expense) of the Nessler Attachment are that 50 mℓ sample tubes (code AF 306) are used, which require the use of 5 DPD tablets per chlorine determination (which has further financial implications), and that the Nessler attachments appeared to be more inconvenient to use than the Lovibond Comparator. These points were reiterated by Mrs S. Freese of Umgeni Water in an Internal Report for Umgeni Water (Freese, 1993). Concentrations below 0.02 therefore could not be measured accurately, except to note that they were between 0 and 0.02 mg/ℓ.

7. When the chlorine solution is too concentrated, it causes bleaching of the solution. When this occurs, the solution in the cell should be discarded and the test re-done with solution diluted with distilled water prior to the addition of the tablet.

The method used for the determination of free and total chlorine using the Lovibond® Comparator is described in **Appendix A.** In addition to using separate glass cells for free and combined chlorine readings to prevent contamination of the samples with potassium iodide, and rinsing the cells thoroughly between readings, separate glass rods were also used for crushing and mixing the tablets in the cells.

The determination of chlorine concentrations will be discussed further in **Section 2.2** in the context of the artificial stream system.

Section 2.2 describes the development of an artificial stream system suitable for testing chlorine tolerance. The original design was that of a recirculating system, but it was subsequently found that, because of the complexities of chlorine chemistry and the duration of the toxicity tests, a constant chlorine concentration could not be maintained without the accumulation of unwanted by-products. The design was therefore changed to that of a flow-through system, which in turn posed new problems with respect to water supply and dosing apparatus for the chlorine. The next section, therefore, deals with the concept of artificial streams, the design alternatives, the problems which were encountered during the development of the artificial streams and the ways in which these problems were solved.

2.2 THE DEVELOPMENT OF AN ARTIFICIAL STREAM SYSTEM FOR ACUTE, CHLORINE TOXICITY TESTING

INTRODUCTION

An artificial stream can be defined as a constructed channel having a controlled flow of water, which is used to study some physical, chemical, or biological property of natural streams (Lamberti & Steinman, 1993). Artificial streams have been used as models of stream ecosystems to investigate a wide range of organisms, populations, community and ecosystem characteristics and functions (Clark *et al.*, 1980), and have been developed in a wide variety of shapes and sizes (Lamberti & Steinman, 1993). The complexity in design of experimental lotic systems (artificial or model streams) seems proportional to the number of people employing them (Clark *et al.*, 1980, Craig, 1993).

The design and location of such systems depends on a number of factors, many of which are, of course, linked. These factors are:

- The location of the system: indoor or out-door.
- The space available for the system.
- The size of the system: large-scale or small-scale.
- The type and size of the test organisms.
- The personnel available to conduct the experiments.
- The position of out-door streams relative to existing waterways: in-stream or out-of-stream.
- The type of system: recirculating or flow-through.
- The nature of the dependent variable (i.e. the condition under study) e.g. a pollutant or toxin: how it reacts with other chemicals in the system.
- The duration of the investigation: long term or short term studies.
- The financial constraints.

The choice of location of an experimental stream system is linked to the proposed size of the system and the amount of space available to accommodate such a system. Artificial streams range in size and shape from large, straight outdoor artificial streams

at the Monticello Ecological Research Station (MERS) which are 520 m long (Arthur, 1988), to miniature, circular laboratory streams (only 150 mm in diameter) powered by a stream of air bubbles (Mackay, 1981).

Large streams have many advantages over small streams e.g.

- they can support multiple trophic levels for long periods of time;
- they may display greater biological realism;
- they can be used to validate laboratory experiments.

Disadvantages of large streams are that

- the cost of construction and maintenance is high; *
- the number of replicates may therefore be low,
- because of stream complexity, cause and effect relationships may be more difficult to discern (Swift *et al.*, 1993)
- the system would require more personnel to operate and for data processing* (Frutiger, 1984).

(*These factors are therefore related to the financial aspect of the project.)

These *artificial* streams have advantages over *natural* systems in that

- they allow for replication of treatments;
- they are generally easier to manipulate.

Outdoor streams have an advantage over *indoor* ones (in laboratories or greenhouses):

- they follow the natural diurnal variations in light, daylength and temperature (Palmer *et al.*, in press).

However it may be more difficult to keep the controlled variables of an experiment constant in outdoor streams.

Large artificial streams would probably be more suitable for long-term experiments, as they allow for the colonisation of the stream by a variety of organisms thereby creating a more "natural" environment than the smaller streams, which would probably be more suitable for short term experiments, e.g. 96 h acute toxicity tests.

Stream size would also depend on the size of the organisms under study - salmon would obviously need larger systems than *Daphnia*. Systems such as the miniature laboratory stream powered by air bubbles would be useful for passive filter feeders e.g. Hydropsychidae which can orient themselves in a uni-directional current (Macay, 1981). An advantage of such a small "stream" is that many can be housed in a small space, and they would be relatively inexpensive to construct and operate, so many replicates could run concurrently.

The decision as to whether to construct a *recirculating* or a *flow-through* artificial stream system depends on what the experimenter intends to do with such a system, and each type of system has its advantages and disadvantages:

The advantages of a *flow-through* system are that there is no accumulation of waste-products from the test organisms or by-products from the reactions of chemicals during the course of the experiment as water enters one side of the stream, flows through the stream then leaves and is discarded down a drain. There would presumably be less microbial habitation of the system: in a recirculating system, the same body of water is present in the system all the time so there is more opportunity for the development of microbial communities over time.

If the water in such a system comes from a river or a dam, the environment would be more "natural" than if treated municipal water were to be used. Also, there is no need to change the water on a daily (or other) basis. If the streams are portable, they could be used in the field either in or next to a real stream or river, and could be colonised by organisms from that stretch of water.

A disadvantage of flow-through systems is that they require a great deal of water. When such a system is used for toxicity testing, it requires continuous dosing of the toxicant, which could prove expensive in terms of the cost of dosing pumps and the chemical under study.

The aim of this study was to design and construct, a medium-sized, laboratory-based, artificial stream system to conduct acute 96 h toxicity tests. The following part of this chapter describes the development of this system from the original recirculating stream system to the final flow-through one.

METHOD DEVELOPMENT

1. THE PROTOTYPE ARTIFICIAL STREAM SYSTEM

A prototype artificial stream system was designed and constructed to determine various factors e.g. the suitability of PVC guttering for such a stream, the shape and size of opening at the outlet, the size of water pump needed to provide adequate water movement and whether or not test organisms would survive in such a system.

The first artificial stream system consisted of a 600 mm length of white PVC flat-bottomed guttering. In cross section, the stream was trapezoidal in shape with a base 91 mm wide, sides 70 mm high, sloping outwards so that the top of the stream was 120 mm across. A 9 mm hole was drilled in the "upstream" stop-end to allow the insertion of a 2 m length of 9 mm clear plastic tubing. A 50 mm diameter hole was cut in the "downstream" stop-end to accommodate a plastic joint which served as the outflow. The stream was placed at an angle of about 5° so that there was a region of shallow, fast-flowing water at the head of the stream and a region of deeper, slower water at the outlet.

A 54 ℓ glass fish tank was used as a sump and to house the water-pump as shown in **Plate. 2.1**. Water from the tank was pumped up to the inlet by a "RENA Powerhead C20" submersible water pump. This pump had an output of 200 ℓ/h and a delivery head of 0.7 m. The water flowed down the stream and fell 500 mm to the water level in the tank, enabling some aeration to occur. To increase the aeration process, two airstones (connected to an aquarium air-pump) were placed in the tank. Ten white kaolinite "stones" (about 60 x 40 x 20 mm) were placed in the stream to provide a substrate for the organisms, as the stream-bottom was rather smooth. A piece of nylon silkscreen mesh (mesh size 0.3 mm) was tied over the outlet tube to prevent the escape of test organisms from the stream into the tank.

Plate 2.1. The prototype artificial stream system.

Laboratory tap water and river water were used in this system. (An analysis of this water, as supplied by Umgeni Water, is provided in **Appendix B**.) This system was a recirculating one as the water flowed down the stream into the tank and was pumped back up to the head of the stream.

Test run

A selection of organisms was collected from a riffle upstream from the outlet of the Darvill Wastewater Works in the Umsunduze River to determine

i) whether or not they would survive in the system and

ii) which organisms would be suitable for use in toxicity testing in such a system.

This was purely a qualitative exercise. The organisms were collected by picking up stones in the riffle, dislodging the animals using a nylon brush and flushing them into a cooler-box with some of the river water. Stones and sand were also collected from the

river to provide a substrate for the organisms. In the laboratory, the organisms were transferred to the artificial stream using a beaker so as to minimise damage to them. River water was used in the system and gradually replaced with de-chlorinated tap water as it evaporated. (The tap water was dechlorinated by allowing air to bubble through it for at least 48 h before being used. A supply of such water was maintained in the laboratory to replenish water lost by evaporation and splashing.)

Among the organisms collected were baetid mayfly nymphs, caddis fly larvae, simuliid larvae, limpets and planarians. Although each toxicity run lasts only six days, these organisms were kept in the laboratory for over a month to determine their survival ability in an artificial stream environment. Commercially available fish flakes (Tetramin) were used as food (Haigh & Davies-Coleman, in press).

The survival of these test organisms was considered to be very good, in that by the end of the month, most of the mayflies had emerged and flown away, (a few were still in the nymphal stage in the tank), the blackflies had pupated, the caddis flies had built new homes, the limpets had survived and the planarians had reproduced.

The mayfly *Baetis harrisoni* looked promising as a test organism for toxicity tests in this system and the choice of this organism will be discussed further in **Chapter 4**.

Although the test organisms survived in the artificial stream, it was felt that the flow-rate with the C20 pump appeared to be a bit too low (in comparison with their natural environment) so it was decided that for the new system, larger pumps would be used. Also, owing to the size and shape of the outlet pipe in the stop-end, eddies formed near the outlet and the water appeared to be moving very slowly there. A number of modifications were made and these are described below.

2. THE UPGRADED, REPLICATED RECIRCULATING SYSTEM

After the apparent success of the prototype artificial stream system, work began on the construction of 12 similar streams. By having 12 streams, 4 systems, (each with 3 replicates), could run concurrently. For each "run" there could therefore be three

different test solutions and a control. The steams were arranged in threes overhanging a 54 ℓ fish tank (300 x 300 x 600 mm). A plastic 300 mm ruler was attached to the side of each tank to facilitate measurement of water depth to calculate the water volume.

A disadvantage of this design was that replicate sets of three channels shared a sump, consequently becoming pseudoreplicates (Hurlbert, 1984). True replicates have no water connection between test containers (APHA, 1992) so each stream needed to have its own separate water system.

The four stream systems each with three replicates are shown in **Plate 2.2** below:

Plate 2.2. The replicated artificial stream systems

Each stream consisted of a 1 m long piece of flat-bottomed PVC guttering, the base of which was 91 mm wide. A 12 mm hole was cut in the upstream stop-end to hold the clear plastic tubing. In order to standardise the water flow in each stream, the length of tubing (which transferred the water from the tank to the stream) was the same length for each stream (2 m). A trapezoidal hole, 20 mm from the edges of the stop-end, was cut in the downstream stop-end to allow a more even water-flow out of the stream. The hole was covered with 0.6 mm stainless steel mesh (which was sealed around the sides with silicone sealant) to prevent the escape of the test organisms.

In order to facilitate more rapid water movement, larger water pumps were purchased. These were the "Rena Powerhead C40" which had an output of 600 ℓ/h and a delivery head of 1.4 metre.

Calibration of the streams

In the context of this project, the term calibration is used to describe the process of defining the experimental conditions in the artificial stream systems. The physical and chemical conditions in these streams were established prior to the addition of chemicals or organisms so as to have a base-line against which to compare the altered water quality.

1. Hydraulic calibration

An Ott propeller meter was borrowed DWAF to measure the flow rate in the streams. However, the smallest propeller available was 30 mm in diameter. This meant that where the water was deep enough for the propeller, the flow was not strong enough to turn it, and where the water was flowing faster, the streams were too shallow to accommodate the propeller. (This propeller has to be totally submerged during measurement of flow rate.) The only place where the meter could be made to turn was directly in the stream of incoming water (at the inlet pipe). However, Mr W Rowlston of DWAF provided the following data about the velocity (v), water depth (y) and cross-sectional area (A) at various points in the artificial stream:

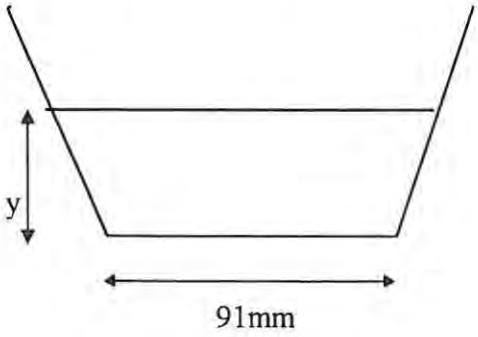
Stream viewed from above:

Distance downstream:	960 mm	750 mm	500 mm	250 mm	0 mm
Velocity:	0.034 m/s	0.036 m/s	0.039 m/s	0.045 m/s	*Jet velocity:* ***1.18 m/s***
Water depth (y)	39 mm	37 mm	34 mm	30 mm	
Area (A):	**3 949 mm^2**	**3 727 mm^2**	**3 398 mm^2**	**2 982 mm^2**	

Cross section of stream:

y = water depth

A = cross sectional area $(91\ y + 0.2632\ y^2\ mm^2)$



2. Ambient temperature

The laboratory in which the stream system was housed initially was not air conditioned, and while this had not posed a problem during the initial stages of the project (since it was winter), in the summer the temperature rose to 30 °C during the day. No cooling facilities (for water or air) were available, and the mayflies emerged and flew away during the course of the experiments. The system was moved to an air conditioned venue where the ambient temperature could be maintained at about 20 °C, which solved the problem of mayfly emergence.

3. Physico-chemical calibration

The following measuring instruments were purchased:

1. WTW Pocket oxygen meter Oxi 92 / SET (for dissolved oxygen and temperature)
2. WTW Pocket conductivity meter LF 92 / SET (for conductivity and temperature)
3. Beckman Φ10 pH Meter (for pH and temperature)
4. Lovibond® 2000 Comparator TK 100 (for free and total chlorine)

All four tanks were filled to the same level (so as to contain 45 ℓ of tap water) and the following parameters were monitored over a period of 6 d (the duration of a test run):

1. amount of water lost by evaporation and splashing.
2. conductivity
3. temperature
4. pH
5. dissolved oxygen

Water loss (ℓ)	Average water loss: 8.3 ℓ (18% of starting volume)
Conductivity ($\mu S.cm^{-1}$)	Average increase: 15 $\mu S.cm^{-1}$
Temperature (°C)	Fluctuated between 19 and 20 °C
pH	Fluctuated between 7.9 and 8.2
Dissolved oxygen (%)	Fluctuated between 99 and 101 % saturation

Table 2.2. Summarised results of the physico-chemical calibration of the four artificial stream systems over a period of 6 d.

The water loss from the tanks owing to evaporation and splashing during the 6 d calibration period is shown in **Fig. 2.2**. On average, it amounted to 3% of the starting volume per day. By having the ruler attached to the side of the tank, it was possible to check the water volume and it would have been possible to maintain a constant water level for the duration of a test-run if these streams had been used for toxicity tests.

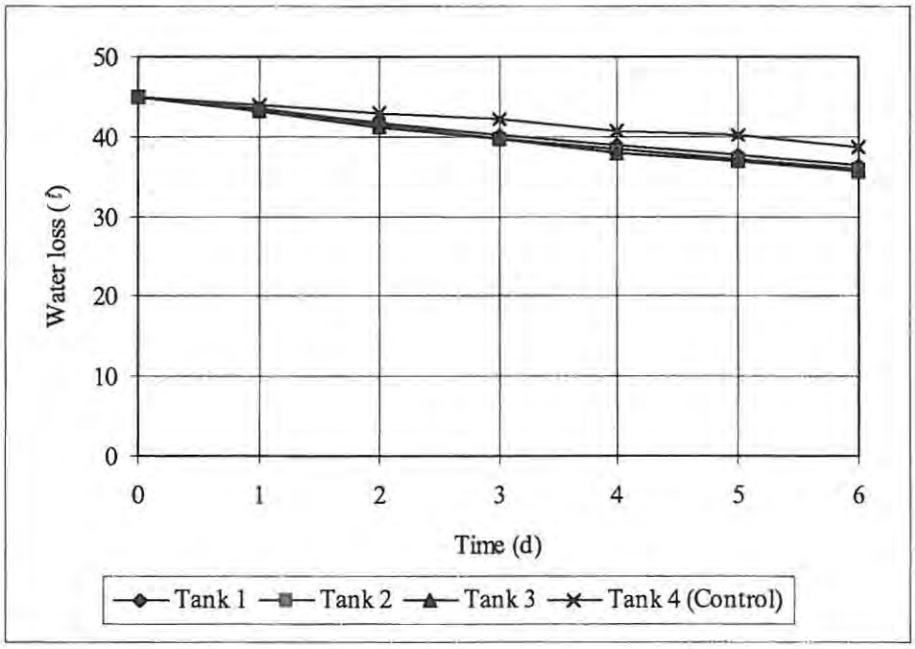


Fig. 2.2. Water loss from tanks over 6 d during calibration of the streams

Chlorine concentrations in the recirculating streams

As explained earlier, the rate of chlorine loss from water depends to some extent on the amount of sunlight present and the temperature of the water. It was known that the chlorine would be lost from the system, but just how fast the chlorine would disappear from the water in the laboratory (where there was no direct sunlight) at a temperature of 20°C was not known. Experiments were therefore carried out to monitor the rate of chlorine loss in the artificial streams over periods of 96 h (the duration of a toxicity test) to determine whether or not the chlorine concentration would remain constant for long enough to conduct the experiments.

Each tank was filled with 45 ℓ of tap water and the water pumps and air pump were switched on. They were left for 48 h before chlorine (in the form of sodium hypochlorite) was added. Three tanks were dosed with 45 mℓ of sodium hypochlorite each and the forth tank was left as a control. (This sodium hypochlorite was in the form of household bleach, with a concentration of approximately 3.4%) The concentrations of free chlorine and combined chlorine were monitored daily for 96 h. **(Fig 2.3.)**

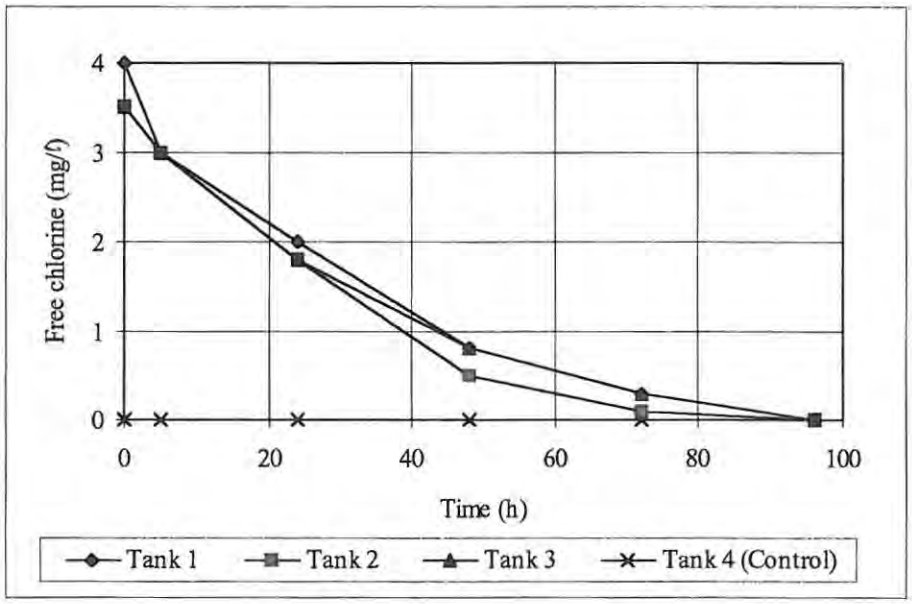


Fig. 2.3. Free chlorine concentration in tanks over 96 h

Free chlorine and total chlorine concentrations were measured at 24 h. intervals. There was no difference between the free chlorine and total chlorine readings, indicating an absence of combined chlorine. This absence of combined chlorine was not surprising as municipal tap water was being used, and it was not expected to have a high chlorine demand.

Since the chlorine had all disappeared before the end of the 96 h test period, it was obvious that sodium hypochlorite would have to be added at regular intervals during the test period to maintain the required chlorine concentrations. However, it was noted that even in the absence of chlorine in the system, the conductivity of the water increased during the 96 h because of the water lost by evaporation (**Fig. 2.4**). This increase in conductivity was more noticeable in the experimental streams where sodium hypochlorite had been added. The continued addition of sodium hypochlorite during the 96 h test period would cause the conductivity to increase to high levels, so that the deaths of the test organisms could be attributed not only to the chlorine in the water but also to the increased conductivity.

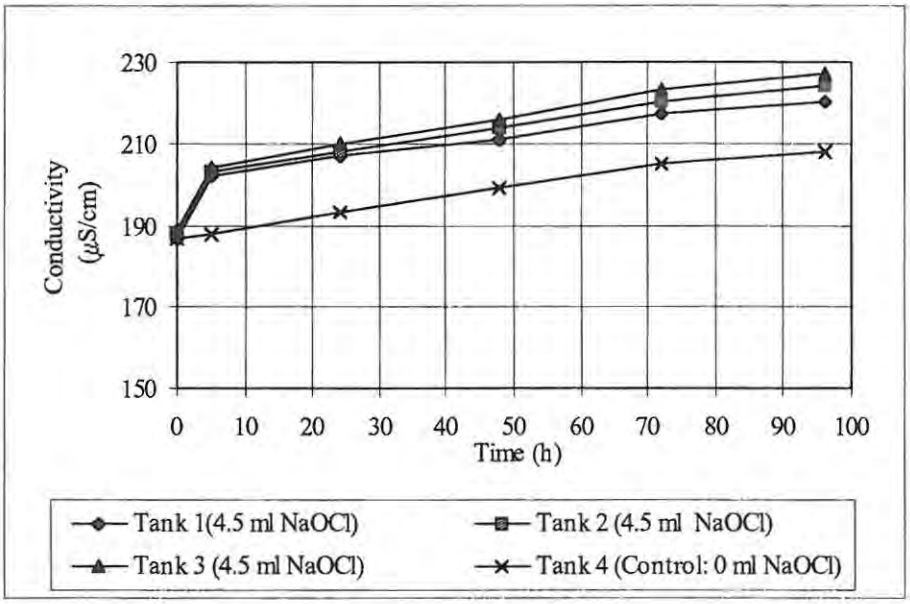


Fig.2.4 Increase in conductivity over 96 h.

Where Time = 0 hours, this is the conductivity prior to the addition of sodium hypochlorite.

Where Time = 5 hours, this is the conductivity after the addition of 4.5 mℓ of sodium hypochlorite to each of the experimental tanks (Tanks 1-3). No sodium hypochlorite was added to the control tank (Tank 4).

The increase in conductivity was then investigated using three different chlorine concentrations: 1 mℓ, 2 mℓ and 3 mℓ of sodium hypochlorite (household bleach, with a concentration of approximately 3.4%) were added to 45 ℓ of water in Tanks 1, 2 and 3 respectively, and Tank 4 served as the control. A clear distinction between the conductivity increases in the four tanks over the 96 h test period can be seen in **Fig. 2.5** below.

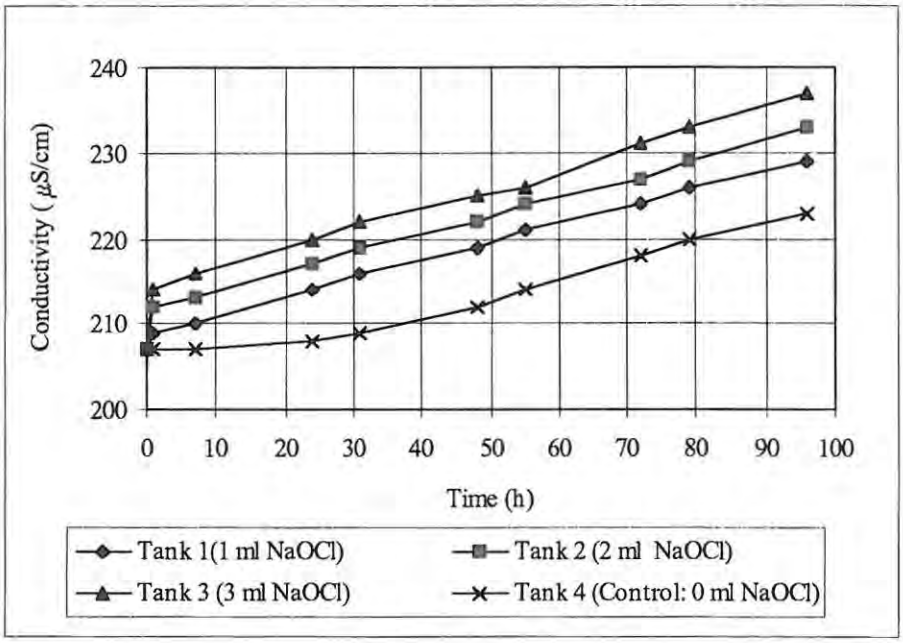


Fig 2.5. Conductivity increases for three different sodium hypochlorite concentrations over 96 h

The **chlorine** concentrations (free and total) which resulted from the addition of 1, 2 and 3 mℓ of sodium hypochlorite respectively are shown in **Fig 2.6**. There was a rapid rate of chlorine loss from the system within the first 24 h. (This experiment was not replicated and no statistical analyses were done as it was only exploratory. This system was not actually used.)

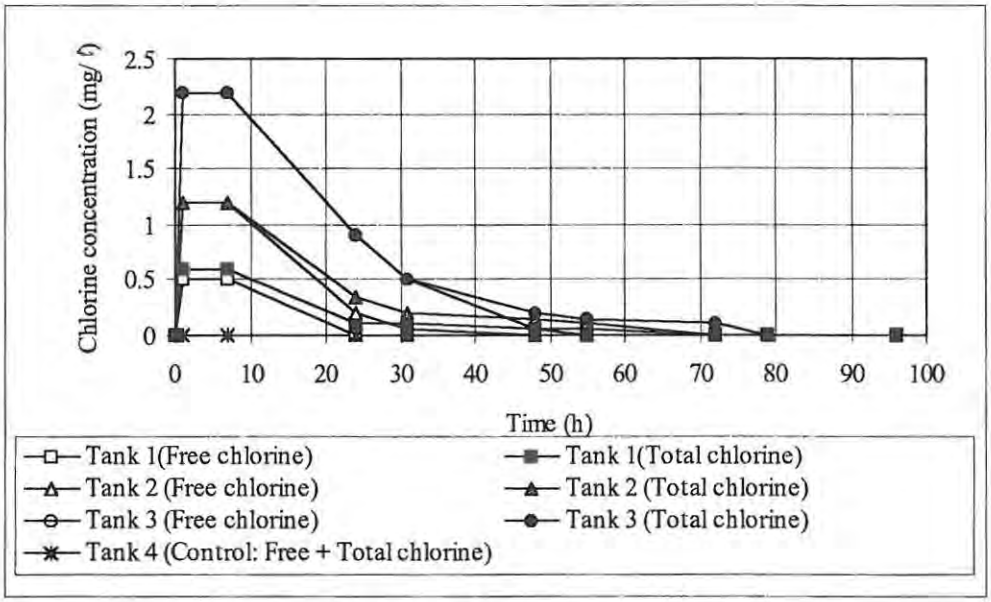


Fig. 2.6. Chlorine loss in tanks over 96 h.

The results of the chlorine calibration indicated that, while such a "recirculating" system would be suitable for testing the toxicity of salts or heavy metals (which remain in the system once added), it was clearly not suitable for a reactive or volatile chemical such as chlorine. A "flow-through" system was therefore required. Since water in a "flow-through" system is used only once before it is discarded down the drain, such a system uses a considerable amount of water. (With 12 pumps each capable of delivering 600 ℓ/h, this would amount to 7 200 ℓ/h or 17.2 kℓ/d.) In addition, the presence of varying chlorine concentrations in the tap water would have an effect on the chlorine concentration required in the experimental streams as it could not be dechlorinated prior to use. Since it was not possible to do this at Technikon Natal, an alternative venue was sought.

3. THE FLOW-THROUGH ARTIFICIAL STREAM SYSTEM

The new venue

Permission was granted to set up the artificial stream system at the Process Evaluation Facility (PEF) at the Umgeni Water Wiggins Waterworks. Wiggins Waterworks is situated in the Cato Manor area of Durban. It is designed to treat raw (untreated) water from the Umgeni River and from Inanda Dam and it supplies potable water to the lower Durban area (Umgeni Water information pamphlet). The PEF is a laboratory in which water treatment pilot plants can be erected and evaluated.

There were several advantages in having the streams at the PEF:

A continuous supply of raw water was available on tap in large quantities. The use of raw water was considered to be advantageous in that it was more *natural* than laboratory water, i.e it was more similar to water which mayflies would encounter in the natural environment than laboratory water which is treated for domestic use and is chlorinated. Also, the water temperature followed that of water in the natural environment (fluctuating between 23 °C in the summer and 13 °C in the winter, with a daily variation not exceeding 1°C) which obviated the need for an air-conditioned venue.

The new system

The conversion of the recirculating system to a flow through one necessitated some major changes. These developments are discussed in the order in which they occurred. The system is shown in **Plate 2.3** below.

Plate 2.3 The Artificial Stream System at Wiggins Waterworks

1. The stand

"Dexion" shelving was used to build a stand which was suitable for supporting the 12 streams (at a height which would allow easy access to all parts of each stream) and from which dosing bags could be hung. The advantage of using this shelving is that it is easily adjustable so at any stage the height of the stand could be changed to obtain the desired slope of the streams. A 3 m length of gutter with a downpipe was attached to the shelving at the front of the stand to collect the waste water and convey it to the drain in the floor below.

2. The water supply.

Raw water is pumped to the PEF from either Nagle Dam or Inanda Dam. Chemical and physical characteristics of the raw water at Wiggins PEF are shown in **Appendix B**. A 40mm hose was connected to the mains supply to direct water into a 200 ℓ PVC drum in which the pumps were placed. A valve was placed between the main shut-off valve and this hose so that the flow of water into the tank could be controlled. By careful manipulation, a balance could be achieved between the inflow into the tank and the discharge from the streams so maintaining a steady water level in the tank. The maintenance of a constant water depth in the tank was important as this had a direct effect on the discharge from the pumps: a higher water level would mean a lower delivery head and therefore a greater discharge from the pumps. Because of the continuous dosing in this system, fluctuations in the flow rate of incoming water would change the concentration of chlorine in the water: the higher the flow rate, the lower the concentration of chlorine in the steam.

The water supply was also used by several water purification pilot plants at the PEF. One plant in particular drew large volumes of water at intervals, interspersed with periods of no water use. This meant that as the various users drew water from the pipeline, the pressure in the pipe was affected which in turn affected the amount of water in the tank - a lower pressure meant that the tank did not fill up fast enough to keep up with the discharge. The resultant lower water level resulted in a lower output from the pumps, which in turn, would result in an increased chlorine concentration in the streams. There was also the danger that the tank would empty completely during

the night, and in the absence of someone to turn the pumps off, they could burn out. (These pumps were designed exclusively for water use, without which they would overheat and be irreparably damaged.) This was obviously not a satisfactory state of affairs.

The problem was solved by the use of a larger (500 ℓ) fibreglass tank borrowed from the PEF. A large float valve was attached to the inlet of the tank so that the first control valve could be left fully open allowing an adequate water supply into the tank to ensure the maintenance of a constant water level regardless of other water users. When the pumps were running, they were 250 mm below water level and the outlet tube (into the stream) was 600 mm above the water level. The float valve prevented overflow when other plants were not drawing water.

3. Dissolved oxygen in the raw water

The streams from which the mayflies were collected had a dissolved oxygen concentration of between 100 and 112% saturation however, the DO of the raw water was only about 75%. As mayflies are sensitive to oxygen concentration, this problem had to be rectified.

The largest aquarium air pump available from the pet shops was purchased and was connected to two 300 mm air stones. However, this pump was found to provide no increase in the DO content of the water. The pump was returned and it was decided to use the compressed air supply at the PEF. In all, eight air stones of the following lengths were purchased: 2 x 300 mm, 2 x 400 mm, 2 x 600 mm and 2 x 800 mm. This amounted to 4.2 m of air-stone. With this air supply, the DO could be increased from 75% to 100% saturation in 35 min. when the water pumps were off (DO Standing), and from 75% to 98% saturation in 95 min. with the water pumps running (DO Running) as shown in **Fig. 2.7**. Once the DO of the water in the tank had been raised, it could be maintained between 98 and 100% saturation with the water pumps running.

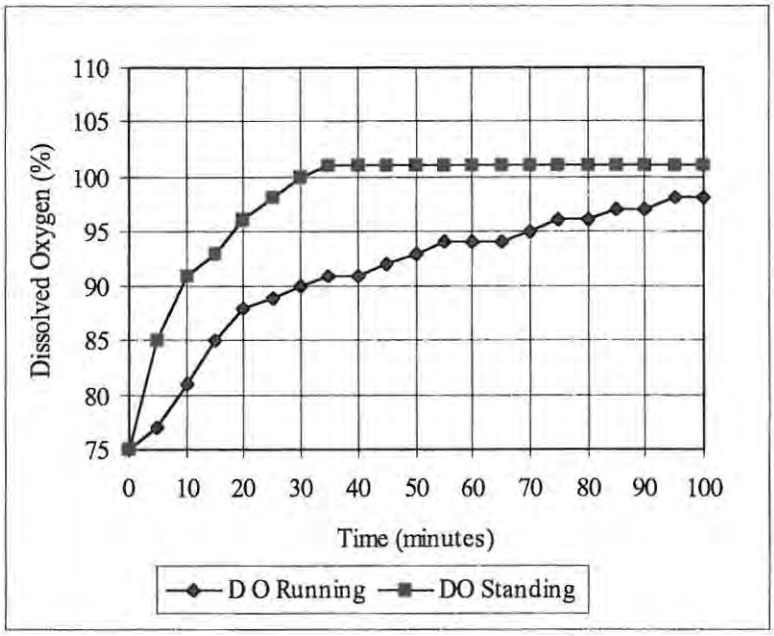


Fig. 2.7 Aeration of the raw water in the tank. (Temperature: 20 - 22 °C.)

4. Trial run with river organisms

A selection of macroinvertebrates was collected from the Umbilo River. As with those collected from the Umsunduze River, they were transported to the laboratory in cooler boxes with ice packs to keep the water cool. Each cooler box had a battery operated air pump to ensure an adequate oxygen supply and to promote water circulation while in transit from the river to the laboratory. (At this stage it had been decided to use organisms from the Umbilo rather than from the Umsunduze River, as discussed in **Chapter 4**.) These organisms were collected from Sample Sites 1 and 2 which were upstream from the effluent outlets.

The organisms collected included mayfly nymphs (almost exclusively *Baetis harrisoni*), dragon fly nymphs (Aeshnidae and Libellulidae), chironomids (Orthocladiinae and Chironominae) and simuliids (mainly *Simulium adersi*). This was not a particularly successful trial run as by the following morning, most of the organisms had escaped through the stainless steel mesh at the outlet. A finer mesh was therefore required.

However, a finer mesh would retard the flow of water out of the streams, so to facilitate more rapid water movement, the holes in the outlet stop-ends were extended to within 10 mm of the edges and the 0.6 mm mesh was replaced with 0.3 mm stainless steel mesh.

Another selection of macroinvertebrates was collected from the river and the streams were restocked with organisms and left overnight. While the smaller mesh solved the problem of organisms escaping through the mesh, it caused another problem. During the night, the mesh clogged up with debris and small crustaceans (which were mainly *Cladocera*) from the raw water. As a result the streams overflowed and, once again, all the organisms escaped !

In order to prevent the cladocerans and debris from getting into the streams, a filter was made by sewing up a bag (250 x 450 mm) of fine silkscreen mesh (mesh size 0.15 mm). A drawstring top allowed quick and easy removal and replacement of the bag for cleaning. Fine fishing line was used to stitch the bag instead of cotton thread as the latter would probably have rotted too quickly. The bag was attached to the pipe at the water inlet so that it hung in the tank. After the installation of this filter bag, no further clogging of the outlet screens occurred.

Two two-way valves and a T-piece were then put into the incoming hose so that the water could be redirected straight from the mains supply down the drain without entering the tank while the filter was removed and rinsed. These valves also allowed the main pipes to be flushed before water was allowed into the tank at the beginning of a run. When no water was being drawn from the main raw water pipe (e.g. over the weekend most of the other plants were not running), mud accumulated in it so that when the main valve to the tank was first opened, the tank would be filled with very muddy water. On the occasions when this happened (prior to the inclusion of the bypass valves) it would take the best part of a day to clean out the tank as the mud would deposit as a thick layer on the bottom.

During these trials, each stream had 10 white kaolinite stones placed in it for the organisms to hold on to. However it was noticed that very few of them actually used the stones. Most of the mayflies held onto the stream bottom or congregated on the stainless steel grid at the outlet. A strip of silkscreen mesh (100 x 1 000 mm) was therefore placed on the bottom of each stream and the mayflies seemed to find this a better substrate. Only four of the "stones" were left in the streams and these were arranged in a staggered pattern (**Plate 2.4**) to form a mixing weir for the chlorine solution in the incoming water.

6. Dosing with chlorine

The dosing of the test chemical is more challenging in a flow-through system than in a recirculating one. In a recirculating system, the appropriate amount of chemical is added to the water and it remains there for the duration of the experiment (unless it is reactive or volatile). However, in a flow-through system, continuous dosing is required. This can be a problem as dosing pumps are very expensive. Also, if the test chemical is expensive, this could also be a problem as a flow-through system would require a large amount thereof. In addition, the problem of the waste-water from the streams arises. As such a large volume of water is required, if it is polluted with some toxic chemical, this could pose a disposal problem.

Investigations into the purchase of dosing pumps for the sodium hypochlorite solutions revealed that they were beyond the financial limits of this project, especially since nine streams would be operating concurrently. Intravenous drip bags were therefore used to provide a steady flow of sodium hypochlorite solution into the incoming water. (Subsequently it was found that Mr R. Birch, from Unilever in the U.K., who came to see the system at the PEF had also made use of intravenous drip bags for dosing of chemicals into test chambers.) Because these drip bags collapse as they deliver their contents, an almost constant drip rate could be maintained. It was only when the bags became almost empty that the drip rate slowed noticeably. Initially the drip rate was controlled with the rolling adjusters which came with the bags. However it was found that after a few hours, the delivery tubing would be squashed so much that the dripping had all but stopped.

Baxter "CONTROL-A-FLO" Regulators (Code 2C 7591), which are made for use with intravenous drip bags, were bought to control the drip rate. The drip rate was set at 15 drops per minute because at this rate , it was calculated that a 1 ℓ drip bag could last from 16h00 (when the PEF closed) to 08h00 the following day (when it opened). All sodium hypochlorite concentrations were calculated according to this drip rate which was kept standard throughout. It was found that once the drip rate for each bag had been set (in itself, a time-consuming process) it remained constant as long as the drip bags did not become too empty. The end of the plastic tubing from which the drops emerged was attached to the incoming water tube in the stream so that the test solution was drawn into the water in a continuous stream rather than pulsing with each drop.

In order to determine whether the test solution from the intravenous drip bags would disperse adequately in the water, dye tests were carried out. Red and green food colouring were placed in separate bags and were released at various drip rates. Visual examination of the water in the streams indicated that mixing was very good and there were no "dead" spots where organisms could escape to a "chlorine-free" area. Without the kaolinite stones, mixing did not appear to be as good and there were small areas near to the upstream end where the colour appeared to be less intense. The tests were very difficult to photograph and a single frame does not give a true reflection of the mixing, but an example is shown in **Plate 2.4**. In this instance, a pulse of colour was released so pale areas in the stream can be seen. However, when a continuous stream of drops was released, no such areas were seen. The position of the kaolinite stones in the photograph (used as mixing weirs) should be noted.

The dye was also used to determine the residence time of the water in the streams. Because of the complex nature of the chlorine reactions, the residence time of the sodium hypochlorite solution in the stream had to be determined as this would be an important factor in the chlorine speciation. Pulses of dye were released, and the times taken for the colour to disappear completely from the stream were recorded. It was found that after a pulse of dye had been released, it took between 25 and 30 seconds for the dye to disappear. (This test was repeated six times.) Subsequent discussions

with Dr P.Wade of Watertek, CSIR (a specialist in chlorine speciation) led to the hypothesis that, owing to the short time interval involved, most of the chlorine in the streams would be present as free chlorine. Usually the measurements of free and total chlorine in the streams using the Lovibond® Comparator showed no difference between these two readings.

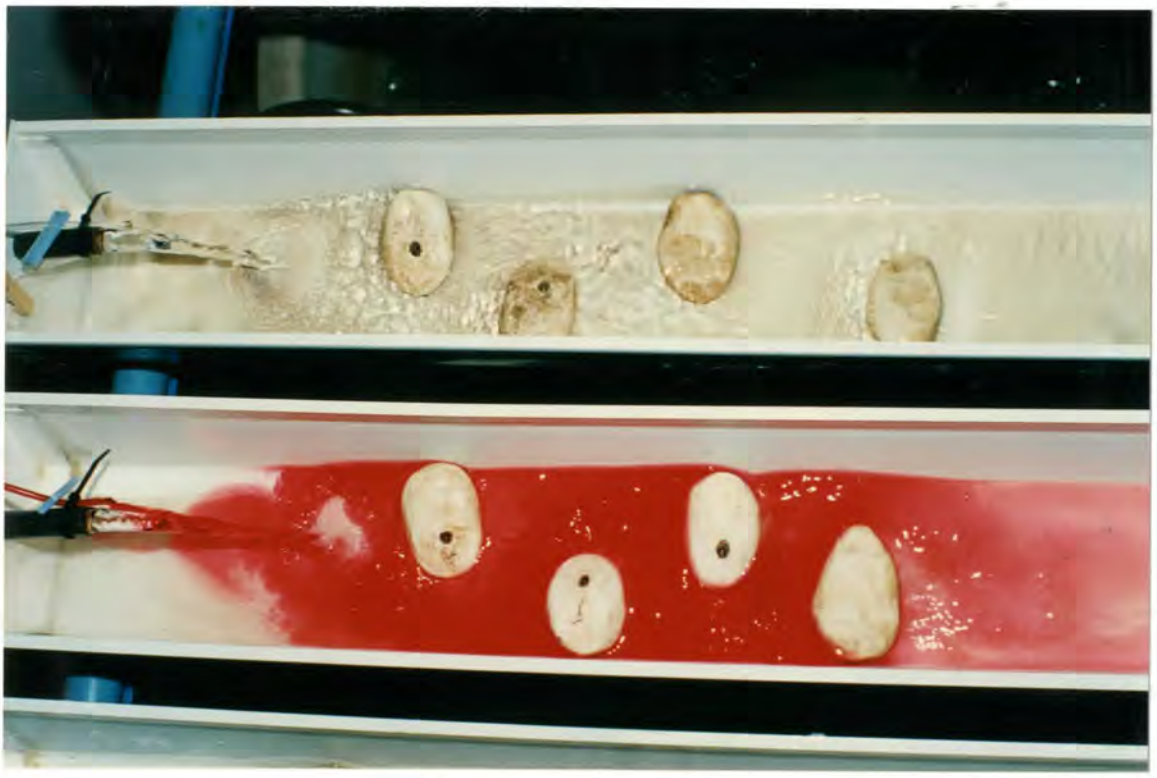


Plate 2.4. Dye test to show mixing of test solution (food colouring) and water.
(Note the narrow, red, test-solution tube next to the wider water inlet tube.)

Chlorine calibration of the flow-through artificial stream system

In order to determine the amount of sodium hypochlorite solution which should be added to the intravenous drip bags, chlorine calibrations were carried out. (The strength of the sodium hypochlorite solution use for this stream system was between 12 and 15% (m/v) at the beginning of the calibrations) The chlorine calibrations were done in the following way : a known aliquot of the stock solution was diluted with enough distilled water to make up 1 ℓ of solution. This solution was allowed to drip at

15 drops per minute into the incoming raw water in the stream. Samples were collected at the outflow and free and combined chlorine concentrations were determined using the comparator.

The first calibration was carried out in January during the developmental stage of the stream system (without organisms being present) and the next was 5 months later in May at the time of the toxicity tests. As mentioned previously, the concentration of the stock solution of sodium hypochlorite decreased over time and this can clearly be seen in the results of the chlorine measurements shown in **Fig. 2.8** below. As there was no difference between the free and total chlorine measurements, only the free chlorine concentrations are shown.

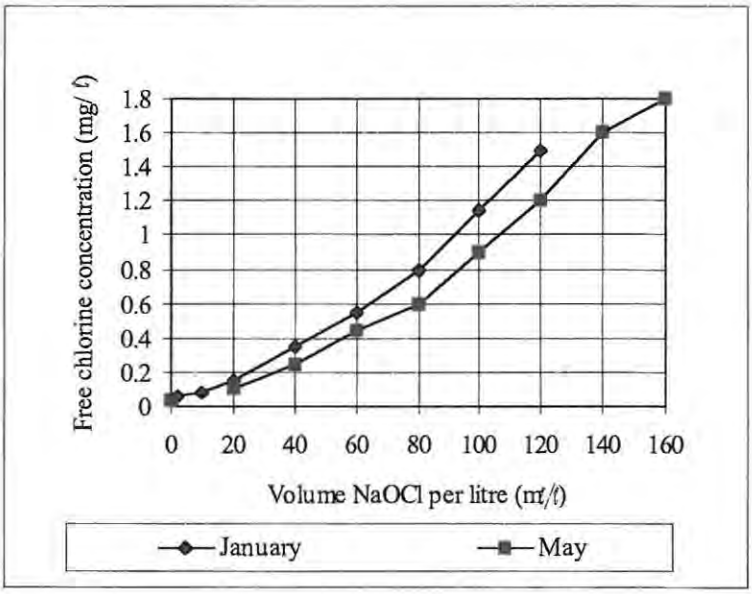


Fig. 2.8. Free chlorine concentrations in the artificial streams which resulted from the dosing of various concentrations of sodium hypochlorite. The decrease in free chlorine concentration from January to May (as a result of decay of the stock solution) is evident.

The reliability of the Lovibond® Comparator

Because of some discrepancies in the readings obtained, an investigation was carried out to compare the readings obtained using the Lovibond® Comparator and those using the Palintest Interface Photometer 7000 which was in the laboratory at the PEF at Wiggins Waterworks.

Sodium hypochlorite test solutions were made by placing 1, 2 and 3 mℓ aliquots of the stock solution into volumetric flasks and diluting them with distilled water (pH 8.0) to make up 1 ℓ of solution (as would be done for the intravenous drip bags for the toxicity tests). These solutions were then transferred to drip bags from which 15 drops (the number of drops released per minute in the artificial streams) were released into a 50 mℓ flask, to be diluted further with either distilled water (with no chlorine demand) or the raw water from the PEF (which would be used in the artificial streams).

Free and total chlorine concentrations were then measured using the Lovibond® Comparator and Palintest Interface Photometer (both of which make use of the DPD tablets). Because the time which the sodium hypochlorite spends in the raw water is important with respect to the formation of combined chlorine, speed was of the essence, and each 50 mℓ dilution was done as quickly as possible and measured immediately. The results are presented in **Appendix C**.

The correlation between the results obtained from the two instruments appeared to be very good. One advantage of the Palintest Photometer is that the values were given to two decimal places whereas the comparator discs 3/40A and 3/40B gave only one decimal place. Only the 3/40E disc gave readings to two decimal places. Also, the photometer does not rely on human judgement for colour-matching, whereas with the comparator, where the colour comparison lies between two colour plates on the disc, the user must estimate the concentration. The concentrations on the discs **A**, **B** and **E** are shown in **Table 2.3** below:

A:					0.1		0.2		0.3	0.4	0.5	0.6	0.7	0.8	1.0				
B:							0.2			0.4		0.6			1.0	1.5	2.0	2.5	3.0	4.0
E:	0.02	0.04	0.06	0.08	0.10	0.15	0.20	0.25	0.30										

Table 2.3 The chlorine concentrations (in mg/ℓ) represented by the coloured glass plates on discs A, B and E of the Lovibond Comparator. The concentrations common to two or more discs and concentrations not present on the discs can clearly be seen.

In similar comparative investigations, Freese (1993, 1994) from Umgeni Water, compared the Lovibond® Comparator with the Hach DR 700 colorimeter and with a Hanna chlorine meter, both of which are portable and suitable for field work. The correlations were good, indicating the validity of using the Lovibond® Comparator for field and laboratory work. However, for low chlorine concentrations, or where greater accuracy is required, (i.e. readings to 2 decimal places), it would be worth considering the use of a photometer where the option is available.

The Palintest photometer was available at the PEF at Wiggins Waterworks for the latter part of the project and it was used for comparative purposes (to verify the Lovibond results) and to try to determine the lower chlorine concentrations. However, because of the low colour intensity of the chlorine/DPD solutions, the Palintest photometer also produced inconsistent readings at concentrations less than 0.05 mg/ℓ, which were encountered in the toxicity tests. For concentrations below about 0.03 mg/ℓ, the same sample inserted into the photometer five times would give as many different readings (in no particular order). The colour of the solution becomes more saturated with time, so it might be expected that the readings on the photometer would increase every time the same sample was reinserted, but this was not the case. The readings showed no pattern (e.g. 0.02, 0.01, 0.02, 0.03, 0.01). For this reason, each graph of the toxicity test results in **Chapter 4** is accompanied by a graph of those chlorine concentrations which could be measured, and the best that could be done for the lower concentrations was to extrapolate back to 0 and read the unknown values from the graph. Conventionally, graphs and tables of the same data are not both presented, however, in this case, the tables were included to indicate when free and total chlorine concentrations varied and when they did not, because the graphs on their own (needed to estimate chlorine concentrations) could be misleading: when the free and total chlorine concentrations were the same, only the total chlorine points were displayed on the graph, although free chlorine appears on the legend.

Titration of the stock solution of sodium hypochlorite.

In a further attempt to determine the lower chlorine concentrations present during the toxicity tests, the "available chlorine" in the sodium hypochlorite stock solution was determined by titration with sodium thiosulphate. The method chosen was the iodometric method which is described in Standard Methods and is provided in **Appendix C**, along with the appropriate calculations. (This method is used routinely at Wiggins.) Unfortunately this method does not distinguish between free and combined forms of chlorine, however it can be used to determine the available residual chlorine.

When a sodium hypochlorite solution containing 1 mℓ of stock solution made up to 1 ℓ with distilled water was used, the calculations based on the titration indicated that the concentration of chlorine in the artificial stream was **0.0056 mg/ℓ**. This was in keeping with the chlorine concentration for 1 mℓ of sodium hypochlorite solution which can be read from the Toxicity Test Graph of 9 May (**Fig. 4.3**) which is between **0.004** and **0.005 mg/ℓ**. This value is important and will be referred to again in **Chapter 4.**

CONCLUSION

A medium-sized, laboratory-based, flow-through artificial stream system had been designed and constructed for conducting 96-h acute toxicity tests. The system was suitable for testing the toxicity of chlorine (in the form of sodium hypochlorite) to riffle-dwelling macroinvertebrates. The toxicity test procedures and results are discussed further in **Chapter 4.**

The design of this artificial stream system was such that it could be dismantled and reassembled reasonably easily and quickly (each process taking approximately a day), so it would be possible to set it up at a sewage works where toxicity testing with treated sewage (in various concentrations) could be carried out. In addition, it would possible at some sewage works (where there is access to the treated sewage prior to chlorination) to conduct toxicity tests with treated sewage to which various doses of chlorine had been added.

CHAPTER 3

THE STUDY AREAS

INTRODUCTION

The effect of chlorinated sewage effluent on benthic macroinvertebrate communities was studied in two rivers in KwaZulu-Natal, the Umsunduze (in the Pietermaritzburg area) and the Umbilo (in the Durban area). Both rivers are severely impacted, the Umsunduze mainly by untreated sewage and the Umbilo mainly from industrial effluent. These rivers are shown on the map of part of KwaZulu-Natal, **Fig 3.1.**

Toxicity tests were conducted on organisms collected from two sources in the Durban area: the severely impacted Umbilo River; and a relatively uncontaminated stream in Westville. These are shown on the map of the Durban area, **Fig. 3.3.**

3.1 THE UMSUNDUZE RIVER

The Umsunduze (Msunduze/Umsinduzi) River is one of the five major tributaries of the Umgeni River. (The other four are the Lions, Karkloof, Umpolweni and Umqeku Rivers.) Together these rivers supply most of the water requirements of the Pietermaritzburg/ Pinetown/ Durban area, the majority of the rural settlements in the catchment, as well as the agricultural and environmental water demands. The Umgeni River catchment covers an area of 4437 km^2 of KwaZulu-Natal (Ninham Shand, 1995).

The Umsunduze River is 123 km long and has a mean annual runoff of 176 million cubic metres. The section under study is shown on the map of the Pietermaritzburg area, **Fig 3.2.**

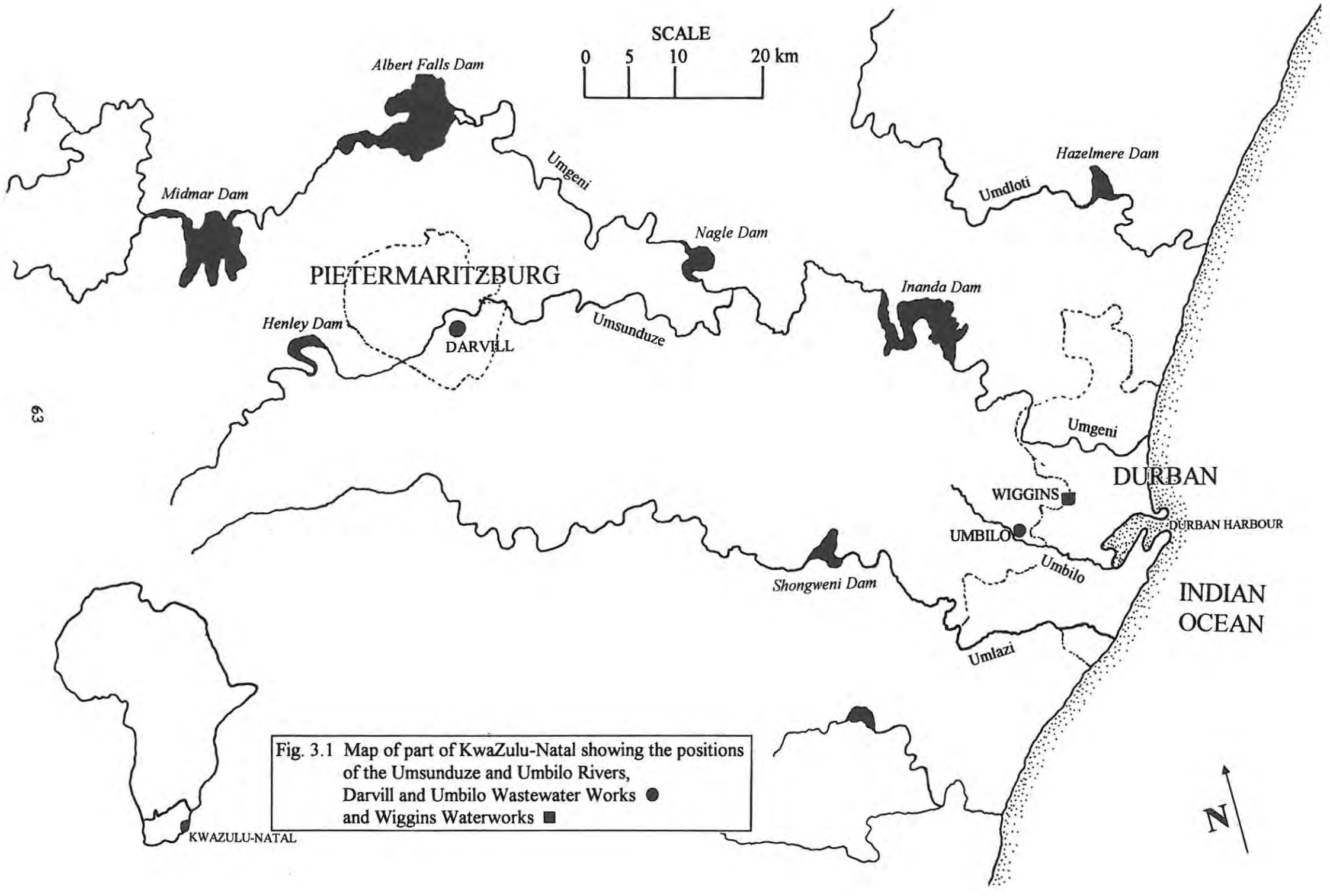


Fig. 3.1 Map of part of KwaZulu-Natal showing the positions of the Umsunduze and Umbilo Rivers, Darvill and Umbilo Wastewater Works ● and Wiggins Waterworks ■

GEOLOGY AND SOILS

The geology of the Umgeni Catchment consists of a series of strata with the Cape and Karoo sedimentary rocks overlying old granites at the base. The upper part of the Umsunduze catchment consists of Beaufort sandstone, Ecca shale and dolerite. Just beyond Pietermaritzburg is an area of Dwyka tillite. From there to the end of the Umsunduze (at its confluence with the Umgeni River) is an area of exposed basement granite.

The soils in the upper parts of the Umgeni and Umsunduze River catchments tend to be deep, permeable, well-drained and fertile yellows and reds. The dolerite sills in the shales and mudstones in the headwater areas provide the highest abstraction potential and result in the surface seeps and springs which are widespread in the upper Umsunduze catchment.

TOPOGRAPHY AND MORPHOLOGY

The channel and catchment characteristics of the Umsunduze, Lions and Karkloof Rivers are similar to the upper Umgeni River, although the catchment and banks of the Umsunduze River above Henley Dam ($5,9 \times 10^6$ m^3) have been severely destabilised, with increased erosion, by informal livestock grazing and watering.

The source of the Umsunduze River is >1700m above sea level. A dolerite dyke forms a small escarpment which runs across the catchment, from the headwaters of the Mpolweni River in the north, through the Karkloof and Howick Falls to the Umsunduze River around Henley Dam in the south. This results in the many waterfalls and steep gorges on the rivers along and downstream of this escarpment. The gradients of the Umgeni and Umsunduze Rivers flatten as they flow onto the plain below this escarpment. The Umsunduze River and its tributaries flowing through Pietermaritzburg have been modified and redirected into grass-lined channels. The weirs forming Camps Drift were constructed to provide a recreational impoundment in Pietermaritzburg.

As the Umgeni and Umsunduze Rivers enter the steep-sided valleys of the Valley of a Thousand Hills, the gradients steepen and the rivers become wide and braided, on coarse sandy beds with rocks and boulders. The incised river banks are covered with dense scrub-bush and dry grass.

RIVER FLOW

The seasonal distribution of natural river flow follows rainfall throughout the catchment, with about two thirds occurring from November to March. The annual river flow, however, is very variable and may be as low as 17% or as high as 240% of the average.

LAND USE

Land use activities have a great impact on the river flow, water use and water quality in the catchment. Land use in the upper Umsunduze catchment above Henley Dam is largely informal rural settlement with mixed crops and livestock. Settlements in and around Pietermaritzburg are a mixture of informal and formal urban, while the lower Umsunduze has some commercial agriculture, but is predominantly rural in character.

WATER USE

Apart from an annual January recreational release from Henley Dam for the "Duzi Canoe Marathon", no other allocations are presently specified for recreation or the natural environment.

Domestic and industrial users in the urban areas are supplied with treated water which is abstracted from the main water supply impoundments. The upper region from Pietermaritzburg to Wartburg and as far as the upper part of Pinetown and Richmond, are supplied by Midmar and Henley Dams.

WATER QUALITY

Both the Umsunduze and Umgeni Rivers are managed by Umgeni Water. Umgeni Water was formed in 1974, its functions being to supply bulk treated water to the Greater Durban and Pietermaritzburg functional regions. At present it undertakes bulk storage, treatment and supply of water to towns and cities in its area of supply,

providing potable water to over 3 million consumers. At the completion of a study of its supply area in 1989 which highlighted the inequities in the provision of services, the organisation set an objective of providing everyone with access to a supply of safe water by the year 2005, and thus the Rural Areas Water and Sanitation Plan was developed (Umgeni Water, 1995).

In the Umgeni River catchment, Umgeni Water undertakes water quality monitoring and performs weekly, monthly or quarterly sampling and analysis for up to 60 water quality indicators (determinands) at over 100 sites in the rivers, impoundments and estuary. Continuous water quality monitoring is being implemented at some sites, while biotic health is monitored on a monthly or quarterly basis at others (Ninham Shand, 1995). The SASS 4 method is used to measure biotic health in order to monitor pollution (Chutter, 1994).

Pollution Sources

Diffuse sources of pollution which could decrease water quality include wash-off from:

- informal and transitional settlements in and around the Pietermaritzburg urban areas, (particularly from Edendale);
- dense rural settlements in the Umsunduze River catchment above Henley Dam;
- formal residential areas around Pietermaritzburg and
- commercial and industrial areas around Pietermaritzburg.

Direct contamination occurs from:

- blocked, damaged or overflowing sewers in the formal residential areas around Pietermarirzburg and
- human and animal use of the rivers near the dense rural settlements in the Umsunduze River catchment above Henley Dam.

The major problems arising from point source pollution are associated with:

- waste water works in Pietermaritzburg (Darvill) and
- illegal industrial discharges and accidental storage and transportation spills in and around Pietermaritzburg.

In general, the worst water quality problems are faecal bacterial contamination, nutrient and sediment loads upstream of Henley Dam, and in addition to organic matter, litter and heavy metals from the areas around Pietermaritzburg (Ninham Shand, 1995).

Numerous streams enter the Umsunduze River and some of these have a detrimental effect on water quality. In the Pietermaritzburg area, the Slangspruit flows into the canal at Camp's Drift, bringing with it effluent and general pollution from squatter settlements. The Foxhill Spruit and the Blackborough Spruit run through residential areas in the south and the water is generally considered to be of good quality. Chutter (1992), disagreed to some extent: with regard to chemical samples, Umgeni Water considered this to be one of their cleanest sampling stations, but according to the SASS 2 scores at the time, "the stream is not all that clean". Between the confluences of these two rivers is that of the Dorpspruit which runs through light industrial area in the north-western side and pollutes the water from time to time. This enters the Umsunduze about 1 km above Site No. 1. Between sites 4 and 5, the Bayne's Spruit joins the Umsunduze. This is regarded as a very poor river and at times it carries vegetable oils from a margarine factory. These streams are shown on **Fig 3.2.**

DARVILL SEWAGE WORKS

Darvill sewage works treats on average 90 Mℓ of water per day. During winter, the treated effluent accounts for more than half of the flow of the Umsunduze River. In the past, situations arose during heavy rains where the sewage works were inundated with more than 300 Mℓ per day, owing to the excess water from the city's stormwater drains. The works were obviously unable to deal with this volume of water, which subsequently overflowed and ran directly into the river, in this way it was possible for raw sewage to contaminate the river.

However, since this study began, Umgeni Water has spent R300 million upgrading the works. A large storage dam has been built to accommodate excess water until it can be treated. Also, the effluent outlet has been moved to a site further downstream from its present site as a "maturation river" has been built. This allows the effluent to

meander down several kilometres of man-made river so that by the time it enters the main river there is no longer any chlorine in it. Recent (unpublished) studies have shown that there is now no difference in the water quality above and below the effluent outlet. (It would be interesting to compare the length of the "maturation river" and the degree of community recovery; with the recovery distance reported in **Chapter 5**; and to use these data to recommend dechlorination procedures for other sewage works). In addition, the phosphorus loading to the Inanda Dam has improved (Dr C. Dickens, Umgeni Water, *pers. comm.*)

The Umsunduze River was initially chosen for this project because it is managed by Umgeni Water and there were comprehensive water quality and biomonitoring data. However, although it was known that the chlorine in the sewage effluent was affecting the aquatic life in the river, it was not known how great this effect was or what concentration of chlorine was lethal. Information obtained from this project would therefore be of assistance to Umgeni Water for setting guidelines for chlorine release in sewage effluent.

It was with some regret that work on this river was discontinued, owing to the tense political situation at the time. The theft of personal belongings and equipment, vehicle damage and the threat to personal safety during one of the field trips led to the decision that it was no longer safe to work in that area.

THE STUDY SITES

The part of the Umsunduse under study in this project extended from the low-level bridge near the Pietermaritzburg SPCA (above the dump) to the low-level bridge at Lincoln Mead, a stretch about 9 km (**Fig. 3.2**).

A number of factors were taken into account in the selection of the study sites:

1. The position of the sites in relation to the effluent outlet

Two sample sites were upstream from the effluent outlet, (as suggested in Standard Methods, (1992)), one at the outlet and five downstream from it.

2. The presence of riffles

The sites were selected carefully so as to minimise differences between them, apart from water quality. The sites chosen had to have suitable riffles (shallow, fast-flowing reaches of a river with turbulent flow and broken water (Dallas & Day, 1993)) where the water was not deeper than 300 mm (the height of the modified "Surber" sampler).

3. Sites monitored by Umgeni Water

Where possible, those sites routinely monitored by Umgeni Water were included as study sites for this project. In this way, pertinent data from Umgeni Water could be used in this study, and the data collected during this study would in turn be useful to Umgeni Water.

4. Accessibility of the sites

Because of the large amount of equipment which had to be taken to each site, the sites had to be reasonably close to the road and require the minimum of bush clearance in order to get to the water.

Selection of the sites took place during a period of fairly low flow, which caused complications for subsequent field trips as some sites could not be sampled. Just prior to the October 1993 trip, heavy rains had fallen in the catchment area. Those stretches of river which had steep banks became very deep and the riffles at the sample sites were totally submerged. The water was too deep for the box sampler at sites 5 and 6, but chemical sampling was still done at these sites. Where the banks were not as steep, the riffles were less affected by water depth, and sampling could be carried out.

Building operations at Darvill prevented access to Site 5 on the second trip and Site 8 was added on this trip as it was noticed from the previous trip that the river did not show much in the way of recovery at Site 7.

The sample sites are shown on the map (**Fig.3.2)** and are described in **Table 3.1.** Sample sites 1 to 6 are shown on the aerial photograph (**Plate 3.1**) at the end of the chapter,

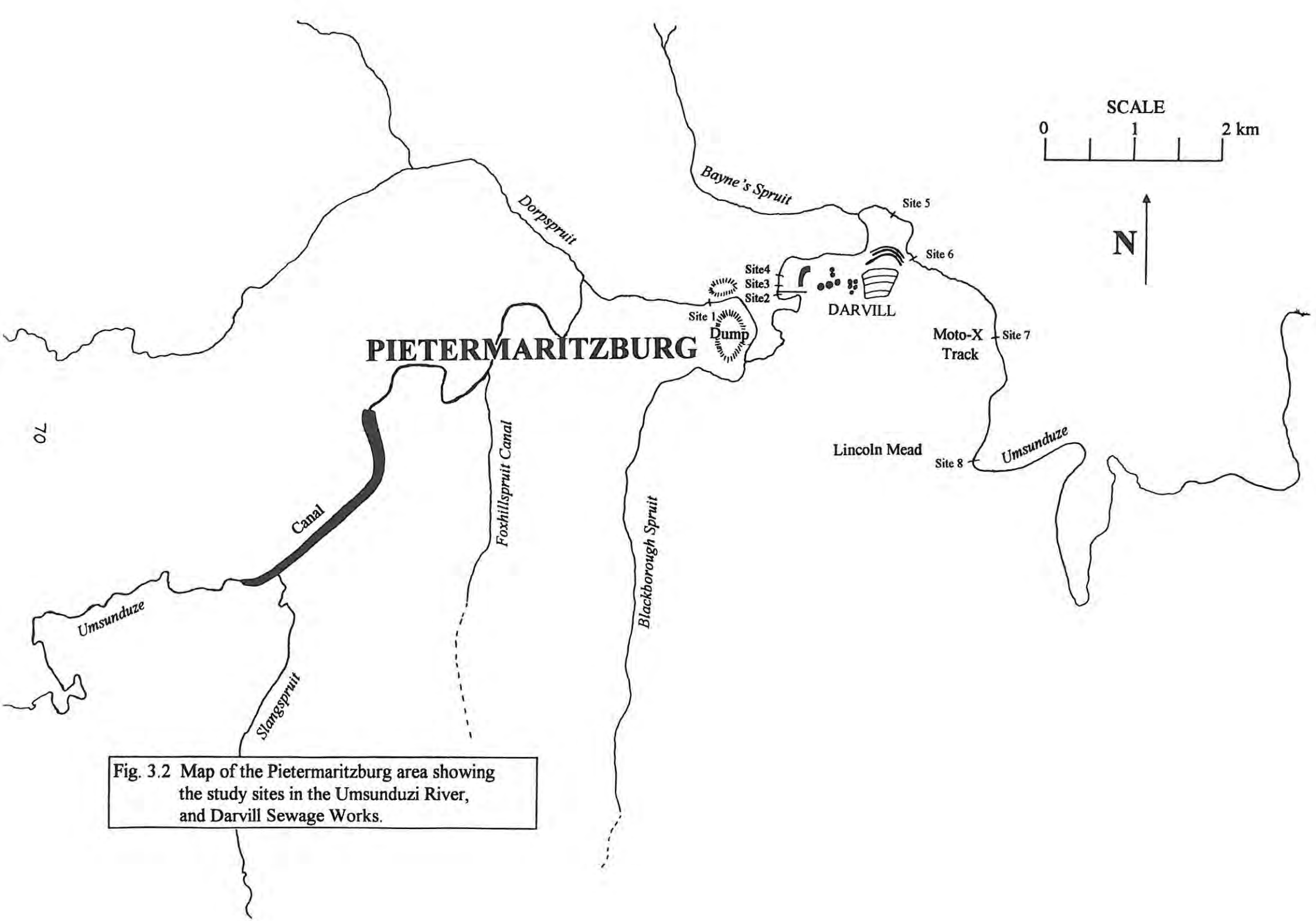


Fig. 3.2 Map of the Pietermaritzburg area showing the study sites in the Umsunduzi River, and Darvill Sewage Works.

Site number	Description
1 Upstream	This was Umgeni Water's sampling site No. 65. It was at the low-level bridge at a municipal dump site 2 km upstream from the Darvill Wastewater Works effluent outlet.
2 Upstream	This was about 5m upstream from the effluent outlet at Darvill Wastewater Works.
3 At effluent outlet	This was at the effluent outlet. Because of the large volume and the high velocity of effluent relative to the river water, the water which flowed over the riffles at this site was almost entirely effluent.
4 Downstream	This was around the first bend about 20m downstream from the effluent outlet, where the effluent and river water appeared to be well mixed.
5 Downstream	This was Umgeni Water's site 66.1, about 2.5 km from the effluent outlet.
6 Downstream	This was at the new effluent outlet which was under construction at the time of this study. It is approximately 3 kilometres from the effluent outlet.
7 Downstream	This was at Umgeni Water's site 67 which is below the concrete weir at the Moto-X track. It is about 5 km from the effluent outlet.
8 Downstream	This was at the low-level bridge at Lincoln Mead, 7 km from the effluent outlet.

Table 3.1. The study sites in the Umsunduze River.

3.2 THE UMBILO RIVER

The Umbilo River was chosen as an alternative study area since it had the same sort of conditions as the Umsunduze, viz. a chlorinated sewage effluent discharge, and it was a much safer site.

The Umbilo River is considerably smaller than the Umsunduze River and is only about 33 kilometres long. It rises at the foot of Field's Hill and winds through Kloof, Pinetown, Paradise Valley Nature Reserve and Queensborough. Just before Carrington Heights is the confluence with the Umkhumbane River. The two rivers flow together into the Umbilo Canal and then join the Umhlatuzana Canal. The combined rivers then all flow into Durban Harbour. The Umbilo River catchment covers an area of 1890 Ha. The rivers can be seen on the map of the Durban area, **Fig. 3.3.**

LAND USE

Land use in the catchment is mainly commercial, industrial and residential, with a small amount of informal settlement in the upper part. There is no commercial agriculture, and the river flows through nature reserves in Paradise Valley and at Roosfontein.

WATER USE

In Pinetown the river is used for recreational purposes, especially at Paradise Valley; and to some extent, the Harbour also uses Umbilo River water.

WATER QUALITY

The Umbilo River is managed by the Pinetown Municipality. Staff at Umbilo Sewage Purification Works (known also as the Wastewater Treatment Works, (WWTW)) monitor water quality at sites approximately every 2 km along the course of the river and the findings are published weekly in the local newspaper, The Highway Mail.

Considering the commercial and industrial activity which takes place along the banks of the Umbilo River, it is not surprising that the water quality is generally not good,

despite diligent monitoring of water quality and seeking out of polluters by dedicated staff at the WWTW.

There are approximately 2500 industries in the Pinetown area, and these affect the Umbilo, Palmiet and Namiet Rivers. The industries and their effluents are described below.

The main industrial areas are Westmead, Westmead Extension, Mahogany Ridge, Maxmead, Hagart Road Industria and parts of the Pinetown CBD.

A large stormwater drain (the Westmead drain) enters the river at the corner of Caversham and Winston Churchill Roads. This drain carries run-off from a large area and contributes a substantial amount to the river. Floor washings from mini-factories, beverage, ice-cream, biscuit and footwear factories and a motor garage run off into the river. Heavy metals (tin, lead, zinc, chromium, cadmium) and cyanide come from electroplating and steel hardening concerns. Occasionally spills or floor washings may run off from manufacturers of adhesives, paint products, battery acid, grouting, polish, toiletries (creams and lotions, plaster of paris), general cleaning chemicals and detergents. In addition there are concrete industries, laundries, laminators, textile industries, warehouses (which have accidental spills during on- and off-loading), injection moulders (which release cleaning and cooling water), and waste-disposal companies which deal with laboratory waste. Many sewers carry effluent into the river and heavy earth-moving equipment compounds the problem by causing siltation of the river.

To add insult to injury, two thirds of the natural wetland in the area has been destroyed.
(Information supplied from WWTW records.)

STUDY SITES IN THE UMBILO RIVER

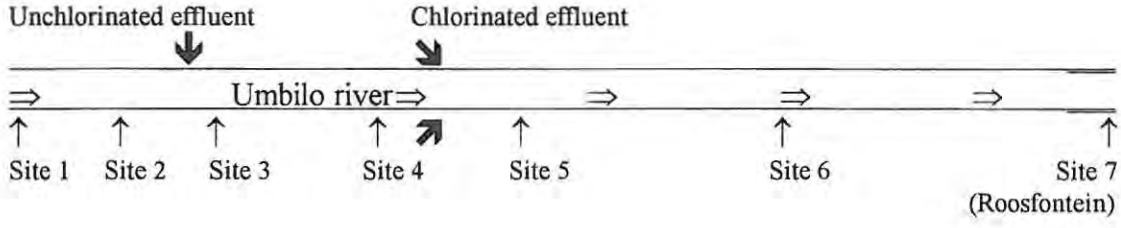
Features similar to those sought in the Umsunduze River were considered in the choice of the study sites in the Umbilo. All sites had to have riffles suitable for using the modified Surber Sampler. The sites are shown on the aerial photographs (**Plates 3.2 and 3.3**) and are described in **Table 3.2** below:

Site number.	**Description**
Site 1	This site was approximately 250 m upstream from the unchlorinated effluent discharge point.
Site 2	This site was approximately 6 m upstream from the unchlorinated effluent.
Site 3	This site was immediately below the unchlorinated effluent discharge.
Site 4	This site was approximately 90 m downstream from the unchlorinated sewage effluent and about 5m upstream from the chlorinated effluent.
Site 5	This site was immediately downstream from the two outlets of chlorinated sewage effluent.
Site 6	This site was approximately 470m downstream from the chlorinated sewage outlet.
Site 7	This site was in the Roosfontein Nature Reserve, about 6.5 km downstream from the chlorinated sewage outlet.

Table 3.2. The study sites in the Umbilo River.

(Sites 1 to 6 are shown in Plate 3.2)

The positions of the sites in relation to the discharges can best be visualised as set out below:



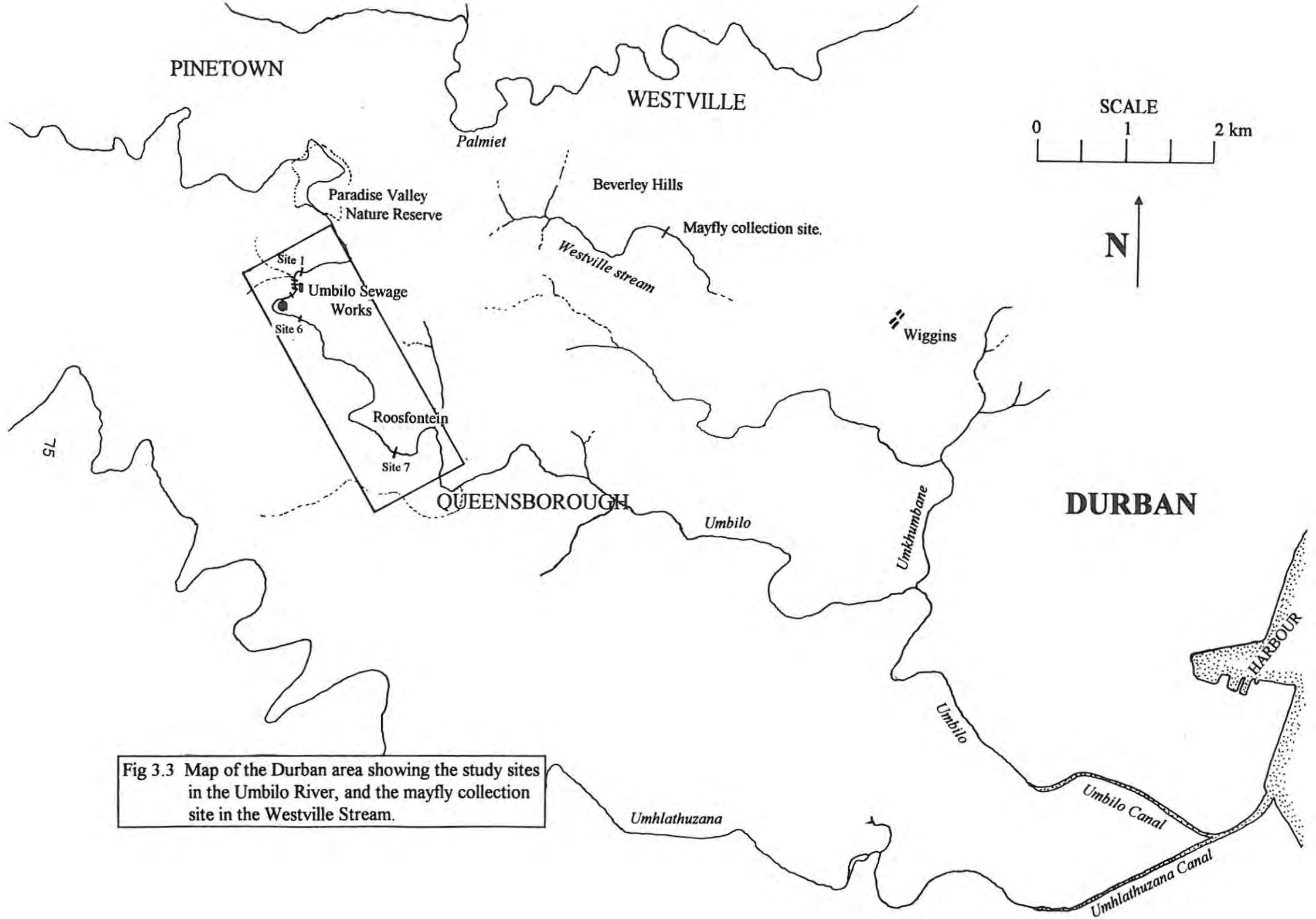


Fig 3.3 Map of the Durban area showing the study sites in the Umbilo River, and the mayfly collection site in the Westville Stream.

3.3 WESTVILLE STREAM

The "Westville Stream", which was the source of mayfly nymphs for the first set of toxicity tests, is a small stream which rises in Beverley Hills, a residential area of Westville. It is shown on the map of the Durban area, **Fig 3.3.** It lies in the area between the Palmiet and Umkumbane Rivers and organisms were collected just downstream from the bridge in Springvale Road. In this area the stream bottom consisted mainly of large expanses of flat bed-rock, teeming with mayfly nymphs, which made collection very easy.

The stream does not flow through any industrial area and shows no indication of industrial pollution. However, the soil is quite sandy in this area and there is some percolation from septic tanks from neighbouring houses (Dr C. Dickens, Umgeni Water, *pers. comm.*).

This stream in Westville was chosen for collection of mayfly nymphs as its community diversity suggested that it was relatively unpolluted. It had a thriving mayfly population, unlike most of the other streams in the Durban area at the time. When the toxicity tests were due to begin, the Umbilo River (previously an abundant source of mayflies) was devoid of almost all macroinvertebrate life, and searches for mayflies in several areas proved fruitless. It would appear that some unidentified pollutant had been down the river, killing almost everything. (A group of post-graduate Chemical Engineering students which had been taken to the Umbilo Sewage Works on one of the sampling trips to learn about macroinvertebrate community changes in relation to sewage effluent were not convinced that *anything* had ever been living in this river. It was , however, a good lesson in the importance of biological monitoring as an indicator of water quality.)

By the time the "Westville mayfly" toxicity tests had been completed, the Umbilo River had been recolonised by a variety of organisms and mayflies were once again present in large enough numbers for toxicity testing.

The following three aerial views were made by scanning aerial photographs into a computer then merging the images and adding the river and labels using the computer programme "Adobe Photoshop".

Plate 3.1. Aerial view of Darvill Wastewater Works in Pietermaritzburg, and the study sites in the Umsunduze River.

Sites 1 and 2 were upstream from the sewage effluent and Sites 3 and 4 were immediately downstream from it.
Site 5 was about 2.5 km downstream, and Site 6 was about 0.5 km further downstream.
Site 7 was about 1 cm beyond the lower right-hand corner of the Plate.
Site 8 was too far away to be on these aerial photographs but it can be seen on the map, **Fig. 3.2.**

The dark L-shaped region to the right of Sites 2, 3 and 4 is the new overflow reservoir which accommodates stormwater to prevent overflow of untreated sewage into the river after heavy rains. The 3 parallel channels near the S of Site 6 are the new "maturation" channels which allow the treated effluent to lose much of its chlorine before being discharged into the river

Scale: 1 : 11 500 (1 cm = approximately 115 m)

Site 1
Site 4
Site 3
Site 2
Site 5
North

Plate 3.2. Aerial view of the Umbilo Sewage Purification Works showing the study sites in the Umbilo River.

Site 1 was about 250 m upstream from the unchlorinated effluent, Site 2 was about 6m upstream and Site 3 was immediately downstream from this effluent.

Site 4 was about 90 m downstream from the unchlorinated effluent and about 5 m upstream from the chlorinated effluent. Site 5 was immediately downstream from the chlorinated effluent, and Site 6 was about 470 m from this effluent..

Site 7 is not shown on this plate as it was too far away, but it is indicated on the map, **Fig. 3.3.**

The Old Works can be seen on the south side of the river, and the New Works are on the northern side.

Scale: 1 : 3 000 (1 cm = 30 m)

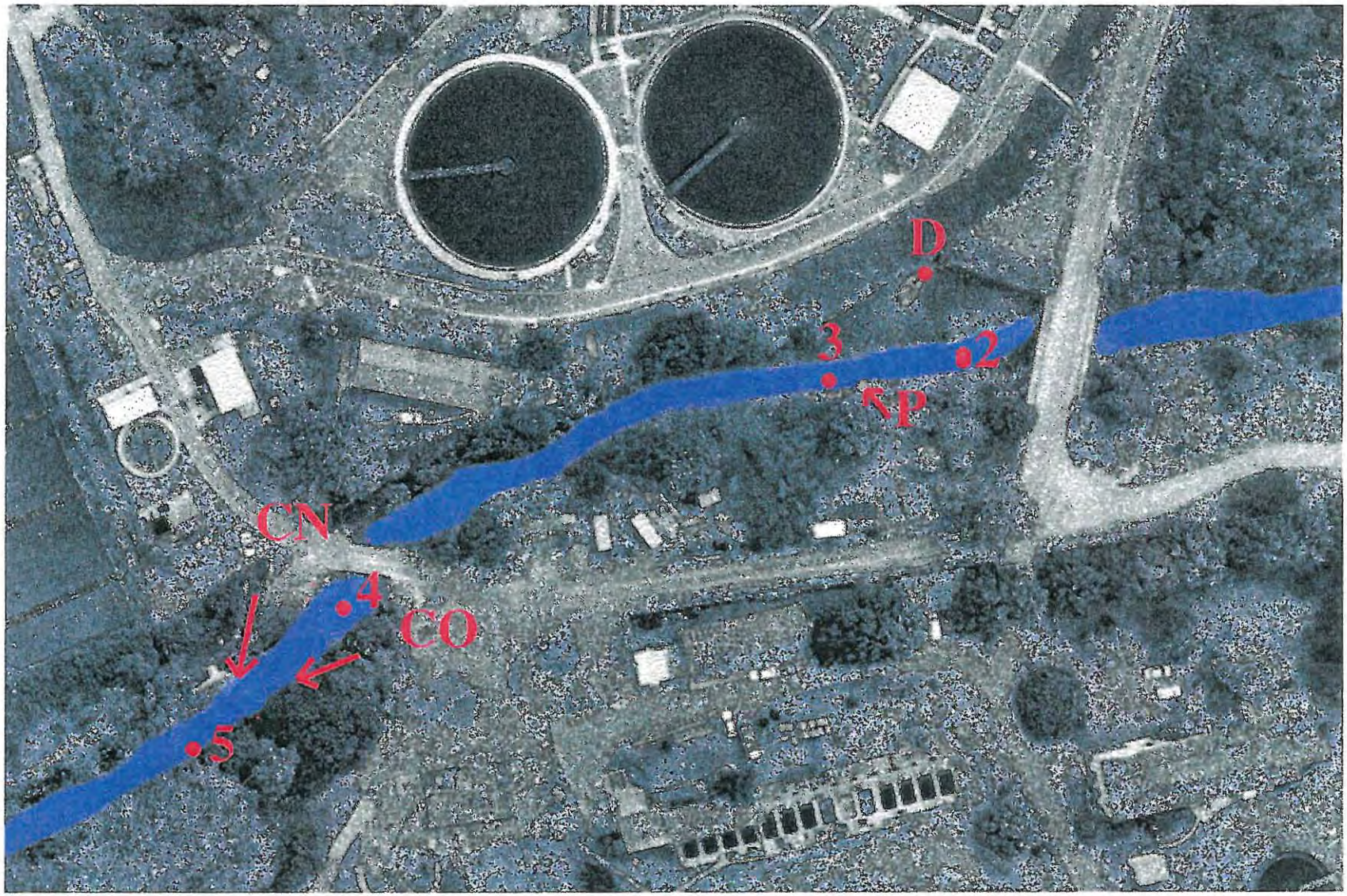
1
2
3
4
5
6
North

Plate 3.3 Aerial view of the Umbilo Sewage Purification Works showing the study sites in the Umbilo River.

This closer view of the works shows some of the other features mentioned in the text:

1) CN indicates the position of the chlorinated effluent from the New Works.
2) CO indicates the position of the chlorinated effluent from the Old Works.
3) D indicates the position of the drain from unused dump site.
4) P indicates the outlet of the plain (unchlorinated) effluent.
5) The area around the O of CO is the area in which building operations were in progress, and which led to the pollution of Site 4, as discussed in **Chapter 5.**

Scale: 1 : 800 (1 cm = 8 m)


D
3
2
P
CN
4
CO
5

CHAPTER 4

ACUTE CHLORINE TOLERANCES OF *BAETIS HARRISONI* FROM THE UMBILO RIVER AND A WESTVILLE STREAM

INTRODUCTION

The aim of this study as a whole has been to investigate the effects of chlorinated, treated sewage effluent on riverine macroinvertebrates. In order to carry out these investigations, it was decided to use both toxicological and ecotoxicological approaches.

The toxicological aspect of the project involved the selection of a macroinvertebrate which was found upstream but not downstream from a point source of chlorinated, treated sewage effluent, and the determination of the acute, lethal response of this organism to chlorine. Acute, 96 h LC_{50} tests for chlorine were carried out using the mayfly *Baetis harrisoni* Barnard as the test organism, and the results are discussed in the context of the development of environmental water quality guidelines.

Toxicity testing and the development of water quality guidelines normally begins with acute toxicity studies, as they indicate individual species sensitivity and lethal concentrations (Buikema *et al.*, 1982).

The 96 h time interval has been recommended for acute toxicity tests for macroinvertebrates (APHA,1992), as it has been stated that organisms should not be fed during short-term tests (APHA,1992; Rosenberg & Resh, 1993) and 96 h has been reported as the limit for non-feeding tests involving invertebrates (Cairns *et al.*, 1976; Arthur *et al.*, 1987; Hickey & Vickers, 1992).

In acute toxicity tests, groups of aquatic organisms are exposed to progressively increasing concentrations of a toxicant in order to estimate the concentration of the test material that is lethal to 50% of the animals of a particular species within a specific time interval (e.g. 96 h) (Gelber *et al.*, 1985). This is then referred to as the 96 h LC_{50} (also written as LC50 in some texts).

Acute toxicity tests begin with range-finding or exploratory tests to determine the range of concentrations to test (Buikema *et al.*, 1982, Parrish, 1985; APHA, 1992). Organisms are exposed to a wide range of concentrations of test substance, thereafter, a geometrically spaced series of concentrations is selected between the highest concentration that killed no, or only a few, test organisms and the lowest concentration that killed most or all test organisms (APHA, 1992). For definitive tests, 4 to 6 different concentrations of the test substance, plus a control, should be used (Buikema *et al.*, 1982, APHA, 1992).

Short-term, acute tests with fish and invertebrates require control survival of 90% or more, to be considered acceptable (APHA,1992), although how the limit of 10% for the control mortality was chosen as the critical level is not clear (Buikema *et al.*, 1982). In South Africa, where *Daphnia* spp. has been used as the test organism, adequate control mortalities have been achieved (Roux *et al.*, 1993). Palmer *et al.*, (in press) found it difficult to achieve control mortalities lower than 20-25% using riverine invertebrates, and this study confirms their finding that handling of test organisms is critical for the achievement of acceptably low control mortalities.

Buikema *et al.*,(1982) reported that of fifteen types of toxicity tests which were evaluated, acute mortality tests were one of the three most highly rated, and were considered to be ecologically significant, the most scientifically and legally defensible, most simple and cost effective, and, although modest in predictive capability, were considered to have the greatest utility. Acute mortality values have also been used extensively in the development of South African environmental water quality guidelines (DWAF, 1995).

The aim of this part of the study was to establish the acute 96 h LC_{50} toxicity of chlorine to *B. harrisoni* mayfly nymphs from a relatively unpolluted stream in Westville and from the severely impacted Umbilo River. Unreplicated range-finding tests were first carried out for both groups and once the tolerance ranges had been established, replicated definitive tests were carried out using the appropriate concentrations of chlorine

This chapter (**Chapter 4**) deals with the process of toxicity testing, and follows directly from **Chapter 2** , in which the development of an artificial stream system which could be used for chlorine tolerance testing, was described. In **Chapter 5** the toxicity approach is extended to an ecotoxicological approach, and toxicity test results are used to develop and test simple hypotheses regarding the responses of riverine macroinvertebrate species and assemblages to chlorine.

MATERIALS AND METHODS

SELECTION OF TEST ORGANISMS

Most toxicity tests have evaluated the short-term lethality of wastes to adult fish, because they are presumed to be the best understood organism in the aquatic environment, and are perceived, by the majority of laymen, as being most valuable (Buikema *et al.*, 1982). (The latter point was made clear in the present study when numerous incredulous people asked the question "So why are you trying to save the mayflies ?") However, although fish have often been the organisms of choice, it is clear that when an effluent enters an aquatic ecosystem, hundreds of other species - especially those at lower trophic levels - may be affected.

Various studies have shown that diatoms and macroinvertebrates are often more sensitive to toxicants than are fish, and often represent a larger majority of the biomass in a natural system than fish do (Buikema *et al.*, 1982). In terms of species richness, relative abundance and productivity, aquatic insects are very important and are potentially the most vulnerable (Sweeney *et al.*, 1993) so should be included in toxicity testing. Invertebrates which are now commonly used for acute toxicity tests include daphnids, amphipods, crayfish, midges and snails (Parrish, 1985).

When selecting test organisms for toxicity tests, the following aspects should be considered:

1) their sensitivity to the factors under consideration;
2) their geographical distribution, abundance and availability within a practical size range throughout the year;
3) their recreational, economic, and ecological importance and relevance to the purpose of the study;
4) their abiotic requirements and whether these requirements approach the conditions normally found at the study site;
5) the availability of culture methods for rearing them in the laboratory and a knowledge of their physical and nutritional requirements;
6) their general physical condition and freedom from parasites;
7) whether or not they give a definite response the test material, and
8) whether they are indigenous to or representative of the ecosystem that may be impacted (Buikema *et al.*, 1982; Rand & Petrocelli, 1985; APHA, 1992).

There is no standard test species that can be used for all ecosystems and the selection will often be based on site-specific considerations (Rand & Petrocelli, 1985).

The choice of test organism for this study

Nymphs of the baetid mayfly *B. harrisoni* were selected as test organisms and this choice is discussed in the light of the above criteria:

1. Preliminary sampling in the Umsunduze river revealed that *B. harrisoni* nymphs were present in large numbers upstream of the effluent outlet, but absent downstream from the chlorinated sewage effluent, which indicated that they were sensitive either to the effluent, or the chlorine, or both.

2. Mayflies are found in almost every type of stream as their nymphs occupy a great variety of habitats, and *B. harrisoni* has been found to be one of the most plentiful of the Natal mayflies (Crass, 1947). This would make them a good choice for toxicity tests as they could presumably be collected from a wide variety of rivers or streams for subsequent studies. In KwaZulu-Natal, mayflies are to be found

throughout the year, and some, notably *B. harrisoni*, are actually more plentiful in cold weather (Crass, 1947). At the upstream site in the Umbilo River and in the Westville stream, they were present in large enough numbers for running a number of batches of toxicity tests. Because of their positions and density on the rocks in both the Umbilo River and the Westville stream, a large number could be collected within a relatively short time for toxicity testing. (It usually took between 1 and 1.5 h to collect about 600.)

3. It could be argued that since trout fishing is a recreational activity in the Drakensberg resorts and other areas in KwaZulu-Natal, and mayflies, especially in the nymphal stage, form an important part of the food of trout, (Barnard, 1932., Crass, 1947), mayfly conservation is important. However, these days there is not as much concern over the survival of trout in Cape or KwaZulu-Natal rivers as there was during the late 19th century as they are alien species (De Moor & Bruton, 1988 *in* Davies *et al.*, 1993). Macroinvertebrates do however form a vital link between the organic matter/bacterial/fungal trophic level and fish, and are important in river processes rather than having direct, individual commercial value.

4. "Bætids, with the exception of the Cloeon group, have immobile gills, and are distinctly intolerant of still water" (Crass, 1947). *B. harrisoni* is a riffle-dwelling organism, although it was not generally found in the fastest flowing parts of the Westville stream or Umbilo River. At one stage of the study, in many of the streams in the Durban area (including the Umbilo River at the beginning of the toxicity testing period) they were not found at all even though there were abundant riffles. (As far as the Umbilo River was concerned, it is assumed that some chemical event was responsible for the eradication of almost all forms of macroinvertebrates at the beginning of March 1995. However, by the end of May, they were again present in large enough numbers for toxicity testing to proceed.) In this study, *B. harrisoni* was found to be well suited to survival in the artificial streams.

5) Standard methods for mass laboratory rearing and experimenting with a wide variety of terrestrial insect species are available but in general there appears to be a paucity of stream insect species available as standard test organisms for laboratory and field bioassay procedures. This is surprising considering that insects are the principal group of consumer organisms in most stream and river ecosystems throughout the world (Sweeney et al., 1993). Although *B. harrisoni* is not cultured at present, it is possible that in future the Standard Laboratory Organism project (Haigh & Davies-Coleman, in press) may investigate this possibility. *B. harrisoni* is a grazer/collector, feeding on diatoms and loose detritus (Palmer *et al.*, 1993), but knowledge of their physical requirements appears to be scarce at this stage.

6. Nothing was known about the general physical condition or parasite content of the test organisms and this lack of information will be discussed further in **Chapter 6**.

7. Death is the criterion for effect most often used in toxicity tests to estimate the LC_{50}, the indicators of death usually being lack of movement and lack of reaction to gentle prodding (Parrish, 1985, APHA, 1992). In some organisms, death is not easily determined so cessation of movement of antennae, mouthparts or other organs may be used (APHA, 1992). In the case of *B. harrisoni*, it was relatively easy to determine whether they were dead or alive. When the mayfly nymphs were alive they generally had their feet firmly on the substrate and their abdomens curved away from the substrate so that their three cerci stuck up. When they had died, they tended to curl themselves inwards in a "foetal" position and usually released their grip on the substrate. Also, if it was not clear whether or not they were still alive, a gentle touch with the tip of fine forceps would send them scurrying off. Those which did not respond at all to touching were considered to be dead.

8. *B. harrisoni* is indigenous to both sample sites, and generally forms a considerable proportion of the communities at the upstream sites (See **Plates 5.1 - 5.6**).

In addition, the following points were taken into consideration:

9. Preliminary investigations with both the recirculating and the flow-through artificial streams indicated the likelihood that these organisms would be preferable to other invertebrates which had been collected (e.g. caddis flies, simuliids, planarians, limpets etc.) because they did not pupate, did not need substrate material to build nests, did not reproduce in the stream and, as long as they were kept at a temperature of about 20°C, did not emerge and fly away

10. Baetid species. have been used for toxicity testing by other researchers and institutions e.g. the United States Environmental Protection Agency (US EPA), the American Society for Testing and Materials (ASTM) (Persoone & Janssen, 1993), the Institute for Water Research, Grahamstown (Palmer *et al.*, in press).

The use of *B. harrisoni* as a test organism could, however, be criticized on the grounds that it is considered to be a relatively tolerant species, found in waters of diverse quality, and toxicity testing should probably be carried out on more sensitive species. However, it was felt that in rivers such as the Umsunduze and Umbilo, if even *B. harrisoni* was absent at some sites, this was indicative of the exceptionally poor quality of the water and cause for concern, so it would be useful to investigate their tolerance limits. Buikema *et al.*, (1982) point out that the selection of test species depends on the objective of the study and while a sensitive species may be appropriate for estimation of probable effects of a new chemical that will be widely distributed, an indigenous species may be appropriate for generating site-specific information for a discharge variance procedure.

A more intransigent problem is the taxonomic recognition of *B. harrisoni* as a single species. The wide distribution of the organism is indicative of what is probably a "sibling species complex". However, taxonomic problems abound in relation to South African mayflies, and lodging specimen samples remains the best defence.

TESTING EQUIPMENT AND WATER SUPPLY

These are described in **Chapter 2.2.**

STATISTICAL ANALYSIS OF THE DATA FROM ACUTE TOXICITY TESTS

While the percentage of animal deaths would probably increase monotonically as the toxicant concentration increases, it is unlikely that one of the concentrations in the experiment will kill exactly 50% of the exposed animals. Therefore, the LC_{50} is estimated either by fitting a smooth parametric function to the observed data or by numerical interpolation. The estimated LC_{50} is based on data from a sample of a particular species, so it is not the *true* LC_{50} (i.e. the concentration which would kill exactly half of the entire species), therefore a confidence interval for the true LC_{50} is usually computed along with its point estimate. This is usually the 95% confidence interval (Gelber *et al.*, 1985, APHA, 1992).

Several methods for the estimation of the LC_{50} and the associated confidence intervals have been proposed in statistical and biological literature. Three independent approaches have evolved: the parametric, the moving average and the nonparametric methods.

The **parametric method** is based on transforming the concentration levels so that the transformed concentration-mortality relationship has a known functional form. This method involves the whole concentration-mortality curve, not just a single point on it (such as the LC_{50}), so can be used for bioassay as well as for acute toxicity testing (Gelber *et al*, 1985). *Probit analysis*, as described by Finney (1971), is an example of a parametric method, and is probably the most widely used LC_{50} calculation procedure (APHA, 1992). Probit values are assigned to the % mortality values so that when plotted against the logarithm of the concentration, a sigmoid-shaped curve is formed. The probit method is disadvantageous in that it requires laborious, time-consuming calculations for the maximum likelihood estimation of the unknown parameters. (Where computer programs are available, these can be used.) Gelber *et al.* (1985) report on methods for modification of the probit method using nomograms to simplify the calculations; a technique using angular arc-sine transformations of the % mortalities to stabilize the variance; moving average interpolation for estimating the LC_{50}; and a combination of the moving average interpolation with an angular transformation of the mortality percentages. Shortcomings of these methods are that

the concentration series of the test must be equally spaced, and the methods cannot be used to calculate an LC value other than the LC_{50} (APHA, 1992).

The most commonly used **non-parametric procedures** are the Spearman-Karber method and the trimmed Spearman-Karber method (APHA, 1992).

LC_{50} can also be estimated by probit method using *graphical analysis*. The dose-response data are plotted manually and a best-fit regression line is drawn by eye. Percentage mortality is plotted on the y-axis and concentration on the x-axis on probit paper. Death is plotted on a probit or probability scale and concentration on a logarithmic scale. A line is fitted to the points by eye and the LC_{50} is read from it. The disadvantage of the graphical method is that confidence limits are not obtained (APHA, 1992).

The statistical methods employed in this study will be discussed further in relation to the results.

THE TEST PROCEDURE

1. Collection of test organisms

The Westville stream from which the mayfly nymphs were collected had large expanses of flat bed-rock on which the organisms were abundant. Two collecting nets were made by sewing a triangle of silk-screen mesh (mesh size 0.15 mm) over a wire coat hanger. The net was held at an angle against the rock and gently pushed upstream, which caused the organisms to be washed onto it (**Plate 4.1**). After each "scoop" the mayflies were gently rinsed off the net with jugs of river water into a 20 ℓ cooler box.

Three cooler boxes were used and each had its own battery operated air pump, two ice packs and about 6 pieces of 10 mm thick foam rubber for the mayflies to hold onto in transit. Approximately 200 organisms were collected in each box. The journey from the river to the artificial streams at the PEF took about 15 minutes.

Each time the mayflies were collected, various determinands were recorded. These were dissolved oxygen (DO), pH, conductivity, temperature and free and total chlorine concentration.

Plate 4.1. Collection of organisms using the *mayfly net.*

2. Transfer to the artificial streams

Mayfly nymphs are extremely sensitive to being handled and die easily if treated roughly, so handling was kept to a minimum and everything was done *gently*. The mayflies were transferred from the cooler box to the streams by using a white plastic jug in which they could be seen clearly and counted as they were poured out. At this stage no attempt was made to identify the mayflies to species as previous investigations of the organisms at the collection sites had shown that usually between 95 and 100% of them were *B. harrisoni*.

(Another method of collection was attempted whereby large stones were picked up out of the water and the mayflies washed off into the cooler box with jugs of water. Once in the laboratory, the organisms were scooped out of the cooler box and poured into a

white plastic tray so that they could be identified. The *B. harrisoni* were then picked out one by one with a modified large-bore Pasteur pipette (as suggested in Standard Methods) and placed in the streams. However, it was found that since most of the mayflies collected were *Baetis* anyway, this method of collection and sorting merely placed unnecessary stress on the organisms and led to a higher mortality rate, as can be seen with the *Control* organisms (**Figs. 4.1B** and **4.8B**).

The organisms were divided equally among the 12 streams by pouring 10 at a time into each until there were none left. Between 35 and 60 were placed in each stream (depending on how many had been caught for a particular 'run'). Those mayfly nymphs which had the dark wing-buds were not used as they were final instar nymphs and would probably emerge and fly away before the end of the test run. Also, the very small nymphs were ignored, so that all the test organisms were approximately the same size.

3. Acclimation

The mayfly nymphs were left in the streams for 48 h to acclimate before the chlorine release began. Any dead organisms were removed prior to the start of the chlorine addition. No attempt was made to transfer organisms from one stream to another to standardize the numbers of organisms in each stream as it was felt that further handling (being sucked up into a Pasteur pipette and then squirted out) would place additional unnecessary stress on the organisms. Also, it was extremely difficult to count the organisms once they were in the streams as they tended to congregate in large numbers and then dart about from group to group. Since the results were to be expressed in terms of "% cumulative mortality" it was decided that where 50 organisms had been placed in each stream, as long as there were between 45 and 50 at the start of the chlorine addition, it is unlikely that these small differences population density would be significant.

4. The 96 h test period

Sodium hypochlorite solutions were prepared by diluting the required number of mℓ of stock solution with distilled water to produce 1 ℓ of test solution. The test solution

was then poured into an intravenous drip bag by means of a funnel which had a specially made spout on the end to enable the solution to enter the small opening of the drip bag.

Dosing with chlorine began at 08h00 on the first day. Drip bags with the appropriate chlorine solution (freshly made up that morning) were hooked onto the stand and connected to the drip tubes. The control valves were set to the correct dose mark allowing the hypochlorite solution to drip in at the specified rate (i.e. 15 drops per minute). The drip rate was checked a number of times daily for each stream to ensure that a constant rate was being maintained.

After 2 h, each stream was checked and any dead organisms were removed, counted and placed in 70% ethanol in a pill vial (a separate vial being used for each stream), for later identification. Each pill vial was labeled with the number of the stream from which the mayflies had come and the time interval at which the deaths had occurred (2 h, 4 h, 96 h, etc.). The numbers of dead organisms were recorded on a data sheet as shown in **Appendix 5.1**. The procedure was repeated after 4, 6 and 8 h on the first day and thereafter at 24, 48, 72 and 96 h as suggested in Standard Methods (APHA, 1992).

Each day at 08h00 and 16h00, the drip bags were replaced with ones containing newly made up sodium hypochlorite solutions and the filter bag in the reservoir tank was emptied and cleaned.

It was noted that a thin, light brown scum developed in the streams overnight. Each morning, this was removed from each stream by carefully skimming over the top of the water near the outlet with a piece of foam rubber (cut to the width of the stream) held at right angles to the water surface. Each morning and evening the stainless steel mesh at the outlet of each stream was brushed gently on the outside of the stream with a nylon brush to dislodge any debris which had accumulated. Failure to do so resulted in clogging of the mesh and overflowing of the stream, which allowed all the organisms to escape which of course led to the abandonment of that run. The experiment would have to be restarted.

At the end of the 96 h test period, after all the dead organisms had been removed, the pumps were switched off and the streams were allowed to drain until only a little water was left. A 10% formalin solution was poured into each stream to kill the surviving organisms. After 5 min., the pumps were switched on again to wash the dead organisms to the end mesh from where they could easily be counted and collected for sorting. In this way the "starting population" of each stream could be ascertained (by adding the total number of dead organisms to those which had survived). The dead organisms were placed in 10 mℓ pill vials with 70% ethanol. The pumps were then switched on again and the streams were cleaned thoroughly to remove all traces of formalin. They were left running until the next batch of organisms was put in.

During the first day of each run, free and total chlorine concentration, DO, pH, conductivity and temperature were measured and recorded. These determinands were checked each day to ensure that they were kept constant. DO, pH, conductivity and temperature were compared to the values recorded by Umgeni Water at the PEF, and where there were any discrepancies, the measurements were redone.

IDENTIFICATION OF DEAD MAYFLY NYMPHS

It was impossible to identify live mayfly nymphs to species, so this was done after they had died. This identification was done with the assistance of Mr. K. Soxujwa of the Institute for Water Research, Rhodes University, and by the use of appropriate texts, e.g. Barnard (1932), Crass (1947), and Macan (1979). Identified specimens are lodged with the Albany Museum, Grahamstown.

CALCULATION OF PERCENTAGE CUMULATIVE MORTALITY

After the test organisms had been identified, those which were not *B. harrisoni* were excluded from the numbers of dead ones in each stream at each time interval. The number of dead *B. harrisoni* was then calculated as a percentage of the total number of *B. harrisoni* for that stream. Any mayflies which had emerged during the course of the experiment were also disregarded.

THE CONCENTRATION RANGE OF TESTS

1. Westville stream mayflies

The range-finding tests were unreplicated and 9 concentrations of sodium hypochlorite were used. These chlorine concentrations resulted from the addition of 5 to 45 mℓ of sodium hypochlorite stock solution to the drip bags, which gave chlorine concentrations which ranged from 0.02 to 0.15 mg/ℓ.

The replicated tests were done in triplicate and volumes of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5 mℓ of sodium hypochlorite were used. (The concentrations of these solutions will be discussed later, as the detection limit for available chlorine was 0.02 mg/ℓ, which was the concentration achieved by the addition of 5 mℓ of sodium hypochlorite.)

2. Umbilo River mayflies

For the unreplicated, range-finding tests, volumes of 1, 2, 3, 4, 5, 10, 15, 20 and 25 mℓ of sodium hypochlorite were used. This gave a chlorine range from 0.02 to 0.08 mg/ℓ.

For the, replicated tests, volumes of 1, 2 and 3 mℓ of sodium hypochlorite were used.

For the first replicated test, an attempt was made to identify the mayflies prior to putting them into the streams. This was done by pouring them into a white plastic tray then removing the *B. harrisoni* with a large bore Pasteur pipette and releasing them into the streams. (It was found that 98 to 100% of the mayflies in the Umbilo at the collection point were *B. harrisoni.*) The results obtained during this run seemed to show some overlap and the mortality rate of the control organisms was unacceptably high. The run was therefore repeated (with the same three concentrations of sodium hypochlorite) but the mayflies were not identified prior to being placed in the streams, so handling (and therefore stress/damage to the organisms) was kept to a minimum. Low control mortalities made this the method of choice.

RESULTS

DATA PRESENTATION

The results of the toxicity tests are presented graphically, first as % Cumulative Mortality vs. Time (**Figs. 4.1** to **4.9**), then as various Concentration-Response Curves (**Figs. 4.10** and **4.11**). (A *concentration-response* relationships is analogous to the *dose-response* relationship employed in mammalian toxicological testing (Rand & Petrocelli, 1985)). Statistical analysis was carried out on the former set of graphs.

As explained in **Chapter 2,** because of the difficulty with the determination of low concentrations of chlorine, each graph of % Cumulative Mortality is accompanied by a table of the measured (or estimated) chlorine concentrations and a graph to show how the estimated values were determined. (By convention, graphs and tables of the same data are not both presented, however, in this case, the tables indicate those instances where free and total chlorine readings differed and when they did not, which is important because where the two values are the same, the graph shows only the total chlorine points, even though the free chlorine points are indicated in the legend. This could cause the reader to think that the readings were only for total chlorine.)

Sodium hypochlorite test solutions which contained 5 mℓ of stock solution per ℓ of solution in the drip bags resulted in a chlorine concentrations of 0.02 mg/ℓ in the streams. This is the limit of detection of both the Lovibond Comparator and the Palintest Photometer. Since the actual concentration of chlorine in the streams could not be measured directly, it was decided that mortalities would be related to the volume (mℓ) of sodium hypochlorite which had been used to make up the test solution, until such time as the concentrations could be determined.

In addition to the chlorine data, each graph is accompanied by a table to show the other determinands from the river at the time of collection, and in the artificial streams during the experiments.

WESTVILLE STREAM MAYFLIES

1. Unreplicated range-finding tests

The results of the range-finding test are shown in **Fig 4.1**. In this test, owing to a technical difficulty, dosing began only at 10h00 on the first day so mortalities were recorded only at 2, 4 and 6 hours. The mortalities were very high in those first 6 h and all the experimental organisms had died within 24 h. The lowest volume of sodium hypochlorite which had been used in this test was 5 mℓ which corresponded to a chlorine concentration of 0.02 mg/ℓ. (There was no difference between the free and total chlorine concentrations during this experiment so the results were expressed as free chlorine.)

The control mortalities of 10 and 13% in two of the streams were almost acceptable, but the 30% mortality in the third control was not. It was not known why the control mortality was so high in this stream.

A more detailed view of the mortalities within the first six hours is shown in **Fig. 4.2**. Within this short time period, a clear distinction among the responses to various doses of chlorine can be seen. The control mortalities at this stage were zero.

2. Replicated definitive tests

In the range-finding test, all the organisms had died within the first 24 h. The lowest volume of chlorine which had been dosed was 5 mℓ so volumes of 0.5 to 5.0 mℓ were used for the first definitive tests. The first set of replicated tests (**Fig 4.3**) showed a clear distinction between the mortalities resulting from the 5 mℓ sodium hypochlorite and the other concentrations. The anomalous situation of the control mortalities being much higher than those in the 0.5 mℓ streams and equal to those in the 2.0 mℓ stream could be attributed to the fact that during the night between the 48 h and the 72 h reading, one of the air stones had become dislodged from its position and had come to rest under the inlets of the control stream pumps. The resultant mass of bubbles prevented the pumps from pumping water into the streams, so that by the next morning, there was very little water in these streams. This could have caused the high mortality of the control stream organisms.

However, despite the poor survival of the control organisms, the results of this test run indicated that the LC_{50} was in the region of 0.01 mg/ℓ available chlorine which was the concentration resulting from the use of 2 mℓ of sodium hypochlorite.

The second replicated test in which volumes of 1, 3 and 4 mℓ of sodium hypochlorite were used indicated that the LC_{50} was probably that of the 1 mℓ solution, which was estimated to be 0.004 mg/l. The results of this test are shown in **Fig. 4.4.**

The third replicated test narrowed the range further by the use of 1.0, 1.5 and 2.0 mℓ of sodium hypochlorite. The results (**Fig. 4.5**) indicate the LC_{50} to be that caused by the use of 1 mℓ of sodium hypochlorite, which is estimated to be a concentration of 0.004 mg/ℓ

UMBILO RIVER MAYFLIES

1. Unreplicated range-finding tests.

The range-finding test (**Fig.4.6**) indicated that the LC_{50} would be between the 1 and 2 mℓ values i.e. a concentration between 0.004 and 0.008 mg/ℓ. As with the range-finding graph of the Westville stream mayflies, the mortality rates in the first 24 hours present an interesting picture and are depicted in **Fig 4.7**. A clear separation of mortalities at the various concentrations can be seen.

2. Replicated definitive tests

In the first definitive test (where an effort was made to identify the mayflies before they were placed in the streams), the LC_{50} appeared to be between the 1 and 2 mℓ marks (between 0.004 and 0.008 mg/ℓ. free chlorine). However, the results show considerable overlap (**Fig. 4.8**) and the control mortalities were too high (between 15 and 25%). These results suggest that the extra handling of the organisms during the identification process may have caused unnecessary stress and they might have been injured when sucked into the Pasteur pipette, making them more vulnerable to the chlorine.

When the replicated test was repeated, the results were far more clear-cut (**Fig. 4.9**) and the control mortalities were down to between 0 and 3%. It would appear from these results that the LC_{50} was between **0.004** and **0.005** mg/ℓ free chlorine.

As explained in **Chapter 2,** an attempt was made to determine the chlorine concentration in the stream (when 1 mℓ of sodium hypochlorite stock solution was in the drip bag) by titration of the stock solution followed by mathematical calculation of the dilution factor. The result of that exercise suggested that concentration of **0.0056** mg/ℓ available chlorine was present in the streams. The two values appear to be quite comparable.

1. Unreplicated, range-finding toxicity tests using *Baetis harrisoni* from the Westville stream.

Table 4.1 A. Chlorine concentrations.
This shows the range of "in stream" chlorine concentrations which resulted from the stated volumes of sodium hypochlorite in 1 ℓ of solution in the drip bags.

Vol.NaOCl/ℓ	Free chlorine	Total chlorine
(mℓ)	(mg/ℓ)	(mg/ℓ)
0	0	0
5	0.02	0.02
10	0.03	0.03
15	0.04	0.04
20	0.05	0.05
25	0.06	0.06
30	0.08	0.08
35	0.1	0.1
40	0.15	0.15
45	0.2	0.2

Table 4.1 B. Comparison between river and laboratory water determinands.

	River	Laboratory
pH	7.7	7.4
Conductivity	472 µS/cm	293-298 µS/cm
DO	95%	83%
Temperature	19.0 °C	22.1 °C

Fig. 4.1 A. Chlorine concentrations for range-finding tests using Westville mayflies.

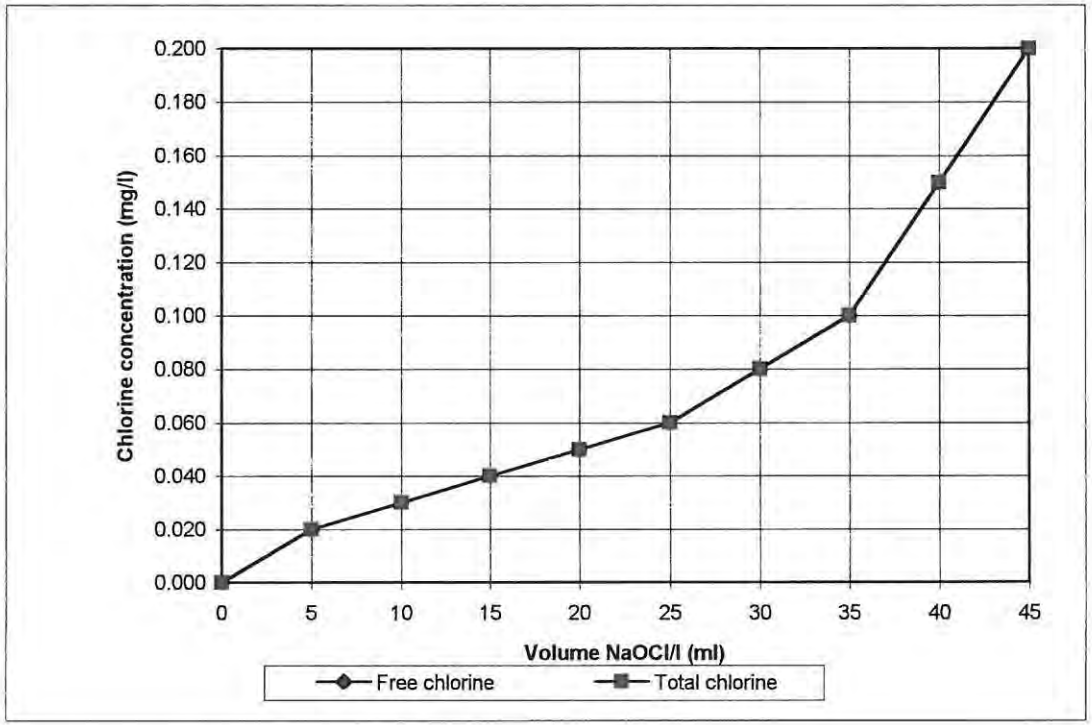
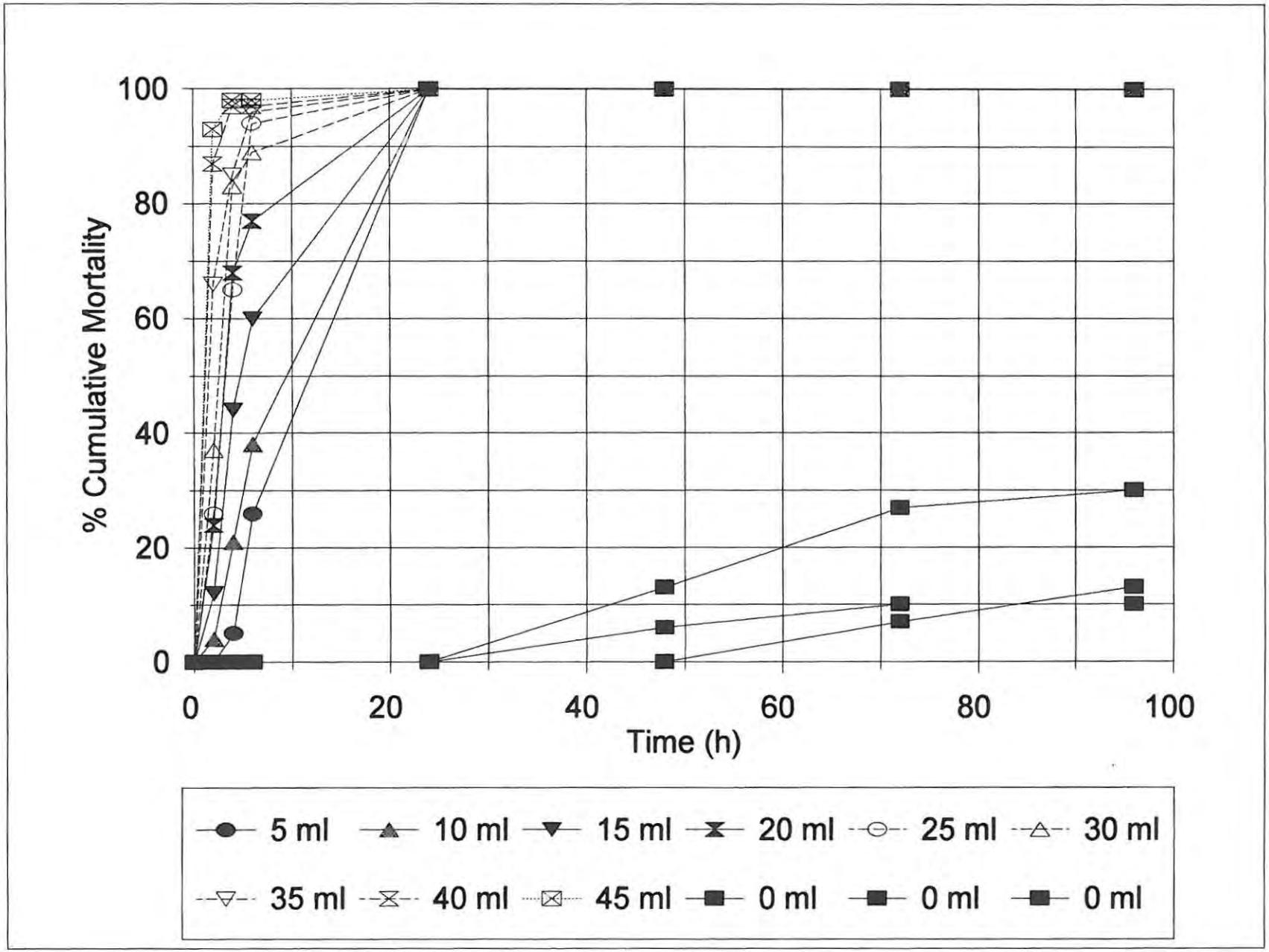


Fig 4.1 B. (Below) Percent cumulative mortalities of *B. harrisoni* over 96 h.

All the test organisms died within the first 24 h at these concentrations, while there were no mortalities among control organisms during this time. The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution. Controls are shown in black as 0 mℓ.

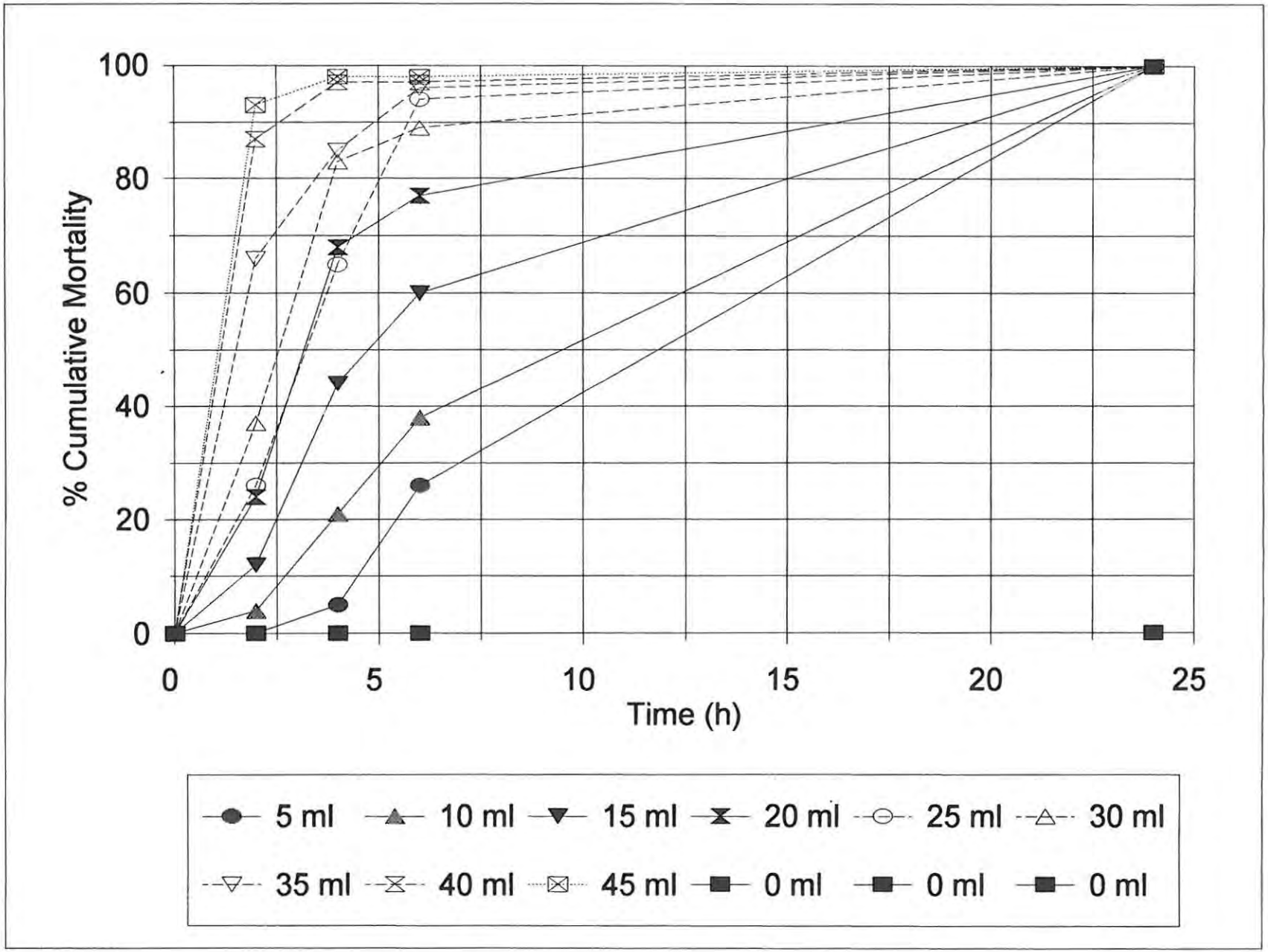

% Cumulative Mortality
100
80
60
40
20
0
0
20
40
60
80
100
Time (h)
5 ml
10 ml
15 ml
20 ml
25 ml
30 ml
35 ml
40 ml
45 ml
0 ml
0 ml
0 ml

2. Unreplicated, range-finding toxicity tests using *Baetis harrisoni* from the Westville stream.

Fig. 4.2 Percent cumulative mortalities of *B. harrisoni* during the first 24 h.

This shows the percentage of organisms which had died after 2, 4 and 6 h exposure to the range of chlorine concentrations shown on the previous page (0.02 to 2.0 mg/l free and total chlorine). Although this was an unreplicated test (so no statistical procedures were carried out) it appears that there was a definite link between the chlorine concentration and mortality rate.
All the organisms which had been exposed to these chlorine concentrations had died within the first 24 hours, but there were no mortalities among the control organisms.

The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution.
Controls are shown in black as 0 mℓ


100
80
60
40
20
0
% Cumulative Mortality
0
5
10
15
20
25
Time (h)
5 ml
10 ml
15 ml
20 ml
25 ml
30 ml
35 ml
40 ml
45 ml
0 ml
0 ml
0 ml

3. Replicated definitive toxicity tests using *Baetis harrisoni* from the Westville stream.

Table 4.3 A. Chlorine concentrations.
This shows the range of "in stream" chlorine concentrations which resulted from the stated volumes of sodium hypochlorite in 1 ℓ of solution in the drip bags.

Vol.NaOCl/ℓ (mℓ)	Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)	
0	0	0	
0.5	0.002	0.002	*
2	0.01	0.01	
5	0.02	0.02	

* Estimated

Table 4.3 B. Comparison between river and laboratory water determinands.

	River	Laboratory
pH	7.7	7.8
Conductivity	490μS/cm	290μS/cm
DO	96%	82%
Temperature	19.2.°C	22.°C

Fig. 4.3 A. Chlorine concentrations for definitive tests using Westville mayflies.

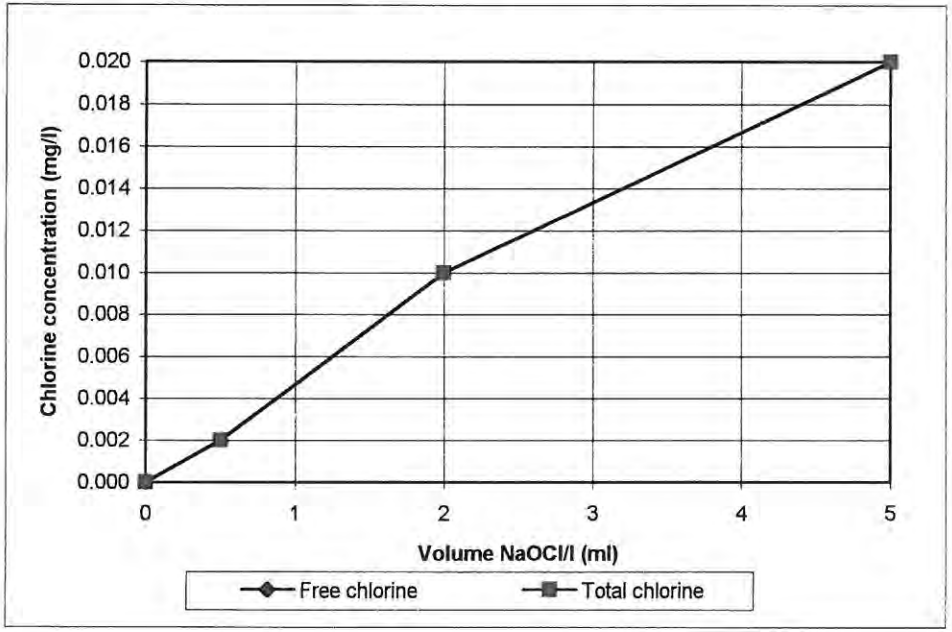
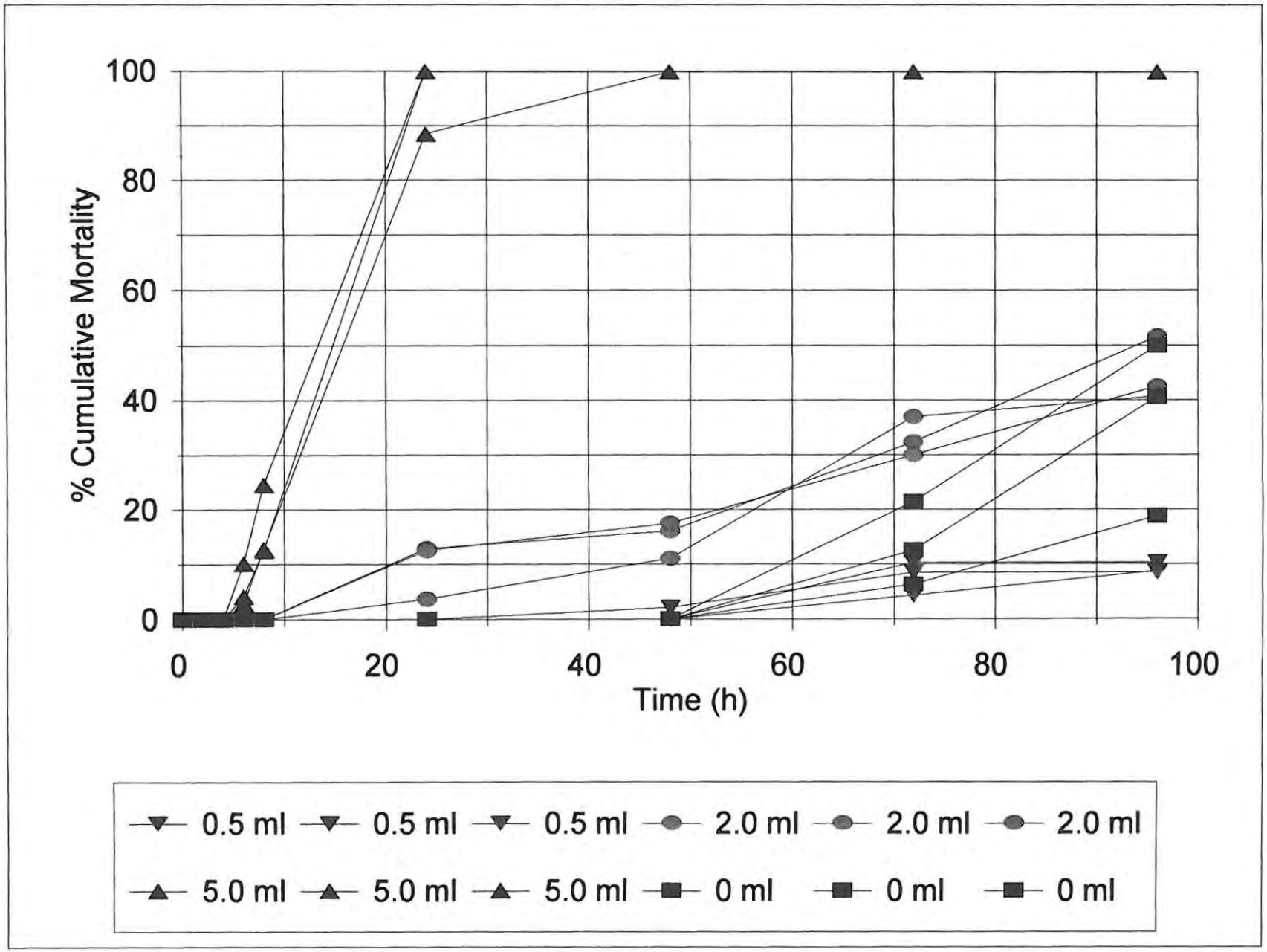


Fig 4.3 B. (Below) Percent cumulative mortalities of *B. harrisoni* over 96 h.

Most of the test organisms exposed to a chlorine concentration of 0.02 mg/ℓ had died within the first 24 h. Although the control mortalities were very high, the results of this test indicate an LC 50 in the region of 0.01 mg/ℓ free chlorine.
The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution.
Controls are shown in black as 0 mℓ.


100
80
60
40
20
0
% Cumulative Mortality
0
20
40
60
80
100
Time (h)
0.5 ml
0.5 ml
0.5 ml
2.0 ml
2.0 ml
2.0 ml
5.0 ml
5.0 ml
5.0 ml
0 ml
0 ml
0 ml

4. Replicated definitive toxicity tests using *Baetis harrisoni* from the Westville stream.

Table 4.4 A. Chlorine concentrations.
This shows the range of "in stream" chlorine concentrations which resulted from the stated volumes of sodium hypochlorite in 1 ℓ of solution in the drip bags.

Vol.NaOCl/ℓ (mℓ)	Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)	
0	0	0	
1	0.004	0.004	*
3	0.012	0.012	*
4	0.016	0.016	*

** Estimated from previous graph*

Fig. 4.4 A. Chlorine concentrations for definitive tests using Westville mayflies.

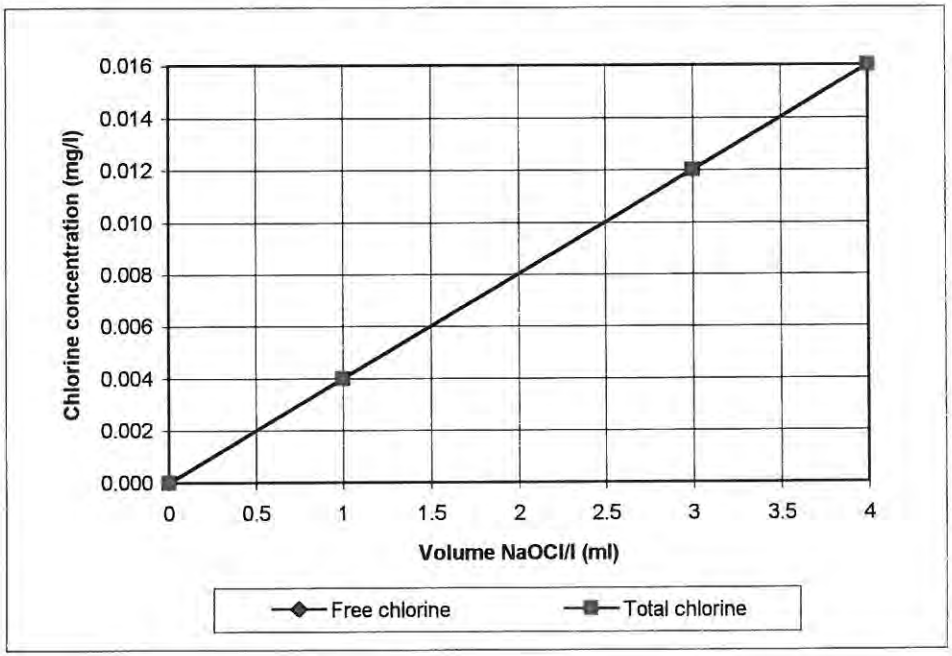
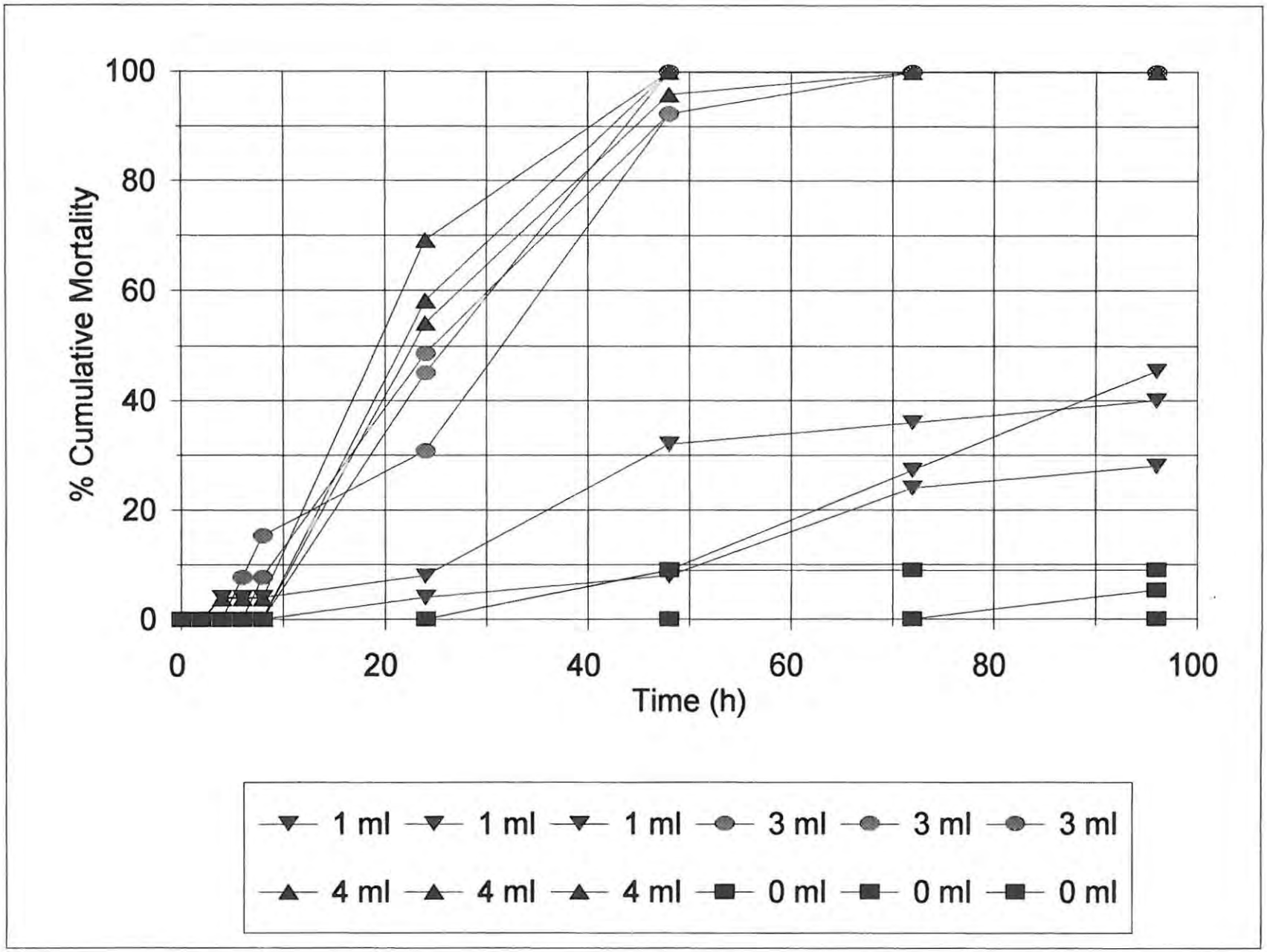


Table 4.4 B. Comparison between river and laboratory water determinands.

	River	Laboratory
pH	7.7	7.8
Conductivity	490μS/cm	290μS/cm
DO	96%	82%
Temperature	17.4°C	21°C

Fig 4.4 B. (Below) Percent cumulative mortalities of *B. harrisoni* over 96 h.

Over 90% of the test organisms exposed to a chlorine concentration of 0.012 and 0.016 mg/ℓ had died within the first 48 h.
The control mortalities had decreased to less than 10%, and the LC 50 appeared to be about **0.004 mg/l** free chlorine.
The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution.
Controls are shown in black as 0 mℓ.


100
80
60
40
20
0
% Cumulative Mortality
0
20
40
60
80
100
Time (h)
1 ml
1 ml
1 ml
3 ml
3 ml
3 ml
4 ml
4 ml
4 ml
0 ml
0 ml
0 ml

5. Replicated definitive toxicity tests using *Baetis harrisoni* from the Westville stream.

Table 4.5 A. Chlorine concentrations.
This shows the range of "in stream" chlorine concentrations which resulted from the stated volumes of sodium hypochlorite in 1 ℓ of solution in the drip bags.

Vol.NaOCl/ℓ (mℓ)	Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)	
0	0	0	
1	0.004	0.004	*
1.5	0.006	0.006	*
2	0.008	0.008	*

* Estimated from previous graph

Fig. 4.5 A. Chlorine concentrations for definitive tests using Westville mayflies.

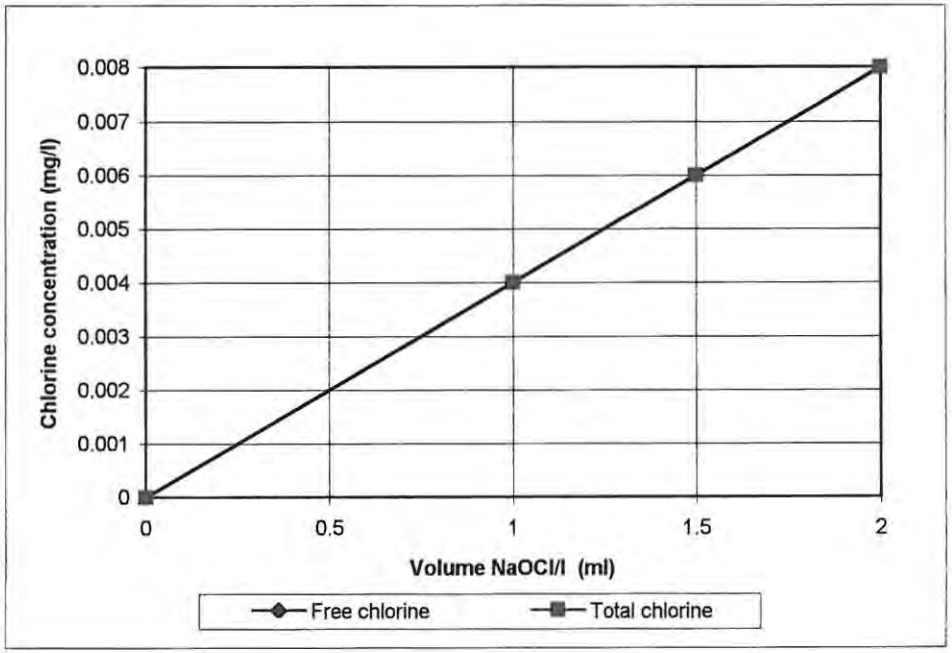
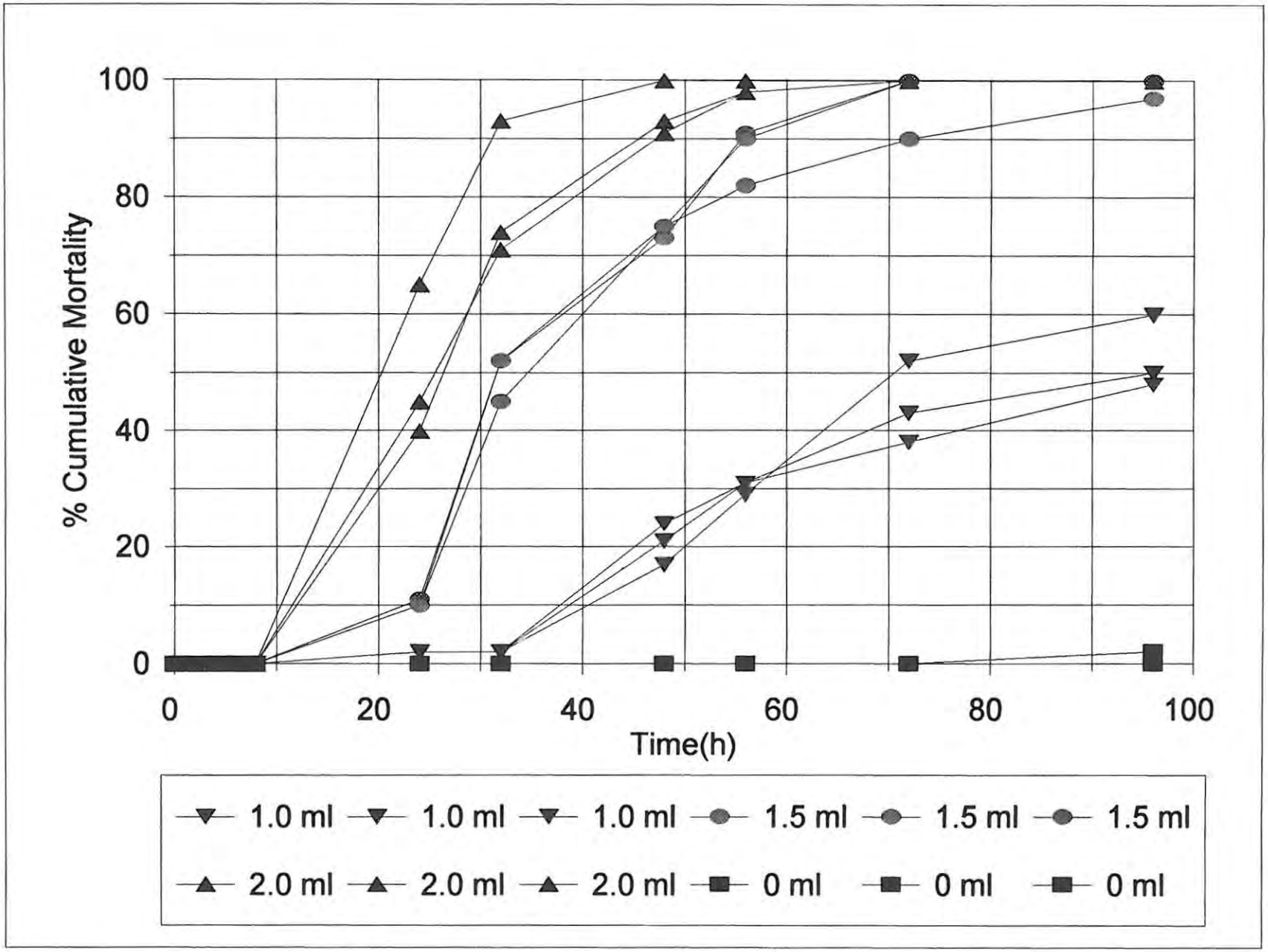


Table 4.5 B. Comparison between river and laboratory water determinands.

	River	Laboratory
pH	8.2	7.9
Conductivity	429μS/cm	299μS/cm
DO	95%	97%
Temperature	15.1°C	16.8°C

Fig 4.5 B. (Below) Percent cumulative mortalities of *B. harrisoni* over 96 h.

The results of this test run indicated an LC 50 of about 0.004 mg/ℓ.
The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution.
Controls are shown in black as 0 mℓ, and in this test, control mortalities of 0 to 3% were achieved.


100
80
60
40
20
0
% Cumulative Mortality
0
20
40
60
80
100
Time(h)
1.0 ml
1.0 ml
1.0 ml
1.5 ml
1.5 ml
1.5 ml
2.0 ml
2.0 ml
2.0 ml
0 ml
0 ml
0 ml

6. Unreplicated, range-finding toxicity tests using *Baetis harrisoni* from the Umbilo River.

Table 4.6 A. Chlorine concentrations.
This shows the range of "in stream" chlorine concentrations which resulted from the stated volumes of sodium hypochlorite in 1 ℓ of solution in the drip bags.

Vol.NaOCl/ℓ (mℓ)	Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)	
0	0	0	
1	0.004		*
2	0.008		*
3	0.012		*
4	0.016		*
5	0.02	0.03	
10	0.03	0.05	
15	0.04	0.08	
20	0.06	0.11	
25	0.08	0.13	

** Estimated from previous graph*

Table 4.6 B. Comparison between river and laboratory water determinands.

	River	Laboratory
pH	7.9	7.7
Conductivity	362µS/cm	292µS/cm
DO	108%	97%
Temperature	14.9°C	16.8°C

Fig. 4.6 A. Chlorine concentrations for range-finding tests using Umbilo River mayflies.

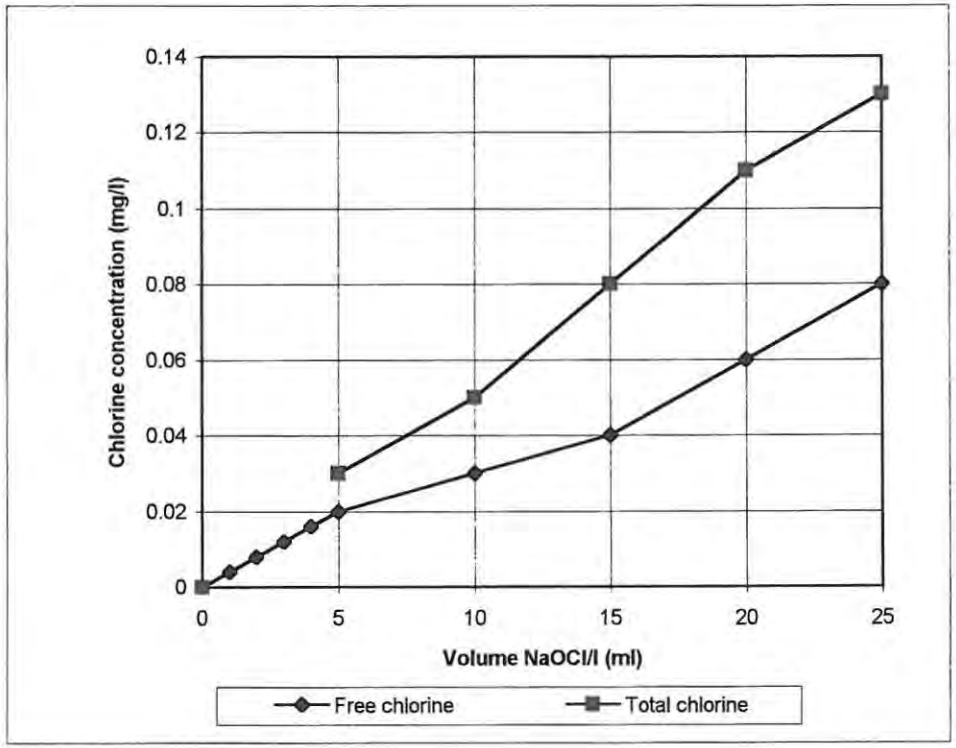
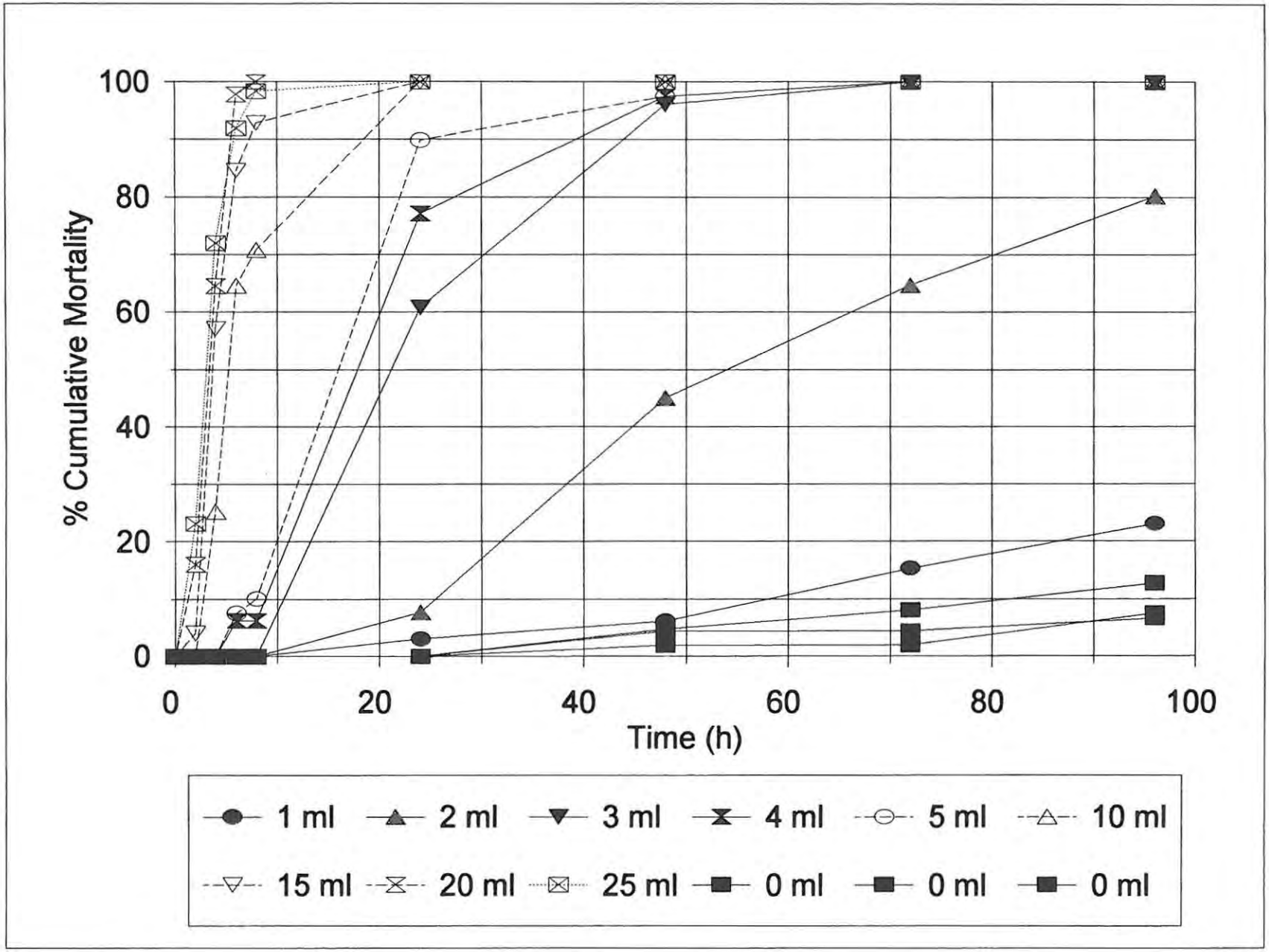


Fig 4.6 B. (Below) Percent cumulative mortalities of *B. harrisoni* over 96 h.

The results of this test indicated an LC 50 between **0.004** and **0.008** mg/ℓ free chlorine.
The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution.
Controls are shown in black as 0 mℓ.

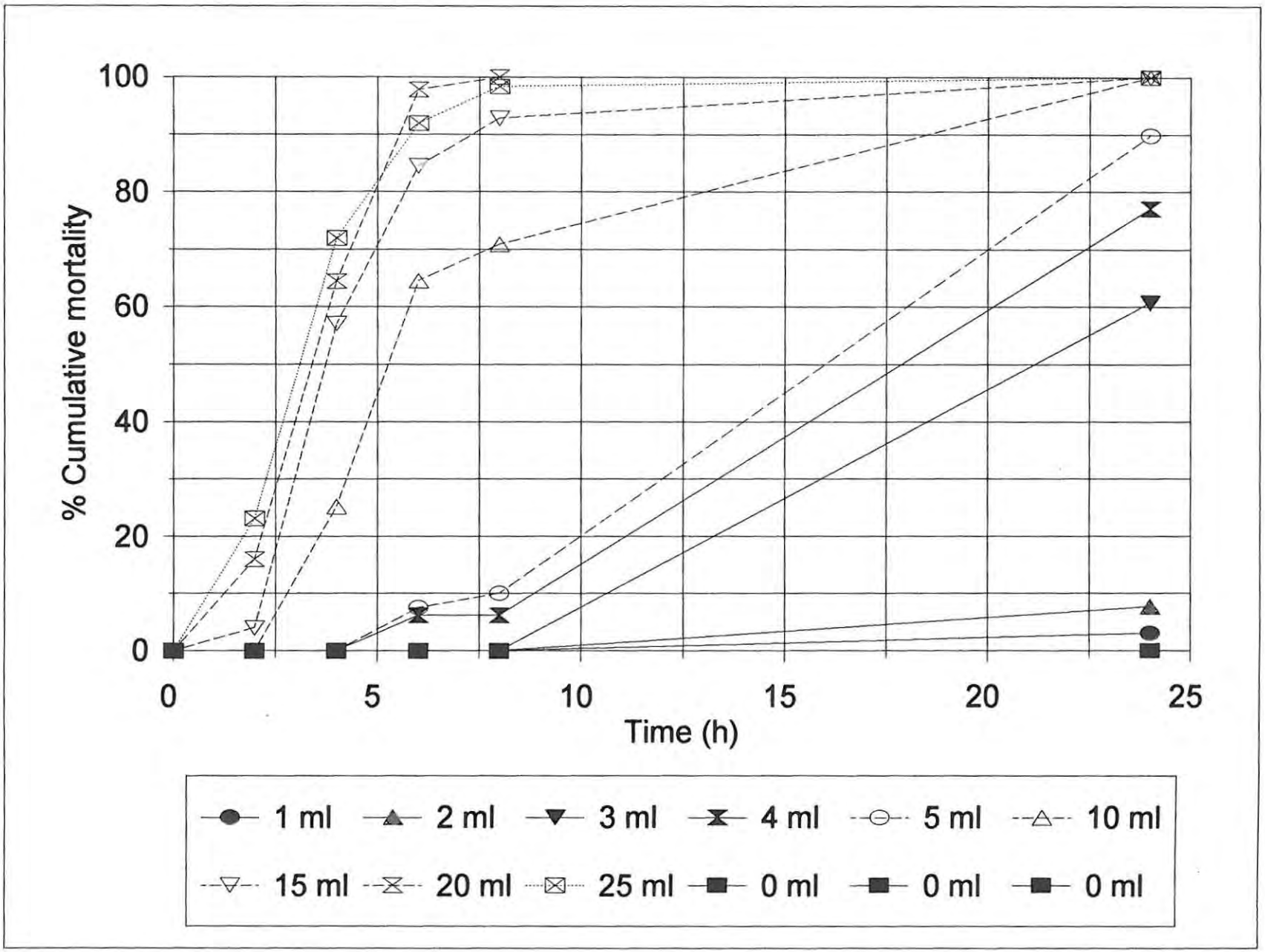

% Cumulative Mortality
100
80
60
40
20
0
0
20
40
60
80
100
Time (h)
1 ml
2 ml
3 ml
4 ml
5 ml
10 ml
15 ml
20 ml
25 ml
0 ml
0 ml
0 ml

7. Unreplicated, range-finding toxicity tests using *Baetis harrisoni* from the Umbilo River.

Fig. 4.7 Percent cumulative mortalities of *B. harrisoni* during the first 24 h.

This shows the percentage of organisms which had died after 2, 4 6 and 8 h exposure to the range of chlorine concentrations shown on the previous page (0.004 to 0.08 mg/l free chlorine and 0.004 to 0.13 mg/l total chlorine). Although this was an unreplicated test (so no statistical procedures were carried out), it appears that there was a definite link between the chlorine concentration and mortality rate. More than 90% of the organisms which had been exposed to chlorine concentrations above 0.04 mg/l (free) and 0.08 mg/l (total) had died within the first 24 hours, but there were no mortalities among the control organisms.

The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution. Controls are shown in black as 0 mℓ


100
80
60
40
20
0
% Cumulative mortality
0
5
10
15
20
25
Time (h)
1 ml
2 ml
3 ml
4 ml
5 ml
10 ml
15 ml
20 ml
25 ml
0 ml
0 ml
0 ml

8. Replicated definitive toxicity tests using *Baetis harrisoni* from the Umbilo River.

Table 4.8 A. Chlorine concentrations.
This shows the range of "in stream" chlorine concentrations which resulted from the stated volumes of sodium hypochlorite in 1 ℓ of solution in the drip bags.

Vol.NaOCl/ℓ (mℓ)	Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)	
0	0	0	
1	0.004	0.004	*
2	0.008	0.008	*
3	0.012	0.012	*

* Estimated from previous graph

Fig. 4.8 A. Chlorine concentrations for definitive tests using Umbilo River mayflies.

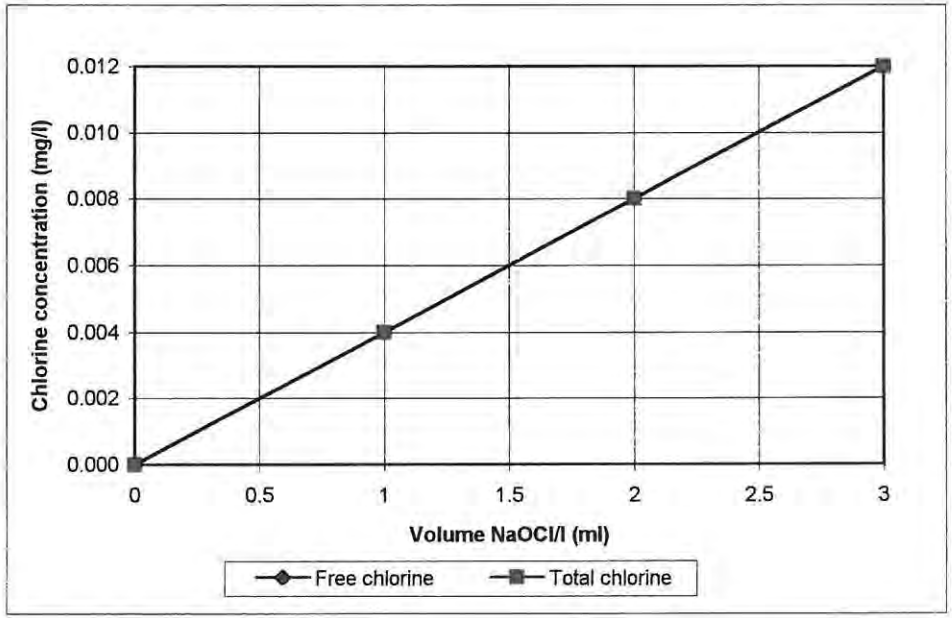


Table 4.8 B. Comparison between river and laboratory water determinands.

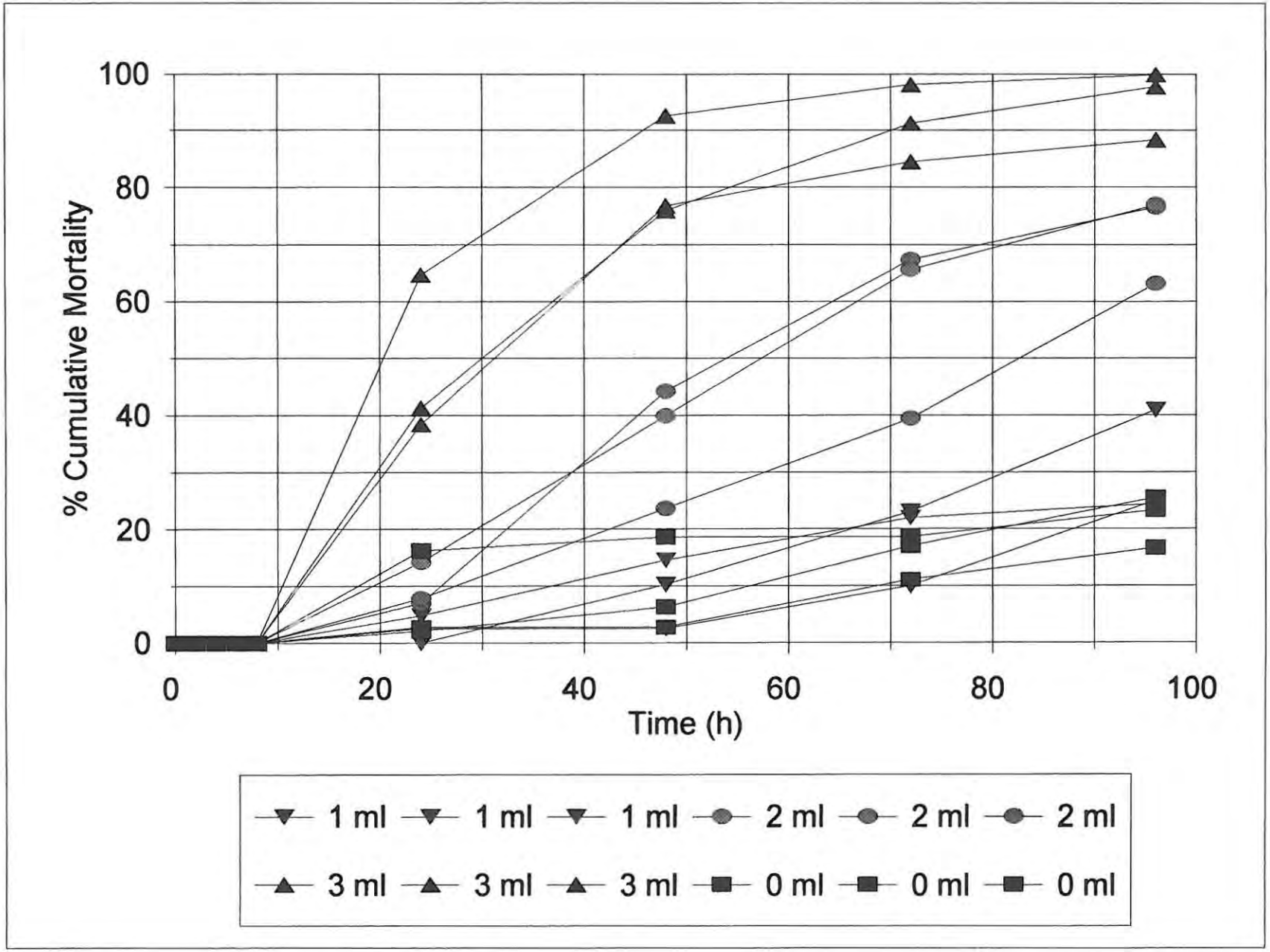
	River	Laboratory
pH	8.2	7.4
Conductivity	374μS/cm	292μS/cm
DO	104%	80%
Temperature	12.3°C	18.9°C

Fig 4.8 B. (Below) Percent cumulative mortalities of *B. harrisoni* over 96 h.

The results of this definitive test also indicate an LC 50 of between **0.004** and **0.008** mg/ℓ free chlorine.

The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution.

Controls are shown in black as 0 mℓ. The high control mortalities may have been the result of the collection technique as the organisms were handled more than before while trying to identify them.


100
80
60
40
20
0
% Cumulative Mortality
0
20
40
60
80
100
Time (h)
1 ml
1 ml
1 ml
2 ml
2 ml
2 ml
3 ml
3 ml
3 ml
0 ml
0 ml
0 ml

9. Replicated definitive toxicity tests using *Baetis harrisoni* from the Umbilo River.

Table 4.9 A. Chlorine concentrations.
This shows the range of "in stream" chlorine concentrations which resulted from the stated volumes of sodium hypochlorite in 1 ℓ of solution in the drip bags.

Vol.NaOCl/ℓ (mℓ)	Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)	
0	0	0	
1	0.004	0.004	*
2	0.008	0.008	*
3	0.012	0.012	*

* Estimated from previous graph

Fig. 4.9 A. Chlorine concentrations for definitive tests using Umbilo River mayflies.

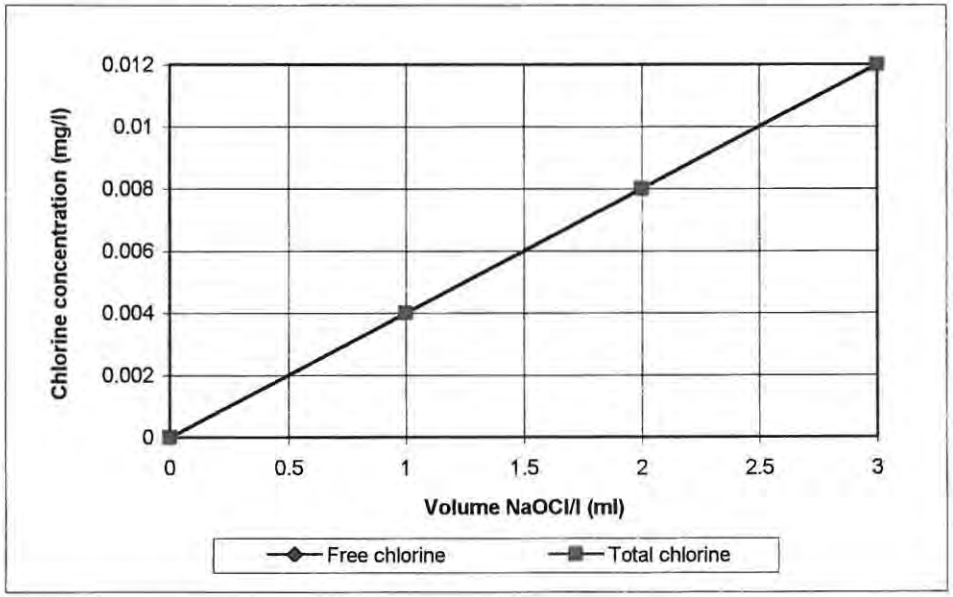
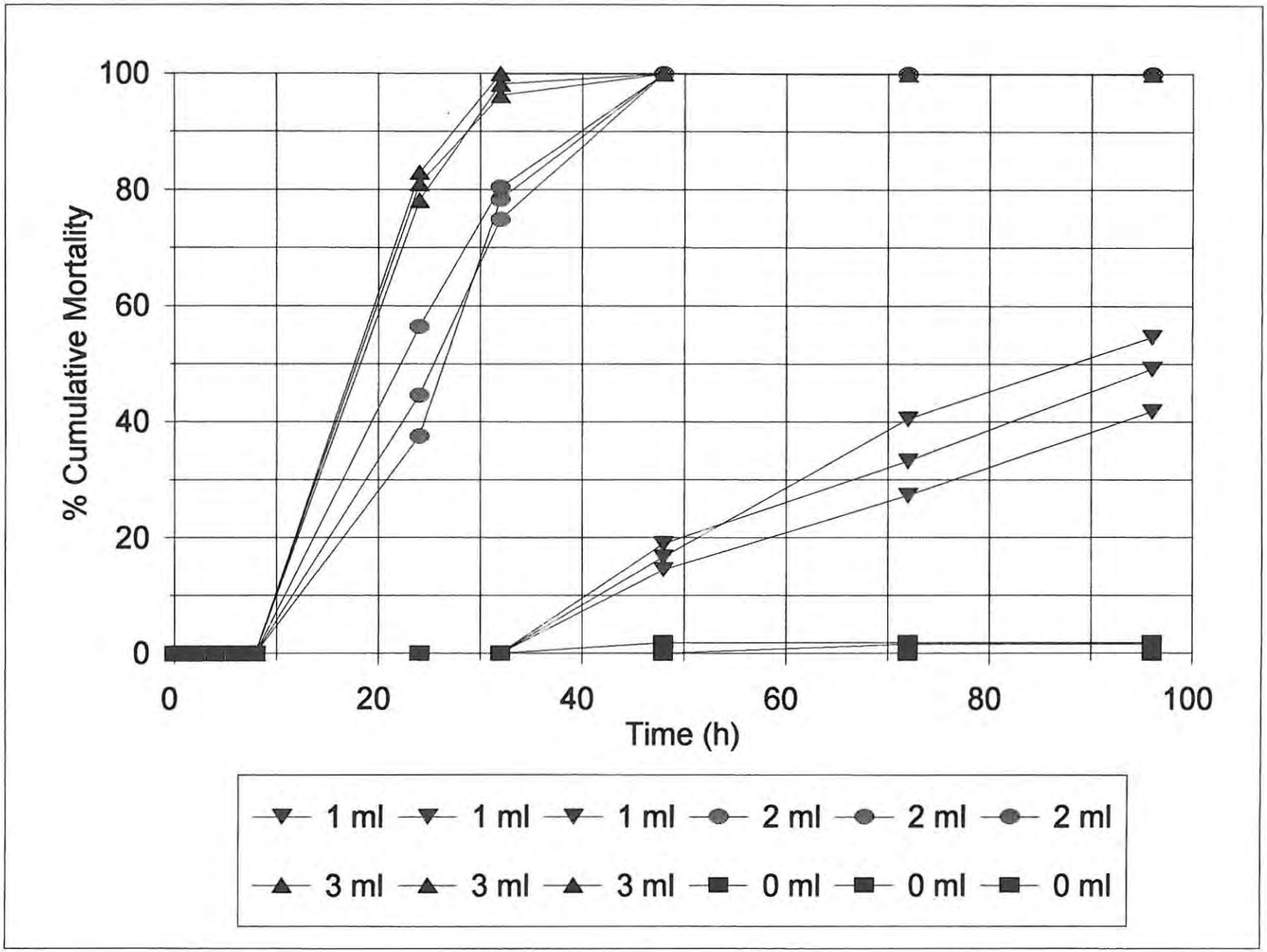


Table 4.9 B. Comparison between river and laboratory water determinands.

	River	Laboratory
pH	8.4	7.8
Conductivity	383μS/cm	298μS/cm
DO	112%	93%
Temperature	13.0°C	16.4°C

Fig 4.9 B. (Below) Percent cumulative mortalities of *B. harrisoni* over 96 h.

This was a repeat of the previous test, where handling of organisms was kept to a minimum, thus reducing the control mortalities.
The results of this test indicate an LC 50 of about 0.004 mg/ℓ free chlorine.
The volumes shown in the legend refer to the amounts of sodium hypochlorite added to distilled water to make up 1 ℓ of solution.
Controls are shown in black as 0 mℓ.


100
80
60
40
20
0
% Cumulative Mortality
0
20
40
60
80
100
Time (h)
1 ml
1 ml
1 ml
2 ml
2 ml
2 ml
3 ml
3 ml
3 ml
0 ml
0 ml
0 ml

STATISTICAL ANALYSIS

Statistical analyses were carried out on the results of the replicated tests to determine whether there were significant differences between:

i) the three replicates of a particular concentration (treatment)

ii) the four treatments in a test run (three chlorine concentrations and a control)

iii) the responses of the organisms from the two rivers

The tests were carried out using the Statgraphics program. Arcsine transformations of the % mortalities were used to stabilize the variance.

i) A One-way Analysis of Variance (ANOVA) was used to determine whether the differences among the three replicates were significant. In all the replicates, the p-value was > 0.05 indicating that the differences between them were not significant at the 95% confidence level. A print-out of the results is provided in **Appendix F**.

ii) A two-way ANOVA and a Multiple range analysis were used to determine whether the differences between treatments (concentrations of chlorine) were significant. A summary of the results and a print-out of the analyses is presented in **Appendix G,** along with means plots of the results. With two exceptions, the differences between treatments (chlorine concentrations) were all found to be significant. The exceptions were differences between:

a) Treatments 1 (0.5 mℓ NaOH) and 4 (Control: 0 mℓ NaOH) of **Fig. 4.3;**

b) Treatments 1 (1.0 mℓ NaOH) and 4 (Control: 0 mℓ NaOH) of **Fig. 4.8;** and

c) Treatments 2 (3.0 mℓ NaOH) and 3 (4 mℓ NaOH) of **Fig. 4.4**

In the first two exceptions (a and b) there was no significant difference between the control mortalities and those of the lowest chlorine concentrations, and in the third exception (c) both chlorine concentrations killed most of the test organisms within 48h.

iii) In the analysis to investigate whether the differences in responses of the organisms from the Westville Stream and the Umbilo River were significant, it was found that the differences between the selected treatments (0 mℓ (Control), 1 mℓ, and 2 mℓ)were significant, but that there was no significant difference between the responses of *B. harrisoni* from the two rivers. The results are shown as the last entry of **Appendix G**.

CONCENTRATION-RESPONSE CURVES

Concentration-response curves for 24, 48, 72 and 96 h were drawn up for the Westville and the Umbilo mayflies and are shown in **Figs. 4.10** and **4.11**. Only volumes of 5 mℓ sodium hypochlorite and below were used as the concentrations above this all resulted in death of all the test organisms within 24 h. The curves were drawn from the pooled data from the test runs.

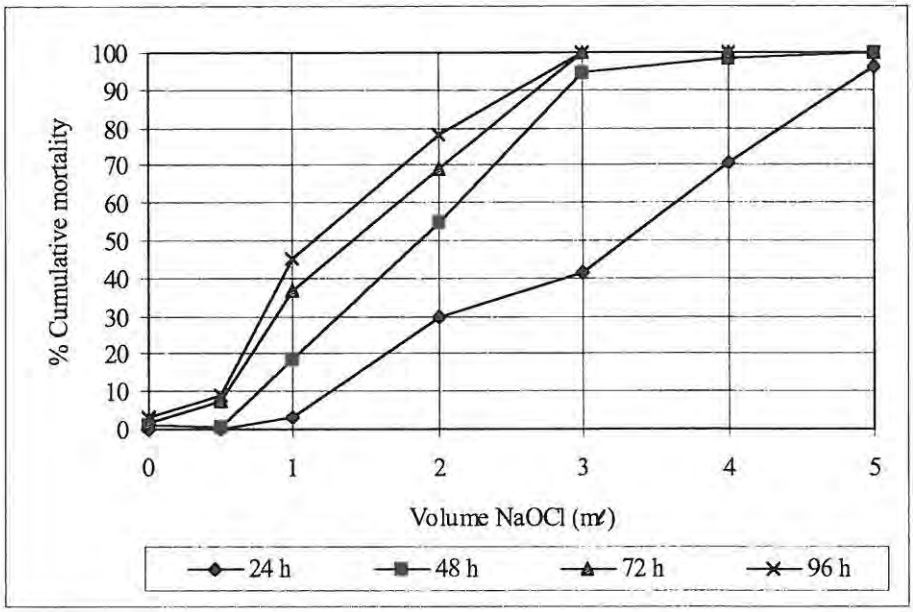


Fig. 4.10. Concentration-response curves for Westville stream mayflies.

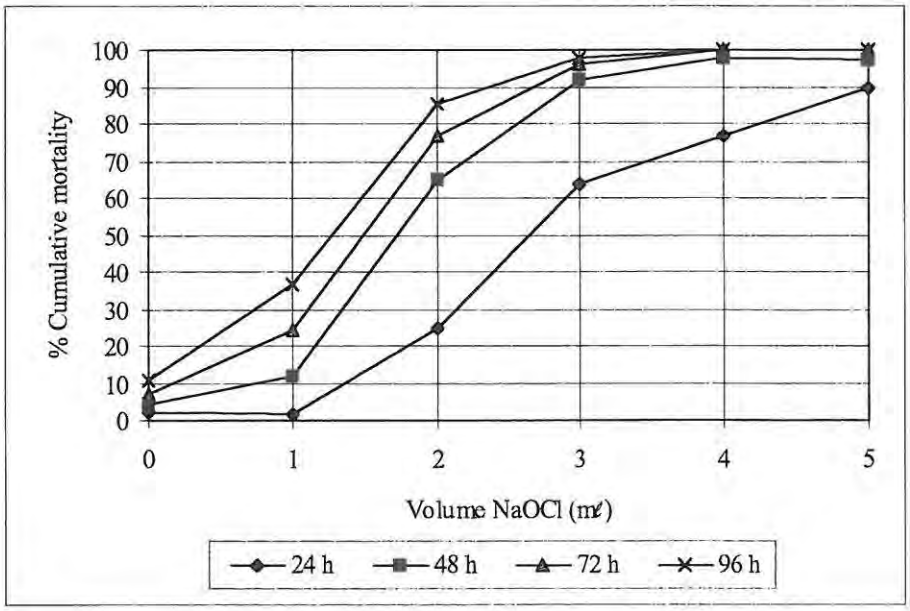


Fig. 4.11. Concentration-response curves for Umbilo River mayflies

The graphs depict the typical sigmoid curves which would be expected from this data presentation, as shown in Finney (1971). Lines drawn from the 50% mark indicated that the 96 h LC_{50} would be in the region of 1.16 mℓ sodium hypochlorite for the Westville mayflies and 1.26 mℓ for the Umbilo mayflies. If 1 mℓ NaOCl corresponded to 0.004 mg/ℓ (or 4 µg/ℓ) available chlorine (as estimated from graphs **4.1A-4.9A**,) then these 96 h LC_{50} values would correspond to values between **4** and **5µg/ℓ**..

The effect of probit transformation was then investigated. The % mortalities were transformed into probit values (as per Table 3.2 in Finney, 1971), and the concentrations were expressed as logarithms. Graphs for the responses of the Westville and Umbilo mayflies using the 96 h time interval were fitted by eye to fit the points as satisfactorily as possible; with extreme values ignored; and attention paid to minimising the vertical deviations from the data points (Finney, 1971; **Fig. 4.12** and **4.13**). The sigmoid shape was transformed into a straight line, and the 96 h LC_{50} value was estimated as shown on **Figs 4.12** and **4.13** i.e a line was drawn horizontally from the 5 mark on the y-axis (the probit transformation of 50% cumulative mortality) to the curve, then vertically down to the x-axis. This showed the log concentration to be 0.65. This was converted to the anti-log value using a calculator, and the estimated LC_{50} for the populations at both these sites, on each of the graphs, was **4.47** µg/ℓ . (The lowest concentration of chlorine which could be measured with any certainty was 0.02 mg/ℓ. or 20 µg/ℓ so an accuracy of 0.01 µg/ℓ cannot be claimed. At best it can only be said that the LC_{50} was in the region of **4 µg/ℓ** , since the low concentrations were estimated by extrapolation from graphs of concentrations of chlorine higher than 0.02 mg/ℓ.)

The various methods of data analysis have never-the-less given very comparable 96 h LC_{50} estimations.

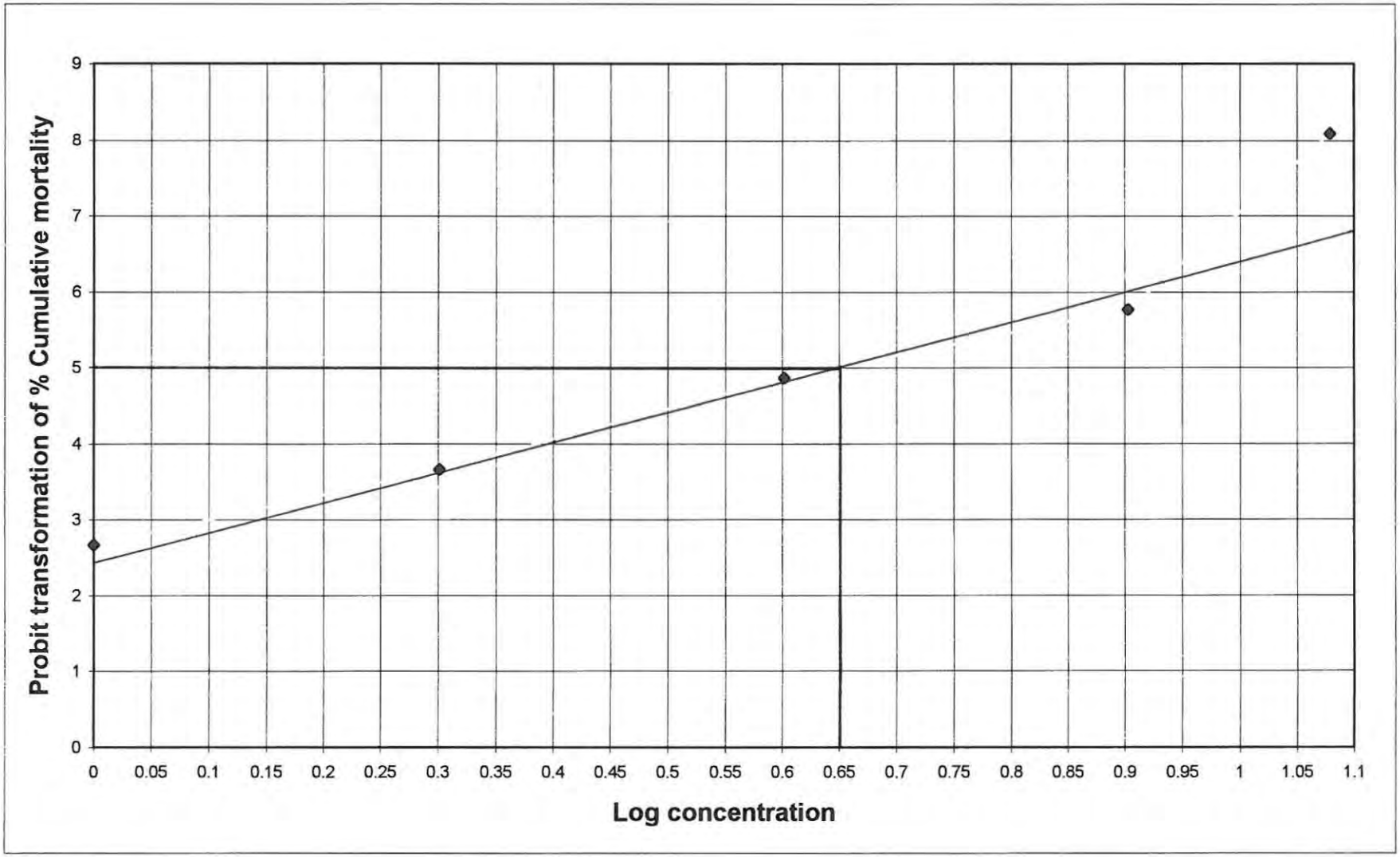


Fig. 4.12 Probit transformation of percentage cumulative mortalities for *B. harrisoni* from the Westville Stream.

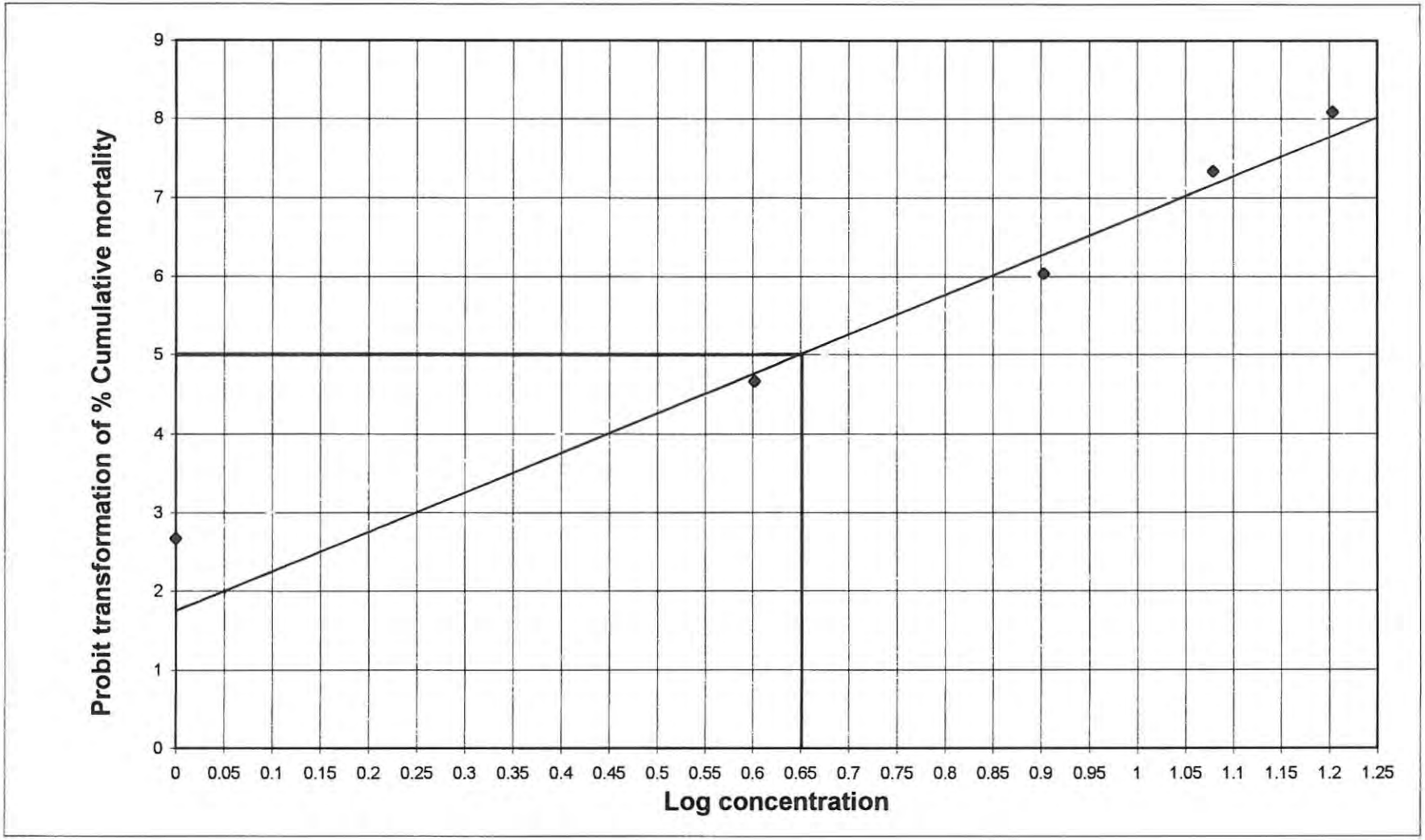


Fig. 4.13 Probit transformation of percentage cumulative mortalities for *B. harrisoni* from the Umbilo River.

DISCUSSION

THE DISTRIBUTION OF *BAETIS HARRISONI* IN POLLUTED WATERS

The mayfly, *B. harrisoni* is regarded as being an organism which is tolerant of organic pollution (Chutter, 1994). However, this field study indicated that while it may have been present in considerable numbers upstream of a chlorinated, treated sewage discharge, it was quite scarce if present at all for a few kilometers downstream. Its absence from downstream sites could have been in response to the sewage effluent or to the chlorine, or both. It was therefore decided that that this would be a suitable organism on which to conduct chlorine toxicity tests.

TOXICITY TESTING

Toxicology began as a formal discipline in the early 1800's in response to the development of organic chemistry. New chemicals were synthesized and their effects were tested to find the potential benefits and drawbacks for any substance, drug or poison. By the 1940's or 1950's biologists had observed striking differences in groups of organisms living in streams that received waste materials from society and those streams that did not. Human judgement that some of these man-made changes were undesirable was followed by the earliest attempts to formulate a management strategy. Surveys of organisms in streams could document damage, but *preventative* measures required an estimation of damage before the fact (Buikema *et al.*, 1982).

Often it is suspected that a certain chemical is harmful to aquatic organisms, but the toxic concentration is not known. It could be said that all substances are toxic, at high enough concentrations. Even something as seemingly innocuous as common salt would prove fatal above a certain concentration, whereas the uptake of minute quantities of toxic chemicals may result in no apparent adverse effect (Rand & Petrocelli, 1985). Paracelsus observed over four centuries ago that :

> *"All things are poisonous, for there is nothing without poisonous qualities.*
> *It is only the dose which makes a thing a poison"*
> (Goldstein *et al.*, 1974 in Moriarty 1983; Roux, 1994).

The objective of measuring the toxicity of a chemical is to estimate as precisely as possible the range of chemical concentrations that produce some selected, readily observable, quantifiable response in groups of the same test species under controlled laboratory conditions (Rand & Petrocelli, 1985). In this study, discussions of toxicity tests will be limited to *aquatic* toxicity tests and the amount of test material which causes a response will be discussed in terms of *concentrations* (in the surrounding water) rather than *dose* (which would be administered by injection or incorporated into food). In the context of aquatic environments, toxicology can be defined as the qualitative and quantitative study of adverse or toxic effects of chemicals on aquatic organisms. These toxic effects may include both lethality (mortality) and sub-lethal effects such as changes in growth, development, reproduction, pharmacokinetic responses, pathology, biochemistry, physiology and behavior (Rand & Petrocelli, 1985).

Toxicity tests are useful for a number of purposes that include the determination of :

1. the suitability of environmental conditions for aquatic life
2. favourable and unfavourable environmental factors such as dissolved oxygen, pH, temperature, salinity or turbidity
3. the effect of environmental factors on waste toxicity
4. the toxicity of wastes to a test species
5. the relative sensitivity of aquatic organisms to an effluent or toxicant
6. the amount and type of waste treatment needed to meet water pollution control requirements
7. the effectiveness of waste treatment methods
8. the permissible effluent discharge rates
9. the compliance with water quality standards, effluent requirements and discharge permits

Toxicity tests differ on the basis of the exposure of the test organisms to the chemical in question. *Acute* (short term) tests last from 8-96 h depending on the type of test organism, (4 d/96 h for fish and macroinvertebrates and 48 h for organisms with a shorter life span), and the effect is usually mortality. *Chronic* (long-term) tests can last

for weeks, months or years, depending on the life span of the organism, and is often one tenth of the life-span or more. In addition to mortality, sub-lethal effects such as reduced growth, reduced reproduction or behavioral changes can be monitored (APHA, 1992; Gerhardt *et al.*, 1994).

The degree to which toxicity test data can be extrapolated to the natural environment is cause for heated debate (Cairns, 1992; 1983; Arthur, 1988; Moriarty, 1983) especially since many predictions of environmental hazard are based on laboratory toxicity tests involving single species (Cairns, 1988a). Interpretation of toxicity test data is made more difficult because different species of aquatic organisms are not equally susceptible to toxic substances nor are organisms equally susceptible throughout their life cycle. Previous exposure to toxicants can alter susceptibility, and organisms can respond differently to the same level of a toxicant from time to time, even when other variables are held constant (APHA, 1992). Some substances are harmful to organisms only at certain stages in their life cycles, some have sub-lethal effects, and some are toxic only in the presence of other substances. This issue is important in the context of this study and will be discussed further in **Chapter 6.**

In regulatory assessments therefore, toxicity test data should be used in conjunction with receiving-water and site-specific discharge data on volume, dilution rates, and exposure times and concentrations (APHA, 1992).

THE LC_{50} OF CHLORINE FOR *BAETIS HARRISONI*

The 96 h LC_{50} of about 0.005 mg/ℓ estimated from this study is considerably lower than the 48 h LC_{50} of 0.357 mg/ℓ Cl_2 for mayfly larvae of the genus *Hexagenia* reported by Ward & De Graeve (1980), who also reported that a few species of macroinvertebrates survived well in effluent with a mean residual Cl_2 levels as high as 1.491 mg/ℓ. (In this study, the 48 h LC_{50} was 50 times lower at about 0.007 mg/ℓ). At first glance, the discrepancy between these values seems absurd, however, the results of this study are based on concentrations of **free** chlorine whereas those of Ward & De Graeve (1978) are for **total** residual chlorine. Free chlorine is 25 times more germicidal than the chloramines (White, 1992). In addition, Rand & Petrocelli (1985)

state that within a laboratory, one investigator may. obtain a series of LC_{50}'s with a range of only about 20% of the median scale, whereas LC_{50}'s from the same laboratory have been known to vary by a factor of 5. Inter-laboratory comparisons may show variation by a factor of only 1.2 or differences as great as 10-fold. An order-of-magnitude variation in toxicity data is to be expected in data gathered from a number of laboratories.

The toxicity of a particular chemical agent is traditionally evaluated on the basis of tests carried out with healthy organisms. Test organisms that are in poor health or are stressed in some other manner, such as by previous or concurrent exposure to other toxicants are, however, likely to be more susceptible to a toxic chemical (Rand & Petrocelli, 1985). In this study, the health status of the test organisms prior to testing was not known. It was known that the Umbilo River is severely impacted by industrial effluent, but it was not known whether the mayflies were very hardy and tolerant of the polluted conditions or whether they were very stressed and therefore the test conditions were the "last straw". However, the comparative study with mayflies from the "relatively clean" Westville Stream yielded similar results to those of the Umbilo River.

The water used in toxicity tests is obviously of utmost importance and it is possible that the use of raw water from a few metres below the surface of a dam placed the test organisms under more stress than water from their respective habitats would have done. However, the water was well aerated before entering the artificial streams.

For comparative purposes, some of the toxicity data from this and other studies involving macroinvertebrates are presented in **Table 4.10** below. The main observations are the lack of standardization of toxicity testing times and the variability of reported LC_{50} results. The results from this study place *B. harrisoni* among the most sensitive of those organisms tested for chlorine. Interestingly the standard test organism *D. magna* has a comparable sensitivity at 24h, but *B. harrisoni* is much more sensitive at 48h.

Reference	Organism	Order	24 h LC_{50} (mg/ℓ)	48 h LC_{50} (mg/ℓ)	96 h LC_{50} (mg/ℓ)
Present study	*Baetis harrisoni*	Ephemeroptera	0.012	0.007	0.005
Gregg, 1975*	*Stenonema ithaca*	Ephemeroptera			0.102
Gregg, 1974**	*Centroptilium* sp	Ephemeroptera	0.071		
	Ephemerella lata	Ephemeroptera		0.027	
	Iron humeralis	Ephemeroptera	0.046 (8 h)		
	Isonychia sp	Ephemeroptera		0.0093	
	Stenonema ithaca	Ephemeroptera	0.502 (8 h)		
Arthur, *et al.* 1975*	*Pteronarcys* sp.	Plecoptera			0.400
Ward & De Graeve,1980 (monochloramine) 1978	*Hexagenia* spp	Ephemeroptera		0.357	
	Larvae (sp?)	Plecoptera		0.781	
	Daphnia magna	Branchiopoda		0.045	
	Daphnia magna	Branchiopoda		0.017	
Cairns *et al.* 1976	*Daphnia magna*	Branchiopoda	0.14	0.116	

Table 4.10. LC_{50} values for chlorine for a number of different macroinvertebrates as reported in other studies.

Source references:

*From US EPA Ambient Water Quality Criteria for chlorine 1984

** From Mattice & Zittel (1976)

The toxicity of chlorine to freshwater aquatic life is usually expressed as the concentration of Total Residual Chlorine (TRC), and the United States Environmental Protection Agency (US EPA) states Water Quality Criteria solely in these terms (Hermanutz *et al.*, 1990). The US EPA freshwater criteria for protection of most aquatic species and their uses are 0.011 mg/ℓ TRC as a 4 d (96 h) average and 0.019 mg/ℓ TRC as a 1 h average. The proposed National Criteria for protecting aquatic ecosystems in South Africa are 0.001 mg/ℓ chlorine for the Acute Effect Value (AEV) and 1×10^{-4} mg/ℓ chlorine for the Chronic Effect Value (CEV) (DWAF, 1995).

The **AEV** refers to the concentration at and above which statistically significant acute adverse effect is expected to occur. It is not a target value or compliance concentration, but rather a danger or reaction level, indicating where acute adverse effects can be expected. Calculation of the AEV is primarily based on results of acute toxicity tests. The **CEV** refers to the concentration limit which is deemed safe for all or most populations even during continuous exposure. As the CEV is exceeded, the risk of ecosystem damage increases (Roux *et al.*, in press).

It is possible that the toxicities of the various forms of TRC are inherently different, but it is also possible that they only have different rates of toxicity. Thus differences in toxicity between components of TRC under very short exposure conditions (a few minutes to a few hours) could be rate dependent (US EPA, 1984; Hermanutz *et al.*, 1990). In some of the literature, it is not absolutely clear what form of chlorine was present, so comparisons of LC_{50}'s may be misleading and it is necessary to clarify some points on chlorine toxicity.

TOXIC FORMS OF CHLORINE

The products which result from the addition of chlorine to water can be grouped into four major categories:

1. Free residual chlorine (*or* free available chlorine residual)
   This is the portion of chlorine injected into water that remains as molecular chlorine, hypochlorous acid (HOCl), or hypochlorite ion (OCl^-) after the chlorine demand has been satisfied.

2. Combined residual chlorine.
   This is the portion of the chlorine injected into water that remains combined with ammonia or nitrogenous compounds after the chlorine demand has been satisfied (chloramines).

3. Total residual chlorine.
   This is the free residual chlorine plus the combined residual chlorine.

4. Chlorine demand.

   This is the difference between the amount of chlorine injected into water and the total residual chlorine remaining at the end of a specified period. The actual products involved are mostly chlorides.

The products which result from chlorine demand are to a large extent not toxic, whereas the free residual and combined residual chlorine are (Mattice & Zittel, 1976).

The various chlorine residuals have different germicidal efficiencies (and hence toxic affects on aquatic life). *Hypochlorous acid* is the most effective germicide of all the chlorine residual fractions. Because its structure is similar to that of water and it has a low molecular weight and is electrically neutral, it can penetrate cell walls/membranes relatively easily. The *hypochlorite ion* is a relatively poor disinfectant because of its negative charge which hinders its passage through bacterial cell walls. The *chloramines* are slower to kill microorganisms than free available chlorine, and it has been estimated that it would take about 25 times more combined available chlorine than free available chlorine to produce the same germicidal efficiency. *Dichloramine* has been estimated to be twice as potent as *monochloramine* as a germicide (White, 1992). The differences in germicidal efficiencies of the various chlorine species may be implicated in the large discrepancies in LC_{50}'s, particularly in the case of Ward & De Graeve (1980) who were working with chlorine in domestic effluent, hence most of the chlorine would probably have been in the form of chloramine, as opposed to the present study in which the chlorine was usually only in the form of free chlorine.

STUDIES WITH OTHER INVERTEBRATES

As mentioned previously, there is a general paucity of comparative data on the toxicity of chlorine to mayflies in particular and stream invertebrates in general. Much of the available information on the effects of chlorine on aquatic animals and plants is concerned with the control of nuisance species in ponds, reservoirs and cooling towers and is not useful for deriving water quality criteria (US EPA, 1984). Many of these studies have involved the use of chlorine, monochloramine and chlorine dioxide to overcome problems of biofouling caused by clams (Mattice & Zittel, 1976; Graney

et al., 1983; Doherty *et al.* 1986; Doherty & Cherry, 1988; Peterson *et al.*, in press) and mussels (van Benschoten *et al.*, 1995). The problem with LC_{50}'s determined for these organisms is that they are able to close their shells tightly and avoid high chlorine concentrations for considerable periods of time (Doherty & Cherry, 1988).

TOXICITY TESTING IN SOUTH AFRICA

Clearly there is considerable scope for macroinvertebrate toxicity testing in South Africa. Roux (1994) reported that at a recent workshop on aquatic biomonitoring in South Africa, a specialist group on toxicity testing concluded that toxicity testing should, in a formal way, become part of local effluent regulation. However, at present, there are few people in South Africa who are competent to carry out toxicity tests, and only two or three laboratories can conduct tests with a representative range of freshwater organisms on a relatively routine basis. A system such as the one developed in this study would be suitable for routine toxicity testing as it is relatively inexpensive to set up, and as mentioned in **Chapter 2**, is relatively portable, so can be used on site where the water used in the artificial stream system is that of the natural environment of the test organisms. At the time of writing, a second artificial system constructed according to the specifications of this one, has been installed in the Eastern Cape Province (Bisho) for similar experimental work.

The results from this study provide a clear indication of the role of such work in the development and refinement of environmental water quality guidelines. In the draft of the aquatic ecosystem guidelines (DWAF, 1995) the Acute Effect Value (AEV) was 0.001 mg/ℓ, however there is an accompanying note: "The data available **did not** satisfy the minimum database requirement for the derivation of the AEV, and a safety factor of 6 was applied."

The acute 96h tolerance of *B. harrisoni* was in the region of 0.004 mg/ℓ. The AEV is half the FAV (Final Acute Value), which is derived directly from acute tolerance data (Roux *et al.*, in press; DWAF, 1995). The FAV would therefore be in the region of 0.002 mg/ℓ, and the experimental results from this study (0.004 mg/ℓ), although double this value, strongly confirm the calculated guideline values, indicating that the

application of the safety factor was successful.. The next step will be the recalculation of the guideline values using these results (which have been made available to DWAF), and possibly to extend the testing to another organism, less tolerant of organic enrichment. This confirms the use of existing results, both local and from elsewhere, to set guideline values, which can be revised and refined as data become available.

CHAPTER 5

RESPONSES OF MACROINVERTEBRATE COMMUNITIES TO CHLORINATED, TREATED SEWAGE EFFLUENT IN THE UMSUNDUZE AND UMBILO RIVERS

INTRODUCTION

The links between toxicology and ecotoxicology are somewhat circular (Dalinger & Rainbow, 1993). Toxicology is the study of individual responses to single toxicants, and there are limitations to the applicability of the results to the complex synergistic, antagonistic, and multi-species nature of the interaction between chemicals and organisms in ecosystems. While the reductionist approach of *toxicology* allows greater precision and replication of results, the study of ecological consequences of pollution, *ecotoxicology*, takes account of the difficulties of environmental complexity and variability, but the results may be specific rather than generally applicable. The circularity arises in the question of whether to use toxicological data to derive hypotheses which can be tested in the field; or whether to survey field conditions and use the results to plan experimental procedures. Ecotoxicological approaches themselves differ, and have involved the manipulation of instream conditions (Shutes *et al.*, 1993); survey-based sampling procedures (Klerks & Levinton, 1993); or the use of experimental microcosms (Kiffney & Clements, 1994).

The experimental investigation of the tolerance of *B. harrisoni* to chlorine presented in **Chapter 4** indicated a 96 h LC_{50} in the region of 0.004 mg/ℓ. Acute tolerance data can be used in the derivation of protective environmental water quality guidelines (Roux *et al.*, in press), but in no way indicate acceptable field conditions, as the LC_{50} value is the concentration at which half the population *dies*. Given that field concentrations of chlorine were in the region of 0.1 mg/ℓ free chlorine and 0.8 mg/ℓ total chlorine at the Darvill sewage works outfall, and 0.15 mg/ℓ free chlorine and 2.0 mg/ℓ total chlorine at

the Umbilo sewage works outfall, it was hypothesised that *B. harrisoni* would be absent immediately downstream of both outfalls, and numbers would increase in response to dechlorination. In addition, because of the sensitivity of a relatively robust organism such as *B. harrisoni* (Chutter, 1994), it was hypothesised that the macroinvertebrate community structure would be altered downstream of chlorinated effluent, as a result of chlorine exposure.

Sampling was planned to test these hypotheses, and both the Umsunduze and Umbilo Rivers were sampled above and below the outfall sites. Since chlorinated sewage effluent by definition comprises both chlorine and treated sewage, it was important to distinguish between the effects of the two. The Umbilo River study provided the opportunity for a field experiment where a portion of the river was exposed to unchlorinated treated sewage effluent. (The unchlorinated stream preceded the chlorinated outfall and posed a minimal health risk).

MATERIALS AND METHODS

STUDY DESIGN

Umsunduze River

Macroinvertebrate community structure at two sites upstream from the outlet of the Darvill Sewage works was analysed and compared to those at five sites downstream from this effluent. Two separate sampling trips were undertaken, one in Spring and the other the following Winter.

The Spring sampling trip took place on the 20th and 21st of October, 1993. The sampling sites had been chosen previously during a time of low flow, but just prior to this sampling trip, the region had good spring rains. While the chemical determinands were recorded at Sites 5 and 6, no riffle samples could be taken as water was waist deep there and flowing very strongly. It was too deep for the Surber sampler (and too dangerous to attempt sampling.).

The Winter sampling trip took place in on the 22nd and 23rd of June, 1994. Site 5 was again not sampled owing to massive earth-moving operations for the upgrading of the Darvill Sewage Works. Because of the presence of heavy duty earth-moving machinery, and a large ditch where the access road had been, it was not possible to get to Site 5 and there was no substitute riffle in that area. However, Site No. 8, further downstream from Site 7 was added as it had been noted from the previous trip that the river fauna present at Site 7 indicated that the river had not recovered from the effects of the effluent.

Umbilo River

Two studies were carried out in the Umbilo River.

The main study was similar to that in the Umsunduze River. Samples were collected on two occasions (two weeks apart) during Winter, 1994 (26th June and 10th July). Sites 1 to 4 were upstream and sites 5 to 7 were downstream from the outlet of treated, chlorinated sewage from Umbilo Wastewater Treatment Works (WWTW). These samples were analysed to determine how the macroinvertebrate community structures differed as a result of being subjected to this chlorinated effluent. This ratio of upstream to downstream sites was chosen because of the secondary study which was carried out.

As mentioned previously, it is not only the chlorine in the sewage effluent which is toxic to aquatic organisms, the effluent itself has a marked effect on the biota. An opportunity to investigate the effect of *unchlorinated* treated sewage effluent on community structure arose. The WWTW was granted permission to discharge unchlorinated, treated effluent at a site upstream from the chlorinated outlet (the *rationale* being that any surviving pathogens would be eliminated by the chlorine 90 m downstream). Although the volume of this unchlorinated effluent was considerably less than that of the chlorinated effluent (which at times contributes about half of the flow of the river) it nevertheless was a source of unchlorinated effluent and it flowed directly into a riffle (Site 3). Staff of the WWTW constructed a channel from river rocks (not ones from the sample sites) to allow this effluent to enter the river just upstream from Site 3.

The aims of this part of the study were to investigate the change in a *particular* community (i.e. that at Site 3) after having been exposed to unchlorinated effluent. (a "before and after effluent" investigation) and to investigate the differences between Site 2 (upstream) and Site 3 (downstream) after the latter had been exposed to unchlorinated effluent (an "upstream and downstream from effluent" investigation).

The study with the unchlorinated effluent began in Spring, 1994. On the 2nd of November, release of this effluent began. One week later (9th November) the whole river (Sites 1 to 7) was sampled again. After a second week (16 November) Sites 1 to 6 were sampled. Site 7 was scheduled to be sampled the following day, but owing to a storm and much rain, it was decided, after looking at the conditions in the river, that the sample would not be useful because of the high turbidity and the sheer volume of water after the rain which had also scoured out the river bed. (In the catchment area upstream from the WWTW, building operations were in progress, and this resulted in heavy siltation every time it rained.)

After the second sampling trip, the unchlorinated effluent flow was temporarily suspended owing to a technical difficulty. It was resumed at the end of January, 1995. Because of the long time interval (10 weeks) without effluent, it was surmised that the community at Site 3 had recovered sufficiently to re-start the experiment.

Effluent release began again on the 30th January, 1995 (Summer). On the 6th February (one week later) Sites 2 and 3 were sampled. These two sites were sampled again after a second week (13th February) and a third time, one month later on the 12th March. The study had to be terminated after this sample because of a drain on the other side of the river. This drain used to leach a small trickle of water which seeped from an unused dump site in Pinetown. The water had a very high conductivity and judging by its colour, some ferrous metal component. (This was later confirmed by WWTW Staff.). Prior to the study involving the release of the unchlorinated effluent, the entry point of the dump-site drain effluent into the river was located, and the path of this effluent in the river was determined by using a conductivity meter. The drain effluent was well clear of the riffle at Site 3 as it was a few metres downstream

and on the other side of the river. However, following unusually heavy rain after the 12th March sample, the flow from the drain was strong enough to cause it to gush straight ahead over the river bank and enter the river upstream from Site 3.

In addition to the problem with the drain, it was noticed that there was a general paucity of organisms in the river when samples were collected on 12th March, which suggested that some toxic chemical may have gone down the river. (This factor will be discussed later in the Results.) These two events meant that changes in community structure could no longer be attributed to the unchlorinated effluent alone. Release of the unchlorinated effluent was then terminated, as was the study.

COLLECTION OF SAMPLES FROM RIFFLES

The sampling net

A modified Surber box-sampler was made to collect organisms from riffles at the study sites (which were described in **Chapter 2.**) The frame was made by welding together iron rods (5 mm in diameter, 300 mm long) to form a cube (300 x 300 x 300 mm^3) Two handles were welded onto the top of the frame so that the sampler could be held firmly in fast flowing water. The frame was covered on three sides with silkscreen mesh (mesh size 0.3 mm) and the top and bottom were left open. On the fourth side the mesh was sewn to form a cone at the end of which was the collecting bottle. At the opening of the bottle, a cut-out screw-top lid allowed for easy attachment to and removal of the bottle from the nylon mesh. At the outlet side of the bottle, a cut-out screw-top lid with the same mesh covering the hole, was attached. This allowed water to flow through the sampler, through the bottle and out through the mesh at the end while retaining the sample organisms.

The traditional Surber Samplers shown in various texts (APHA, 1992) showed the sampler to have triangular mesh sides. It was decided that a cube enclosed with mesh on three sides would be better as it would prevent organisms from upstream washing down into the sample.

In retrospect, with regard to the size of the sampler, it would have been better to have made the sides 3.162 mm as this would provide an area of one tenth of a square metre

instead of 0.09 m^2, which would have made calculation of the numbers of organisms per m^2 easier. In this study, therefore, the numbers of organisms in a given sample are quoted as the number per 0.09 m^2 and not per m^2

Use of the sampler

Samples were collected from **three places** in a riffle at each sampling site. The sample areas were chosen carefully so that they all had similar characteristics (e.g. water velocity, presence of rocks, sandy bottom beneath the rocks etc.). The sampler was placed on the stream bed around a cluster of rocks and where necessary, stones were packed around the base to prevent organisms from washing away under the net. The sampler was placed such that the cone was facing down-stream. Each stone within the 300 x 300 mm base of the net was picked up and gently scrubbed with a nylon brush to remove the organisms on it. The water flowing through the sampler washed these organisms down the cone into the collecting bottle. After each stone was scrubbed and checked for organisms, it was placed outside the net and scrubbing continued until all the stones had been cleaned. At sites where there was a sandy bottom under the stones, this was agitated with the handle of the brush to dislodge any organisms. These, too, were carried into the sample bottle by the water current.

The sample bottle was removed from the net and the water was allowed to drain from it (through the mesh at the other end). The contents were then tipped into a labelled 600 ml clear plastic honey jar. By repeated rinsing with river water, all the organisms trapped within the net were also removed. (This rinsing was done by tipping jugs of water onto the *outside* of the net so as to avoid adding more organisms to the sample.) Any organisms which still adhered to the mesh were removed with forceps (this was particularly true of the leeches which were very difficult to dislodge.) Excess water was carefully tipped out of the sample bottle (through the silkscreen mesh) when necessary.

The organisms were then killed by the addition of a 10% formalin solution. After 48 h, the formalin was replaced with a 70% ethanol solution.

COLLECTION OF SAMPLES FROM POOLS

In the Umbilo River, samples were collected from slow-flowing pools with sandy bottoms as well as from the riffles at each sampling site. The same sampler was used in order to standardise the sampling area. Because the current was not strong enough to wash the organisms into the sample bottle, a different technique was used. With the sampler pushed firmly into the sand, the substrate was stirred up with the handle of the brush and a household sieve was used to scoop up the disorientated organisms floating in the water above. Afterwards, the sieve was used as a scoop to pick up loads of sand which were then dipped repeatedly in the water within the sampler, allowing the sand to wash through while retaining the organisms. As the organisms were collected they were placed straight into the sample bottle. Where there was evidence of organisms small enough to pass through the sieve, a current was created within the sampler after the sand had been stirred up and the organisms were washed into the net. As with the riffle samples, the organisms were killed with formalin (which was replaced with ethanol after 48 h.)

WATER CHEMISTRY

At each sample site the following determinands were recorded using meters and apparatus as described in **Chapters 2** and **4** :

- Free chlorine
- Total chlorine
- Dissolved oxygen
- pH
- Conductivity
- Temperature

SORTING OF SAMPLES

Each sample bottle contained a certain amount of sand and other debris along with the organisms, so the first step was to separate the organisms from the unwanted matter. This was done by tipping the contents of a bottle into a rectangular "Tupperware" container (330 x 220 x 60 mm). Those organisms which were easily visible to the naked eye were picked out using fine forceps (0.05mm) and placed in ice trays

according to families or major groups. The container was then placed under a dissecting microscope and the smaller organisms were picked out at magnifications of 10 X and then at 30 X.

Each group or family of organisms was then sorted to species level where possible, otherwise to genus, family or order, then stored in 70% ethanol, and the collection lodged with the Albany Museum, Grahamstown. The numbers of each species/family were recorded on data sheets using one data sheet per sample.

For samples which had very high numbers (i.e. hundreds or thousands) of a species, e.g. the chironomids, sub-sampling was done. This was done in the following way:

1. The organisms (e.g. all the small chironomids) were picked out of the sample in the "Tupperware" tray and placed in a pill vial/s as before. (During this process, all the other organisms were also separated from the debris and placed in their respective pill vials.)

2. The chironomids were then poured out into a separate rectangular clear perspex tray which had a grid marked on the bottom. A little ethanol was poured into the tray so that the organisms could be spread out evenly over the tray bottom. A hundred organisms were then picked out at random and put to one side. These were later identified to Order or Family level as appropriate with the aid of a dissecting microscope.

3. Of the remaining organisms in the perspex tray, the organisms in each of 10 of the 100 squares were counted, added together and multiplied by 10 to get the total number in the perspex tray. The number of organisms in the sub-sample (i.e. 100) was then added to this to determine the total number for that sample. By using the figures from the identification of the organisms in the sub-sample, the ratios of each order/family in that sample could be calculated (Palmer & O'Keeffe, 1990).

Some of the samples had considerable amounts of plant matter in them, particularly bits of filamentous algae, which made separation of the organisms from the plant matter very difficult.. It was therefore decided that in such samples, the whole sample (plants, debris and organisms) would first be sub-sampled, then in the sub-sample, the organisms would be extracted from the debris and identified. The numbers would then be multiplied appropriately to obtain the total number of each species in the sample. The sorted, identified organisms were then placed in pill vials with ethanol as before.

DATA PREPARATION, EVALUATION AND PRESENTATION

The number of each species/family/order (where applicable) on each data sheet was entered into a computer in a spreadsheet (Quattro Pro) for analysis.

Two basic approaches can be used in evaluating effects of pollutants on aquatic life:

1. Qualitative analysis of flora and fauna "above and below" or "before and after", thereby determining species present and absent.
2. Making a quantitative inventory of the number of specimens, species and structure of the aquatic community affected by the pollutant and comparing it to reference information.

Qualitative data evaluation

No two aquatic organisms react identically to a pollutant because of complex interrelationships between genetic factors and environmental conditions. Certain groups are intolerant of pollution, but operculate snails, immature stages of certain mayflies, stoneflies, caddisflies and riffle beetles are sensitive to most pollutants. Pollution-tolerant macroinvertebrates such as certain sludgeworms, midge larvae (bloodworms), leeches, pulmonate snails and some polychaetes usually increase in number under organically enriched conditions. Facultative organisms (those that tolerate moderate pollution, include most snails, sowbugs, scuds and blackfly larvae. Tolerant organisms may be found in either clean or polluted situations so that their presence is not definitive. However, a population of tolerant organisms combined with an absence of intolerant ones is a good indication of the presence of pollution (APHA,1992).

Quantitative data evaluation

Cairns & Dickson (1971) suggest that one way to present these sorts of data for interpretive purposes is to graph the number of kinds of organisms (A) and the total number of specimens (B) found at each site. If the values for A and B for the upstream site are similar to the values for A and B for the downstream site, then little or no damage has occurred.

Swift *et al.*. (1993) used pie-charts to show the relative proportions of the kinds of organisms found at each site. Changes in the proportions of organisms found at various sites can be indicative of the effects of pollutants.

Statistical analyses of biological data are important and commonly include determining the mean and confidence interval and use such tests as chi-square, Student's *t*, regression, correlation, one- and two-way analysis of variance, robust analyses, and numerous non-parametric tests. The use of mathematical expressions of community structure to derive numerical indices of diversity of aquatic communities is based on the general, though not invariably true, assumption that the greater the diversity of aquatic life, the greater the structural and functional stability of the system.

Diversity indices, although limited, condense considerable biological data into a single numerical value. More sophisticated multivariate statistical analyses, such as cluster and ordination, principal component, MANOVA, and discriminant analyses generally are more appropriate and less subject to criticism.

The sources of variability commonly found must be identified in order to evaluate statistically the data collected in pollution surveys. Variability in macroinvertebrate data comes from the sampling methods and organism distribution. The major source is probably sampling error. Organisms are usually clustered in relation to habitat distribution, therefore random samples often show high variability among replicates. In statistical analyses of quantitative data, large numbers of samples often are required to detect statistically significant differences. Care should be exercised when using parametric statistical methods because the basic assumption of normal distribution is

not always true. Data often have to be transformed before being tested. It should not be assumed that a statistically significant difference is ecologically significant. (APHA, 1992).

Multivariate analysis

The data used in this part of the study were collected from several sites (multi-spatial) and on successive sampling occasions (multi-temporal). Many taxa were collected, and several environmental variables were measured simultaneously for each sample. In an effort to detect patterns which may have been related to the downstream chlorine gradient, a multivariate technique was used. This is indirect gradient analysis (Whittaker 1973). There are several indirect gradient analysis methods available (Gaugh 1982), the simplest of which is classification.

Two-way indicator species analysis (TWINSPAN) (Hill, 1979) is a classification procedure which arranges sample sites into two distinct groups along the first axis of a reciprocal averaging ordination. Further dichotomous divisions are achieved using the presence, absence and relative abundances of indicator species. TWINSPAN simultaneously produces groups of species with similar distribution among sites. TWINSPAN is an improvement upon the original indicator species analysis (Hill 1973) in that species are classified as well as samples.

RESULTS

In this study, community structure data were analysed and presented as:

1. percentage occurrence pie charts (**Plates 5.1** to **5.7**);
2. graphs of average number of taxa per site (**Figs.5.1A** to **5.7A**); and average number of individuals per site (**Figs. 5.1B** to **5.7B**); and
3. a multivariate analysis (**Fig 5.10**)

In addition, because of the particular interest in *B. harrisoni*, graphs were drawn to show the average number of this species at each site, to compare the effects of chlorinated and unchlorinated sewage effluent (**Figs 5.8** and **5.9**)

PRESENTATION OF DATA

As suggested by Swift *et al.* (1993) pie charts were drawn to show the relative proportions of the kinds of organisms found at each site. The pie charts show the average number of organisms per sample for each set of 3 riffle samples at a site (**Plates 5.1** to **5.7**). The colour scheme was kept as consistent as possible for each Plate (e.g. in Plate 5.1, *Baetis harrisoni* (Barnard) was always red and *Chironomus* sp. always yellow). The average number of organisms per sample is indicated above each chart. It is important to look at these numbers, as the pie charts could otherwise be misleading. As an example, first glance at Site 5 of **Plate 5.2,** , suggests that *B. harrisoni* was unaffected by the effluent and contributed 67% to the sample. It is only when the *number of organisms per sample* is consulted that it is noticed that of the **6 organisms** found at Site 5, **4** were *B. harrisoni.*

THE UMSUNDUZE RIVER

The results of the two sampling trips (Spring, 1993 and Winter 1994) are shown as pie charts in **Plates 5. 1** and **5. 2** and graphs of average number of taxa per sample (**A**) and average numbers of individuals per site (**B**) are shown in **Figs.5.1 A** and **B,** and **5.2 A** and **B**.

Spring, 1993

During the collection of these samples, the low numbers of species and individuals suggested that the water quality was not good, and because of the pollution and risk of infection, protective clothes (trout waders and long rubber gloves) were worn on all sampling trips.

The poor quality of the water was confirmed later on analysis of the riffle samples. Sites 1 and 2 (upstream from the chlorinated effluent) were dominated by Orthocladiinae, with a few Burnupia at Site 1 and some oligochaetes at Site 2. By looking at the numbers of taxa (**Fig. 5.1A**) and individuals (**Fig. 5.1B**), it can be seen that even upstream from the effluent, there were very few organisms.

At Site 3, immediately downstream from the effluent, the water was clear and smelt strongly of chlorine. The rocks and stones were devoid of any signs of living organisms.

At Site 4, the chlorine could no longer be smelt and a few *Chironomus* and Trichoptera were present (but only about 46 organisms per sample).

Box samples were not collected from sites 5 or 6, but on a previous trip to select the sample sites, it was found that *Chironomus* was the only organism present at these sites. Although they where not collected and counted, it was noted on that occasion that there were about 100 to 200 in the area sampled (i.e. the $0.09m^2$ of the box sampler). At Site 7, the river showed some sign of recovery in that the average number of taxa had increased to 6 and the average number of organisms had increased to 233.

B. harrisoni is a particularly resilient mayfly (Chutter, 1994) and the degraded nature of the river generally is evidenced by its presence at the upstream sites. However, while *B. harrisoni* had been noticed on the previous trip, only one or two individuals were collected during this sampling trip, suggesting that the water quality in the stretch of river under study had deteriorated even further.

Winter, 1994

Numbers of organisms at Site 1 were about half the number collected in spring, but the number of taxa had almost doubled (**Fig. 5.2A**), and the samples were dominated by *B. harrisoni* instead of Orthocladiinae (**Plate 5.2**).

The average number of individuals at Site 2 was much higher (118) than it had been in spring (33), and higher than that at Site 1 (76). Because of the close proximity of Sites 2 and 3, the difference between samples collected at these sites gave the clearest indication of the destructive effect of chlorinated effluent At Site 3, the average number of organisms had been reduced to 26, 18 of which were *Chironomus*. At this site, the free chlorine concentration was 0.1 mg/ℓ and the total chlorine, 0.8 mg/ℓ.

Site 4 was typical of the effect of sewage effluent in that the samples consisted entirely of *Chironomus*, which is adapted to withstand low oxygen conditions owing to the presence of haemoglobin. The free chlorine concentration at this site had decreased to 0.06 mg/ℓ and the total chlorine had decreased to 0.8 mg/ℓ.

Although Site 5 was again not sampled, it was probably also dominated by *Chironomus*, as was the case at Site 6.

By Site 6, conditions had hardly improved except that in addition to *Chironomus*, two other species were present: leeches (Hirudinea) and Oligochaetes, but neither of these is indicative of good quality water. By Site 7 there was a marked increase in both species number and number of organisms, but *Baetis harrisoni* was still not present.

Site 8 was dominated by *Chironomus* and an alga which was growing about 50 mm thick on the rock. At Site 8 there was still no sign of *B. harrisoni* which suggested that 7 km from the effluent outlet, the river had still not recovered from the chlorinated sewage effluent.

The absence of *B. harrisoni* at Sites 7 and 8 may have been because of the presence of chlorine: the free chlorine concentration was 0.02 mg/ℓ, at Site 7 and 0.01 mg/ℓ at Site 8, which was much higher than the LC_{50} of 0.004 mg/ℓ recorded in the toxicity tests.

THE UMBILO RIVER

Winter 1994 (26 June and 10 July)

The results of the first two sampling trips are shown as pie charts in **Plates 5.3** and **5.4** and graphs of average number of taxa per sample (**A**) and numbers of individuals per site (**B**) are shown in **Figs.5.3 A** and **5.3 B** and in **Figs 5.4A** and **5.4B**.

By June the average numbers of organisms per sample at Sites 1 to 4 showed some differences, with Sites 2 and 3 having about half the numbers recorded for Sites 1 and 4 (**Plate 5.3**) However all four upstream sites had higher numbers of individuals and taxa than the downstream sites 5 and 6, with Site 4 having the highest number of taxa and individuals. As Sites 4 and 5 were so close together, differences between community structures at these two sites give the clearest indication of the effects of the chlorinated sewage effluent. Site 4 had an average of 12 taxa and 227 individuals per sample whereas Site 5 had only 2 taxa and 6 individuals. First glance at the pie chart for Site 5 on **Plate 5.3** appears to indicate a large presence of *B. harrisoni* and *Chironomus,* however when the *sample size* is noted, there were only an average of **6** organisms per sample, and how close to death these specimens were at the time of collection was not known..

Sites 1, 2 and 3 showed substantial increases in the number of organisms collected from June (**Plate 5.3**) to July (**Plate 5.4**) sampling trip. Site 1 was the most consistent in terms of species composition even though the number of organisms had doubled. Both sets of Site 1 to 4 samples were dominated by *B. harrisoni* and Orthocladiinae. There appeared to be a distinct difference in species number at Sites 3 and 4 between the first and second sample. At Site 4 on the second trip, an average of only 20 organisms were present in the sample. This could probably have been attributed to the building operations which were taking place at the WWTW at the time. Site 4 was just below a causeway across the river and while samples were being collected it was noticed that one of the builders was dumping building rubble (cement attached to rusty wire) in the river. How long this dumping of building rubble had been in progress, and what had been dumped prior to the sampling trip, was not known, but it certainly had an effect on the Site 4 communities.

The high values for both free chlorine and combined chlorine for the first samples (**Table 5.3**) should be noted. They were, however particularly low on the second sampling trip (**Table 5.4**) as there had been a problem with the dosing of the chlorine at the old works and the effluent from one part of the works had not been chlorinated for some time prior to the sampling trip. This could account for the unusually high number of organisms found at Site 5 on this occasion.

On both occasions there were almost no organisms at Site 6. The average of **3.6** organisms found during the first trip should be noted. No organisms were found at Site 6 during the second trip.

On both occasions the river had shown signs of improvement by Site 7. There was a notable increase in both species number and number of organisms. However, in contrast to the upstream sites (1 to 3) which had doubled in number in the two weeks between samples, Site 7 numbers had halved. It was also noticed that, unlike the Umsunduze, Site 8 (about 7 km downstream) *B. harrisoni* was present in the last Umbilo downstream site (about 6.5 km downstream).

Spring 1994 (9 and 16 November)

There were several aspects to be investigated from these two sampling trips. The first spring trip was one week after the release of unchlorinated effluent had begun, and the second was a week later. The following aspects were investigated:

i) As with the previous investigations, the effect of chlorinated treated sewage effluent on the macroinvertebrate community composition at Sites 5 to 7 was assessed.

ii) Sites 2 and 3 were compared to see whether there were differences in community structure which could be attributed to release of unchlorinated effluent.

iii) Site 3 results from the first spring trip were compared to those of the second spring trip to see if the unchlorinated effluent had had any noticeable effect on community structure.

The results of the two spring sampling trips are shown as pie charts in **Plates 5.5** and **5.6** and graphs of average number of taxa per sample (**A**) and numbers of individuals per site (**B**) are shown in **Figs. 5.5 A** and **B** and in **Figs. 5.6 A** and **B**.

i) *Effect of chlorinated effluent*

With reference to **Plate 5.5** and **Table 5.5**, the high free and total chlorine concentrations (0.2 and 1.5 mg/ℓ) recorded in the effluents and in the water at Site 5 probably account for the complete absence of organisms at this site. At Site 6, the chlorine concentrations had dropped from 0.2 to 0.08 mg/ℓ for free chlorine and from 1.5 to 0.6 mg/ℓ for total chlorine, and the conductivity was still almost 4 times that of the upstream sites, however conditions appeared to be suitable for a substantial population of Chironomini and Orthocladiinae. Although number of species was low at Site 6, there was a high average number of organisms per sample.

High species number and average number of organisms per sample at Site 7 again suggested that the river had undergone some recovery. *B. harrisoni* were also present at this site.

One week later, chlorine concentrations at Sites 5 and 6 were lower (**Table 5.6**) than they had been the previous week and these sites were both dominated by Chironomini and Orthocladiinae (**Plate 5.6**) as Site 6 had been the previous week. The free chlorine concentration of 0.06 mg/ℓ at Site 5 was a bit lower than had previously been recorded at Site 6, which would probably explain how the organisms were able to survive.

ii) *Differences between Sites 2 and 3 as a result of unchlorinated effluent*

With reference to **Plate 5.5** (one week after unchlorinated effluent), although there was a decrease in numbers of organisms at Site 3, this was only a 32% difference, and in the previous sample (in winter, before the unchlorinated effluent was released), there was a decrease of almost 20% from Site 2 to Site 3. In addition, in this spring sample there was a decrease of 49% between Sites 1 and 2, so it is unlikely that the unchlorinated effluent was responsible for the decrease or that this difference was significant. On average there were 36 *B. harrisoni* at Site 2 and 27 at Site 3 (compared to 34 at Site 1) so it would appear that at this stage the unchlorinated effluent had had little effect.

With reference to **Plate 5.6**, there was a large decrease in average number of organisms from 307 at Site 2 to 81 at Site 3. This was a decrease of 74%. (However, there was also a decrease from 750 at Site 1 to 307 at Site 2, a difference of 59%.) The 74% decrease from Site 2 to 3, and the 64% increase in number of organisms at Site 4 (which would probably not have been affected by the relatively small volume of unchlorinated effluent upstream) suggests that the unchlorinated effluent may have caused a reduction in *numbers* of organisms, although *the number of taxa* did not appear to have decreased.

iii) *Comparison of Site 3 communities after 2 weeks of unchlorinated effluent*

The average number of organisms per sample (89 and 81) and the number of taxa were very similar (**Plates 5.5 and 5.6**). The percentage of *B. harrisoni* had increased marginally from 31 to. 42%, and almost half of the Orthocladiinae had been replaced by Chironomini. There did not appear to be any real change at Site 3 after 2 weeks exposure to unchlorinated effluent.

Summer 1995

Sites 2 and 3 were sampled after 1, 2 and 6 weeks of exposure to unchlorinated effluent. The results of the three summer sampling trips are shown as pie charts in **Plate 5.7** and graphs of average number of organisms per sample (**A**) and numbers of species per site (**B**) are shown in **Fig. 5.7A** and in **Fig. 5.7 B**.

i) *After 1 week*

The average number of organisms at Site 3 was 50% lower than that at Site 2. There had also been some change in species composition with a decrease in Orthocladiinae and an increase in Chironomini. In addition, there was a large population of *Chironomus*. The *B. harrisoni* population was investigated in more depth and a Student t test showed that the difference in numbers between Sites 2 and 3 was not significant. The unchlorinated sewage appeared to have caused a decrease in numbers of organisms and a change in species composition, but in the absence of statistical analysis, it is not possible to assess whether these differences were significant.

ii) *After 2 weeks*

The difference in average numbers of organisms between Sites 2 and 3 was only 5.6%, but there did seem to be a difference in community structure. *B. harrisoni* decreased from 55% of the population at Site 2 to only 18% of the population at Site 3 (a decrease from 275 to 81 organisms per sample) There was an increase in Chironomini from 0.22% to 43 02%, and the Orthocladiinae almost doubled from 17 % to 35%. However, there had been a decrease of 66% in the population number at Site 2 between the first and second summer samples. When these two Site 2 samples were studied further, it was noticed that the decrease in numbers was caused by a sharp decrease in numbers of Orthocladiinae, from 1298 to 81 per sample. The *B. harrisoni* had in fact increased in number (from 102 to 276) during the week, and the *Simulium* population had remained constant at 102 organisms per sample. It was clear that in addition to the unchlorinated effluent there were other factors in the river which were affecting the numbers of individuals and species. (The *B. harrisoni* and *Simulium* numbers endorse the idea that with these pie charts it is important to look at the average number of organisms per sample.)

It would appear that after 2 weeks exposure to unchlorinated effluent there had been a slight decrease in numbers of organisms per sample and a change in community structure. A Student t test showed that differences in numbers of *B. harrisoni* at these sites were not significant.

iii) *After 6 weeks*

The samples collected after 6 weeks suggest that some significant pollutant had been down the river. The average numbers of organisms per sample were 35 for Site 2 and 13 for Site 3. The other sites in the river were visited on the same day and there were almost no signs of life at any of the sites. At a site between Sites 5 and 6 there was a pool which was usually teeming with *Chironomus* to the extent that they could be scooped by the hundred in a household sieve, but on this occasion they had all disappeared.

Umsunduze								
21 10 1993			*Chlorinated*					
Sites	**1**	**2**	*effluent*	**3**	**4**	**5**	**6**	**7**
Location relative to effluent	2 km U	5 m U		5 m D	20 m D	2.5 km D	3 km D	7 km D
Free chlorine (mg/ℓ)	0.05	0.05	*0.12*	0.10	0.50	0.10	0.10	0.00
Total chlorine (mg/ℓ)	0.10	0.15	*1.20*	0.90	0.65	0.30	0.20	0.02
pH	7.7	7.5	*6.9*	7.0	7.2	7.6	7.5	7.3
Conductivity (μS/cm)	229	219	*685*	629	485	297	294	428
Temperature (°C)	24.1	23.9	*23.0*	23.0	23.6	22.9	22.5	23.1
Dissolved oxygen (ppm)	8.9	8.2	*8.9*	8.9	8.6	7.7	7.8	5.6
						*	*	

*River
too deep
for box samples

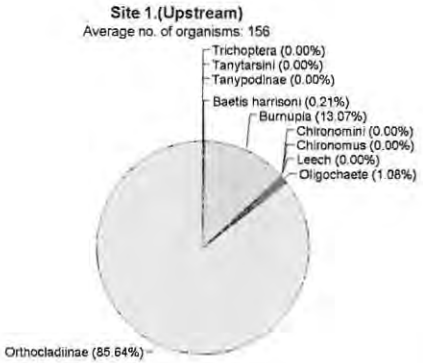
Table 5.1. Determinands recorded at sample sites at the time of collection of box samples.

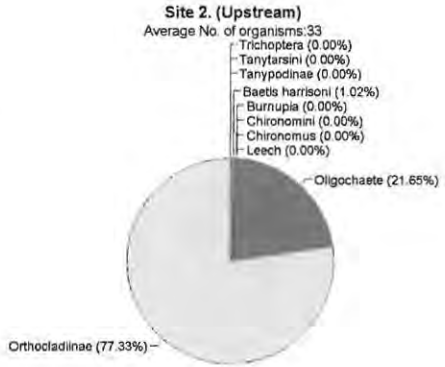
The letters U and D refer to whether the sites were Upstream or Downstream from the chlorinated sewage discharge point.

Plate 5.1. (Below) Relative proportions of the kinds of organisms found at each site.

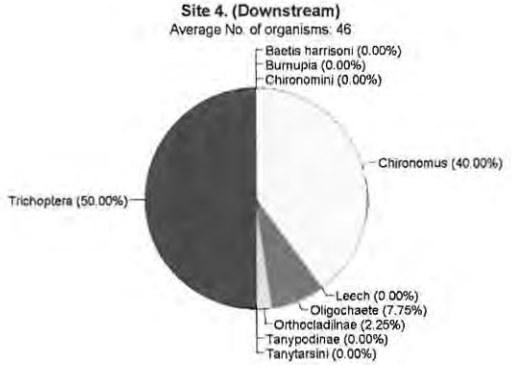
These samples were collected on the 20 and 21 October, 1993.
Each pie chart shows the average number of organisms per sample for each set of 3 riffle samples at a site.
At Site 3, immediately downstream from the chlorinated sewage out fall, no organisms were found in any of the samples.
Sites 5 and 6 could not be sampled on this occasion owing to the high water level following heavy rain.

UMSUNDUZE RIVER
OCTOBER 1993. (SPRING)





Site 3.
No organisms present
in samples.



Sites 5 and 6.
No box samples taken.

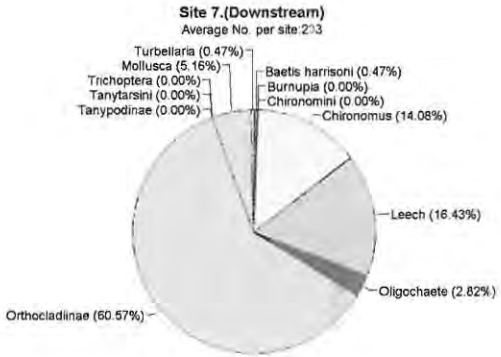


Fig. 5.1 A Average number of taxa at each site.
Each bar shows the average of three riffle samples collected at a site in the Umsunduze River.
Spring, 21 October, 1993.

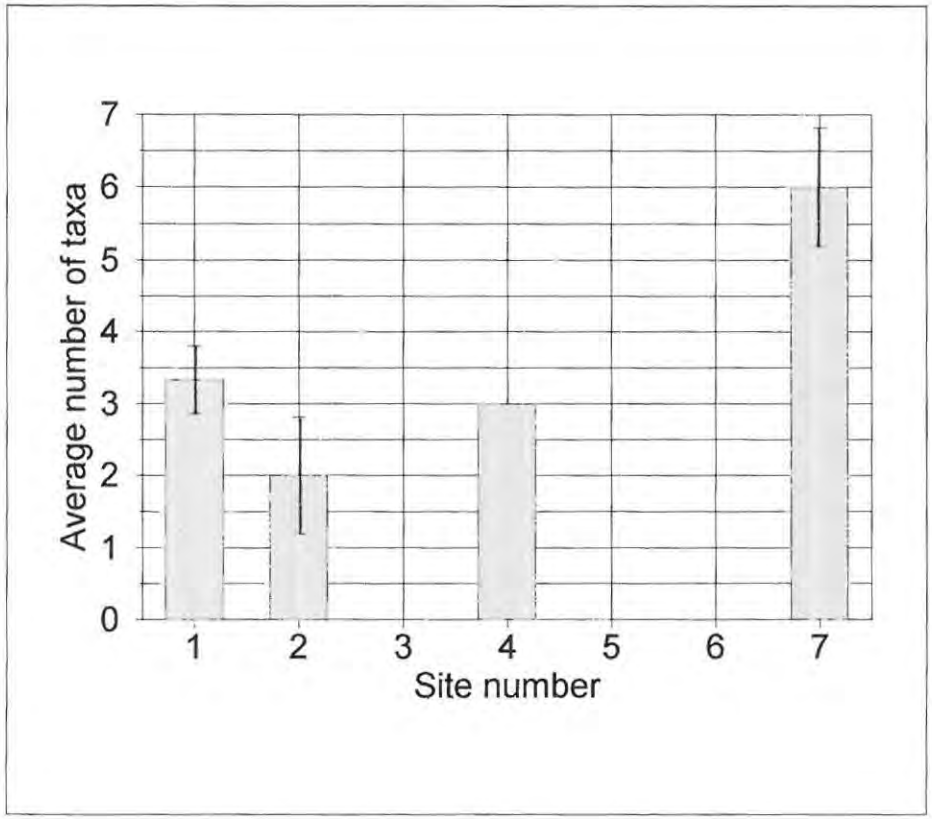
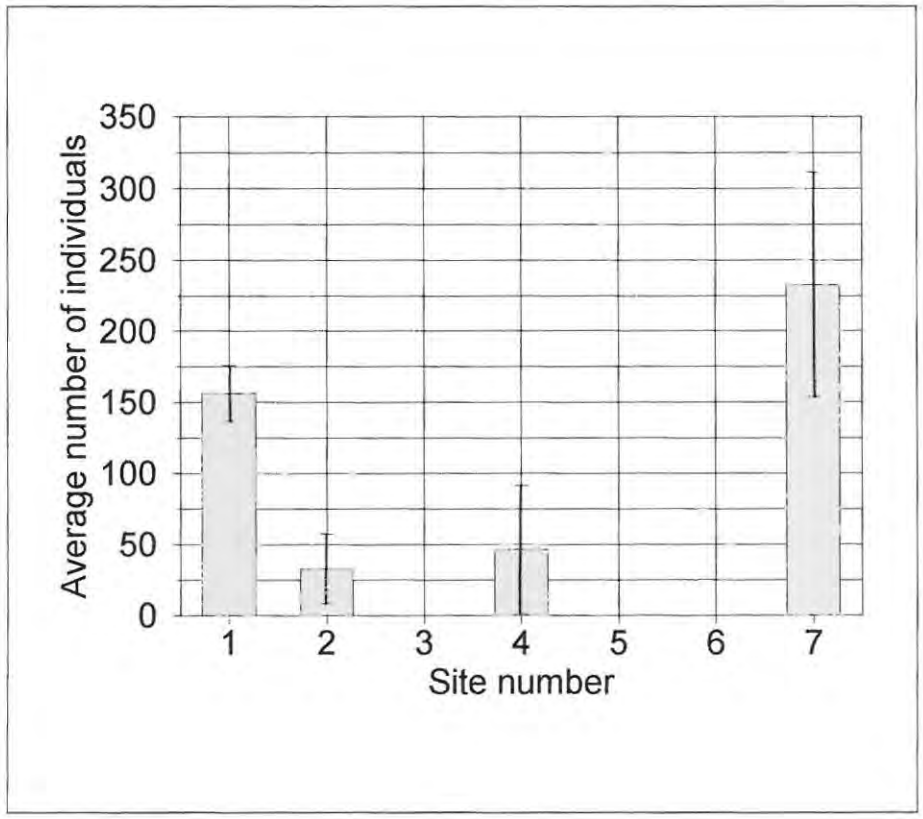


Fig. 5.1.B Average number of individuals per site.
Each bar shows the average of three riffle samples collected at a site in the Umsunduze River.
Spring, 21 October, 1993.



No box samples were taken at Sites 5 and 6.
I-Beams on both graphs represent 2 x Standard Deviation.

Umsunduze									
22 06 1994			*Chlorinated*						
Sites	**1**	**2**	*effluent*	**3**	**4**	**5**	**6**	**7**	**8**
Location relative to effluent	2 km U	5 m U		5 m D	20 m D	2.5 km D	3 km D	5 km D	7 km D
Free chlorine (mg/ℓ)	0.02	0.04	*0.12*	0.10	0.06	0.02	0.02	0.02	0.10
Total chlorine (mg/ℓ)	0.02	0.08	*1.10*	0.80	0.70	0.10	0.04	0.05	0.09
pH	8.3	8.9	*6.8*	7.0	7.1	6.8	7.3	7.2	7.0
Conductivity (μS/cm)	220	220	*680*	669	535	524	440	516	668
Temperature (°C)	12.2	11.5	*18.0*	16.0	16.2	16.1	15.0	15.0	17.7
Dissolved oxygen (ppm)	9.1	11.7	*9.9*	9.9	11.2	8.3	6.3	6.8	9.7

Table 5.2. Determinands recorded at sample sites at the time of collection of box samples.

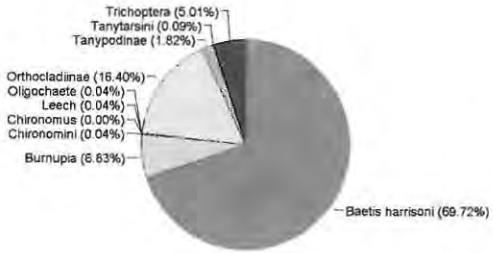
The letters U and D refer to whether the sites were Upstream or Downstream from the chlorinated sewage discharge point.

Plate 5.2. (Below) Relative proportions of the kinds of organisms found at each site.

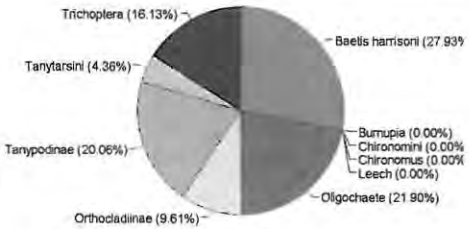
These samples were collected on the 22 and 23 June, 1994.
Each pie chart shows the average number of organisms per sample for each set of 3 riffle samples at a site.
At Site 3, immediately downstream from the chlorinated sewage out fall, a few organisms were found in the samples.
Site 5 could not be sampled on this occasion owing to the building operations for upgrading Darvill Sewage Works.

UMSUNDUZE RIVER
WINTER 1994

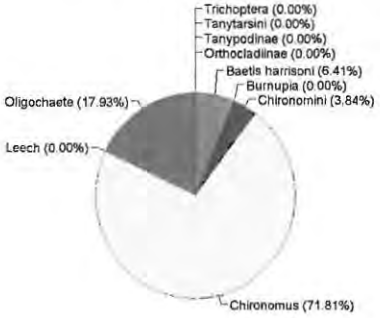
Site 1 (Upstream)
Average No. of organisms: 76



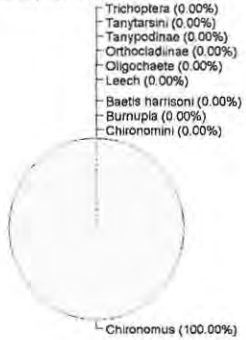
Site 2 (Upstream)
Average No. of organisms: 118



Site 3. (At outlet)
Average No. of organisms : 26

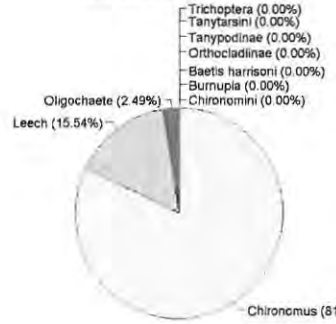


Site 4. (Downstream)
Average No. of organisms: 206

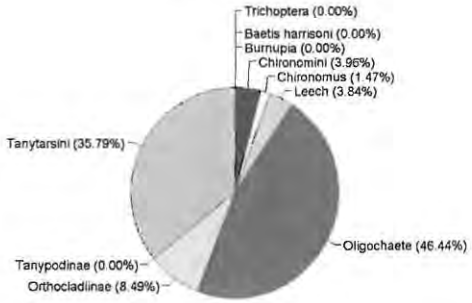


SITE 5 NOT SAMPLED

Site 6. (Downstream)
Average No. of organisms:147



Site No. 7 (Downstream)
Average No. of Organisms :623



Site 8. (Downstream)
Average No of organisms: 1780

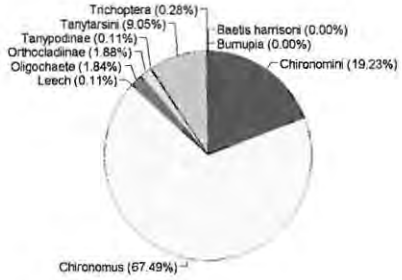
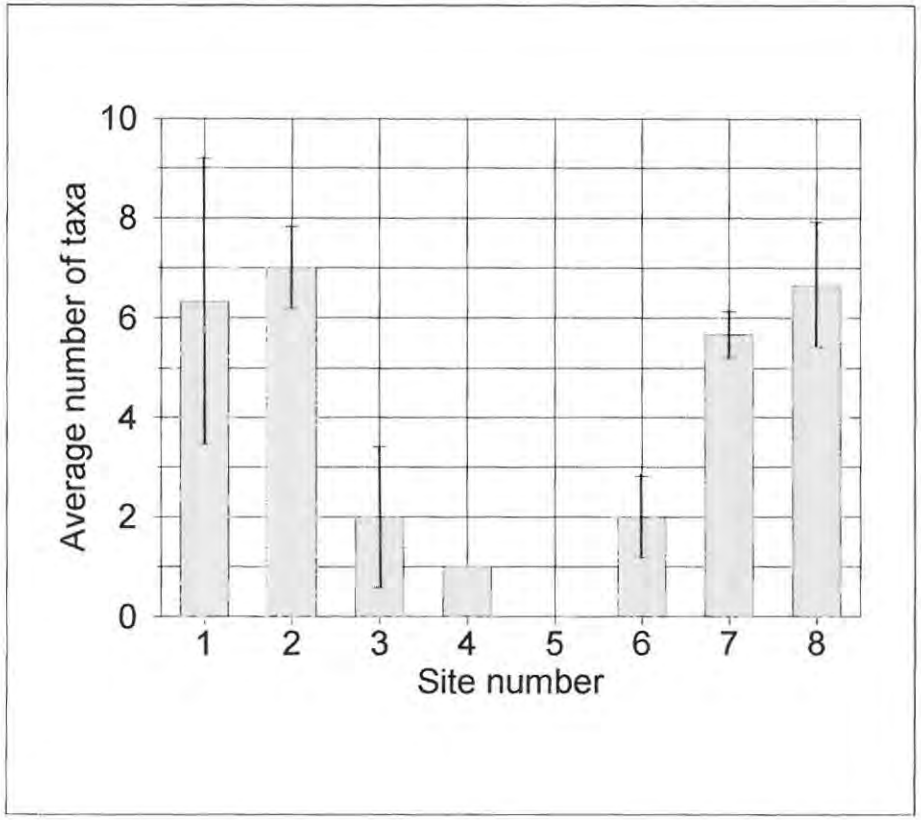


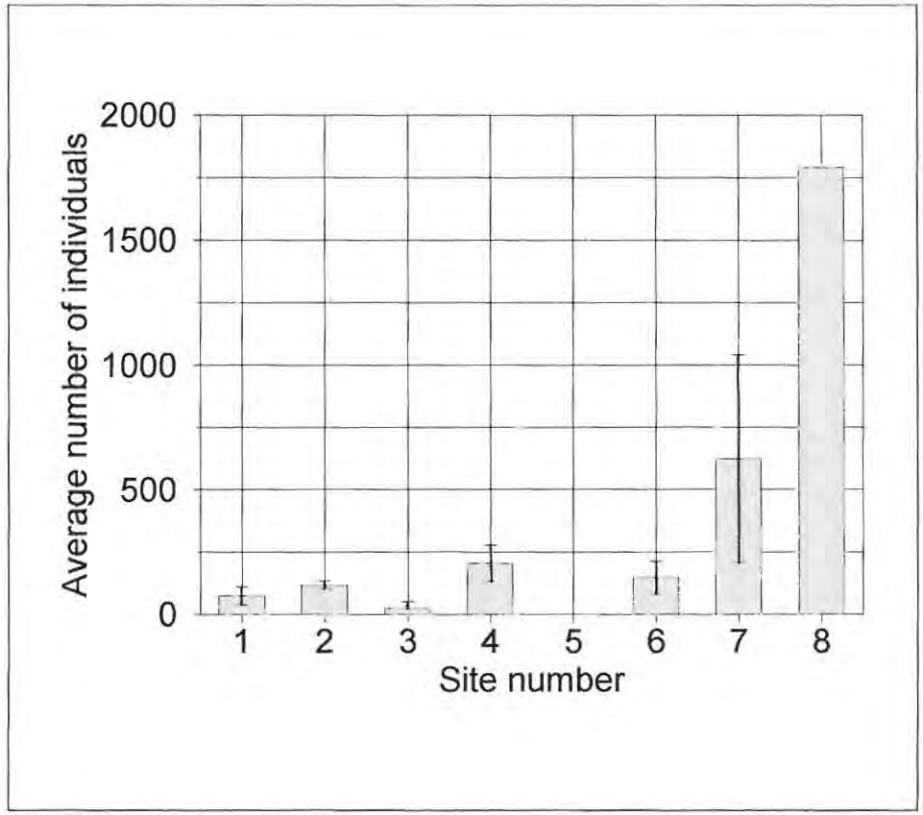
Fig. 5.2 A Average number of taxa at each site.
Each bar shows the average of three riffle samples collected at a site in the Umsunduze River.
Winter: 22 June,1994.



I-Beams on both graphs represent 2 x Standard Deviation.

Site 5 was not sampled owing to inaccessibility of this part of the river.

Fig. 5.2.B Average number of individuals per site.
Each bar shows the average of three riffle samples collected at a site in the Umsunduze River.
Winter: 22 June,1994.



Note: Site 8 standard deviation bar was not included as it caused the y-axis scale to extend to 4000, which made the values at Sites 1 to 4 difficult to see.

Umbilo										
26 06 1994						*New*	*Old*			
Sites	**1**	**2**	Unchloreff	**3**	**4**	*Chlor. effluent*		**5**	**6**	**7**
Location relative to effluent	250 m U	6 m U		1 m B	90 m B			5-10 m D	470 m D	6.5 km D
Free chlorine (mg/ℓ)	0	0	x	0	0	*0.15*	*0.30*	0.10	0.08	0.00
Total chlorine (mg/ℓ)	0	0	x	0	0	*3.00*	*6.00*	2.50	2.00	0.04
pH	8.6	8.5	x	8.8	8.0	*7.5*	*7.2*	7.5	7.9	7.7
Conductivity (µS/cm)	382	396	x	392	202	*1102*	*1111*	1010	994	944
Temperature (°C)	13.8	15.0	x	15.1	11.7	*20.0*	*19.0*	18.5	18.3	15.3
Dissolved oxygen (ppm)	12.8	11.2	x	10.7	11.5	*8.2*	*8.8*	9.2	10.1	9.0

Table 5.3. Determinands recorded at sample sites at the time of collection of box samples.

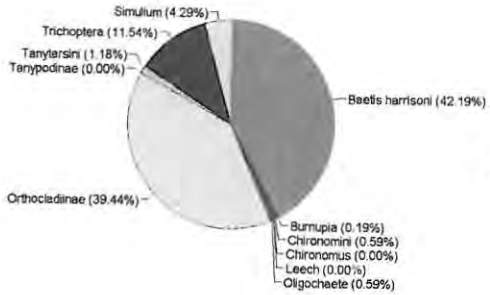
The letter U indicates that the sites were Upstream from the unchlorinated, treated sewage effluent.
The letter B indicates that the sites were Between the unchlorinated and chlorinated outfalls.
The letter D indicates that the sites were Downstream from the chlorinated, treated sewage effluent.
Unchloreff. represents the unchlorinated effluent.
Chlor.effluent represents the chlorinated effluent.
New / Old refer to the new and old sewage plants

Plate 5.3. (Below) Relative proportions of the kinds of organisms found at each site.

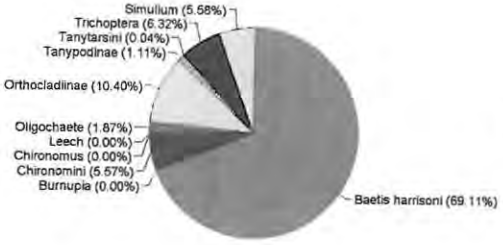
These samples were collected in winter on the 25 and 26 June, 1994.
Each pie chart shows the average number of organisms per sample for each set of 3 riffle samples at a site.
At Sites 5 and 6 downstream from the chlorinated sewage out fall, very few organisms were found in any of the samples.

UMBILO RIVER
JUNE 1994 (Winter)

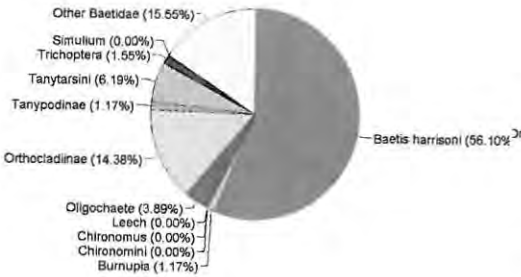
Site 1 (Upstream)
Average no of organisms:193



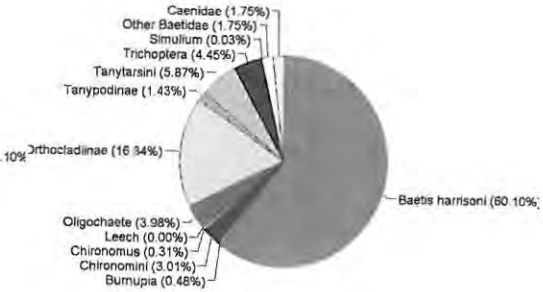
Site 2 (Upstream)
Average no. of organisms:99



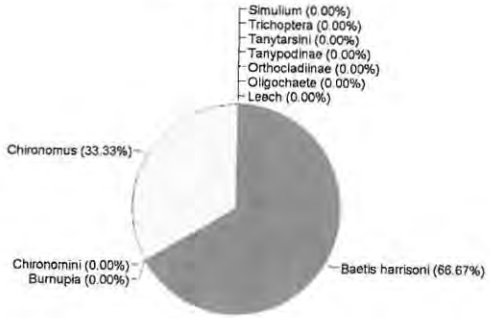
Site 3. Upstream
Average no. of organisms: 101



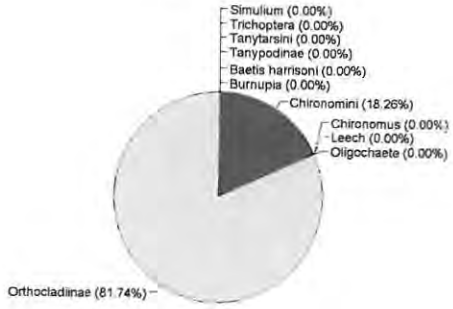
Site 4. Upstream
Average No. of organisms :227



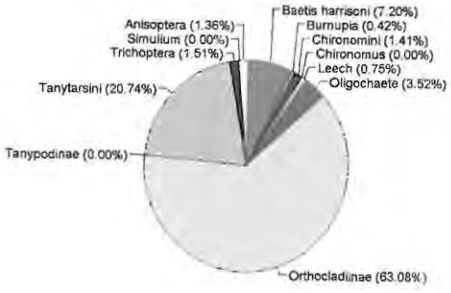
Site 5. (Downstream)
Average no. of organisms: 6



Site 6. (Downstream)
Average no. of organisms: 3.7

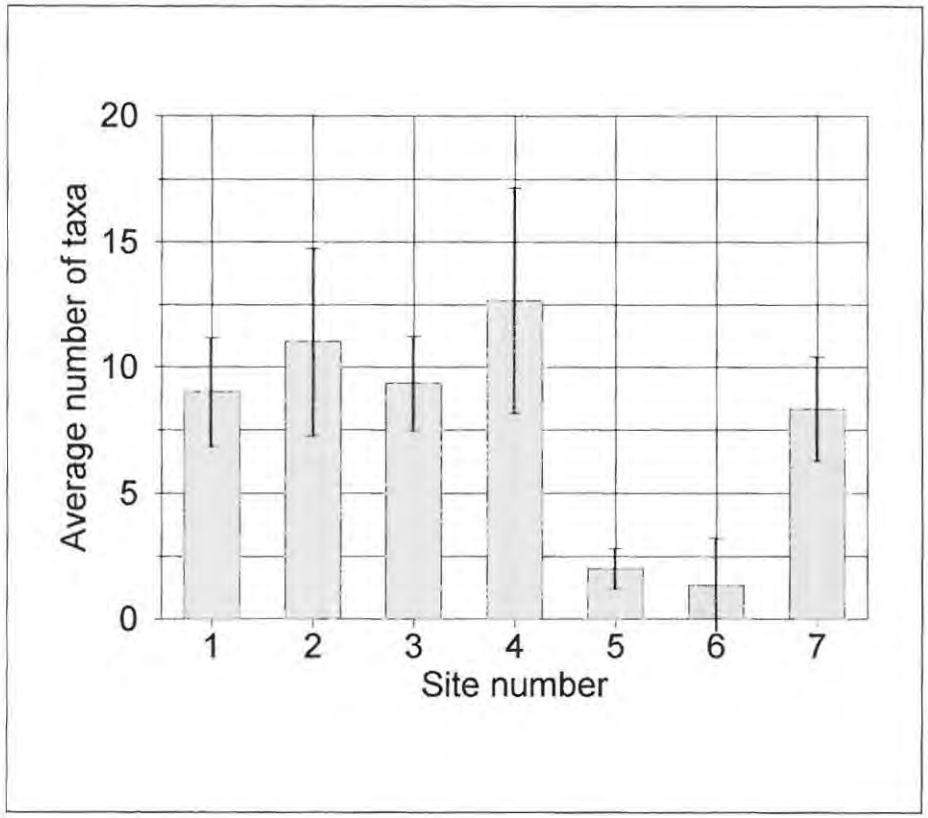


Site 7. (Downstream)
Average no. of organisms : 710



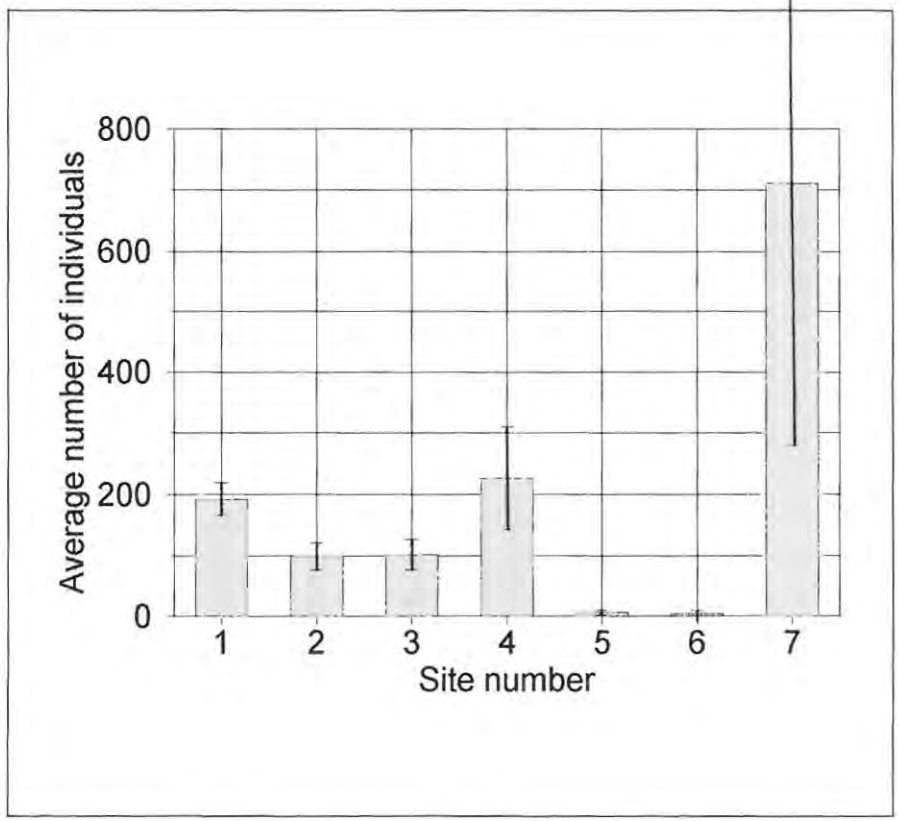
N.B. Note the average number of organisms per sample at each site.

Fig. 5.3 A Average number of taxa at each site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Winter: 27 June, 1994.



I-Beams represent 2 x Standard Deviation.

Fig. 5.3.B Average number of individuals per site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Winter: 27 June, 1994.



Note: Site 7 standard deviation was not included as it caused the y-axis scale to extend to 2000, which made the values at Sites 1 to 6 difficult to see.

Umbilo										
10 07 1994						*New*	*Old*			
Sites	**1**	**2**	Unchloreff	**3**	**4**	*Chlor. effluent*		**5**	**6**	**7**
Location relative to effluent	250 m U	6 m U		1 m B	90 m B			5-10 m D	470 m D	6.5 km D
Free chlorine (mg/ℓ)	0.02	0.02	x	0.02	0.02	*0.10*	* *0*	0.02	0.02	0.02
Total chlorine (mg/ℓ)	0.03	0.02	x	0.06	0.04	*0.10*	* *0*	0.09	0.09	0.09
pH	8.5	8.8	x	8.7	8.8	*7.4*	*7.6*	7.7	7.7	7.6
Conductivity (μS/cm)	413	420	x	418	416	*1187*	*1170*	1040	1021	995
Temperature (°C)	12.3	14.0	x	14.7	14.2	*21.0*	*20.5*	20.6	19.5	14.4
Dissolved oxygen (%)			x					98		96
Dissolved oxygen (ppm)	12.0	13.2	x	12.1	12.5	*8.2*	*8.6*	9.2		10.1

** Chlorinator not working*

Table 5.4. Determinands recorded at sample sites at the time of collection of box samples.

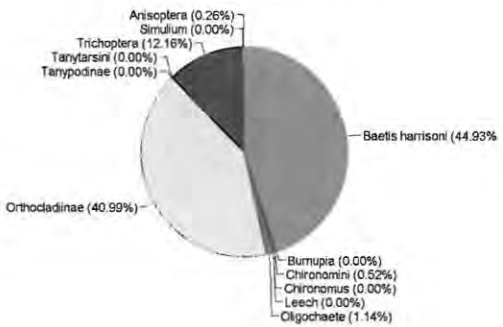
The letter U indicates that the sites were Upstream from the unchlorinated, treated sewage effluent.
The letter B indicates that the sites were Between the unchlorinated and chlorinated outfalls.
The letter D indicates that the sites were Downstream from the chlorinated, treated sewage effluent.
Unchloreff. represents the unchlorinated effluent.
Chlor.effluent represents the chlorinated effluent.
New / Old refer to the new and old sewage plants

Plate 5.4. (Below) Relative proportions of the kinds of organisms found at each site.

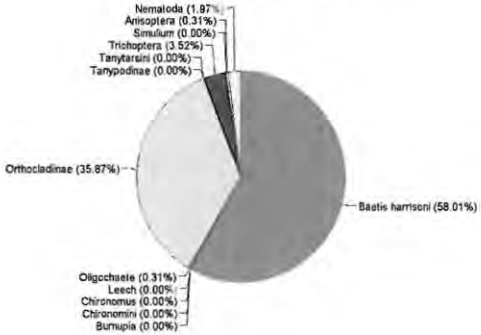
These samples were collected in winter on the 9 and 10 July, 1994.
Each pie chart shows the average number of organisms per sample for each set of 3 riffle samples at a site.
At Site 6 downstream from the chlorinated sewage out fall, no organisms were found in any of the samples.

UMBILO RIVER
JULY 1994 (Winter)

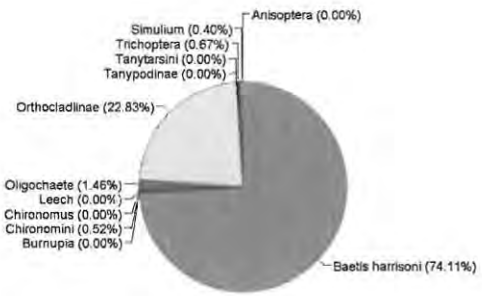
Site 1 (Upstream)
Average No. of organisms : 395



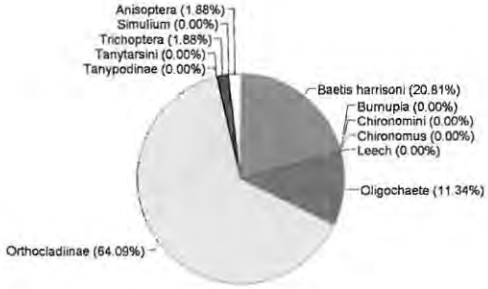
Site 2. (Upstream)
Average No. of organisms : 326



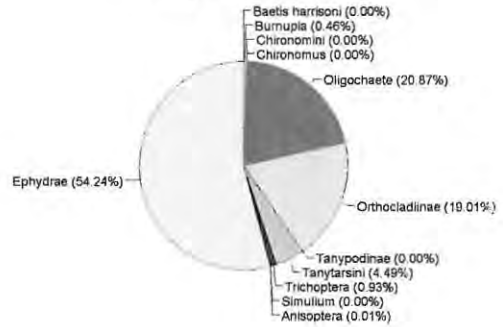
Site 3 (Upstream)
Average No. of organisms : 263



Site 4 (Upstream)
Average No. of organisms : 20



Site 5. (Downstream)
Average no. of organisms : 217



Site 6:
Nothing found in sample

Site 7. (Downstream)
Average No. of organisms : 360

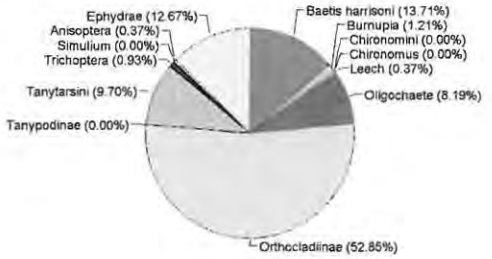


Fig. 5.4 A Average number of taxa at each site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Winter: 10 July, 1994.

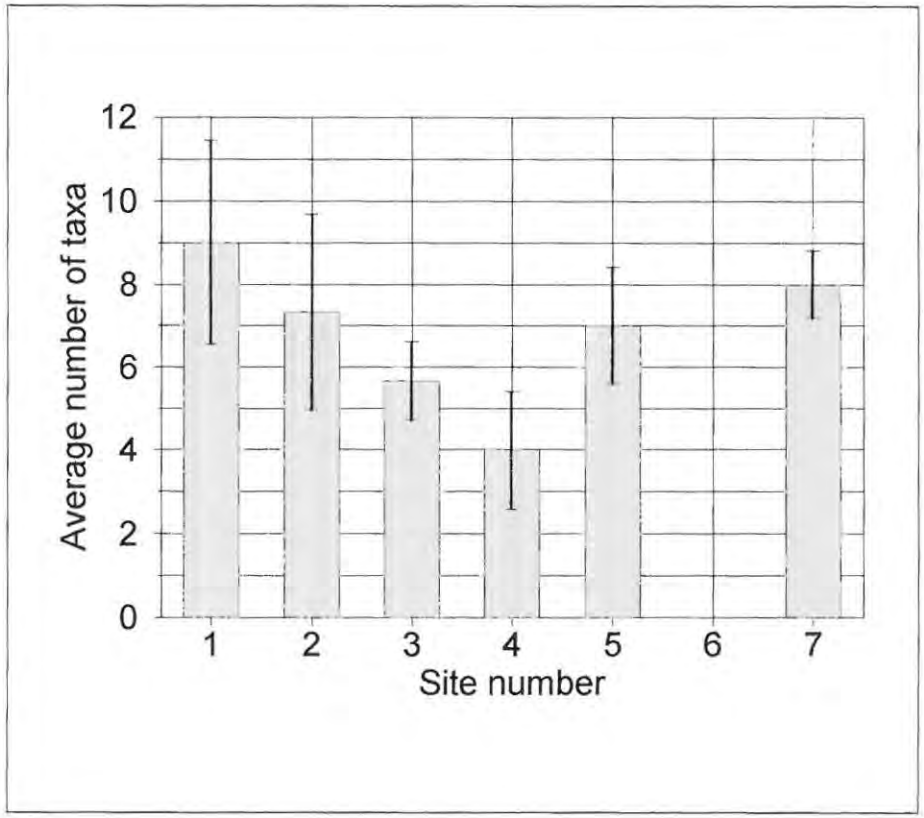
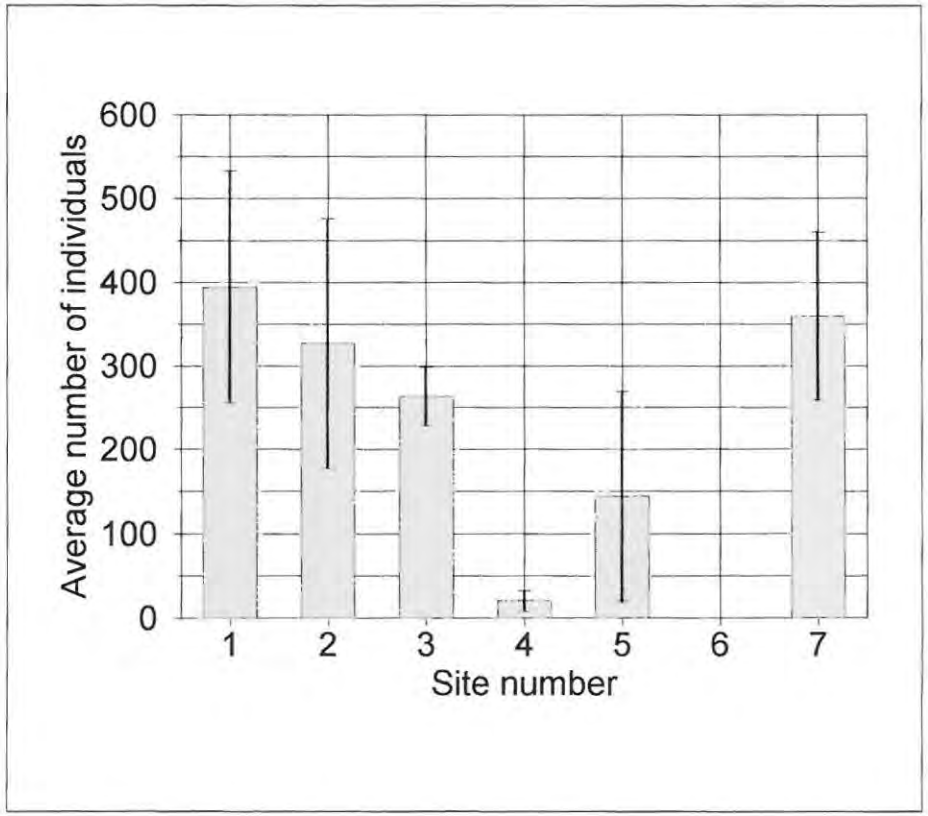


Fig. 5.4.B Average number of individuals per site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Winter: 10 July, 1994.



I-Beams on both graphs represent 2 x Standard Deviation.

Umbilo											
09 11 1994						*New*	*Old*				Drain
Sites	**1**	**2**	Unchloreff	**3**	**4**	*Chlor. effluent*		**5**	**6**	**7**	
Location relative to effluent	250 m U	6 m U		1 m B	90 m B			5-10 m D	470 m D	6.5 km D	
Free chlorine (mg/ℓ)	0.07	0.04	0.00	0.04	0.04	*0.20*	*0.25*	0.20	0.08	0.04	0.00
Total chlorine (mg/ℓ)	0.27	0.12	0.00	0.12	0.09	*2.00*	*2.00*	1.50	0.60	0.08	0.00
pH	8.9	9.0	8.1	9.1	9.0	*7.8*	*7.4*	7.8	7.9	8.1	7.6
Conductivity (µS/cm)	285	330	1324	293	311	*1280*	*1292*	1218	1190	586	804
Temperature (°C)	24.9	25.6	27.0	25.7	26.3	*27.8*	*27.2*	26.3	27.0	24.0	26.0
Dissolved oxygen (%)	121	103	63	107	117	*100*	*102*	94	98	99	92
Dissolved oxygen (ppm)	10.1	8.4	5.1	8.6	9.4	*7.8*	*8.0*	7.5	7.8	8.4	7.4

Table 5.5. Determinands recorded at sample sites at the time of collection of box samples.

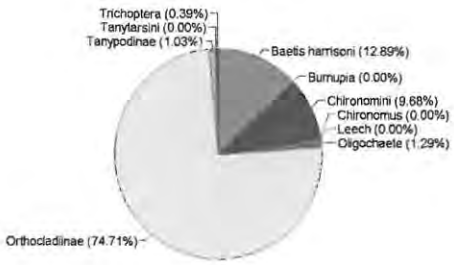
The letter U indicates that the sites were Upstream from the unchlorinated, treated sewage effluent.
The letter B indicates that the sites were Between the unchlorinated and chlorinated outfalls.
The letter D indicates that the sites were Downstream from the chlorinated, treated sewage effluent.
Unchloreff. represents the unchlorinated effluent.
Chlor.effluent represents the chlorinated effluent.
New / Old refer to the new and old sewage plants

Plate 5.5. (Below) Relative proportions of the kinds of organisms found at each site.

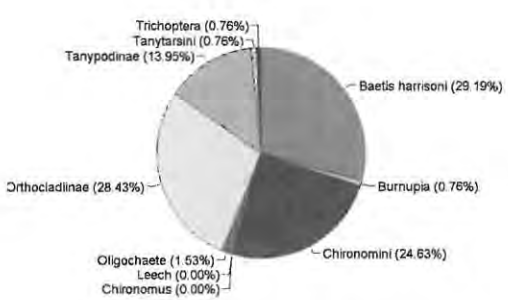
These samples were collected in winter on the 9 and 10 November, 1994.
Each pie chart shows the average number of organisms per sample for each set of 3 riffle samples at a site.
At Site 5 downstream from the chlorinated sewage out fall, no organisms were found in any of the samples.

UMBILO RIVER
NOVEMBER 1994 (Spring)

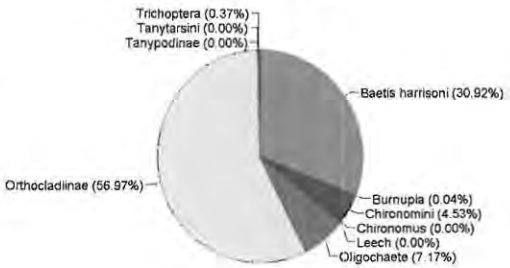
Site 1. (Upstream)
Average No. of organisms : 259



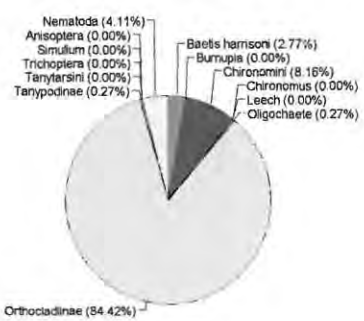
Site2. (Upstream)
Average No. of organisms : 131



Site 3. (Downstream plain effluent)
Average no. of organisms :89

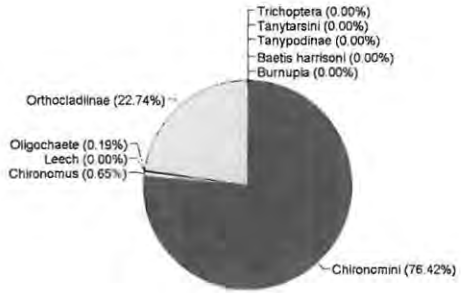


Site 4. (Downstream plain effluent)
Average No. of organisms : 374



Site 5: Nothing found in sample.

Site 6. (Down from chlorine effluent)
Average No. of organisms : 1034



Site 7. (Downstream)
Average No. of organisms : 1702

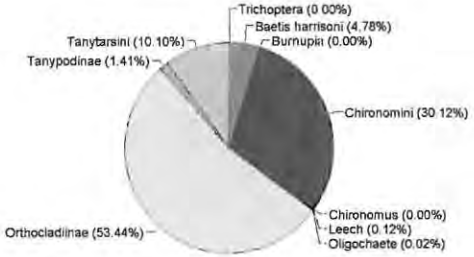


Fig. 5.5 A Average number of taxa at each site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Spring:9 November 1994.

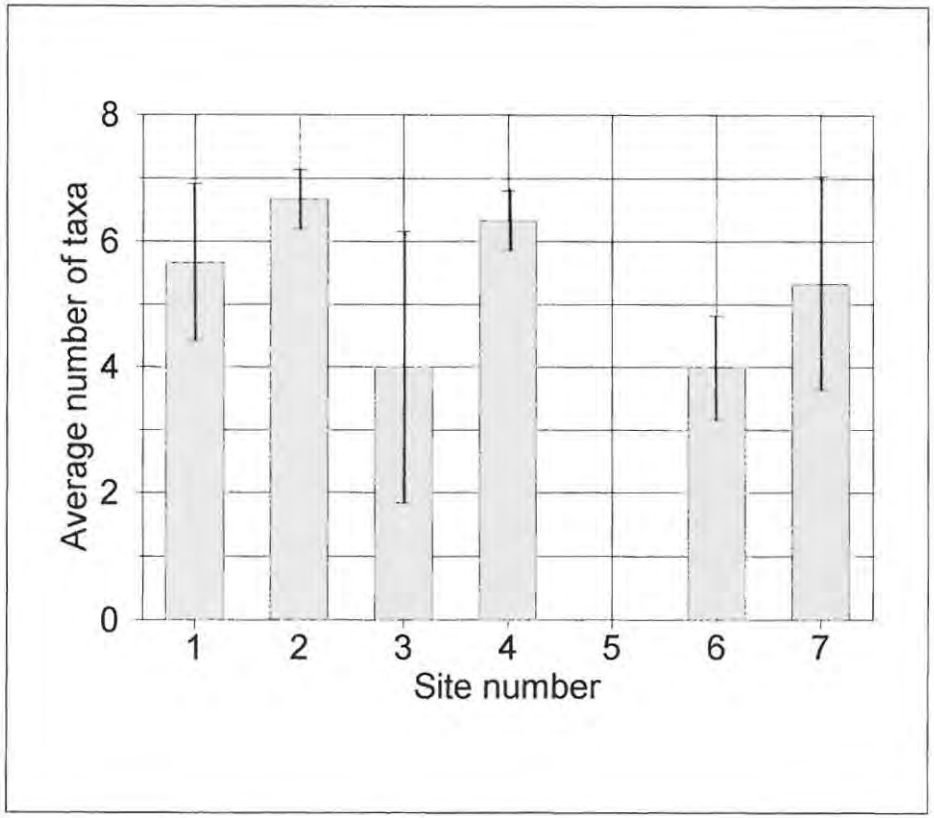
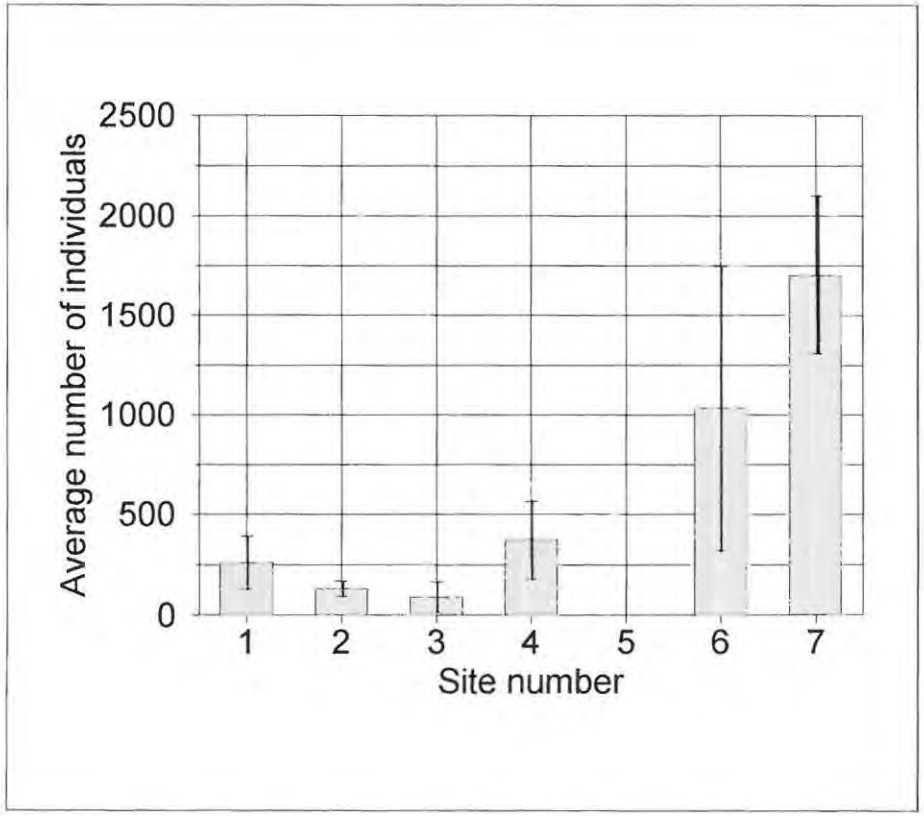


Fig. 5.5 B Average number of individuals per site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Spring:9 November 1994.



I-Beams on both graphs represent 2 x Standard Deviation.

Umbilo											
16 11 1994						*New*	*Old*				Drain
Sites	**1**	**2**	Unchloreff	**3**	**4**	*Chlor. effluent*		**5**	**6**	**7**	
Location relative to effluent	250 m U	6 m U		1 m B	90 m B			5-10 m D	470 m D	6.5 km D	
Free chlorine (mg/ℓ)	0.02	0.00	0.00	0.00	0.02	*0.10*	*0.08*	0.06	0.07		0.00
Total chlorine (mg/ℓ)	0.10	0.04	0.00	0.04	0.06	*0.90*	*0.80*	0.90	0.30		0.00
pH	8.7	8.5	8.0	8.3	8.5	*7.8*	*7.7*	7.8	7.9		7.5
Conductivity (µS/cm)	259	260	1327	340	283	*1427*	*1444*	1103	1111		450
Temperature (°C)	26.9	21.5	24.7	21.5	27.0	*27.9*	*26.6*	25.0	27.0		24.7
Dissolved oxygen (%)	116	97	70	95	101	*98*	*104*	96	98		81
Dissolved oxygen (ppm)	9.2	8.6	5.8	8.4	8.0	*7.6*	*8.3*	7.8	7.7		7.3

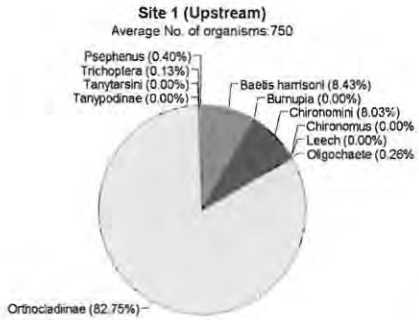
Table 5.6. Determinands recorded at sample sites at the time of collection of box samples.

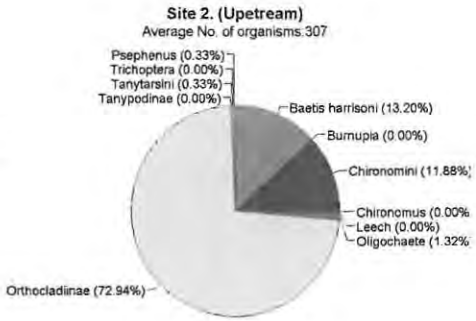
The letter U indicates that the sites were Upstream from the unchlorinated, treated sewage effluent.
The letter B indicates that the sites were Between the unchlorinated and chlorinated outfalls.
The letter D indicates that the sites were Downstream from the chlorinated, treated sewage effluent.
Unchloreff. represents the unchlorinated effluent.
Chlor.effluent represents the chlorinated effluent.
New / Old refer to the new and old sewage plants

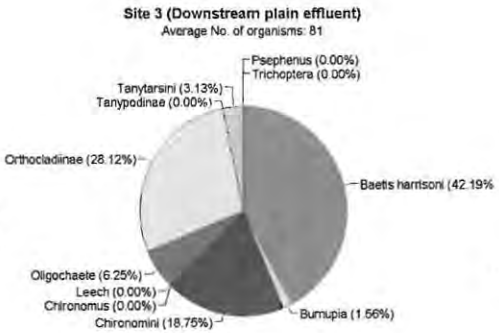
Plate 5.6. (Below) Relative proportions of the kinds of organisms found at each site.

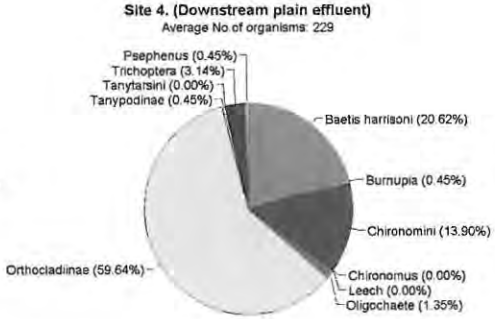
These samples were collected in winter on the 16 and 17 November, 1994.
Each pie chart shows the average number of organisms per sample for each set of 3 riffle samples at a site.
At Sites 5 and 6 downstream from the chlorinated sewage out fall, very few organisms were found in any of the samples.
Site 7 was not sampled owing to heavy rain.

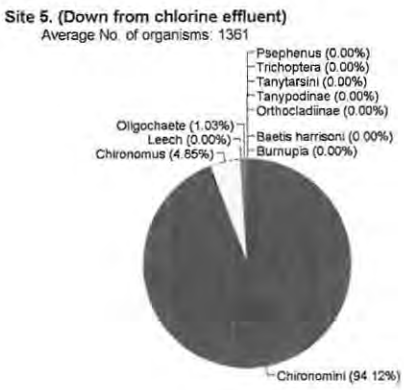
UMBILO RIVER
NOVEMBER 1994 (Spring) (One week later)

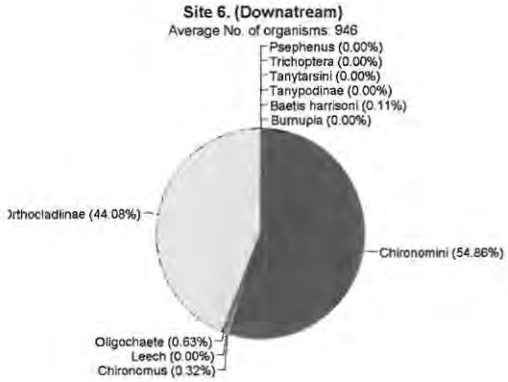












Site 7 not sampled owing to heavy rain.

Fig. 5.6 A Average number of taxa at each site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Spring: 16 November, 1994.

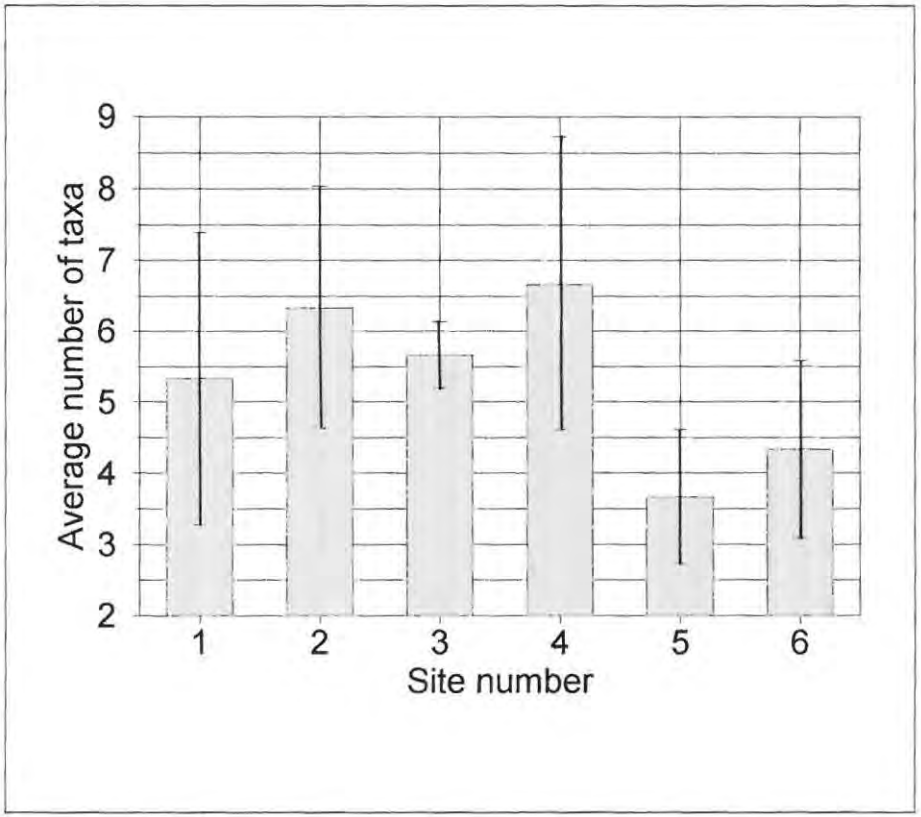
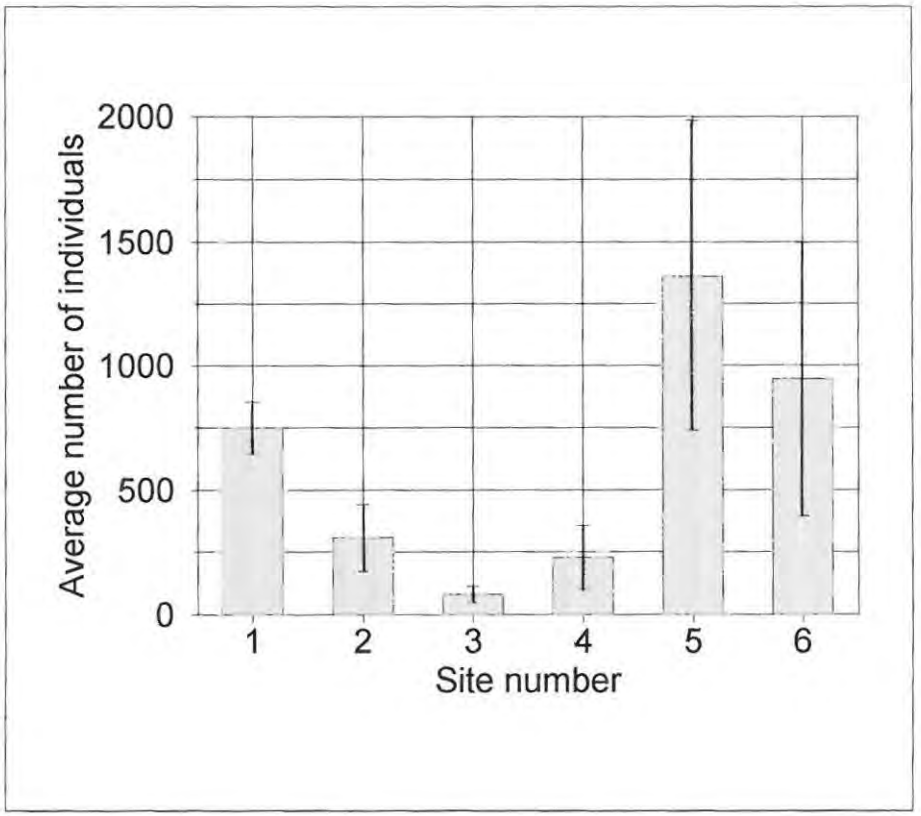


Fig. 5.6.B Average number of individuals per site.
Each bar shows the average of three riffle samples collected at a site in the Umbilo River.
Spring: 16 November, 1994.



I-Beams on both graphs represent 2 x Standard Deviation.

After 1 week (6 Feb.)				
Sites	**2**	Unchlorinated	**3**	
		effluent		
Free chlorine (mg/ℓ)	0	0	0	
Total chlorine (mg/ℓ)	0	0	0	
pH	9.0	8.1	8.8	
Conductivity (μS/cm)	312	1095	340	
Temperature (°C)	29	29.4	29	
Dissolved oxygen (%)	131	83	107	
Dissolved oxygen (ppm)	10.1	6.1	8.2	

After 2 weeks (13 Feb.)				
Sites	**2**	Unchlorinated	**3**	Drain
		effluent		
Free chlorine (mg/ℓ)	0	0	0	0
Total chlorine (mg/ℓ)	0	0	0	0
pH	8.9	7.9	8.2	8.3
Conductivity (μS/cm)	336	1208	630	400
Temperature (°C)	27.8	28.1	27.9	27.4
Dissolved oxygen (%)	125	76	107	84
Dissolved oxygen (ppm)	9.8	5.8	8.4	7

After 6 weeks (12 March.)				
Sites	**2**	Unchlorinated	**3**	Drain
		effluent		
Free chlorine (mg/ℓ)	0	0	0	0
Total chlorine (mg/ℓ)	0	0	0	0
pH	8.0	7.8	7.9	7.4
Conductivity (μS/cm)	319	1149	388	649
Temperature (°C)	21.3	27.0	21.4	23.8
Dissolved oxygen (%)	105	60	93	79
Dissolved oxygen (ppm)	9.3	4.9	8.3	6.7

Table 5.7 Determinands recorded at sample sites at the time of collection of box samples.

"Drain" refers to the outlet from the dump-site, which overflowed causing the cessation of this study.

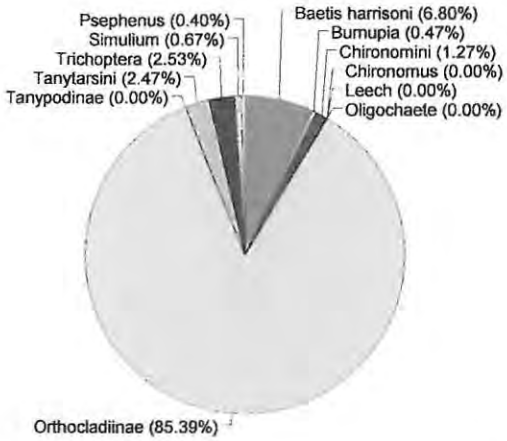
Plate 5.7 (Opposite) Relative proportions of organisms found at Sites 2 and 3 after 1, 2 and 6 weeks exposure to unchlorinated, treated sewage effluent.

UMBILO RIVER

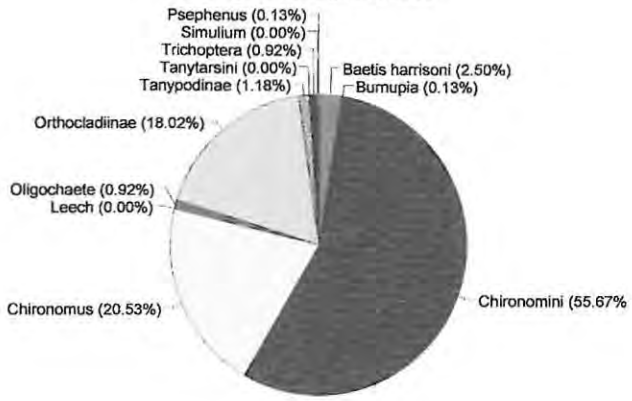
Sites 2 and 3: Sampled after exposure to unchlorinated effluent

After 1 week

Site 2 (Upstream) 6.2.95
Average No. of organisms:1527

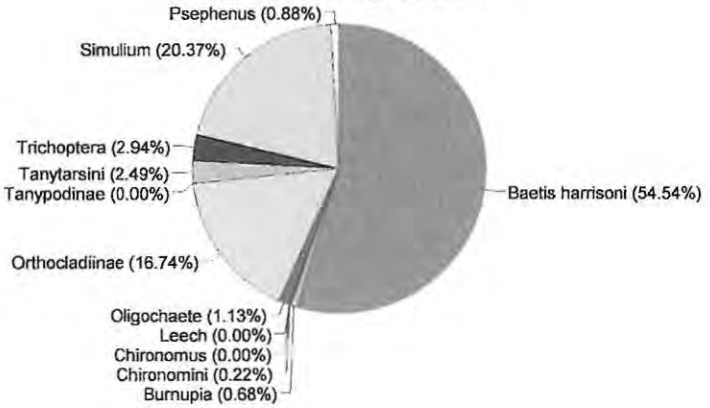


Site 3 (Downstream) 6.2.95
Average No. of organisms:760

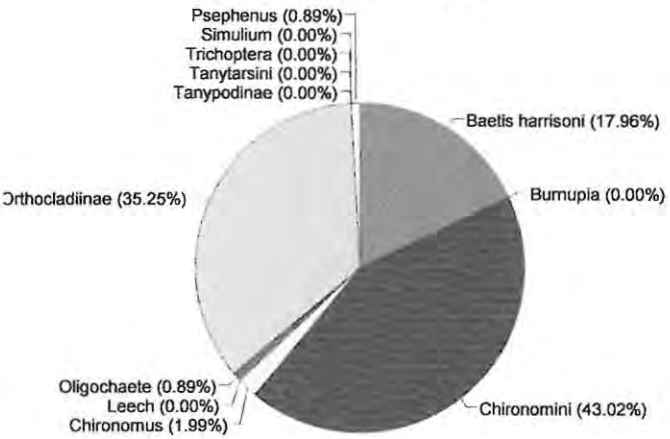


After 2 weeks

Site 2 (Upstream) 13.2.95
Average No. of organisms:511

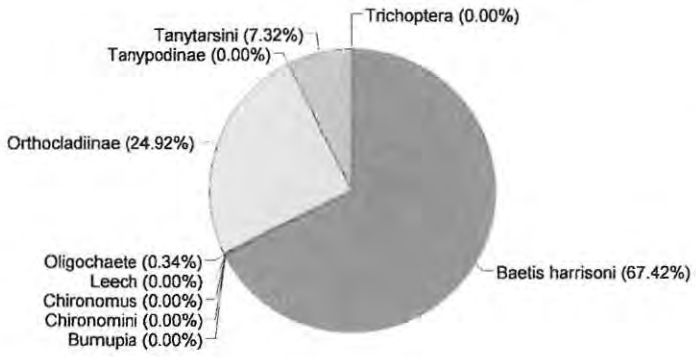


Site 3 (Downstream) 13.2.95
Average No. of organisms: 482



After 6 weeks

Site 2.(Upstream) 12.3.95
Average No. of organisms: 35



Site 3 (Downstream) 12.3.95
Average No. of organisms: 13

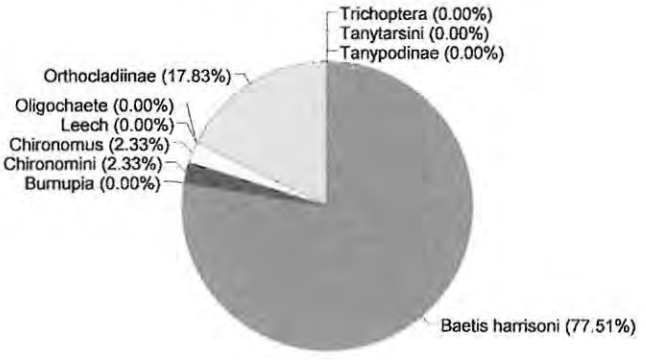


Fig. 5.7 A Average number of taxa at Sites 2 and 3.
This shows the numbers of taxa after 1, 2 and 6 weeks exposure to unchlorinated effluent.

Each bar shows the average of three riffle samples collected at a site in the Umbilo River.

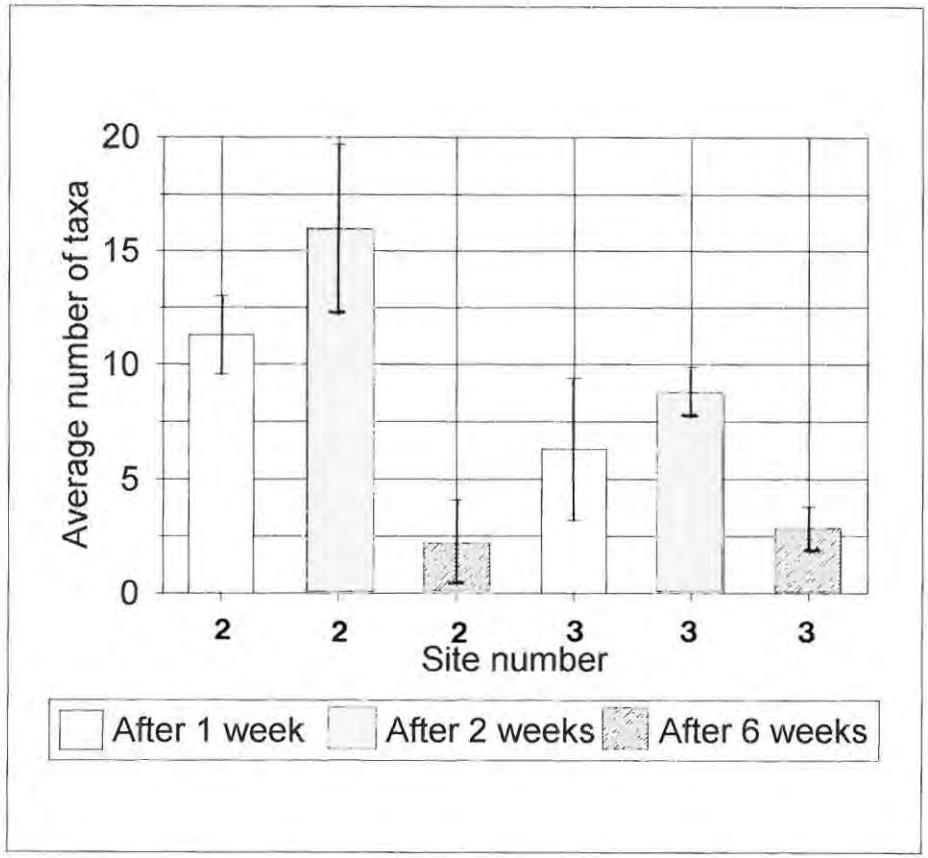
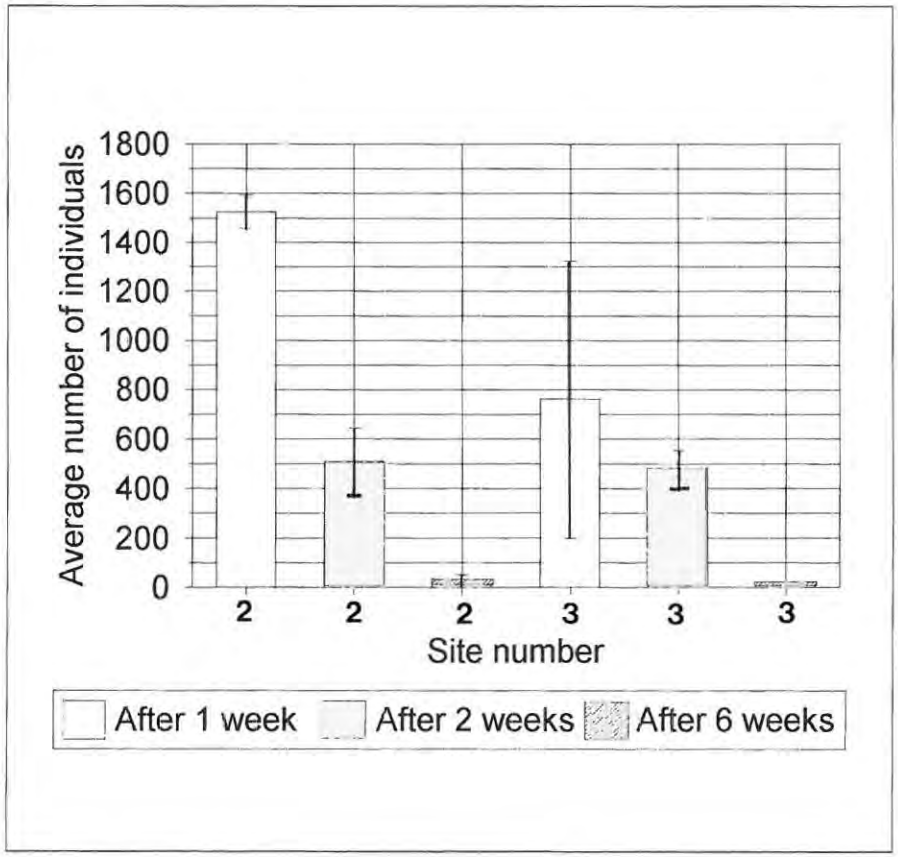


Fig. 5.7.B Average number of individuals per site.
This shows the numbers of organisms after 1, 2 and 6 weeks exposure to unchlorinated effluent.

Each bar shows the average of three riffle samples collected at a site in the Umbilo River.



I-Beams on both graphs represent 2 x Standard Deviation.

Fig. 5.8 Average number of B.harrisoni at each site before release of unchlorinated effluent at Site 3

This shows the average no. of B. harrisoni at each of the four upstream sites (1 to 4) and three sites downstream (5 to7) from the chlorinated effluent in the Umbilo River.

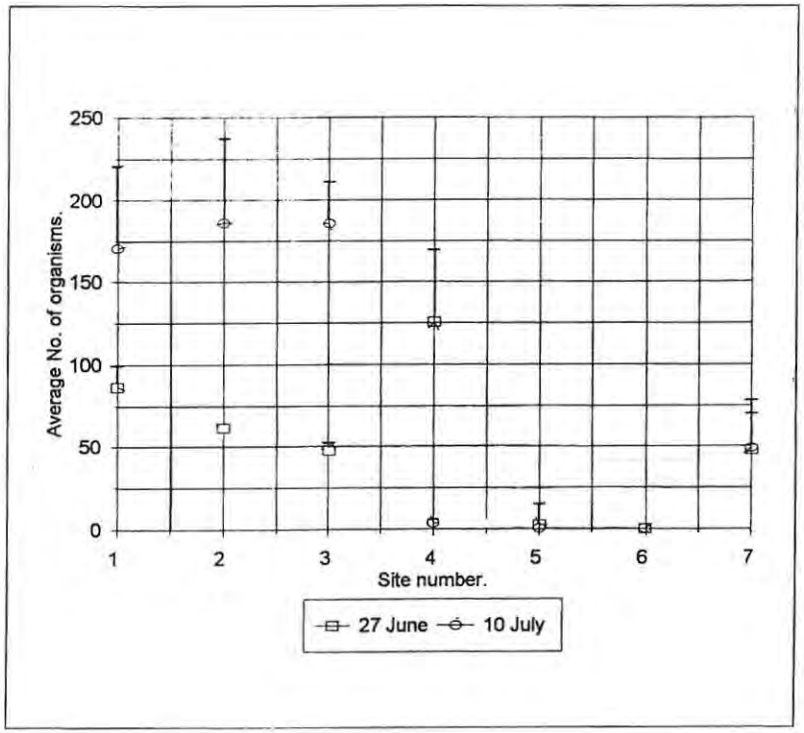
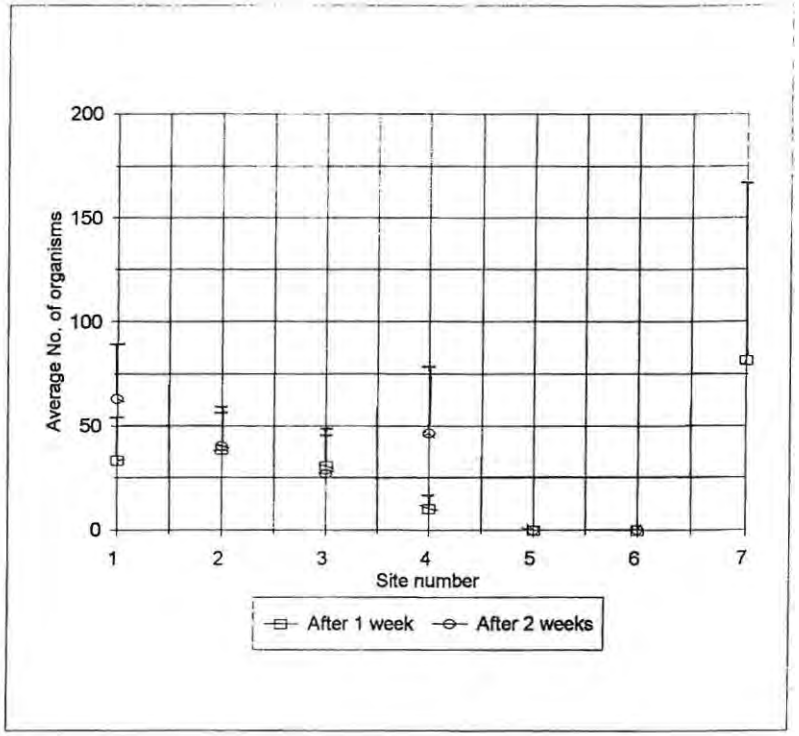


Fig. 5.9 Average number of B harrisoni at each site after release of unchlorinated effluent at Site 3

This shows the average no. of B. harrisoni at each site after 1 and 2 weeks exposure to the unchlorinated effluent released between Sites 2 and 3. Chlorinated effluent was released between Sites 4 and 5.



Each point represents the average of 3 samples per site.
Horizontal lines represent 1 X Standard Deviation.

MULTIVARIATE ANALYSIS

A total of 150 samples from the Umbilo River were classified, and 56 taxa were identified (**Fig. 5.10** and **Appendix I**). The classification was taken to two levels and at Level 2, the four resultant groups were related to the number of taxa per group. At the first level the primary distinction was between samples collected in different biotopes: riffles, as distinct from pools. At the second level the effects of chlorination became apparent. Riffle samples from Sites 1, 2, 3 and 4 (above the chlorinated effluent inflow) and Site 7 (6.5 km below the effluent outfall) were classified together in Group IA, and distinguished from Group IB with samples from Sites 5 and 6 (immediately downstream of the effluent). Pool samples collected from sites from above and below the effluent stream were also clearly distinguished. Group IIA comprised samples from site 1, 2, 3, 4, and some site 7 samples; whereas Group IIB comprised samples from Site 5 and 6, with some Site 7 samples. The number of taxa per group decreased across the classification gradient. Riffle samples unaffected or recovered from chlorinated effluent had 55 taxa; riffle samples downstream of the effluent had 17 taxa; pool samples above the effluent had 14 taxa, and pool samples downstream of the effluent had 8 taxa.

The groups were characterised by the following faunal assemblages;

Group I A (Riffles, upstream of, and recovered downstream of chlorinated effluent)

B. harrisoni and Orthocladiinae dominated these samples, and *Cheumatopsyche afra* (Mosely) was characteristically present. This group had the highest diversity of taxa (55). (Although all the riffle samples were classified in the group, a few samples collected from pools at the sites above the effluent discharge (Site 1-4) were also included. These were pool samples dominated by *B. harrisoni*).

Group I B (Riffles immediately downstream of chlorinated effluent)

These samples were characterised by intermediate diversity (17 taxa in the group); presence, but lack of dominance by *B. harrisoni*; and the presence of *Chironomus* sp. and Chironomini were common in both this group and Group IIA.

Group II A (Pools upstream of chlorinated effluent)

The most characteristic feature of these samples was the presence of dragonfly nymphs (Aeshnidae, Gomphidae and Anisoptera). Of these, the Gomphidae were dominant. The group was intermediate in diversity (14 taxa) and *B. harrisoni* and *Centroptilum excisum* (Barnard) were present.

Group II B (Pools downstream of chlorinated effluent)

The samples had the lowest diversity (8 taxa) and were dominated by oligochaetes and *Chironomus*.

Samples from Site 7 pools were spread between IIA and IIB, depending on the presence of *B. harrisoni*. The dominance of oligochaetes and lower diversity in all the pool samples made distinctions less obvious than in the riffle samples.

SUMMARY OF RESULTS

The results of both the Umsunduze and the Umbilo river samples indicate that chlorinated sewage effluent can kill an entire community at the point of discharge, and it is some time before the "sewage community" is restored and can begin cleansing the river of the organic load. In the Umsunduze River the community structure 7 km from the sewage outlet was still very different from the communities upstream from the effluent. In the Umbilo River, the communities 6.5 km downstream from the chlorinated effluent outlet had shown some form of recovery.

The study with the unchlorinated effluent suggested that while this effluent did not kill the entire community, it could have caused some changes in community structure. (However, the limitations of this study must be remembered as the volume of unchlorinated effluent in no way paralleled that of the chlorinated effluent downstream.)

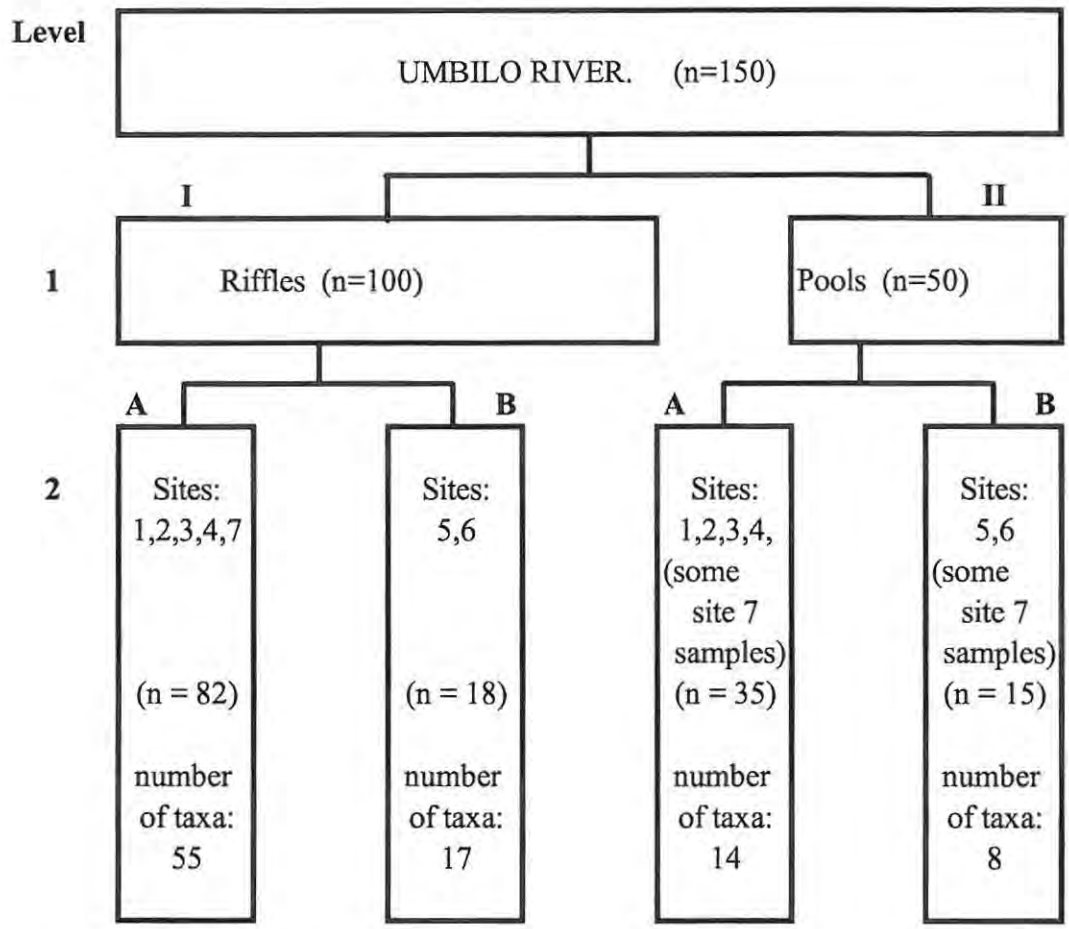


Fig 5.10. The results of a TWINSPAN (Hill, 1979) classification of the benthic riffle and pool fauna collected from the Umbilo River (1994, 1995).

At Level 1, riffle and pool samples were distinguished; and at Level 2 within each biotope, Sites 1 - 4 (upstream of the chlorinated effluent outfall) were distinguished from Sites 5 and 6 (immediately downstream of the outfall). Site 7 riffles were grouped with the upstream sites, but pools were split between upstream and downstream sites.
(n = number of samples)

DISCUSSION

As mentioned in **Chapter 1,** South Africa has a critical water shortage and in order to supplement water quantity, treated sewage is released back into the natural environment. However, although the *quantity* of water is increased in this way, there are serious implications for water *quality,* particularly when this effluent is chlorinated. This part of the study has involved the investigation of the effects of chlorinated, treated sewage on communities of aquatic organisms.

MACROINVERTEBRATE COMMUNITY STRUCTURE AND ECOTOXICOLOGY

According to Moriarty (1983), the term *ecotoxicology* was coined by Truhaut in 1969 as a natural extension from toxicology (the science of the effects of poisons on individual organisms, as described in **Chapter 4**) to the *ecological* effects of pollutants: ecotoxicology is concerned with effects on *populations* of individuals. The transition from the study of single organisms to that of ecosystems has, however, brought complexities which do not yet appear to be fully appreciated. Moriarty (1983) goes on to note that in some studies, it would appear that the only difference between toxicology and ecotoxicology is the species selected for the toxicological tests: where acute toxicity is measured on an organism found in the natural environment, e.g. the water flea, instead of being measured on the standard laboratory test organism, i.e. the laboratory rat, this does not constitute an ecotoxicological study, it is merely a toxicity test on a different organism. In this way the essential difference between the two sciences is missed and it would appear that many writers do not make a clear distinction between toxicology and ecotoxicology, and appear to use the two terms interchangeably. In this study, the two concepts were seen as being distinct.

The shift of emphasis from toxicology to ecotoxicology has many implications e.g.

1. The range of variables that affect population responses includes, but is greater than, the range that affects the individual responses to pollution.
2. Sublethal effects on individuals may be as important as lethal effects.

It is widely accepted in toxicology that different individuals of one species will not react in an identical way to a toxin. In ecosystems different populations of one species

may not all react in an identical manner to a pollutant, and, inter-species interactions such as predation and competition operate in an ecological context and are affected by responses of individuals to pollutants.

THE EFFECT OF TREATED SEWAGE EFFLUENT ON WATER QUALITY

Organic enrichment e.g. effluents from domestic sewage, food processing plants, animal feedlots and abattoirs, causes decreases in dissolved oxygen levels and increases in nutrient, turbidity and suspended solid levels in the receiving waters. (Dallas & Day, 1993).

A useful measure of organic enrichment is that of the biological oxygen demand (BOD) which is measured as the rate at which oxygen disappears from a solution in a sealed bottle kept in the dark (to avoid photosynthesis) for 5 d at 20 °C. The BOD values range from 0.5 to 7.0 mg/ℓ in natural water but in treated sewage effluent, the range is from 3 to 50 mg/ℓ. The BOD of raw sewage can be as high as from 200 to 800 mg/ℓ (Hellawell, 1986 *in* Dallas & Day, 1993). Organic enrichment is one of the most common types of pollution occurring in rivers and has been well documented (Watson, *et al.*, 1982; Newman & Perry, 1989; Whitehurst & Lindsey, 1990; APHA, 1992; Reynoldson & Metcalfe-Smith, 1992; Dallas & Day, 1993). Both the nutrient increases (increased nitrates (NO_3^-), phosphates (PO_4^{3-}) and ammonia (NH_3 and NH_4^+) and the decrease in dissolved oxygen have marked effects on the riverine biota (Odum, 1971; Davies & Day, 1986; Dallas & Day, 1993).

THE EFFECT OF TREATED SEWAGE EFFLUENT ON AQUATIC LIFE

While an organic discharge itself may not be directly toxic to aquatic life, it may cause a significant change to community structure and biological processes (Dallas & Day, 1993). The immediate effects of the entry of sewage effluent into rivers appear to be those associated with low oxygen levels (Whitton, 1975), because, in the presence of a high organic load, "sewage fungus" (bacteria, fungi, algae and protozoans) develops. The rapid decomposition of the organic matter leads to oxygen depletion (Dallas & Day, 1993) and may cause anaerobic production of hydrogen sulphide, (hence the smell associated with this effluent) so the environment is unsuitable for most benthic

macroinvertebrates.

In unperturbed environments, the composition and density of macroinvertebrate communities in streams, lakes, estuaries and marine waters are reasonably stable from year to year. (Seasonal fluctuations may, however, result in extreme variation at specific sites during the course of the year.) In general, undisturbed environments with acceptable water quality and substrate conditions tend to be characterised by high species diversity or richness and an even distribution of individuals among species. (This is not true of environments such as deserts or saline lakes.) Pollution by sewage causes adjustments in community structure (usually a decrease in species diversity) because sensitive species are lost and tolerant species increase in number (Cairns & Dickson, 1971; Ward & Stanford, 1983; Karr, 1991; Reynoldson & Metcalfe-Smith, 1992; APHA, 1992). The distribution of benthic macroinvertebrates is therefore generally considered to be indicative of the chemical quality of surface waters (Sloof, 1983), organisms will be absent where conditions exceed their tolerance limits (Whitton, 1975). In habitats which are dominated by a few species, changes in their relative numbers may, however, not be indicative of changes in water quality (APHA, 1992).

Macroinvertebrate community responses to environmental perturbations are also useful in assessing the impact of industrial, oil and agricultural wastes, and impacts from other land uses on surface water bodies. Patterns of macroinvertebrate community structure change have been documented in situations such as organic loading, substrate alteration and toxic chemical pollution (APHA, 1992).

Rivers are often able to recover from the effects of organic pollution, provided that they remain uncanalised and that other human intervention is not too severe. Because they are moving water masses and because of their plant, animal and microbial communities, they can clean themselves of their organic loads (Odum, 1971; Davies & Day, 1986; Whitehurst & Lindsey, 1990). This cleansing can be either on a temporal scale (over a period of time, organisms can clean the river after a "spill" of organic material e.g. if sewage works overflow during heavy rains as has happened at Darvill Wastewater Works on occasions) or it can be on a spatial scale (where the water

becomes cleaner further downstream from the source of effluent). However, as explained in **Chapter 2**, for human health reasons, treated sewage effluent is usually chlorinated to kill pathogenic bacteria. When chlorine enters aquatic ecosystems, it is able to kill a large proportion of the plant, animal and microbial communities. If this happens, the cleansing process is retarded, and the river retains its organic load for a longer stretch. The chlorine present in wastewaters has been cause for much concern and has initiated a considerable amount of research in the fields of biological monitoring, toxicity testing and ecotoxicology (Mattice & Zittel, 1976; Cherry *et al.*, 1977; Ward & De Graeve, 1980; Giattina *et al.*, 1981; Mattice *et al.*, 1981; Watson *et al.*, 1982; Heckman, 1983; Newman, *et al.*, 1987; Newman & Perry, 1989; Zhulidov & Pokarzhevskiy, 1990; Camargo, 1991; Van Benschoten *et al.*, 1995).

The assessment of the effects of a pollution source generally involves the comparison of macroinvertebrate communities and their physical habitats at sites influenced by pollution with those collected from adjacent unaffected sites. The basic information required is a count of individuals per species, from which communities can be characterised according to community structure, density, biomass, diversity or other analyses. The procedure includes sampling and analysing both affected and unaffected communities and determining whether the presumed pollution-affected communities differ from the non-affected ones. The basic information required for most community structure analyses is a count of individuals per species. From the count data, the communities can be characterised and compared according to community structure, density, biomass or other analyses. Information regarding physical and chemical features of the sites are also desirable (APHA, 1992).

The results of this study demonstrated unequivocally, the deleterious effect of chlorinated, treated sewage on riverine macroinvertebrate community structures. The clearest indications of this change were to be seen in the comparisons between Sites 2 and 3 in the Umsunduze River and Sites 4 and 5 in the Umbilo River. Graphs showing the average number of taxa at each site (**Figs. 5.1** to **5.7** **A**) generally showed a sharp decrease in the number of taxa from the upstream to the downstream site. (An exception was **Fig. 5.4A,** where building operations had all but destroyed the

community at Site 4, and one of the chlorinators was not working (**Table 5.4**), so the chlorine content of the effluent was unusually low.) Graphs of the average number of individuals per site (**Figs. 5.1** to **5.7 B**) also showed a decrease in the number of individuals at the downstream sites. Again, **Fig. 5.4** was an exception because of the building, as was **Fig. 5.6**, where a large number of Chironomini was present at Site 5. These Chironomini had colonised the areas within the space of a week, as the previous week, there had been no organisms at this site.

Closer inspection of the free chlorine concentration shows that when there were no organisms at Site 5 (**Figs. 5.3 and 5.5**) the free chlorine concentration had been 0.1 and 0.2 mg/ℓ respectively, whereas on those occasions when organisms were present at Site 5, the free chlorine concentrations had been much lower at 0.02 and 0.06 mg/ℓ respectively. The total chlorine concentrations followed the same pattern as the free chlorine with concentrations of 0.25 and 1.5 mg/ℓ being recorded when no organisms were present, and 0.09 and 0.9 mg/ℓ when organisms were present. It is unlikely that there were major fluctuations in the composition of the treated sewage effluent over the course of a week whereas there were differences in the chlorine concentrations, so it is possible that the presence or absence of organisms at these downstream sites is related to the chlorine concentrations. As mentioned previously, free chlorine is far more toxic than combined chlorine, so it is possible that the presence of free chlorine has the greatest effect on the riverine organisms.

The results of the TWINSPAN analysis of samples from the Umbilo River confirm the adverse effects of chlorine in sewage effluent. Palmer & O'Keeffe (1991) showed clearly that biotopes in the Buffalo River (eastern Cape) were characterised by particular macroinvertebrate assemblages. In the Umbilo River the difference in assemblage composition between riffle and pools was even more distinctive than the effects of effluent discharge and was responsible for the primary classification split.

However, within both riffle and pool samples, the effects of chlorination were clearly evident. Samples from Site 5 and 6 had few organism, low diversity and were

dominated by *Chironomus*. It is interesting that those samples collected at Site 3 and 4 when they were exposed to unchlorinated effluent were not distinguishable on the basis of faunal composition. This is clear evidence of the effects of chlorination as distinct from sewage effluent.

The dominance of *B. harrisoni* at all sites unaffected by chlorination confirms its tolerance of sewage effluent (Chutter, 1992), and can be related to its sensitivity to chlorine reported in Chapter 4.

Site 7, 6.5 km downstream indicates a chlorination recovery distance. Dechlorination by running effluent along a "natural' channel before discharge into a river, as has now been done at Darvill sewage works is an effective approach to remediating the instream consequences of the discharge of chlorinated effluent.

CHAPTER 6

CONCLUDING DISCUSSION

The use of chlorine in water treatment, the validity of toxicity test data and the use of artificial streams for toxicity testing are all contentious issues. This study embraced all three. Some aspects of these issues have been mentioned in previous chapters but others demand further attention.

The formation of disinfection by-products is one of the major issues facing present-day water treatment technology and drinking water quality control (Meintjies & Murphy, 1994) and chlorine is one of the culprits. The benefit of chlorine to human health is beyond question and Shuval *et al.*, (1994) go as far as to state that since it was introduced for disinfection of potable water in 1908, it has probably saved more lives than penicillin and all the modern antibiotics combined. However, in the light of more recent discoveries, its continued use is being questioned. The use of chlorine in water treatment came under fire in 1974 when Rook demonstrated the presence of by-products such as chloroform and other trihalomethanes (THM) in chlorinated drinking water, and these were later implicated as possible carcinogens (Johnson & Jolley, 1990; Orme *et al.*, 1990; Shuval *et al.*, 1994; Chang *et al.*, 1994; Baxter, 1994). These THM's form as a result of chemical reactions of chlorine with humic substances e.g. humic acid, fulvic acid and hymatomelanic acid; algal materials and an assortment of aromatic substances which occur naturally in raw water (Muttamara *et al.*, 1994). Here, the use of chlorine is being questioned because of implications in terms of *human* health, but what of *environmental* health?

In addition to the threat to human health, the discharge of toxic disinfectants into the environment is detrimental to ecosystem integrity. The present study has shown that the chlorine present in treated sewage effluent has a distinct effect on macroinvertebrate community structure. It probably also has a devastating effect on the natural microbial communities. Therefore, if chlorine forms carcinogenic THM's

and poses a pollution threat to the environment, alternative disinfection techniques should be investigated. At present, what are the disinfection alternatives?

Various other methods of disinfection have been investigated and some are in use at present in various parts of the world (South Africa included), and each has its advantages and disadvantages, e.g.:

- **Chlorine dioxide**: There are a number of advantages to using chlorine dioxide as opposed to chlorine for disinfection (Freese & Knobel, 1994). It has proven to be an effective disinfectant with nearly 2.5 times the oxidising power of chlorine (Orme *et al.*, 1990), it does not result in the formation of THM's, it is excellent for the control of phenolic taste and odour problems, it does not react with ammonia nitrogen in the water (White, 1992; Freese & Knobel, 1994) and it provides a measurable residual (Aieta *et al.*, 1984). Unfortunately there are also a number of disadvantages to using chlorine dioxide for disinfection (Freese & Knobel, 1994) as it is dangerous and troublesome to produce (Brits *et al.*, 1994). As it is explosive it cannot be transported so has to be generated on site. In addition it is an expensive disinfectant (which South Africa could probably not afford at present) and there is the possible toxicological effect of both the chlorine dioxide itself, and its by-products, chlorite (ClO_2^-) and chlorate (ClO_3^-) ions (Gordon & Tachiyashki, 1991; White, 1992; Freese & Knobel, 1994). Chlorine dioxide does not seem to be a viable alternative at present.

- **Chloramines**: Although free chlorine is a powerful disinfectant, it remains effective for only about 8 h. Monochloramine, however, is a less effective disinfectant and is much slower, but it remains effective for much longer periods so is used as a secondary disinfectant to prevent aftergrowth of micro-organisms in the distribution system. It also has the advantage in that the formation of THM's is limited (Acton & Joubert, 1994). There are, however chloramination by-products e.g. nitrogen trichloride (NCl_3) (which causes taste and odour problems) and dichloramine ($NHCl_2$) (Shuval *et al.*, 1994). [Shuval *et al.*, (1994) also mention *trichloramine* with a formula $\underline{NH}Cl_3$, but this was not found in other reputed texts e.g. Handbook of Chlorination (White, 1986) or Standard Methods for the

Examination of Water and Wastewater (APHA, 1992), which refer to NCl_3 as trichloramine or nitrogen trichloride.] Chloramines also are not a viable alternative.

- **Ozone:** This is an effective oxidising and disinfection agent, and with the progress of ozone technology, it is now used successfully in many water treatment plants all over the world (Shadiakhy, & Blankenfeld, 1994) e.g. it has been widely used for several years in Europe, Canada, France, Switzerland and U.K. (White, 1992; Erni *et al.*, 1994; Carnimeo *et al.*, 1994 ; and Martin *et al.*, 1994). However, like chlorine dioxide, it must be generated on site (White, 1992). Its primary limitation is the lack of a long acting disinfection residual, and it also forms disinfection by-products in natural waters which contain bromide ions, e.g. inorganic hypobromite, bromate, bromoform and many brominated organic compounds which are suspected to be carcinogenic (Shuval *et al.*, 1994). In South Africa ozone is used at a few wastewater treatment plants (Mr B. Rowlston, DWAF. *pers. comm.*)

- **Bromine:** Bromine and bromine chloride are relatively soluble in water, but bromine is too hazardous to handle in the treatment of wastewater. In 1953, Kamlet (*in* Ward & DeGraeve, 1978) advocated the use of bromine chloride as a water and wastewater disinfectant on the basis of its greater disinfection effectiveness and economy when compared with either bromine or chlorine. Bromine chloride is easier to handle than bromine, but materials and equipment different from those for chlorine are required for feeding and metering of this gas. While bromine compounds appear to be less toxic to aquatic life than chlorine in that the residuals die away rapidly, this characteristic makes bromine or bromine chloride residual control virtually impossible. In addition, the bromine compounds are more expensive (these days) than chlorine (White, 1992) and Shuval *et al.*, (1994) state that various toxic brominated disinfection by-products e.g. bromine hypobromite and hypobromous acid are formed, so the use of bromine is not recommended as an alternative to chlorine.

- **Iodine:** Iodine is only slightly soluble in water and has been used in emergencies for water treatment. Little is known about it as a wastewater disinfectant and it is very expensive so it is not really viable as a wastewater disinfectant (White, 1992).

- **Ionising radiation:** Gamma radiation is an effective sterilant and gamma rays are more penetrating than UV. However the cost of radiation energy is high so gamma radiation is not economically competitive as a disinfection process for wastewater (White, 1992).

- **Silver:** Shuval *et al.*, (1994) suggested the use of oligodynamic silver (silver in very small concentrations) used with hydrogen peroxide (because of their synergistic effects) as an alternative to chlorine disinfection, and although the results of their experiments were promising, they were still in the preliminary stages of investigation.

- **Ultra-violet light:** U.V. on its own has the disadvantage of not having any residual disinfectant properties, and, because of its poor penetration ability, lethal action in wastewater cannot be exerted through more than a few centimetres. For effective exposure of sewage effluents containing varying amounts of interfering suspended solids and ordinary turbidity, a very thin sheet of water of uniform thickness must be maintained (White, 1992). As far as the residual disinfectant properties are concerned, Carnimeo *et al.*, (1994) reported some success when U.V. is used in combination with hydrogen peroxide (H_2O_2), so that the purely physical, reportedly non-disinfection by-product forming action of UV is combined with a persistent chemical disinfectant, hydrogen peroxide.

- **Bromochlorodimethylhydantoin**: This is a broad-spectrum biocide which has been used effectively to disinfect vast quantities of service water underground in the Vaal Reefs Gold Mine. It that context, it was cheaper to use than the alternative which was calcium hypochlorite solution (HTH) (Brits *et al.*, 1994) but no mention was made of its suitability for wastewater disinfection.

Disinfectants are by their very nature toxic, and it is clear that feasible alternatives to chlorine should be sought because of the actual or potential threats to human and environmental health. Several factors need to be assessed, e.g. whether or not the THM's are in fact carcinogenic is also controversial, A comprehensive review carried out by the World Health Organisation in 1992 led to their statement that there is insufficient evidence to classify chlorine in drinking water as a carcinogen, this despite many studies carried out in the area (Baxter, 1994). Although there is growing concern about the possible links between THMs and cancer in humans as they get older, one must not lose sight of the fact that in Third World countries especially, chlorination is at present the only economically viable way to disinfect drinking water and wastewater. If this is not done, the people will succumb to water-born diseases in infancy or early childhood and will not live long enough to develop cancer.

In South Africa, the law requires that all wastewater be returned to the rivers, from where it is used by other people downstream. Therefore the effluent standard as far as microbial pathogens are concerned is that the *E. coli* count must be zero,("The wastewater or effluent shall contain no typical (faecal) coli per 100 ml" (DWAF, 1991)) and it is important that the disinfection practices are adhered to. (An anomaly in this regard is that the Umgeni Water Site Specific Wastewater Standard for sewage works effluent is 500 *E. coli* per 100 ml.) In some countries where wastewater in the rivers is not used for human consumption downstream, disposal of biologically treated effluents containing a maximum of 200 faecal coli per 100 ml is permitted in certain areas. In California, a level of 23 total coliform per 100 ml is permitted for irrigation of golf courses, parks, and pastures grazed by milking animals, and for groundwater recharge and direct irrigation of food crops, a level of 2.2 total coliforms per 100 ml is permitted (Narkis *et al.*, 1990). However such standards would not be appropriate for the South African situation, where raw water *is* used downstream. As it is, in South Africa at present, the infant and childhood mortality rates (owing to water-born diseases) among those living in informal settlements are very high (Mr T Jackson, MRC. *pers. comm.*) It appears, therefore, that disinfection of treated sewage effluent is necessary and chlorine appears to be the most appropriate disinfectant for use in South Africa at present.

But what of the environment?

As mentioned in **Chapter 1,** rivers are the source for most water users and failure to maintain them in a healthy state would mean failure to meet the needs of these users. This study has shown clearly that the use of chlorine in the disinfection of treated sewage effluent poses a threat to environmental integrity. If chlorine is the only affordable disinfectant in South Africa at present, what can be done to minimise its adverse effects on the environment?

Dechlorination of wastewater is a possible option. Wastewater dechlorination systems are designed to produce a zero chlorine residual in the effluent before it leaves the plant. This can be achieved either by the use of sulphur dioxide, or by using a holding pond (aeration and holding lagoons) or by carbon adsorption (using granular activated carbon (GAC)) (White, 1992).

In 1980, Chen & Gan (*in* White, 1992) reported that of the three dechlorination methods, the sulphur dioxide process was the most cost effective. As mentioned in **Chapter 3,** Umgeni Water is now practising the holding pond method at Darvill Wastewater Works, where a long stream has been built to allow the treated sewage to meander along to eliminate the chlorine prior to the entry of the effluent into the Umsunduze River. (The new stream is visible as three long, curved, parallel dark areas on **Plate 3.1**.) The GAC method has been successful as a dechlorinating agent for potable water but not for wastewater treatment. (White, 1992). In general, however, dechlorination is seldom practised in South Africa.

If chlorine is the best option for wastewater disinfection at present, how can its toxic effects be quantified in order to set Water Quality Guidelines?

Toxicity tests have been used widely throughout the world to determine the effects of toxic substances on aquatic animals (APHA, 1992; Dallas & Day, 1993; Roux, 1994) but for some time their value has been questioned as there are certain limitations to the validity of data collected from such tests. Some of the problems which are associated with toxicity tests are that, apart from fish, no riverine organism is commonly used and

none is available as a standard laboratory animal (Dallas & Day, 1993). Cairns (1983, 1986b, 1988a, 1988b) has questioned the fact that most toxicity tests are *single species* tests. In single species tests, cause-and-effect relationships can be established easily because of the degree of control over laboratory conditions. The tests are easy to conduct, and many are standardised and can be replicated (Rand & Petrocelli, 1985). However, although single species toxicity tests have dominated the field of environmental toxicology since it began, the deficiencies of these tests were recognised from the outset, e.g. sometimes the test species selected may not inhabit the water body in which the toxicants occur. Cairns (1983, 1986a, 1988a, 1988b) therefore suggests that *multispecies* testing would be preferable as it can provide valuable additional evidence for protection of the environment. Multispecies tests and ecosystem tests can also be conducted in the laboratory (Rand & Petrocelli, 1985), however, most of these multispecies tests are bacterial/algal/protozoan systems and may not give an accurate insight into interactions higher up the food chain.

Another limitation of toxicity tests is that the extrapolation from laboratory data to field conditions is difficult, if at all reliable: firstly it is possible that organisms would respond differently in laboratory systems (that are low in environmental realism) and field conditions; and secondly, toxicity tests provide data about the responses of individual organisms, not higher levels of biological organisation, e.g. aquatic communities and ecosystems in complex, highly variable natural systems., (Moriarty, 1983; Cairns, 1988b; Dallas & Day, 1993). In general, field validations of laboratory-derived criteria are rare (Cairns, 1983, 1986a) yet in the absence of other information, the data from these tests are used to set water quality guidelines. In defence of single-species tests, Rand & Petrocelli (1985) reported that many single species tests have yielded results that are well correlated with the observed ecological effects of chemicals.

The general consensus, therefore, appears to be that while toxicity testing is a valuable tool, water quality guidelines should not be based on the results from such tests alone (Dallas & Day, 1993; Cairns, 1988a; b), e.g. research by Hickey (1995) included field monitoring around discharges, experimental manipulation using chemically-dosed sites

and laboratory based toxicity bioassays with effluents and sediments for the assessment of biological toxicity, which would provide numeric guidelines for standards for receiving waters.

The third contentious issue in this study is the use of artificial streams. A substantial amount of toxicity testing has taken place in artificial stream systems of numerous shapes and sizes, so the two fields are inextricably linked. Along with the questions regarding the validity of laboratory-based toxicity testing is the question "How valid is the use of artificial streams in toxicity testing?"

In 1992, a symposium was held at the annual meeting of the North American Benthological Society in Louisville, Kentucky to address the problem that, although there had been increased use of artificial streams in aquatic research over 20 years, this had not been accompanied by a systematic, critical analysis of their advantages and disadvantages. The symposium covered diverse aspects of artificial stream research, including the use of such streams for *ecotoxicological* research. The major conclusions which emerged from the symposium were that: (1) there was no single best design for artificial streams; the stream design depended on the aim of the experiment being conducted; (2) artificial streams were suitable for research geared to mechanistic understanding of lotic processes; and (3) testable hypotheses could be generated and could then be validated in natural stream ecosystems (Lamberti & Steinman, 1993).

Toxicity testing in natural streams is not practical because of the environmental harm caused by the studies themselves. Artificial streams provide greater complexity and realism compared to bioassays so it is assumed that their results would be more reliable predictors of the potential for *in situ* effects (Guckert, 1993). Artificial streams therefore have a role to play in environmental decision-making (Guckert, 1993; Roux, *et al.*, in press). What, then, are the implications of this study for Water Quality Management?

According to the Compilation of Water Quality Guidelines & Standards. (Report No. WQP 4/95. Water Quality Department, Scientific Services, Umgeni Water.), the previous Water Quality Standards in South Africa for chlorine were:

DWAF General and Special Effluent Standards 1984: General - 0.1 mg/ℓ
Special - 0.0 mg/ℓ

An Umgeni Water Standard relevant to this study was the following:
Site Specific Wastewater Standards for chlorine for Works Operated by
Umgeni Water, 1995: Darvill Effluent : 0.1 mg/ℓ
(Most other Umgeni Wastewater standards are 0.3 mg/ℓ)

These were considerably higher values than the Canadian Water Quality Guidelines for Freshwater Aquatic life - 1987 which stipulates 0.002 mg/ℓ for residual chlorine (Umgeni Water, 1995).

According to the draft of the new South African Water Quality Guidelines (DWAF, 1995), the criteria for chlorine in aquatic ecosystems are:

Criteria	**Chlorine concentrations (mg/ℓ)**
Target Water Quality Range (TWQR)	5×10^{-5}
Chronic Effect Value (CEV)	1×10^{-4}
Acute Effect Value (AEV)	1×10^{-3}

The TQWR is a management objective which has been derived from numerical or narrative criteria, and is not a water quality criterion. The DWAF will strive to protect South Africa's water resources by maintaining water quality within the "no effect range" (NER), so the TQWR is set equal to the NER.

The CEV is defined as that concentration or level of a constituent above which there is a significant risk (>5%) of chronic toxic effects on the most sensitive organisms in aquatic ecosystems.

The AEV is defined as that concentration or level of a constituent above which there is a significant risk (> 5%) of acute toxic effects on the most sensitive organisms in aquatic ecosystems.

These values are more stringent than any of the previous standards, and this can only benefit the natural environment.

In this study, the 96 hour LC_{50}'s for *B. harrisoni* from both the Umbilo River and the Westville stream were in the region of 0.004 mg/ℓ, and *B. harrisoni* is not considered to be a sensitive species. Field validation of the toxicity test results indicated that *B. harrisoni* was sensitive to low doses of chlorine as it was not found at sites where a detectable concentration of chlorine was present, while it *was* still found at a site contaminated with unchlorinated, treated sewage effluent. The results of this study therefore support the reduction of the residual chlorine standard to 0.001 mg/ℓ for the AEV. (However, on a technical note, with the experience of the difficulty of measuring very low chlorine concentrations, the TWQR of 5×10^{-5} mg/ℓ, is puzzling. This is a *very* low concentration and the limit of detection for most chlorine determination methods is in the order of 0.01 mg/ℓ or 10 µg/ℓ.)

It is hoped that the information derived from this study will be of use in the setting of Water Quality Guidelines, and that the artificial stream system which was developed will continue to be used for further toxicity testing or other artificial stream research.

COMMENTS AND SUGGESTIONS FOR IMPROVEMENT

The use of *Baetis harrisoni* as a test organism

The mayfly *B. harrisoni,* turned out to be a most suitable test organism in this study. In this artificial stream system they were easy to find and the dead ones could be seen and removed from the system with little disturbance to other organisms. They appeared to respond well to the test environment, and on several occasions, a 0% mortality was achieved in the control streams.

As the collection method and test procedure became more streamlined, the cumulative mortality results became more consistent, the best being those of **Fig 4.9**.

The suitability of the artificial stream system

The artificial stream system appeared to be most suitable for conducting acute 96 h toxicity tests. However, for future work a number of modifications could be made to streamline the test procedure, and these are outlined below:

Dosing pumps

Although dosing pumps are expensive, if such streams were to be used regularly for conducting toxicity tests, it would be worth the investment. Filling the nine intravenous drip bags was a laborious and time-consuming process (> 1 h) as it took a long time for the litre of solution to run into the bags. In the event of dosing pumps being too expensive, the process could be shortened by the construction of a stand which could support the 9 bags and their funnels while the test solution ran in. The Baxter Control-a-flow Regulators served their purpose, but they, too, required constant monitoring as on one occasion, one of these valves clogged and the solution stopped dripping for a few hours. This is not to say that dosing pumps are trouble-free, but they would probably be an improvement. Ramsay *et al.* (1988) report the use of a peristaltic pump capable of delivering a sodium hypochlorite solution at the rate of between 0.3 and 1.8 mℓ/min. which would have been suitable for this project in which approximately 1 mℓ/min. was dosed.

Killing of survivors

At the end of the 96 h test period, the survivors had to be killed for counting and speciation. Formalin was used as it was a quick method of killing. It would probably have been better to have used either 70% ethanol or hot water (Crass, 1947) because, despite the thorough washing of the streams after a test run, it was not known whether there was any residual contamination.

Measurement of chlorine concentration

One of the principal problems with this series of toxicity tests was difficulty experienced in the determination of the chlorine concentrations below 0.02 mg/ℓ in the streams, with both the Lovibond Comparator and the Palintest Photometer. Mattice *et al.*, (1981) reported a detection limit of 0.01 mg/ℓ using a Wallace and Tiernan amperometric titrator and Cairns *et al.* (1990) report a reading of 0.0063 mg/ℓ with the same instrument. Newman & Perry (1989) used an IBM EC/250 series chlorine analyzer and report readings of 0.01 mg/ℓ TRC with this instrument. There are, therefore alternatives for chlorine measurement, although the more sophisticated the measuring apparatus, the more costly they are. A relatively new instrument on the market is the Hach DR/2000 photometer which may be a suitable alternative.

Comparison of experimental results and proposed AEV (Acute Effect Value)

The AEV for chlorine in the draft of the South African Water Quality Guidelines (DWAF, 1995) has been directly compared with the acute 96 h tolerance results from this study. It is important to note that the AEV is half the FAV (Final Acute Value), which is directly calculated from acute LC_{50} values (DWAF, 1995). The final guideline document has not yet been published so comparisons have been limited to the AEV rather than to the calculation and derivation of this value. The results from this study have been made available to DWAF, and they could be used to recalculate the guideline values.

Other general criticisms of the study

The development of the artificial stream system and the time taken to refine the design meant that once it was running well, time for experimental runs was limited - it would have been interesting to investigate the tolerance of a stream invertebrate less tolerant than *B. harrisoni*.

The move from the Umsunduze to the Umbilo for safety reasons also necessitated a change in direction and interrupted the continuity of field sampling. This however provided the opportunity of comparing two rivers, which has proved an advantage.

The non-chlorinated sewage experiment was interrupted by a technical fault which also interrupted the continuity of the experiment, but the restart again allowed the collection of comparative data.

In all cases continuing either sampling or experimental procedures was frustratingly interrupted but there has been an attempt to turn these to advantage.

SUMMARY

The aims of the study have been met as follows:

1. To develop an appropriate artificial stream system, and use it to establish experimentally the acute toxicity of chlorine to an organism tolerant of sewage effluent.

After several trial designs it became clear that the chemistry of chlorine necessitated a through-flow system. Through access to on-tap raw water at the Process Evaluation Facility, a through-flow system was designed, built and operated.

The baetid mayfly nymph *B. harrisoni* is well known as a organism that is tolerant of organically enriched conditions and was selected as a test organism. Acute 96 h tolerance testing was conducted, and an LC_{50} value in the region of 0.004mg/ℓ established. This value was the same for individuals collected both from a small unimpacted stream, and from the impacted Umbilo River. (Graphical estimates of LC_{50} usually are very close to those obtained by formal probit analysis with a computer, but confidence limits are not obtained by graphical interpolation (APHA, 1992))

2. To use the results to hypothesise in-stream effects of chlorine both in respect of the test organism and the macroinvertebrate community.

From these results it was hypothesised that *B. harrisoni* would not be present immediately downstream of the sewage outfalls in the Umsunduze and Umbilo Rivers;

that there would be a significant change in community structure downstream of the chlorinated outfall; and that community recovery would be linked to dechlorination.

B. harrisoni was indeed absent immediately downstream of the chlorinated effluent; both composition and numbers of organisms changed dramatically downstream of the outfall, and the multivariate analysis linked community structure to absence of chlorinated sewage.

3. To investigate the effects of chlorinated sewage effluent on a riverine macroinvertebrate community in the field, and to attempt to distinguish the effects of chlorine from those of sewage effluent.

The clearest indication of the difference between the effects of chlorinated and unchlorinated sewage come from the grouping of samples (on the basis of taxonomic presence, absence, and abundance) collected from sites exposed to unchlorinated sewage with those from upstream of the effluent outfall, in the multivariate analysis. The experimental flow of unchlorinated sewage may not have run for sufficiently long to draw conclusions, but the indication is that chlorination has a much more dramatic impact on invertebrate communities than treated sewage effluent.

4. To use the tolerance data and the field study to assess the newly developed environmental water quality guideline for chlorine (DWAF, 1995).

It would appear from the results that the target water quality range for chlorine of 5×10^{-5} mg/ℓ in the DWAF (1995) guidelines for aquatic ecosystems would be conservative of diversity and ecosystem function. It would however be impossible to measure to that degree of accuracy. No chronic tests were done so it is difficult to comment on the Chronic Effect Value, but the Acute Effect Value, calculated using inadequate data and a safety factor (0.001 mg/ℓ), was the same order of magnitude as the experimental results (0.004 mg/ℓ).

This experimental approach has been successful, and these sort of data should prove useful in the development and refinement of environmental water quality guidelines.

APPENDIX A

DPD/LOVIBOND® METHOD FOR CHLORINE (3)

(As per information sheet supplied with Comparator)

PRINCIPLE OF THE METHOD

DPD indicator is specific for free available chlorine at a controlled pH. Subsequent addition of a small amount of potassium iodide immediately causes monochloramine to produce colour. Further addition of excess potassium iodide causes a rapid response from dichloramine. Interference from copper and dissolved oxygen is prevented by the use of EDTA which is incorporated in the tablet reagents.

TECHNIQUE

LOVIBOND COMPARATOR, USING DISCS 3/40 A AND 3/40 B

FOR FREE CHLORINE

1. Place a 13.5 mm/10 ml moulded cell, containing the sample, in the left hand compartment of the Comparator.
2. Rinse out another cell with sample and leave a few drops in the bottom.
3. Add to this cell a DPD No.1 tablet and crush with a stirring rod.
4. Make up the volume to 10 ml with sample, mix and place the cell in the right hand compartment of the Comparator.
5. Hold the Comparator against a source of white light and rotate the disc until a colour match is obtained. Match at once. Record this reading as free chlorine.

FOR DIFFERENTIAL ESTIMATION OF FREE AND COMBINED CHLORINE

1. Determine free chlorine as above.
2. After recording the disc reading, add a DPD No.3 tablet to the coloured liquid in the right hand cell and mix to dissolve. Allow to stand for two minutes.
3. Rotate the disc and match the colours again. This second reading represents total residual chlorine. By deducting the first reading (free chlorine), the combined chlorine value is obtained.

LOVIBOND COMPARATOR USING DISC 3/40 E

For the measurement of lower chlorine concentrations:

The same general procedure is followed as above but using 40 mm/20 ml cells and two of each tablet instead of one.

APPENDIX B

ANALYSIS OF WATER USED IN THE STUDY

(AS SUPPLIED BY UMGENI WATER ANNUAL REPORT, 1995)

CHEMICAL AND PHYSICAL CHARACTERISTICS OF WATER (cont)

TABLE 20
WIGGINS WATERWORKS

	UNITS	RAW Max.	RAW Min.	RAW Average	FINAL Max.	FINAL Min.	FINAL Average
Chlorophyll 'A'	µg/ℓ	7,17	0,04	1,46	-	-	-
Free Chlorine	mg/ℓ	-	-	-	1,80	0,10	0,95
Total Chlorine	mg/ℓ	-	-	-	2,00	0,30	1,19
pH		8,40	7,10	-	8,10	7,60	-
Colour	'H	29,50	2,00	7,62	3,97	1,00	1,29
Turbidity	NTU	94,20	1,26	19,62	3,18	0,01	0,16
Conductivity	mS/m	32,90	9,43	23,62	33,60	7,48	26,86
Total Aluminium	µg/ℓ	606,00	10,00	161,63	107,00	10,00	35,13
Aluminium S	µg/ℓ	-	-	-	10,70	10,00	10,04
Alkalinity	mg/ℓ $CaCO_3$	74,70	32,10	58,32	70,60	41,50	63,39
Total Hardness	mg/ℓ $CaCO_3$	69,37	40,22	56,44	71,50	62,67	66,70
Calcium	mg/ℓ	15,30	8,24	11,74	16,20	11,80	14,36
Magnesium	mg/ℓ	7,82	4,58	6,50	8,30	6,79	7,39
Sodium	mg/ℓ	30,10	13,00	25,42	31,80	27,50	29,59
Potassium	mg/ℓ	3,77	2,14	3,09	3,78	2,80	3,41
Iron	mg/ℓ	1,79	0,05	0,69	0,06	0,02	0,02
Manganese	mg/ℓ	0,31	0,01	0,11	0,06	0,01	0,01
Silica	mg/ℓ	4,80	0,50	2,70	5,60	0,40	2,26
Nitrate	mg N/ℓ	0,77	0,05	0,40	1,57	0,05	0,46
Nitrite	mg N/ℓ	0,05	0,05	0,05	0,05	0,05	0,05
Chloride	mg/ℓ	38,60	15,30	31,24	45,80	35,10	39,00
Fluoride	µg/ℓ	75,00	75,00	75,00	106,00	75,00	78,68
Sulphate	mg/ℓ as SO_4	18,30	8,55	13,78	19,60	11,50	16,18
Totally Dissolved Solids	mg/ℓ	186,00	82,10	133,70	181,50	53,10	146,39
Suspended Solids	mg/ℓ	49,50	4,00	17,18	-	-	-
Total Organic Carbon	mg/ℓ as C	5,81	2,19	3,50	4,94	0,85	3,07
Total Trihalomethanes	µg/ℓ	4,44	0,8	1,26	76,30	32,90	48,22

TABLE 19
DURBAN HEIGHTS WATERWORKS

	UNITS	RAW Max.	RAW Min.	RAW Average	FINAL Max.	FINAL Min.	FINAL Average
Chlorophyll 'A'	µg/ℓ	7,45	0,29	1,77	-	-	-
Free Chlorine	mg/ℓ	-	-	-	1,20	0,70	0,99
Total Chlorine	mg/ℓ	-	-	-	1,50	0,90	1,20
pH		8,40	7,10	-	8,30	7,60	-
Colour	'H	39,9	5,85	11,99	4,41	1,00	1,20
Turbidity	NTU	99,60	7,26	24,72	0,45	0,01	0,14
Conductivity	mS/m	20,1	8,86	14,38	27,50	9,89	16,19
Total Aluminium	µg/ℓ	893,00	44,00	216,18	145,00	18,00	46,00
Aluminium S	µg/ℓ	-	-	-	24,00	10,00	11,05
Alkalinity	mg/ℓ $CaCO_3$	47,60	29,00	39,16	49,70	32,60	41,20
Total Hardness	mg/ℓ $CaCO_3$	45,61	32,34	39,61	50,75	38,49	45,09
Calcium	mg/ℓ	9,16	6,17	7,91	11,5	8,53	10,02
Magnesium	mg/ℓ	5,50	4,06	4,76	5,64	4,12	4,81
Sodium	mg/ℓ	16,50	8,16	12,63	17,80	9,68	14,01
Potassium	mg/ℓ	2,54	2,03	2,27	2,63	1,82	2,28
Iron	mg/ℓ	1,46	0,37	0,69	0,09	0,02	0,02
Manganese	mg/ℓ	0,15	0,01	0,05	0,03	0,01	0,01
Silica	mg/ℓ	7,00	2,40	3,85	3,20	3,20	3,20
Nitrate	mg N/ℓ	0,59	0,10	0,30	0,67	0,23	0,38
Nitrite	mg N/ℓ	0,05	0,05	0,05	0,05	0,05	0,05
Chloride	mg/ℓ	22,70	10,60	15,42	27,00	17,40	20,75
Fluoride	µg/ℓ	92,00	75,00	76,42	80,80	75,00	75,48
Sulphate	mg/ℓ as SO_4	10,10	5,10	7,67	10,40	5,88	8,36
Totally Dissolved Solids	mg/ℓ	108,20	35,20	76,02	129,60	48,80	94,08
Suspended Solids	mg/ℓ	58,60	4,00	18,94	-	-	-
Total Organic Carbon	mg/ℓ as C	5,70	1,67	3,20	3,76	1,31	2,53
Total Trihalomethanes	µg/ℓ	4,76	0,80	1,69	84,00	46,30	63,45

APPENDIX C

COMPARISON OF CHLORINE CONCENTRATIONS DETERMINED USING A LOVIBOND COMPARATOR AND A PALINTEST PHOTOMETER.

Volume NaOCl added to 1 ℓ distilled water		Lovibond Comparator		Palintest photometer	
		Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)	Free chlorine (mg/ℓ)	Total chlorine (mg/ℓ)
1 mℓ	Distilled water	0.8	0.8	0.82	0.82
		0.7	0.7	0.75	0.75
		0.8	0.8	0.80	0.82
	Raw water	0.5	0.7	0.57	0.77
		0.5	0.8	0.57	0.80
		0.5	0.7	0.57	0.79
2 mℓ	Distilled water	1.5	1.5	1.54	1.55
		1.5	1.5	1.45	1.47
		1.5	1.5	1.52	1.53
	Raw water	0.9	1.5	0.87	1.56
		1.0	1.5	0.92	1.54
		1.0	1.5	0.97	1.58
3 mℓ	Distilled water	2.5	2.5	2.43	2.44
		2.5	2.5	2.40	2.37
		2.5	2.5	2.42	2.43
	Raw water	1.0	2.5	1.01	2.43
		1.0	2.4	1.02	2.52
		1.0	2.5	1.03	2.48

APPENDIX D

WIGGINS BLEACH ANALYTICAL TEST PROCEDURE

TEST No 007/1 **To determine available chlorine in sodium hypochlorite.**

SOLUTIONS REQUIRED :

1. 16% potassium iodide (KI) [16g KI in 100mℓ solution]

2. 20% acetic acid [20 mℓ in 100 mℓ solution]

3. 0.1 M sodium thiosulphate [24.8g in 1000 mℓ solution]

METHOD

1. Pipette 1 mℓ sample of sodium hypochlorite into a conical flask.

2. Add 10 mℓ potassium iodide solution.

3. Add 10 mℓ acetic acid solution.

4. Titrate with sodium thiosulphate solution until clear.
(Starch solution may be added to see end point more clearly)

5. Sample calculation:

Available chlorine:

$$\frac{\text{Vol. thiosulphate x molarity x 35.45}}{\text{Vol. of hypochlorite}}$$

$$\frac{10{,}9\ \text{mℓ} \times 0{,}1\ \text{M} \times 35{,}45}{1\ \text{mℓ}}$$

38,64 mg/ℓ or ppm Cl_2

Calculation for standardising chlorine for temporary standards.

This was the method used to determine the concentration of the stock solution of sodium hypochlorite which was used in drip bags to dose the artificial streams. Once this concentration was determined, the theoretical concentration of chlorine in the streams could be calculated using the dilution factor.

$$\text{mg Cl as } Cl_2/m\ell = \frac{\text{Volume of thiosulphate x molarity x 35.45}}{\text{Volume of hypochlorite}}$$

$$= \frac{10.5\ m\ell \times 0.1\ M \times 35.45}{1.0\ m\ell}$$

$$= 37.22\ \text{mg}/m\ell$$

i.e $37.22 \times 1000 = 37\,220\ \text{mg}/\ell$

For the Toxicity Tests, one mℓ (or multiples thereof) of this stock solution would have been diluted to 1 ℓ with distilled water so the concentration in the drip bag would be 37.22 mg/ℓ.

The chlorine solution entered the raw water in the streams at 15 drops per minute, during which time the pumps delivered 5.83 ℓ of water. One mℓ of solution was equivalent to 17 drops, so 15 drops would be 0.88 mℓ. The 15 drops would contain

$$\frac{0.88 \times 37.22}{1000}$$

$$= 0.0328\ \text{mg of chlorine}$$

This would be diluted with 5.83 ℓ of water, so the concentration should be

$$\frac{0.0328}{5.83}$$

$$= \mathbf{0.0056\ mg}/\ell$$

APPENDIX E

TOXICITY TEST DATA SHEET

This is the data sheet which was drawn up to record the determinands and mortalities during the toxicity tests.

TOXICITY TESTS ***Drip rate :*** 15/min Umbilo ***Date :***

Stream No	Chlorine Conc. Vol Jik per litre	Chlorine Conc. ppm	pH	Cond. µS/cm	D.O.	Temp °C	Load	0 hrs	24hrs	32 hrs	48 hrs	56 hrs	72 hrs	96 hrs D	96 hrs A
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															

APPENDIX F

ONE WAY ANALYSIS OF VARIANCE FOR COMPARISONS AMONG REPLICATES

This was done to determine whether the results of the three replicates of each chlorine concentration were significantly different.

Means plot: LSD Confidence level: 95 % Range test: LSD

Analysis of variance

Data:	Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig.level
arcsin						
9MC1	Between groups	0.0029789	2	0.0014894	0.088	0.9161
9MC2	Between groups	0.0066775	2	0.003387	0.029	0.9716
9MC3	Between groups	0.025168	2	0.012584	0.022	0.9783
9MC4	Between groups	0.0176993	2	0.0088497	0.155	0.8575
22MC1	Between groups	0.1145686	2	0.0572843	0.832	0.4472
22MC2	Between groups	0.004207	2	0.0021035	0.004	0.9959
22MC3	Between groups	0.053963	2	0.0269817	0.049	0.9519
22MC4	Between groups	0.0501601	2	0.02508	2.576	0.0969
3AC1	Between groups	0.001633	2	0.0008165	0.008	0.9922
3AC2	Between groups	0.02866	2	0.0143299	0.035	0.9655
3AC3	Between groups	0.053565	2	0.0267827	0.051	0.9503
3AC4	Between groups	0.0012203	2	0.0006101	0.5	0.6115
7JC1	Between groups	0.0119652	2	0.0059826	0.114	0.893
7JC2	Between groups	0.0329083	2	0.0164541	0.093	0.9118
7JC2	Between groups	0.0715721	2	0.035786	0.089	0.915
7JC4	Between groups	0.0290302	2	0.0145151	0.34	0.7154
27JC1	Between groups	0.0046625	2	0.0023312	0.025	0.9751
27JC2	Between groups	0.00272	2	0.0013598	0.003	0.9975
27JC3	Between groups	0.002921	2	0.0014607	0.002	0.9976
27JC4	Between groups	0.008328	2	0.004164	1.763	0.1907

KEY:

9MC1 represents 9 May, concentration 1. Concentrations 1 to 3 were chlorine concentrations and 4 was the control where no chlorine was present.

Similarly, 22M represents 22 May, 3A was 3 August, 7J was 7 June and 27J was 27 July.

APPENDIX G

SUMMARY OF ANOVA AND MULTIPLE RANGE ANALYSES FOR COMPARISONS BETWEEN TREATMENTS (CHLORINE CONCENTRATIONS) AND RIVERS.

The results refer to the following graphs: Fig. 4.3 (9May), Fig.4.4 (22 May), Fig. 4.5 (3 August), Fig. 4.8 (7 June) and Fig. 4.9 (27 July)

Printouts of the statistical analyses are shown on pages 205 and 206 (No. 1 - 5). No. 6 shows the comparison of the results from the two rivers.

Means plots of the statistical analyses are shown on page 207.. An overlap of the I-Beams indicates no significant difference between treatments/rivers.

Fig 4.3 Treatments:

1	0.5 ml sodium hypochlorite	The only difference which was not significant
2	2.0 ml sodium hypochlorite	was that between treatments 1 and 4.
3	5.0 ml sodium hypochlorite	
4	0.0 ml sodium hypochlorite (Control)	

Fig 4.4 Treatments:

1	1.0 ml sodium hypochlorite	The only difference which was not significant
2	3.0 ml sodium hypochlorite	was that between treatments 2 and 3.
3	4.0 ml sodium hypochlorite	
4	0.0 ml sodium hypochlorite (Control)	

Fig 4.5 Treatments:

1	1.0 ml sodium hypochlorite	All differences were significant.
2	1.5 ml sodium hypochlorite	
3	2.2 ml sodium hypochlorite	
4	0.0 ml sodium hypochlorite (Control)	

Fig 4.8 Treatments:

1	1.0 ml sodium hypochlorite	The only difference which was not significant
2	2.0 ml sodium hypochlorite	was that between treatments 1 and 4
3	3.0 ml sodium hypochlorite	
4	0.0 ml sodium hypochlorite (Control)	

Fig 4.9 Treatments:

1	1.0 ml sodium hypochlorite	All differences were significant.
2	2.0 ml sodium hypochlorite	
3	3.0 ml sodium hypochlorite	
4	0.0 ml sodium hypochlorite (Control)	

1

Analysis of Variance for SG9MAY.arcsin - Type III Sums of Squares

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. lev
MAIN EFFECTS					
A:SG9MAY.time	10.793187	8	1.3491484	259.755	.0000
B:SG9MAY.conc	8.082641	3	2.6942137	518.724	.0000
INTERACTIONS					
AB	7.1764444	24	.2990185	57.571	.0000
RESIDUAL	.3739628	72	.0051939		
TOTAL (CORRECTED)	26.426235	107			

0 missing values have been excluded.
All F-ratios are based on the residual mean square error.

Multiple range analysis for SG9MAY.arcsin by SG9MAY.conc

Method: 95 Percent Tukey HSD

Level	Count	LS Mean	Homogeneous Groups
1	27	.0703274	X
4	27	.1122199	X
2	27	.2478144	X
3	27	.7567977	X

contrast	difference	+/-	limits	
1 - 2	-0.17749		0.05159	*
1 - 3	-0.68647		0.05159	*
1 - 4	-0.04189		0.05159	
2 - 3	-0.50898		0.05159	*
2 - 4	0.13559		0.05159	*
3 - 4	0.64458		0.05159	*

* denotes a statistically significant difference.

2

Analysis of Variance for SG22MAY.arcsin - Type III Sums of Squares

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. lev
MAIN EFFECTS					
A:SG22MAY.time	18.301571	8	2.2876963	229.711	.0000
B:SG22MAY.conc	7.044159	3	2.3480528	235.771	.0000
INTERACTIONS					
AB	8.3438306	24	.3476596	34.909	.0000
RESIDUAL	.7170509	72	.0099590		
TOTAL (CORRECTED)	34.406611	107			

0 missing values have been excluded.
All F-ratios are based on the residual mean square error.

Multiple range analysis for SG22MAY.arcsin by SG22MAY.conc

Method: 95 Percent Tukey HSD

Level	Count	LS Mean	Homogeneous Groups
4	27	.0424585	X
1	27	.2212795	X
2	27	.6160937	X
3	27	.6369642	X

contrast	difference	+/-	limits	
1 - 2	-0.39481		0.07144	*
1 - 3	-0.41568		0.07144	*
1 - 4	0.17882		0.07144	*
2 - 3	-0.02087		0.07144	
2 - 4	0.57364		0.07144	*
3 - 4	0.59451		0.07144	*

* denotes a statistically significant difference.

3

Analysis of Variance for SG3AUGUS.arcsin - Type III Sums of Squares

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. lev
MAIN EFFECTS					
A:SG3AUGUS.time	20.970209	10	2.0970209	617.857	.0000
B:SG3AUGUS.conc	10.039783	3	3.3465943	986.026	.0000
INTERACTIONS					
AB	9.9495643	30	.3316521	97.717	.0000
RESIDUAL	.2986740	88	.0033940		
TOTAL (CORRECTED)	41.258230	131			

0 missing values have been excluded.
All F-ratios are based on the residual mean square error.

Multiple range analysis for SG3AUGUS.arcsin by SG3AUGUS.conc

Method: 95 Percent Tukey HSD

Level	Count	LS Mean	Homogeneous Groups
4	33	.0085998	X
1	33	.2616881	X
2	33	.5764612	X
3	33	.7179383	X

contrast	difference	+/-	limits	
1 - 2	-0.31477		0.03756	*
1 - 3	-0.45625		0.03756	*
1 - 4	0.25309		0.03756	*
2 - 3	-0.14148		0.03756	*
2 - 4	0.56786		0.03756	*
3 - 4	0.70934		0.03756	*

* denotes a statistically significant difference.

4

Analysis of Variance for SG7JUNE.arcsin - Type III Sums of Squares

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. lev
MAIN EFFECTS					
A:SG7JUNE.time	12.767146	8	1.5958932	243.435	.0000
B:SG7JUNE.conc	2.276797	3	.7589324	115.766	.0000
INTERACTIONS					
AB	3.0889790	24	.1287075	19.633	.0000
RESIDUAL	.4720122	72	.0065557		
TOTAL (CORRECTED)	18.604934	107			

0 missing values have been excluded.
All F-ratios are based on the residual mean square error.

Multiple range analysis for SG7JUNE.arcsin by SG7JUNE.conc

Method: 95 Percent Tukey HSD

Level	Count	LS Mean	Homogeneous Groups
4	27	.1583073	X
1	27	.1586073	X
2	27	.3149581	X
3	27	.5117276	X

contrast	difference	+/-	limits	
1 - 2	-0.15635		0.05796	*
1 - 3	-0.35312		0.05796	*
1 - 4	0.00030		0.05796	
2 - 3	-0.19677		0.05796	*
2 - 4	0.15665		0.05796	*
3 - 4	0.35342		0.05796	*

* denotes a statistically significant difference.

5

Analysis of Variance for SG27JULY.arcsin - Type III Sums of Squares

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. lev
MAIN EFFECTS					
A:SG27JULY.time	21.443569	9	2.3826188	1966.438	.0000
B:SG27JULY.conc	10.911567	3	3.6371889	3001.867	.0000
INTERACTIONS					
AB	12.032283	27	.4456401	367.798	.0000
RESIDUAL	.0969314	80	.0012116		
TOTAL (CORRECTED)	44.484351	119			

0 missing values have been excluded.
All F-ratios are based on the residual mean square error.

Multiple range analysis for SG27JULY.arcsin by SG27JULY.conc

Method: 95 Percent Tukey HSD

Level	Count	LS Mean	Homogeneous Groups
4	30	.0219124	X
1	30	.1810088	X
2	30	.6541738	X
3	30	.7290219	X

contrast	difference	+/-	limits	
1 - 2	-0.47316		0.02358	*
1 - 3	-0.54801		0.02358	*
1 - 4	0.15910		0.02358	*
2 - 3	-0.07485		0.02358	*
2 - 4	0.63226		0.02358	*
3 - 4	0.70711		0.02358	*

* denotes a statistically significant difference.

6

Multiple range analysis for RIVERS.arcsin by RIVERS.conc

Method: 95 Percent Tukey HSD

Level	Count	LS Mean	Homogeneous Groups
3	60	.0156861	X
1	60	.2052716	X
2	60	.6481430	X

contrast	difference	+/-	limits	
1 - 2	-0.44287		0.02002	*
1 - 3	0.18959		0.02002	*
2 - 3	0.63246		0.02002	*

* denotes a statistically significant difference.

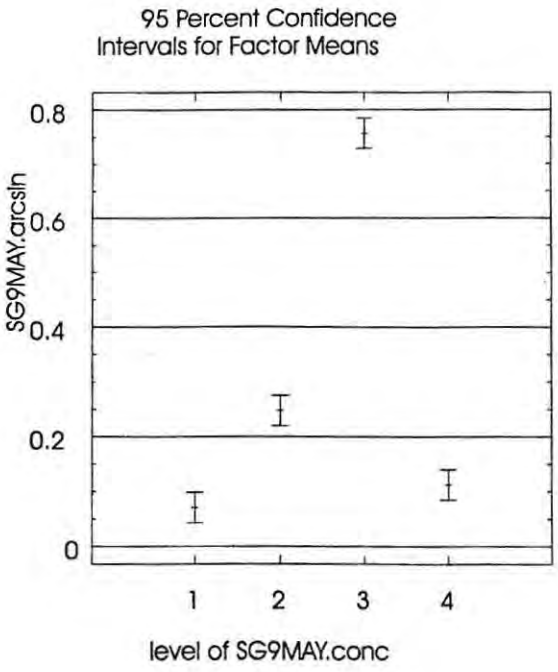
Multiple range analysis for RIVERS.arcsin by RIVERS.river

Method: 95 Percent Tukey HSD

Level	Count	LS Mean	Homogeneous Groups
2	90	.2856983	X
1	90	.2937022	X

contrast	difference	+/-	limits
1 - 2	0.00800		0.01364

* denotes a statistically significant difference.


95 Percent Confidence
Intervals for Factor Means
SG9MAY.arcsin
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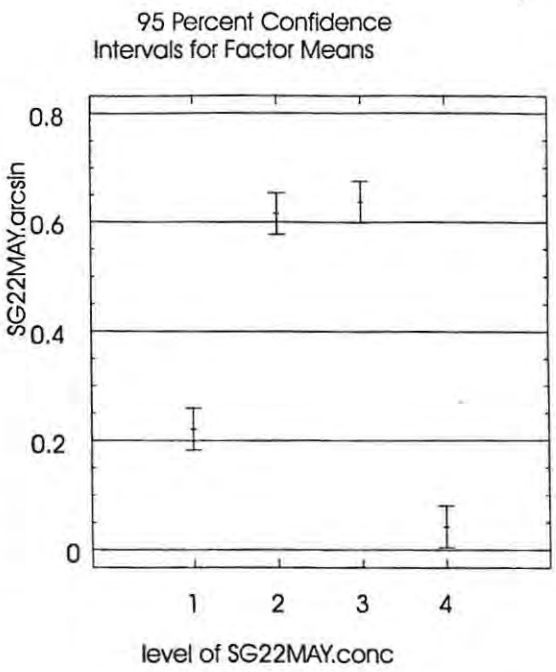
0.6
0.4
0.2
0
1
2
3
4
level of SG9MAY.conc


95 Percent Confidence
Intervals for Factor Means
SG22MAY.arcsin
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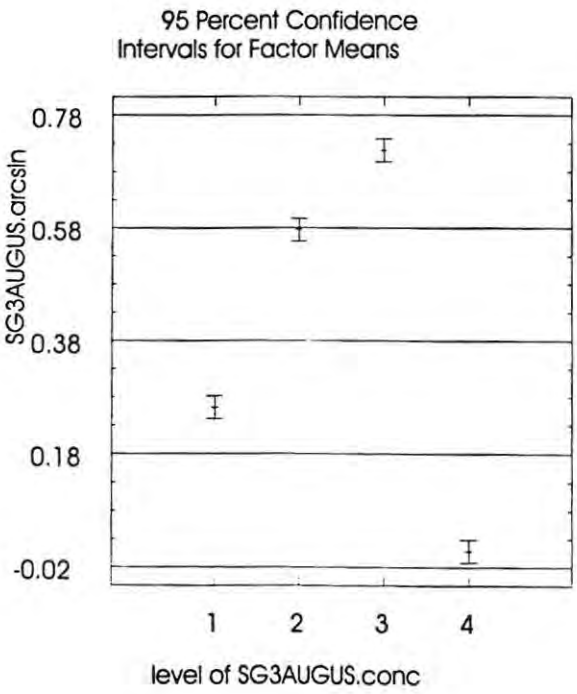
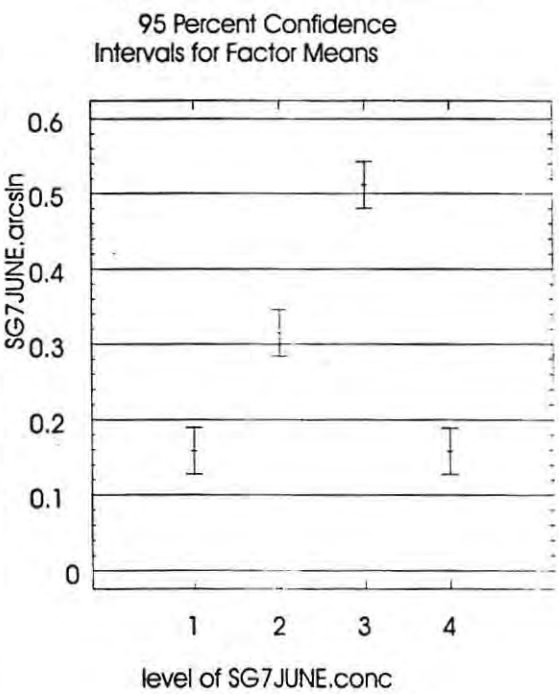
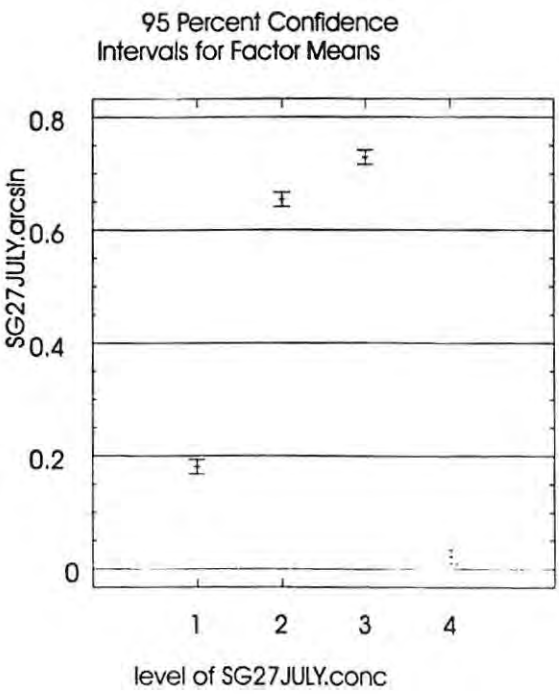
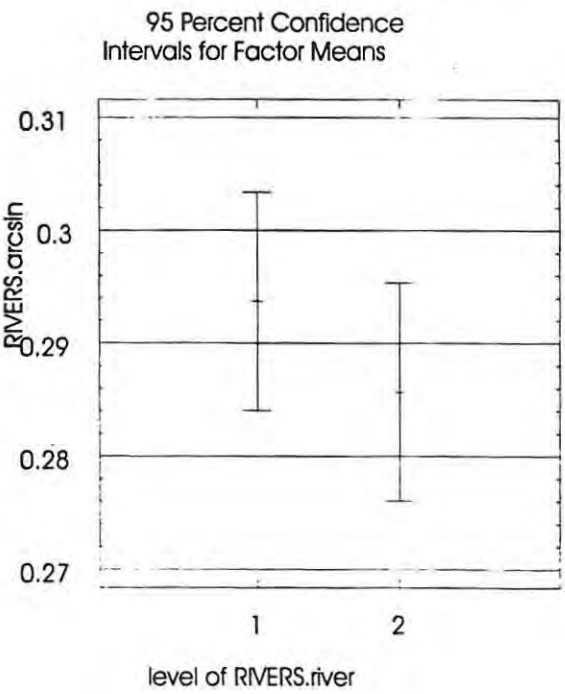
0.6
0.4
0.2
0
1
2
3
4
level of SG22MAY.conc


95 Percent Confidence
Intervals for Factor Means
SG3AUGUS.arcsin
0.78
0.58
0.38
0.18
-0.02
1
2
3
4
level of SG3AUGUS.conc


95 Percent Confidence
Intervals for Factor Means
SG7JUNE.arcsin
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
level of SG7JUNE.conc


95 Percent Confidence
Intervals for Factor Means
SG27JULY.arcsin
0.8
0.6
0.4
0.2
0
1
2
3
4
level of SG27JULY.conc


95 Percent Confidence
Intervals for Factor Means
RIVERS.arcsin
0.31
0.3
0.29
0.28
0.27
1
2
level of RIVERS.river

APPENDIX H

TWO SAMPLE ANALYSIS FOR OCCURRENCE OF *B. HARRISONI* AT SITES 2 AND 3 IN THE UMBILO RIVER FOLLOWING EXPOSURE TO UNCHLORINATED EFFLUENT

The numbers of *Baetis harrisoni* (in 3 samples) at Site 2 was compared to those at Site 3 after 1, 2 and 6 weeks of exposure to unchlorinated effluent in the Umbilo River.

After 1 and 2 weeks, the differences between the numbers found at Sites 2 and 3 were found to be not significant at the 5% level (p-values were 0.026994 and 0.00769943 respectively: i.e. they were < 0.05.

After 6 weeks, the difference was found to be significant (p-value 0.119365) which is > 0.05.

KEY:

U1	(Upstream 1)	Site 2 after 1 week
D1	(Downstream 1)	Site 3 after 1 week
U2	(Upstream 2)	Site 2 after 2 weeks
D2	(Downstream 2)	Site 3 after 2 weeks
U6	(Upstream 1)	Site 2 after 6 weeks
D6	(Downstream 1)	Site 3 after 6 weeks

Two-Sample Analysis Results

		DATAA.U1	DATAA.D1	Pooled
Sample Statistics:	Number of Obs.	3	3	6
	Average	102.333	18.6667	60.5
	Variance	2494.33	382.333	1438.33
	Std. Deviation	49.9433	19.5533	37.9254
	Median	74	17	56

Difference between Means = 83.6667

		95 Percent	
Conf. Interval For Diff. in Means:			
(Equal Vars.)	Sample 1 - Sample 2	-2.33903 169.672	4 D.F.
(Unequal Vars.)	Sample 1 - Sample 2	-24.0659 191.399	2.6 D.F.

Ratio of Variances = 6.52398

Conf. Interval for Ratio of Variances: 0 Percent
Sample 1 + Sample 2

Hypothesis Test for H0: Diff = 0 — Computed t statistic = 2.70189
vs Alt: GT — Sig. Level = 0.0269941
at Alpha = 0.05 — so reject H0.

Two-Sample Analysis Results

		DATAA.U2	DATAA.D2	Pooled
Sample Statistics:	Number of Obs.	3	3	6
	Average	212	80.6667	146.333
	Variance	2308	837.333	1572.67
	Std. Deviation	48.0416	28.9367	39.6569
	Median	196	66	144

Difference between Means = 131.333

		95 Percent	
Conf. Interval For Diff. in Means:			
(Equal Vars.)	Sample 1 - Sample 2	41.401 221.266	4 D.F.
(Unequal Vars.)	Sample 1 - Sample 2	33.1247 229.542	3.3 D.F.

Ratio of Variances = 2.75637

Conf. Interval for Ratio of Variances: 0 Percent
Sample 1 + Sample 2

Hypothesis Test for H0: Diff = 0 — Computed t statistic = 4.05604
vs Alt: GT — Sig. Level = 0.00769943
at Alpha = 0.05 — so reject H0.

Two-Sample Analysis Results

		DATAA.U6	DATAA.D6	Pooled
Sample Statistics:	Number of Obs.	3	3	6
	Average	20.6667	9.66667	15.1667
	Variance	137.333	52.3333	94.8333
	Std. Deviation	11.7189	7.23418	9.73824
	Median	16	6	14

Difference between Means = 11

		95 Percent	
Conf. Interval For Diff. in Means:			
(Equal Vars.)	Sample 1 - Sample 2	-11.084 33.084	4 D.F.
(Unequal Vars.)	Sample 1 - Sample 2	-12.9398 34.9398	3.3 D.F.

Ratio of Variances = 2.6242

Conf. Interval for Ratio of Variances: 0 Percent
Sample 1 + Sample 2

Hypothesis Test for H0: Diff = 0 — Computed t statistic = 1.38343
vs Alt: GT — Sig. Level = 0.119365
at Alpha = 0.05 — so do not reject H0.

APPENDIX I

LIST OF ORGANISMS IDENTIFIED IN SAMPLES FROM THE UMSUNDUZE AND UMBILO RIVERS AND TWINSPAN CLASSIFICATION DATA

(Names of authors are provided and the codes used in the TWINSPAN analysis are shown alongside)

LIST OF ORGANISMS	TWINSPAN CODES ↓
PHYLUM: ARTHROPODA	
CLASS: INSECTA	
ORDER:EPHEMEROPTERA	
Family: Baetidae	
Baetis harrisoni Barnard	B. harr
Baetis latus Agnew	B. latus
Afroptilum excisum (Barnard)	C. exci
Afroptilum parvum (Crass)	C.parv
Baetid pupae:	Baetidp
Family: Leptophlebiidae	
Adenophlebia sp. Eaton	Adenop
Family: Caenidae	Caenid
ORDER: TRICHOPTERA	
Family: Hydropsychidae	
Cheumatopsyche affra (Mosely)	C. afra
Cheumatopsyche thomasseti (Ulmer)	C. thoma
Family: Hydroptilidae	
Hydroptila sp	Hydrop
Hydroptila pupa	Hydropp
ORDER: DIPTERA	
Family: Blepharoceridae	Bleph
Family: Simuliidae	Simulid
Simulium adersi Pomeroy	S. aders
S. adersi pupae	S. adpup
Simulium bequaerti Gibbons	Bequae
Simulium damnosum Theobold	S. damn
Simulium dentulosum Roubaud	S. dent

Simulium medusaeforme Pomeroy	S. med
Simulium nigritarsis Coquillett	S. nigr
Simulium ruficorne Macquart	S. rufi
Simulium vorax Latrielle	S. vorta
Simulium sp.	Simulls
Family: Chironomidae	
Subfamily: Orthocladiinae	Orthoc
Subfamily: Tanypodinae	Tanypo
Subfamily: Chironominae	
Tribe: Chironomini	Chimini
Chironomus	Chimus
Tribe: Tanytarsini	Tanyta
Family: Ceratopogonidae	Cerato
Family: Tabanidae	Tabanid
Family: Tipulidae	Tipuli
Family: Ephydridae	
Ephydra	Ephydr
Family: Rhagionidae	
Atherix sp.	Anther
ORDER: ODONATA	
Suborder: Zygoptera	Zygopt
Suborder: Anisoptera	Anisop
Family: Aeshnidae	Aeshni
Family: Gomphidae	Gomph
Family: Libellulidae	Libell
ORDER: HEMIPTERA	Hemipt
Family: Naucoridae	Mauro
Family: Pleidae	Pleidae
ORDER: COLLEMBOLA	Collem
ORDER: COLEOPTERA	Coleop
Family: Gyrinidae	
Aulonogyrus	Gyrinus
Family: Psephenidae	Psephe

CLASS: ARACHNOIDEA
SUBCLASS: ARACHNIDA

ORDER: Acarina	
Family: Hydracarinidae/Hydracnellae	Hydrac

CLASS: CRUSTACEA
ORDER: DECAPODA
Family: Potamonidae
Potamon Potam

PHYLUM:MOLLUSCA

CLASS: GASTROPODA
ORDER: PULMONATA
Family: Ancylidae
Burnupia sp. Burnup

PHYLUM: ANNELIDA

CLASS: HIRUDINEA Leech

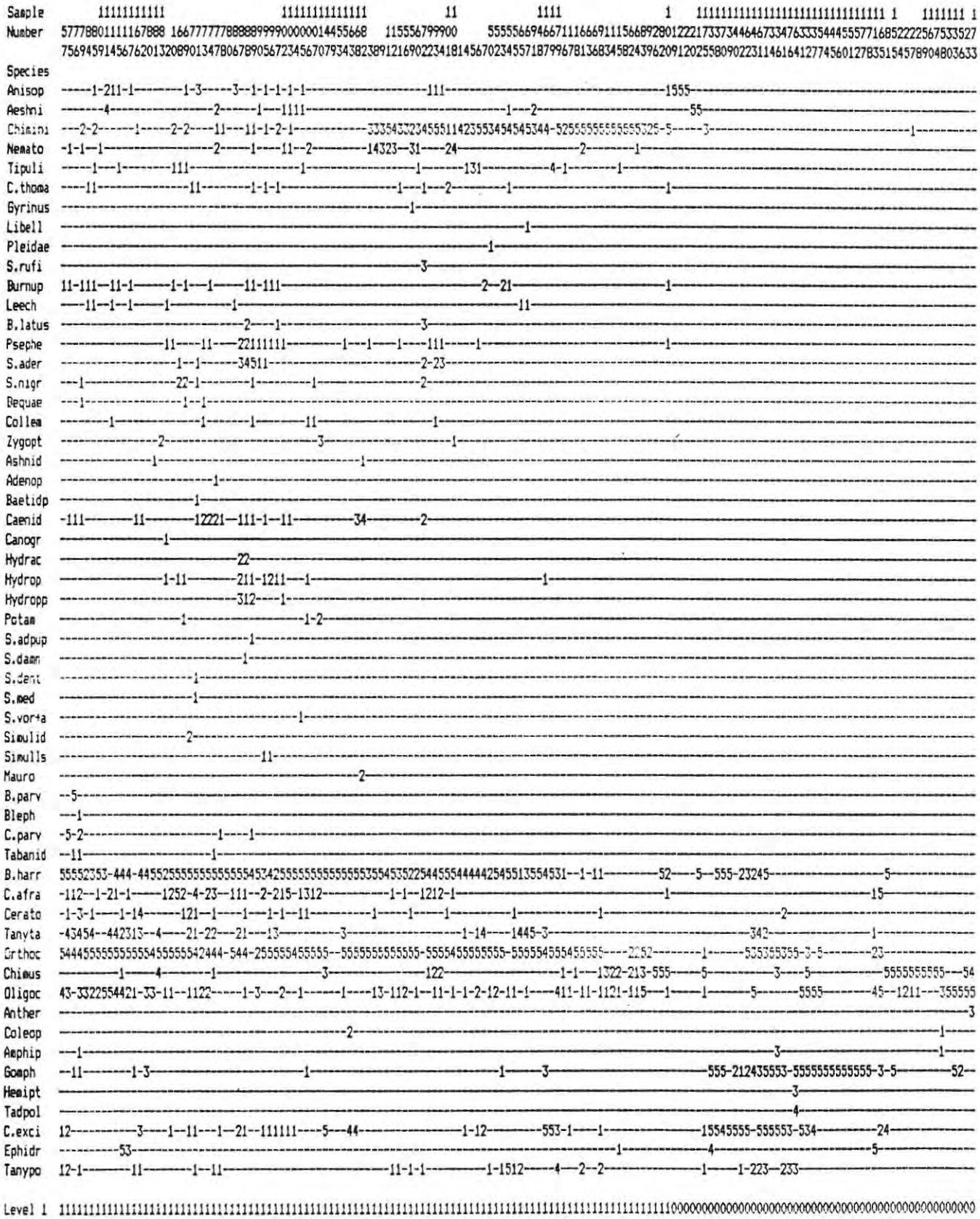
CLASS: OLIGOCHAETA Oligoc

PHYLUM: NEMATODA Nemato

PHYLUM: CHORDATA
SUBPHYLUM: VERTEBRATA

CLASS: AMPHIBIA Amphip/Tadpol
(Tadpole)

TWINSPAN ANALYSIS



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