Development of a Recirculating Artificial Stream System to Investigate the Use of Macro-Invertebrates as Water Quality Indicators

CG Palmer • PA Goetsch • JH O'Keeffe

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DEVELOPMENT OF A RECIRCULATING ARTIFICIAL STREAM SYSTEM TO INVESTIGATE THE USE OF MACRO-INVERTEBRATES AS WATER QUALITY INDICATORS

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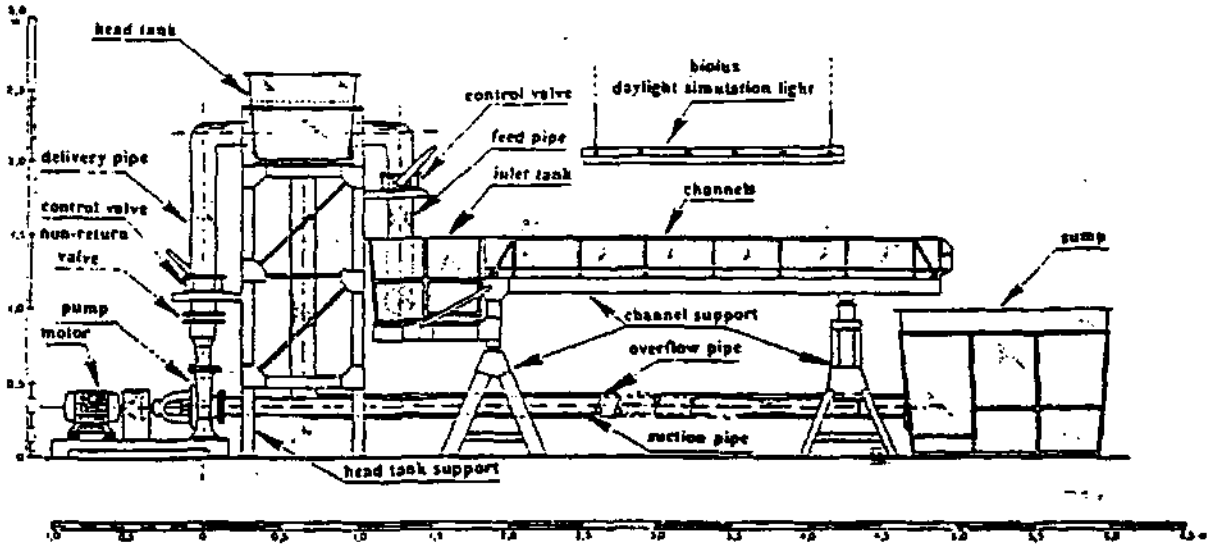

Figure A
AN ELEVATION OF ONE OF THREE 3-CHANNEL ARTIFICIAL STREAM UNITS
Water is pumped at a constant 20 l/sec. through a non-return valve (which prevents back-flow through the pump after switch-off or electrical failure) and a control valve, up the delivery pipe into the head tank. Water flows to the perspex channels through a second control valve, via a perforated diffuser pipe in the inlet tank, which contains a shaped tank-to-channel transition to ensure equal delivery to each channel. If the second control valve is set to deliver less than 20 l/sec. the excess water overtops an internal weir in the head tank, and is returned direct to the sump via the overflow pipe. The suction pipe delivers water from the sump to the pump. The channel gradient can be varied from level to 4% via the adjustable channel support, and a wide range of hydraulic conditions can be achieved by varying discharge and slope.

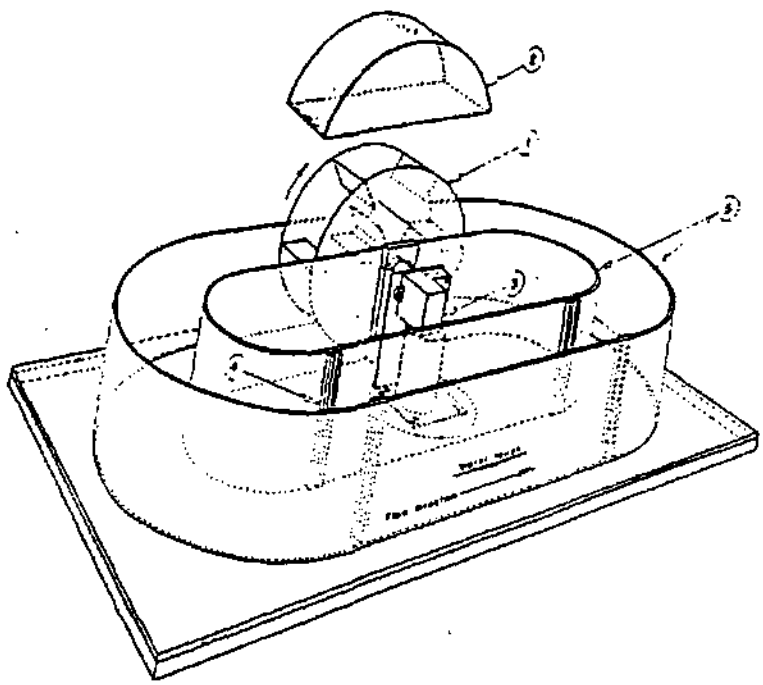

Figure B
SMALL-SCALE RACEWAY
One of the 15 perspex portable raceways used both in the artificial stream laboratory and in the field. 1: paddle wheel, 2: splash hood, 3: windscreen-wiper (DC or AC) motor, 4: slots for nets to contain test animals in the parallel part of the channel, 5: perspex walls.

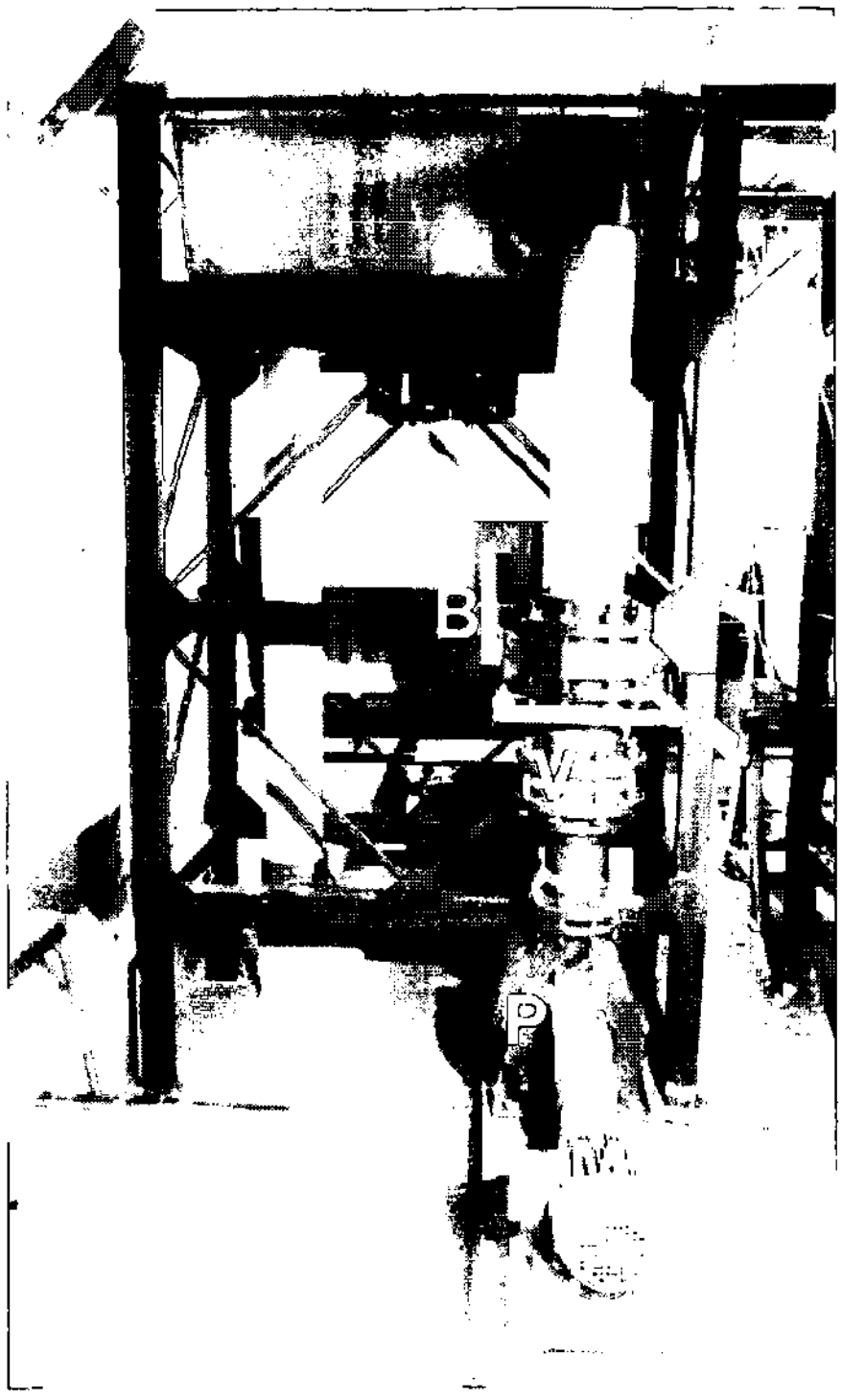

Figure C
PUMP, MOTOR AND DELIVERY PIPEWORK

Pump (P): Sulzer Bros. AZG type horizontal spindle centrifugal pump - with 125mm suction, 100mm delivery and 180mm impeller. The design delivery is 20 l/sec. against a total head of 2.5m.

Motor (M): 1.1kW, 6 pole, 380 volt 3 phase, 950 rpm.

The uPVC delivery pipework is tapered up to accommodate a 150mm disc-type non-return valve (V), and 150mm butterfly control valve (B).

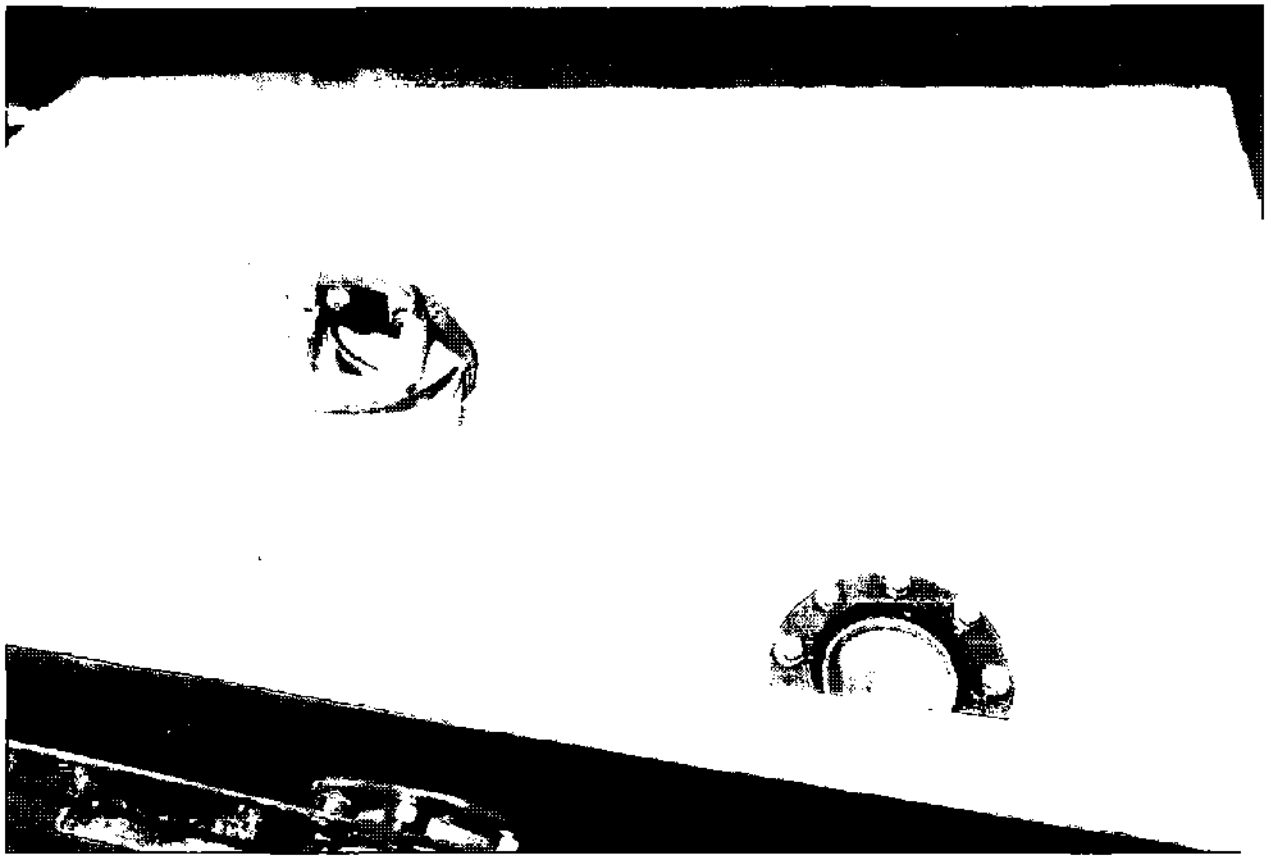

Figure D
HEAD TANK
The internal weir (W) ensures that the pump delivery head stays within acceptable limits. It also helps to smooth out any fluctuations in the delivery rate to the channels caused by unsteady pump delivery rate (due, for instance, to variations in power supply). When the water level is at weir crest level 20 l/sec. is delivered to the channels, but when the flow rate to the channels is reduced, the excess pumped flow spills over the weir and is rejected back to the sump by an overflow pipe. The head tank is fibreglass.

Figure E
CHANNEL FEED PIPEWORK, DIFFUSER AND INLET TANK
A 150mm butterfly valve, calibrated for 5, 10, 15 and 20 l/sec. controls the discharge from the head tank to the channels. A horizontal, perforated uPVC pipe (P) in the fibreglass inlet tank dissipates much of the energy of the incoming flow, and distributes the flow evenly into the tank. A transition section (T) between the tank and the channels ensures equal flows into all three channels, and reduces turbulence in the upstream part of the channels.

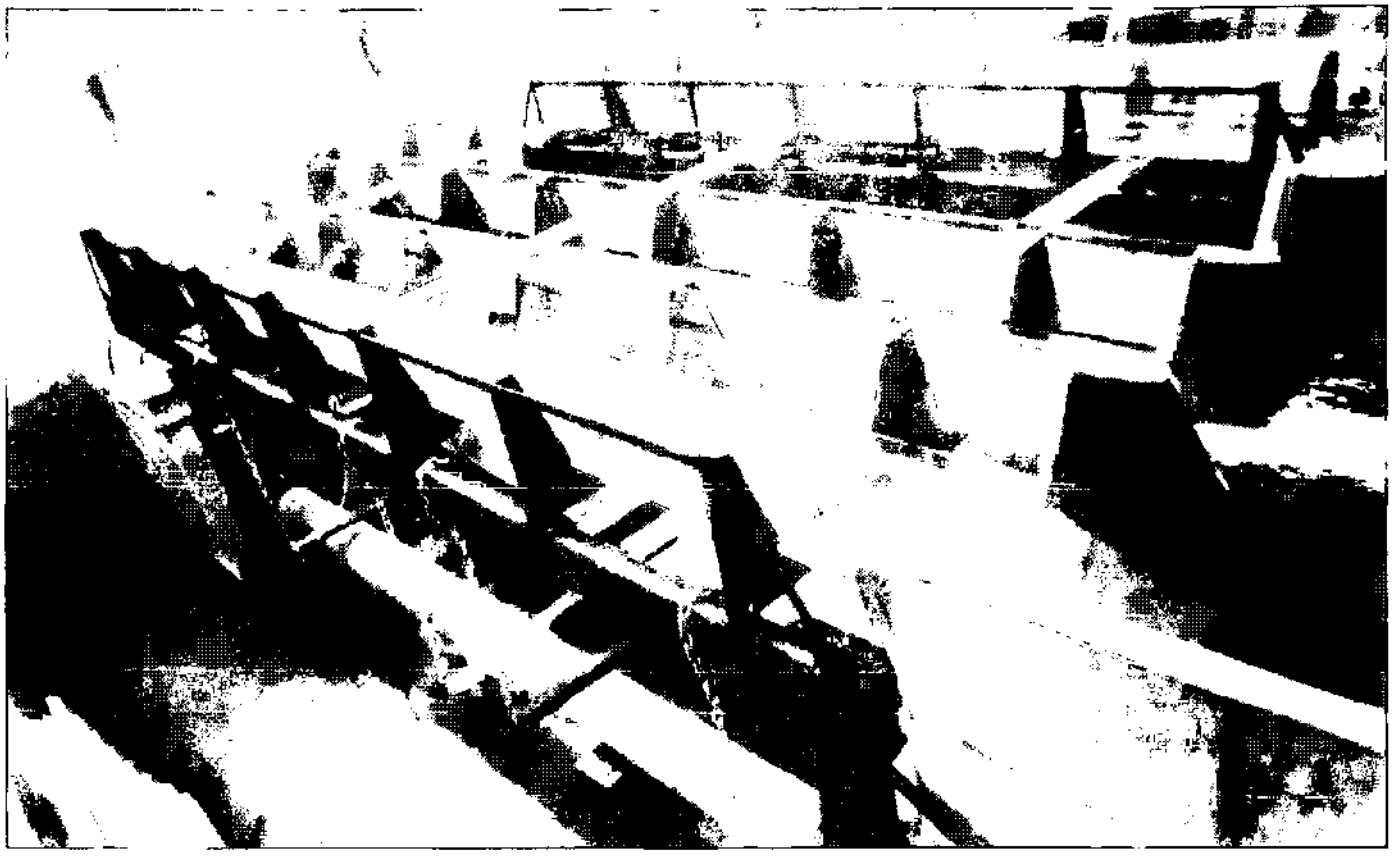

Figure F
CHANNELS
Three identical trapezoidal channels (200mm base width, 200mm deep with 70° side angle, 3.2m long), fabricated in perspex for full visibility into the flow, are provided for replication of conditions with the stream unit.

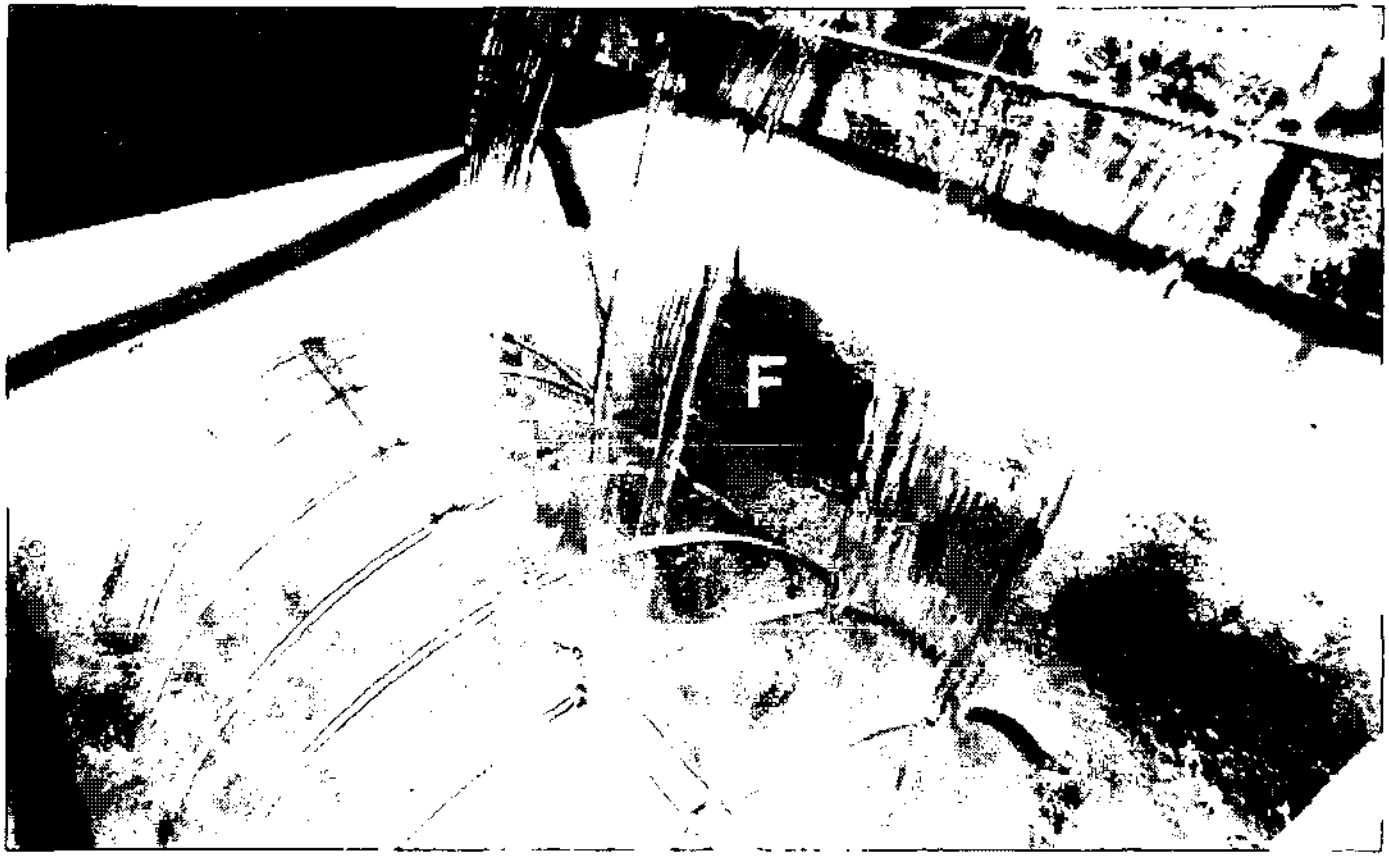

Figure G
SUMP, COOLING COIL AND FLOAT SWITCH
The fibreglass sump has a total capacity of 1580 litres. The stainless steel cooling coil(s) is capable of reducing the temperature of the water to 9°C, and maintaining it at this temperature when water is flowing. The cooling unit is thermostatically controlled. In the event of serious leakage from any component of the stream unit, the float switch (F) will stop the pump motor when the level of water in the sump drops 100mm below its normal operating level, preventing damage to the motor if the pump runs dry.

EXECUTIVE SUMMARY

1. INTRODUCTION AND PROJECT AIMS

The Department of Water Affairs and Forestry (DWAF) faces two major water management issues: water quantity and water quality. Recently, water quality management changed from the uniform effluent standard approach to the Receiving Water Quality Objectives (RWQO) approach, which aims to manage water bodies in a state "fit for use", as defined by five recognised water users: industry, agriculture, domestic supply, recreation and the natural environment. This was followed by the publication of water quality guidelines for all the users except the natural environment. In terms of water quantity it was envisaged that the five users would compete for scarce water resources and each user would have to motivate for its share.

There is a fundamental problem with the concept of the natural environment as a competing user, as it is the resource, and requires assured maintenance before other users can apply for allocations. What is needed is a management approach which formally recognises the aquatic ecosystem as the resource, so both research and management can focus on the maintenance of aquatic systems in a functional state. The need for this approach has been articulated in the recently published leaflet "You and Your Water Rights" calling for public response to water issues (DWAF, 1995).

Research on water quantity for environmental flow requirements for rivers is well advanced. The need for experimental research on the water quality requirements of riverine fauna was highlighted in 1989 at a Kruger National Park Rivers Research Programme (KNPRRP) workshop which was held to try to establish environmental water quality guidelines for the KNP rivers. It became apparent that no data existed on the water quality requirements or tolerances of any indigenous riverine organisms.

There are a variety of data sources for the development of environmental water quality guidelines: using the historical water quality record to calculate, for example, the 90th percentile of naturally occurring concentrations, using the concentrations in water bodies where populations and communities thrive naturally, existing international guidelines, and experimentally determined tolerance values.

The artificial stream project aims to use the experimental method of ecotoxicology to contribute to water quality management, including providing data for the development and refinement of environmental water quality guidelines. In South Africa, riverine macroinvertebrates have most frequently been collected from riffle habitats, which are also the main habitat sampled in bioassessment studies. In order to relate experimental data to field distributions and bioassessment data, the artificial stream system was designed for the use of riffle organisms as test animals. Since these current-dwelling organisms have specific hydraulic habitat requirements, the major specification for an experimental system was for a flowing water system where the range of hydraulic conditions could be accurately described.

The long term aim of this project is:

To use an artificial stream system to measure the tolerances of selected stream macroinvertebrates to a range of environmental variables under controlled conditions, so as to use them as water quality indicators.

The immediate aims of this project were:

1. To synthesise information on the design, construction, operation and research planning of experimental stream systems worldwide.

    * Several designs and uses were seen in England, Australia, Canada and the USA. The literature was reviewed, and is summarised in Chapter 2.

2. To design, plan and build a recirculating artificial stream at Rhodes University, together with smaller scale, portable, mesocosm laboratory systems.

    * The artificial stream laboratory, with large and small-scale models, is fully equipped and functional. The design and potential use are presented in Chapter 2.

3. To establish procedures for the selection and maintenance of stream macroinvertebrates, and to design appropriate experimental procedures.

    * This aim was transferred to the Standard Laboratory Organism project which will end in December 1995. Prof. Jay O'Keeffe and Dr. Carolyn Palmer are project leaders, and the research officer is Mrs. Lil Haigh.

4. To calibrate the experimental system by monitoring physico-chemical changes in channels containing only water, and water with substrate.

    * Hydraulic, temperature and water quality calibrations are complete, and behavioural calibration has been initiated. These are reported in Chapter 3.

5. To conduct experiments on the tolerances of selected species to salinity, initially in the smaller laboratory recirculating systems, and then in the experimental stream.

    * Tolerance experiments have been conducted in the small-scale raceways and are presented in Chapter 4. Tolerance testing in the large systems will commence as soon as behavioural calibration is satisfactory.

6. To initiate a country-wide collaboration between researchers involved in programmes on water quality and assimilative capacity.

    * Collaboration has been extensive and is reported in Chapter 5.

2. DESIGN AND CONSTRUCTION OF AN ARTIFICIAL STREAM LABORATORY

This section has been published:
Palmer C G, Rowlston W S, Jezewski W A and Skotnicky P I (1994) The design and research potential of an artificial stream system for the investigation of macroinvertebrate water quality tolerances. Water SA 20:247-258.

During the course of this project an artificial stream laboratory has been developed at Rhodes University. Design and construction were contributed by the Department of Water Affairs and Forestry. The laboratory provides a controlled environment, with a defined and replicated range of hydraulic conditions, so that experimental research can be conducted using rheophilous organisms. In the first instance, research has focused on the response of riverine macroinvertebrates to changes in water quality. The system is also ideal for the investigation of hydraulic requirements of riverine taxa. In Chapter 2 we briefly review artificial stream designs and provide design details of the artificial stream system which has been built.

One of the most obvious features of natural ecosystems is the complexity of interactive effects between abiotic and biotic variables. In an experimental approach, the general aim is to maintain certain variables constant while investigating the effects of others by altering them within controlled limits. Artificial stream research is based on the further premise that the provision of a flowing water environment is fundamental to any experimental research on riverine organisms.

Stream design involved choices between alternatives, or selection over several gradients: 1) "naturalness" to complete operational control (indoors vs outdoors); 2) large to small; 3) recirculating or through-flow. Each choice has advantages and disadvantages, and decisions depend on the research question. Further considerations are the three major criticisms that have been levelled at artificial stream research: 1) the limited degree to which results are applicable to the natural environment; 2) the physical scale of many artificial streams is too small for natural processes to occur; and 3) they are usually inadequately replicated, so it becomes impossible to assess within-system variability, and to evaluate between-treatment experimental results in comparison.

The artificial stream laboratory was designed with these options and criticisms in mind. We accept that no indoor system can mimic the natural world, but argue that the operational control gained allows valuable opportunities for consideration of both single variables, and complex effluent effects. The system comprises models at two scales. There are three large systems, each with three channels (Figure A), and 15 small-scale models constructed on a raceway design (Figure B). The large 3-channel units are accommodated lengthwise in the laboratory, and three raceways are associated with each unit. This allows each experiment to run at two scales. Single variables can be selected and maintained at different experimental levels at both scales; and portable raceways can subsequently be taken into the field, and the experiment repeated under river-side conditions using river water and freshly collected animals. In this way the small-scale raceways are used for "field verification" of the large-scale channel systems, with laboratory raceways providing a comparison of scale effects.

The hydraulic and structural design of each unit proceeded from the specification of the required flow regime in the channels. So as to be able to work with the whole range of rheophilous organisms, flows from low velocities (0.3 $msec^{-1}$) to flows in excess of 1 $msec^{-1}$ were required: for example the pest species of blackfly *Simulium chutteri* is only found in velocities greater than 1 $msec^{-1}$. From these flow rate requirements, appropriate pipe sizes, tank dimensions and the overall dimensions of the unit were progressively calculated.
The large units recirculate 2000 litres of water, and the raceways 25 litres.

In addition to the hydraulic control offered within each unit, other factors such as temperature, daylength and light can also be controlled in the laboratory. Light is provided by OSRAM "biolux" tubes which provide a full spectrum of wavelengths, similar to sunlight. Intensity is controlled by suspending the lights on chains, so their height above the channels can be adjusted. Lights are on a daylength adjustable timer. Air temperature control is provided by two air conditioners which allow control within 1°C, between 16 and 26°C. Water circulated through the channels can be heated to 26°C, as the pumps heat the water, and cooled to 14°C by epoxy coated copper cooling coils in the sump through which coolant is circulated by a compressor. For any experimental period at a particular temperature setting, water temperature is maintained within a 2°C range.

In experiments where water quality variables are altered, the starting water quality must be specified. There is a range of alternatives. The laboratory is supplied with municipal tap water, there is a 10 000 litre rain tank outside, and local river water can be brought in by water tanker.

3. CALIBRATION OF THE STREAM SYSTEM

The term calibration is used here to describe the process of defining experimental conditions in the artificial stream laboratory. The objective of calibrating the streams was to establish the physical and chemical conditions in the streams before organisms were introduced; then to monitor the behaviour of organisms in the streams before water quality conditions were altered. Chapter 3 deals with hydraulic, temperature, water quality and behavioural calibration.

Hydraulic calibration
The first step was hydraulic calibration. This involved confirming that hydraulic conditions in each of the channels, and in the three systems, were the same for any given discharge and slope setting. Hydraulic conditions were measured for a range of slope, discharge and substrate settings so experimental conditions could be selected and accurately described.

Each of the three 3-channel stream units is designed so that the flow delivered to the feed tank is distributed equally among the three channels; that is to say for any combination of discharge and channel gradient, the average velocity and depth of flow at any point in one channel should be the same at the equivalent points in the other two channels. Furthermore, the design of each of the three stream units is identical, so that the same discharge/gradient combination in all three units should produce the same hydraulic conditions in all nine channels.

However, fabrication and constructional tolerances, together with slight differences in pump, motor and valve characteristics among units, combine to make this ideal intra and inter-unit

similarity of hydraulic conditions unlikely to be achieved in practice. One objective of the hydraulic calibration was therefore to assess, against accurately metered flow rates, the accuracy of the three-way distribution of flow among the three channels of a stream unit. The suitability of the stream units for the purposes of the research project was based to a large extent on the ability of the channels to produce a specified range of velocity/depth conditions in the channels, as predicted by a 1-dimensional analysis of the channel hydraulics. A second objective was therefore to compare the hydraulic conditions obtaining in the units with those calculated, and to assess the success of the predictions. Since budget limitations precluded the incorporation of formal flow measuring devices into the stream units, the third objective was to establish control valve settings in each of the three units installed in the Grahamstown laboratory, so that the flow rate delivered to the channels was known as accurately as possible, and so that hydraulic conditions in each of the three stream could be set as nearly as possible the same.

Flow distribution among the channels of individual stream units: In the calibration of the first stream in Pretoria the flow distribution among the three channels was such that velocity at any point varied by a maximum of about 3% about the ideal mean: this is considered acceptable. In the commissioning tests in Grahamstown the flow distribution was less good, resulting in a maximum velocity variation among the three channels of any one unit of about 6% about the ideal mean: this is less acceptable, and some additional calibration work is recommended.

Flow conditions among stream units: The three similar tests which form the basis of this comparison indicate an unacceptably high velocity variation of about 12% about the ideal mean across the nine channels of the three units. Flow rates in the three units were however, probably not exactly the same. The flow control valves are not sufficiently precise to set exactly equal flow rates in the streams, and it is recommended that depths of flow are set as near as possible equal in the channels before commencing experimental work.

Prediction of hydraulic condition: The 1-dimensional computer program CFP (Channel Flow Profiles) adequately simulated depths and average velocities of flow in the channels.

Hydraulic conditions with substrate: The reduction in velocity and increase in depth were much as expected. Hydraulic variables need to be determined for each specific case, as the possible variations of substrate size and pattern of distribution on the channel floor make calibration for a general case impractical.

Temperature calibration

The system has a combination of temperature control options. The laboratory is air conditioned, and each set of channels has a water chiller. The air conditioners were capable of maintaining the laboratory temperature between 16 and 26°C, within 1°C. Water temperature in the channels, when the pumps were not running, was maintained at 1-2°C lower than the laboratory temperature over a period of 10 days. Running the pumps raised the water temperature by 2.8°C. The chillers reduced the water temperature by 2.8°C ± 2°C.

Water quality calibration

Since the artificial streams were designed to conduct invertebrate water quality tolerance experiments it was important to establish the range of water quality variation in the systems when they were run over periods of time longer than the envisaged experimental times. Acute

96 hour experiments were planned, so water quality calibration experiments were run over seven days. Systems were monitored with water, then with substrate added, and finally with substrate and organisms (to ascertain the effects of test populations on water quality). Water quality calibration proved to have additional significance in the context of the ultimate statistical analysis of experimental data.

The three channels in each system were designed as replicates, but since they share the same water source they are not in fact independent replicates. Each 3-channel system is independent of the others, but channels can only be regarded as pseudoreplicates. However, cost precluded nine separate systems and the nine channels could not be routed randomly between the three pumps. Three separate test populations are better than one, even with a shared water source, and since it can be shown that water quality and hydraulic conditions in the systems are not significantly different, the pseudoreplicate status of the channels is less problematic.

Bearing in mind the lack of within system independence, the aim of the water quality calibration in the large systems was twofold: 1) to describe between system variation; and 2) to describe within system variation over 7 days. The raceways were independent, so they were monitored for between raceway variation, also for seven days.

Water quality calibration experiments were conducted in both the large stream systems and in the small-scale raceways. When streams were filled with municipal water they were run for 48 hours to remove chlorine, as they would be in a tolerance experiment. Day 1 commenced after this dechlorination period, but when rainwater was used monitoring commenced immediately. Temperature, pH, and conductivity; as well as nutrient concentrations (ammonium, nitrite, nitrate and phosphate), were measured three times per day for seven days. The raceways were filled with municipal water (dechlorinated using a carbon filter), or rainwater, and the same variables were measured once per day for seven days. The stream system and raceway runs were not conducted simultaneously, but were both conducted in the artificial stream laboratory.

Water quality was stable in the three stream systems with regard to pH and conductivity, with any variability present following the same magnitude and pattern in all three systems. Nutrient concentrations increased over time, but were undetectable over at least a four day period. The pattern and magnitude of this increase was the same in each of the systems. These variables will be monitored during the course of each tolerance test. Since the water volumes in the raceways are smaller and there is more potential variability in nutrient concentrations during 96 hour experiments than in the large systems, raceways have been run as recirculating renewal systems during toxicity testing i.e. a portion of the test medium is replaced daily.

Biological calibration

It was necessary to understand test organism responses to the experimental system before the water chemistry was changed, as these are the conditions that would be the controls during an experiment. The process of defining organism response was termed behavioural calibration. Since an exhaustive approach was not feasible, a small selection of slope, discharge and substrate conditions were selected, and organism responses observed. Behavioural calibration in the large streams has been largely unsuccessful, and more research is required to optimize these systems.

One of the requirements for ecotoxicity testing is a maximum of 10% mortality in the control population. So far this has been impossible to achieve at either scale using wild populations of riverine macroinvertebrates. In the first sets of raceway experiments control mortality was 40-60%. It is now possible to keep control mortality between 10% and 20% in the raceways. Methods for achieving this differ for different test organisms, but one of the most important factors is handling. Limpets especially have to be introduced into the test system on a substrate, rather than pried loose and put into the test system.

Techniques for the use of the large stream systems for tolerance testing are still at the exploratory stage. Three experimental runs have been undertaken with the aim of establishing which substrate/flow combination gave the best animal retention and survival results. None of the results were sufficiently satisfactory to allow tolerance testing in the large streams to commence. Many of the test organisms either drift or are washed into the sump bag and rapidly die, but most are put into the stream and never seen again (a direct consequence of a larger scale system). However the ultimate success in using the raceways is encouraging, and perspex-frame net (0.05mm) "baskets" are being constructed to fit into each channel, to retain test organisms.

Holding test populations in raceways and channels under control conditions has led us to several important conclusions:

1. The Bloukrans River baetid population are inappropriate test organisms for three reasons: 1) The river is too polluted to be an adequate site for the collection of tolerance test organisms as experimental responses could be influenced by previous environmental stresses, 2) the baetids are a multi-species population and there is no method of selecting only one species for toxicity experiments, and 3) the baetids are too small, they are difficult to contain, and difficult to find during experiments. It has proved difficult to achieve adequate survival in the controls of experiments, either in the raceways or in the large streams.
2. The work of the Standard Laboratory Organism project proves to be essential for the success of the artificial stream work. We are dependent on adequate test populations and it is not feasible to depend on riverine populations.
3. Until the breeding programme is underway at an adequate rate, tolerance testing must be timed to coincide with the highest populations of the leptophlebiid mayfly *Adenophlebia auriculata*, in the Palmiet River. The nymphs of this mayfly are an ideal size for a test organism, they respond well to artificial holding conditions, and the Palmiet River is unpolluted. Populations from the upper reaches of the Buffalo River could also be used.
4. Other rivers in a reasonable radius around Grahamstown must be reconnoitred for populations of *A. auriculata*.
5. Perspex frames covered in 0.5mm mesh net to form closed "baskets" (1m in length) will be fitted snugly into the large stream channels to contain test populations.

4. EXPERIMENTAL TOLERANCE TESTING

Chapter 4 describes the experimental determination of salinity tolerances of indigenous riverine macroinvertebrates. Salinisation has been widely described as posing a serious threat to South African freshwaters, and was therefore identified by the artificial stream project as the first variable to be investigated during this study. Portable perspex raceways were

designed as experimental chambers in which toxicity testing was carried out. These systems provide a recirculating aquatic environment in which riffle-dwelling organisms can be tested. Macroinvertebrates are generally regarded as useful test animals due to their abundance in unpolluted streams and their sensitivity to low concentrations of pollutants. This methodology was therefore developed to investigate the feasibility of using riverine macroinvertebrates for ecotoxicity studies, rather than laboratory-reared organisms such as *Daphnia* widely used in regulatory testing. Single-taxon (using a baetid population during Eastern Cape studies) or single-species (using *Tricorythus* sp. during Mpumalanga studies) acute tests were employed as these tests are routinely used to establish water quality criteria and to predict the environmental impact of hazardous materials on aquatic communities. Four day (96 hour) tests were carried out. Mortality was the criterion used for determining toxicity and the LC_{50}, i.e. the concentration of pollutant responsible for the death of 50% of the population, was the standard measure of response.

One of the aims of this research was to establish a guideline value for salinity (measured as conductivity) for the protection of the aquatic community, and subsequently the natural environment. Two salts, sodium chloride and sodium sulphate, were selected for tolerance testing at a range of conductivities and concentrations. Experiments were carried out in the artificial stream laboratory in Grahamstown (Eastern Cape) using a baetid population from the nutrient-enriched Bloukrans River, and in the Kruger National Park (Mpumalanga) using the mayfly *Tricorythus* sp. from the unpolluted Sabie River. Salinity tolerances of the Bloukrans River limpet *Burnupia stenochorias*, and the Sabie River caddisfly *Chimarra* sp., were briefly investigated in range-finding experiments. A 96 hour experiment was also carried out to investigate the tolerance of Sabie River *Tricorythus* sp. and the leptophlebiid *Choroterpes* sp., to water of the polluted Selati River. Water chemistry and mortalities were monitored throughout the experiments.

Mortalities observed for the baetid population in Bloukrans River water were linked to high conductivities of sodium sulphate (900 - 1 000 $mS.m^{-1}$ i.e. 16.0 $g.l^{-1}$) and sodium chloride (1 000 - 1 200 $mS.m^{-1}$ or 8.0 to 9.8 $g.l^{-1}$). Difficulties were encountered with low invertebrate abundances in Eastern Cape rivers, necessitating the use of a mixed baetid population from the polluted waters of the Bloukrans River. Control mortalities were above the recommended 10% and replicability was difficult to achieve, possibly due to the effect of Bloukrans River water quality and the variability of the test population.

Sabie River results showed *Tricorythus* sp. tolerance to higher conductivities of sodium chloride than sodium sulphate. As the effect of sodium chloride on the test population was not clearly evident, probit analysis of cumulative mortality data assigned the LC_{50} value to a range between 400 and 800 $mS.m^{-1}$ i.e. above 2.10 $g.l^{-1}$. The effect of sodium sulphate on *Tricorythus* sp. was immediate, with the LC_{50} apparent at 100 $mS.m^{-1}$, or 0.66 $g.l^{-1}$.

Sabie River *Tricorythus* sp. and *Choroterpes* sp. showed contrasting tolerances to Selati River water. The effect of test water on *Tricorythus* sp. was immediate, with high mortalities after only 12 hours. The adaptability of *Choroterpes* sp. was evident as mortality could not be linked to test water and similar survival was seen in both Sabie and Selati River waters. *Choroterpes* sp. has been documented in both the Olifants River (of which the Selati River is a tributary) and Sabie River systems (Noble, 1970).

Despite the difficulties encountered during this method development phase. it presents a unique opportunity to experimentally observe and monitor the behaviour of riverine organisms under conditions of salinity stress. Toxicity testing using riffle-dwelling macroinvertebrates in recirculating raceways appears to present a useful contribution to the science of ecotoxicity testing, and to the development of water quality guidelines for the protection of the natural aquatic environment.

5. COLLABORATION AND PROJECT TEAM ACTIVITIES

One of the most exciting aspects of this project has been contact, workshops and collaborative research with researchers and managers in the fields of water quality and riverine ecology.

The project team as referred to in this section:

Prof Jay O'Keeffe	JO'K
Dr Carolyn Palmer	CGP
Dr Patsy Goetsch	P-AG
Mr Qurban Rouhani	QAR
Mr Brenton Maart	BM

Collaboration

Department of Water Affairs and Forestry (DWAF)

DWAF have provided comprehensive support in every stage of the project, specifically in the following areas:

* Design and construction of artificial streams
* Planning of the direction and scope of the second phase of artificial stream research (IWQS)
* Analysis of water samples (IWQS)
* Comparative *Daphnia* tolerance tests (IWQS)

Kruger National Park Rivers Research Programme (KNPRRP)

* In August 1992 CGP took the first raceway to the KNP for a trial run. At the April 1992 artificial stream workshop it was recommended that an experimental facility be built at the KNP. The feasibility of this was assessed by CGP during the August 1992 visit.
* A paper on the development of the artificial stream system to investigate the use of macroinvertebrates as water quality indicators was presented by CGP at the KNPRRP Second Annual Conference in Pretoria in June 1992.
* Tolerance experiments have been conducted by P-AG and QAR in the KNP on two occasions during this method development project (Chapters 4 and 8). In the second phase of artificial stream research, collaboration with the KNPRRP will remain a priority.
* The water quality sub-programme of the KNPRRP has identified the investigation of the tolerances of organisms from the KNP rivers as one of the priority research needs (JO'K is water quality sub-programme leader, and CGP has attended meetings).
* In July 1994 CGP and JO'K attended a workshop run under the auspices of the KNPRRP on a catchment process model named HSPF (Barnwell and Johanson, 1981). The workshop was presented by Dr Rob Johanson (Pacific University, California). The link between this and the tolerance research is that HSPF can be used to describe the

likely duration of particular conditions in a particular river. If this can be linked to tolerances of organisms it makes a possible connection between laboratory tolerance testing, conditions in the river, and catchment processes. CGP has arranged to work with Dr Mark Dent (Computer Centre for Water Research, University of Natal) on using tolerance data from Sabie River invertebrates in an initial modelling exercise for the planned Sabie river IFR workshop in 1995.

Water Quality Guidelines

* The results of tolerance testing are directly applicable to the development and refinement of environmental water quality guidelines. CGP and P-AG have been sub-contracted to assist with the guideline project. As tolerance results become available they will be incorporated into the development and refinement of environmental water quality guidelines.

Chemical Speciation Modelling

* Metals have consistently been identified as an area which should be considered for tolerance testing. Any attempt to establish the toxicity of metal ions to South African macroinvertebrates will require that metal speciation be taken into account. In preparation for work on the toxicity of metals in the second phase, we have made contact with the Pollution Research Group, Natal University, led by Prof Chris Buckley. Prof Buckley has completed a WRC-funded project to make the chemical speciation model MINTEQA2 accessible to researchers, and visited Rhodes first in February 1994, and then in May 1994 to present a MINTEQA2 course.
* Dr Peter Wade (Watertek, CSIR) is a speciation specialist, and uses the CSIR speciation model JESS, which has a greater modelling range and capacity than MINTEQA2. He is available as an advisor and critic.
* Dr Ansie de Kok (PE Technikon) is also available for consultation on chemical matters.

Artificial stream for microbiological research

* The International Centre for Waste Technology (Natal University) has a WRC-funded project on the development of an artificial stream system for microbiological-scale research on the breakdown of xenobiotic compounds. The project researcher, Mr Charles Hunter, visited the Rhodes University Artificial Stream Unit in 1993, during which there was a useful exchange of ideas.

Water quality interest group

* Within the South African Society of Aquatic Scientists (SASAQS), CGP has initiated the water quality interest group. Through this group Dr Steve Mitchell (WRC) organised a workshop on toxicity methods, this initiative was furthered at a workshop on aquatic toxicity at the SASAQS conference in July 1994, and in 1995 an e-mail interest group - ECOTOXA was established. In June 1994 CGP co-ordinated a one-day session at the Afriwater '94 conference on riverine water quality, which served as a useful forum for contact and communication.

Blackfly growth

* Dr Robert Palmer (Agricultural Research Council) has a WRC- funded project to investigate the control of problem blackfly in the Orange River. The artificial stream

system can provide the high velocities *Simulium chutteri* requires. Dr Palmer spent 10 days in July 1994 familiarising himself with the artificial stream laboratory and conducted preliminary growth experiments.

Fish swimming speeds

* Prof Tom Hecht (Department of Ichthyology and Fisheries Science. Rhodes University) has an honours student investigating the maximum speed mullet can swim, in order to make recommendations for the design of fish ladders.

Training

Two students have registered with the IWR in association with the artificial stream project, under the supervision of CGP and P-AG. In both cases the students are full-time employees upgrading their qualifications with the support of their employers.

Chlorine tolerance testing

Ms Margot Williams (Natal Technikon) is investigating chlorine toxicity, and the effects of chlorinated sewage on macroinvertebrate communities.

Whole effluent testing with textile effluent

Mrs Tandiwe Zokufa (Dept. Public Works, Water Affairs, Bisho) will investigate the tolerances of invertebrates from the Buffalo river to treated and untreated textile effluent from the Da Gama textile mill outside King William's Town. This will be the first use of artificial streams for whole effluent testing.

Research team activities

These are recorded in detail in Chapter 5 and include:

* Water Research Commission steering committees
* External contracts
* Participation in, and/or organisation of DWAF, WRC and FRD workshops
* Courses attended and/or organised
* International travel and contacts
* Publications and presentations

6. EVALUATION - PITFALLS AND PROGRESS

In any project which involves pioneering development, the list of "what not to do" is often longer than the final protocols. Despite every effort to ask questions, and read the literature, there are things that in retrospect would have been better done differently. Chapter 6 is therefore a record of the project with notes on what to avoid, and what was learned.

7. CONCLUSIONS

The primary aim of this project was the development of a facility for experimental research, under controlled conditions, into the water quality tolerances of organisms which live naturally in flowing water. This has been achieved and is the major accomplishment of the project.

The next aim was the development of methods for tolerance testing. In this regard a significant step forward came with an independent project on the rearing of test populations.

This has meant a relatively ready supply of limpets for our experiments, and advice on successful transport of animals from the field. The further development of these production methods until two taxa can reliably be supplied is **essential** for further work in the artificial stream laboratory.

Despite technical problems with the pumps in the large systems, and incompetent service from the suppliers which resulted in the loss of 3 months experimentation time, methods have been developed for the use of both the portable raceways and the large streams. Both systems have been calibrated. An important discovery has been the susceptibility to handling distress of limpets used in experiments. Protocols for minimal disturbance during collection and experimentation have been developed.

The results of salinity tolerance testing have contributed both data and method protocols. Information has been generated concerning the best type of riverine organisms to use for toxicity studies, as well as the water quality of choice. Comparative studies using a mixed baetid population from the polluted Bloukrans River (Eastern Cape), and the mayfly *Tricorythus* sp. from the Sabie River (Mpumalanga), have clearly shown the advantages of using a clean water system and a reliable source of similar-sized invertebrates. Results generated have clearly shown that salinity tolerance is salt-specific, with a higher mortality response to sodium sulphate than sodium chloride. The extent of mortality appears to be linked to the natural habitat of the test organism. Water chemistry results indicate that mortality may not only be linked to high total dissolved solids (TDS) concentrations, but also to the identity and strength of the anion. Physiological studies will be necessary before this hypothesis can be adequately investigated.

In the immediate future the most useful application of this research is in the development and refining of environmental water quality guidelines. It is important to recognise that there was previously no knowledge whatsoever of the tolerances of indigenous riverine fauna to any water quality variables, and that without such information we are limited in our ability to set realistic environmental guidelines. We are therefore at a stage in the development of environmental guidelines in which <u>any</u> results represent an advance in our knowledge, and it is in this respect that this project has been most successful.

As the role of artificial stream research in ecotoxicology is evaluated on an ongoing basis it will be important: 1) To recall the difficulty in developing environmental water quality guidelines in the face of the spatial and temporal complexity of water quality, 2) to ascertain the relative cost-effectiveness of using indigenous riverine taxa in tolerance testing, and 3) to add chronic testing to the repertoire of methods developed.

8. RECOMMENDATIONS FOR ARTIFICIAL STREAM RESEARCH

The artificial stream laboratory is designed to meet the needs of a wide range of research applications, both applied and in the field of general stream ecology. However, the most useful immediate application of tolerance testing results is the development and refinement of environmental water quality guidelines. In order to achieve this, the greatest research need is continued work on the supply and rearing of test organisms, and continued tolerance testing. In addition, experimental scales need to be evaluated, cost-effectiveness calculated, and clients and markets identified. The project also has the potential integration of water quality into the

in-stream flow requirement (IFR) method. Finally, a range of general research possibilities are listed, and the contribution the artificial stream laboratory could make to training is described.

Environmental water quality guidelines

The development of the first set of national environmental water quality guidelines is well underway. The method which will be used is based on results from tolerance testing using a wide range of biota, potentially including riverine invertebrates. There will frequently be insufficient data, and in these instances safety factors will be imposed. The results from the artificial stream tolerance testing can be used to reduce or remove safety factors.

Recommendations are therefore:

* to complete salinity tolerance testing, and go on to testing a selected metal ion (planned for Phase 2),
* to calculate guideline values for salinity using the data from the artificial stream project, and so evaluate the contribution of the stream data, and
* to repeat this procedure after metal ion results are available from Phase 2 of the stream project.

Once the national guidelines have been published, the next step is to legislate compliance in terms of tolerance testing results.

The recommendation is:

* to collaborate with DWAF, so that when compliance is required of an industry in terms of tolerance testing, there is the suggestion on comparing standard test organisms and riverine fauna.

Catchment modelling and IFR methods

One of the most successful applications of river ecology to water management has been the implementation of the Building Block methodology, in the process of IFR assessments. This process has concentrated on the water quantity requirements of rivers, particularly in the context of the development of impoundments for water supply. However it is of little use to ensure an adequate supply of water, if water quality impairment renders it unfit for use. It is therefore important to include the assessment of water quality requirements in the IFR methodology in rivers with water quality problems.

The recommendation is:

* to pursue the investigation initiated informally between Dr Mark Dent (CCWR) and CGP to attempt to apply the HSPF model incorporating tolerance data from the Sabie River.

Standard Laboratory Organism (SLO) project

The SLO project is not yet at a stage where two test species can be supplied in sufficient numbers on demand. This is an essential step for progress with artificial stream tolerance testing research.

A **critical** recommendation is:

* to support SLO research for another 3 year period.

Comparison with *Daphnia*

Results of tolerance testing with standard test organisms and riverine organisms is an essential step in evaluating both cost-effectiveness and value of both methods.

The recommendation is therefore:

* to compare the results of tolerance testing (salinity, followed by metal toxicology) using *Daphnia* and stream invertebrates on a cost-effective basis, and then define the role of tolerance data generated from indigenous fauna.

Tolerance testing

Recommendations for further tolerance testing include:

* undertaking chronic and well as acute tolerance testing,
* planning multi-generation testing, and
* evaluating the effects of both scales of model, and defining the roles of the raceways and the large streams.

Clients and markets

The WRC has funded artificial stream research in two phases: 1) 1992 - mid-1995: development of the stream laboratory, and methods for its use, and 2) mid-1995 - mid-1998: data collection on tolerances to salinity and a metal ion, and comparison of results with *Daphnia*. In addition the SLO project (1993-1995) has been an essential part of artificial stream research. Continued efforts in the SLO project are essential, and although it is unlikely that by 1998 all the problems will have been solved, it will certainly be possible to define data that can be generated, together with costs and applicability.

Once we have demonstrated the value of the data, procedures will need to be repeated as data on a wide range of variables and organisms are collected. In addition, whole effluent testing should also be developed. The question arises as to the funding for this continued research. It is important to identify future clients and markets: the objects of "technology transfer".

If the ongoing development and refining of environmental water quality guidelines is recognised by DWAF as a priority, the Department could be one such client. If tolerance testing becomes part of effluent permit requirements, and we can demonstrate the value of site-specific testing with riverine organisms, then industry becomes a potential client.

So far we have experienced difficulties in involving industry, even after making presentations to Sappi Ltd. and Richards Bay Minerals. There are many potential clients but this kind of initiative is time-consuming, and seems to have a low rate of immediate, direct success. The recognition of markets and promotion of research products is never articulated as an aim within the research programme - there is a real need to define who should do this, how, and when.

We recommend that the next steps should be:

* to approach DWAF and investigate the possibility of their being a client for the generation of tolerance data for the development and refining of environmental water quality guidelines, and
* to organise a workshop/course in Grahamstown on the application of artificial stream research specifically for potential clients in industry and the government sector.

General

Hydraulic habitat assessment: The great strength of the large stream systems is their engineering rigour and the potential for accurate definitions of hydraulic conditions. They are ideal for research on the hydraulic habitat requirements of riverine biota. both invertebrates and small fish. IFR assessments would ideally use data on the flow requirements of key taxa, in all their life history stages. We recommend that collaborative research be encouraged, where the artificial streams are used as an experimental complement to field research.

Other potential directions and applications include:

* investigation of fish swimming speeds for fish ladder design,
* algal-nutrient dynamics - information on algal responses to nutrient enrichment could be applied to eutrophication problems in rivers,
* macroinvertebrate growth - an applied example is the need for growth studies of blackfly larvae in the blackfly control programme on the Orange River, and
* contributions to general stream ecology in the fields of grazer-algal interactions, fish ecology, and disturbance theory.

Training and net-working

The field of ecotoxicology is embryonic in South Africa. IWQS (DWAF), Watertek (CSIR), Rand Water Board and Umgeni Water are the main organisations which routinely undertake water-related toxicity testing. If toxicity testing is integrated in effluent discharge permits, the demand for testing will escalate. There will be a tremendous need for trained personnel. Training should become one of the major interests associated with the artificial stream laboratory. In the future it would certainly be possible to run specialist training courses and this should be pursued.

GLOSSARY

acclimate	to accustom test organisms to different environmental conditions, such as laboratory conditions
acute tests	short-term 48 - 96 hour toxicity tests
aquatic ecosystems	all the interacting members of an aquatic biological system
benthic	living on the bottom
bioavailability	the extent to which a particular pollutant is available to the biota
biomonitoring	the systematic use of biological responses to evaluate changes in the environment
caddisflies	a group of insects with aquatic larvae e.g. order Trichoptera, families Philopotamidae and Hydropsychidae
catchment	area in which rainfall collects to form the supply of a river
chemical species	the various chemical forms (e.g. ionic forms) of an element
chronic tests	longer-term (7 days to several weeks) toxicity tests
definitive tests	replicated toxicity tests at a defined range of concentrations
DWAF	Department of Water Affairs and Forestry
EC	electrical conductivity i.e. the measure of electrical current conducted which depends on the ions in solution, and is therefore proportional to the total quantity of salts dissolved in a sample of water
geomorphology	the gross structure of land forms
hydraulics	describing the movement of water in channels, particularly in terms of depth and velocity, and in pipes
isotonic	no swelling of shrinkage of a cell in solution, i.e. at equilibrium
IFR	Instream Flow Requirements
IWQS	Institute for Water Quality Studies (DWAF, Pretoria)
IWR	Institute for Water Research (Rhodes University)
KNP	Kruger National Park
KNPRRP	Kruger National Park Rivers Research Programme
larva	the immature stage (no wings) of those insects that have a pupal stage in the life-cycle e.g. caddisflies
LC_{50}	the concentration of a toxin which causes the death of 50% of the test population
macroinvertebrates	invertebrates that are retained by mesh sizes ≥200 to 500µm
mayflies	a group of insects with aquatic nymphs, generally sensitive to polluted conditions e.g. order Ephemeroptera (mayflies), families Leptophlebiidae, Tricorythidae and Baetidae
NOEL	no observed effect level
nymph	the immature stage (no functional wings) of insects that do not have a pupal stage in the life-cycle e.g. mayflies
MATC	maximum acceptable tolerance concentration. This value is bounded at the lower end by the highest tested concentration having no observed effect (NOEL), and at the higher end by the lowest tested concentration having a significant toxic effect (LOEC) in a partial or full life-cycle
MLA	maximum level of acceptability

mesocosm	an artificial. experimental stream or lake
planktonic	small organisms found in the water column
pseudoreplicate	lack of independent replication; the replicates are inter-dependent
range-finding test	a test conducted to estimate the concentrations to be used for definitive tests
rheophilic	current-dwelling
RWQO	receiving water quality objectives i.e. the water quality towards which a regulatory body strives
salinisation	the process whereby the saltiness of soils and rivers increase
salinity	saltiness of water
TDS	total dissolved solids in the water column
tolerance	the ability of an organism to withstand the effect of a pollutant
toxicity test	tests measuring the degree of response produced by exposure to a specific concentration of chemical
water quality	the combined effect of the physical attributes and chemical constituents of a sample of water
water quality variable	any attribute or constituent which may affect the quality of the water

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ACKNOWLEDGEMENTS

The completion of this project required taking to heart an adage I first heard from Mr Charel Bruwer (DWAF): "Eat the elephant a bite at a time..." However, regardless of the enormity of the task, we have experienced fantastic support in every phase of the project, which has, in every sense, been a collaborative effort.

From the first proposal we sought the support of the Department of Water Affairs and Forestry, and from that stage on received enthusiastic assistance. Mr Wouter van der Merwe (Manager, Scientific Services) has provided encouragement and direction since the project's inception. Design and construction was supported by Mr Hendrik Best (Managing Engineer, Development) and Dr Jan Jordaan (Chief Engineer, Design Services). Engineers from Hydraulics Studies designed and built the streams, and conducted the hydraulic calibration: Mr Bill Rowlston (Deputy Chief Engineer, Strategic Planning), Mr Witek Jezewski (Principal Engineer, Hydraulic Services) and Mr Peter Skotnicky (Control Industrial Technician, Hydraulics Laboratory). Once the streams were commissioned, the project team was challenged as to the selection of field sites, and variables for testing. After correspondence with Mr. van der Merwe and Mr Sakkie van der Westhuizen (Director, Water Quality Management), a Technical Advisory Committee from the Institute for Water Quality Studies (IWQS) was established to ensure the results of the project, and plans for future directions would provide the results most useful to DWAF. Members of the committee are: Dr. Henk van Vliet (Director, IWQS), Dr. Phillip Kempster, Mr. Dirk Roux, Mr. Sebastian Jooste, and Mr. Dana Grobler.

From this list special mention must be made of Bill Rowlston and Dirk Roux both of whom have so generously contributed of their considerable professional expertise, and beyond that, of their personal encouragement and friendship.

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We have received considerable assistance with travel funding from Anglo American Chairman's Fund, Sappi Ltd. and Richard's Bay Minerals, and have been hosted with great hospitality in England, Australia, Canada and the United States. The design for the large stream was based on an Australian system, and the raceways on a Canadian design.

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During the construction phase various people from Rhodes University were particularly helpful: Mr Martin Davies offered part of a DIFS shed and Prof Tom Hecht agreed, enabling the favourable siting of the laboratory. Prof Hecht also provided a link to DIFS compressed air supply. Mr Ken Murdoch (Estates Division) designed the laboratory and co-ordinated building, Mr Andy Hatting (Building Maintenance Services) supervised construction. Mr Hendrik Rautenbach and Mr Jack Atherfold (Electrical Section) saw to the electrical requirements including back-up power and several changes of design. Mr A. J. Lisher fitted the interior, and Mr Patrick Jordaan sub-contracted the building.

The budget and administration of the project account was ably tackled by Mrs Ann McKane who has always been patient in the face of our ignorance; and Mrs Sarah Radloff has always been available with assistance and advice on statistical procedures.

Within the Institute for Water Research, the project team has changed during the course of the work, and to Brenton Maart and Qurban Rouhani who left before the end, we extend very grateful thanks for their efforts. Qurban participated in the extended field study in the KNP, and was a stalwart in the construction and leak-fixing phase. Kekere Soxujwa has contributed to every phase of the project, with special expertise in aquatic organism identification and accurate counting of organisms in tolerance tests. Dr Gay Youthed worked briefly on the "stream team" and has also offered considerable help as the IWR Principal Technical Officer. Gay has smiled her way through glue and other nasties, always cheerful, and always with an offer of a better way to do a task. Mrs Margi Rogers has undertaken all sorts of typing and administration and without her help this report would not be complete. Other IWR members offered support, encouragement and an editorial eye: Denis Hughes, Lil Haigh, Dez Weeks, Mandy Uys, Heather Davies-Coleman and Nkosinathi Mtwa.

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No professional undertaking happens without impacts at home, so thanks to family and friends: Tony, Richard, Nicola, William, Thomas, Lucy, Arusha, Vanessa, Christine, Mike, Larry, Chloe, Barry, Clive, Joe, Mandy, Lorna, Janet, Margot, Mark, Monika and Brian.

Finally, within the research team there has been considerable support and mutual assistance.

PREFACE

The aim of this research is to contribute information which will assist in water quality management in South Africa. Both the research and management implications are awesome. Water quality is not a single factor. It is the sum total of chemical processes that are continually shifting through time and space as a myriad of dissolved, suspended and deposited particles interact.

One of the persistent questions and criticisms of experimental research is the degree to which it can simulate the "real world", and therefore the extent to which results can be confidently used for management.

The answer is uncertain. If we could easily define the "real world" we would have less need of experimental research. It is precisely because the aquatic environment is so complex that we attempt to isolate single variables for investigation. Experimental conditions will never mirror the natural environment. However, the small, discrete pieces of information from experiments can be used as "windows" to see different aspects of the vast complexity "out there".

Management can also make use of other approaches in combination with experimental results, such as field research which can, after multivariate analysis, offer a less precise, but more holistic and integrated interpretation.

So, the data and the experimental method in this report are offered in humility in the face of an infinitely complex reality - but also with conviction that the windows of understanding that they provide are useful.

Streams - Natural and Artificial

Tumbling freedom
wayward water
unfettered. Bridal
in its fern fringed
white frothed
loveliness.

Free.
Free, but threatened.
Virginal clarity
too soon sullied.
Used, diverted, entrapped,
held, passed, purified.
Mouths, sewers, dams,
sprinklers and long-drops.

Torn, tattered, and raped.
Contained and canalised
an aquatic lobotomy

And we
in steel and perspex
will dare to channel, monitor
and simulate
your heartbeat

an aquatic laboratory.

C G Palmer

CHAPTER 1

INTRODUCTION

Summary Water quality management in South Africa is based on the Receiving Water Quality Objectives approach, which aims at the maintenance of water in a state that is "fit for use". The water quality requirements of the natural environment need to be established to fulfil this aim. In order to provide a flowing water experimental facility where the water quality requirements of riverine organisms can be investigated, an artificial stream laboratory has been developed. The aims of the project are given, and related to the various Chapters in this report.

1.1 MANAGEMENT CONTEXT

1.1.1 Water quality management

The Department of Water Affairs and Forestry (DWAF) takes responsibility for the management of South Africa's scarce, essential water resources, and in so doing faces two major water management issues: water quantity and water quality. Although these are often treated separately they are intricately linked. An important philosophical change in water quality management came with publication of the Receiving Water Quality Objectives (RWQO) approach (Van der Merwe and Grobler, 1990). This caused a move away from the uniform effluent standard approach, and focused attention on five recognised water users: industry, agriculture, domestic supply, recreation and the natural environment.

Water quality management goals were stated in terms of the maintenance of water resources in a state that is "fit for use" by any or all the designated users. This was followed by the publication of water quality guidelines for all the users except the natural environment (DWAF, 1993). Information needs for RWQO management include stated water quality requirements for each user group of a water body, with the aim of managing water bodies for the most sensitive user. This is achievable for the four human users, but the natural environment has no way of articulating its water quality requirements other than through the efforts of environmental scientists.

In terms of water quantity it was envisaged that the five users would compete for scare water resources and each user would have to motivate for its share (DWAF, 1992). The fundamental problem with this approach is that the natural environment should not be viewed as a competing user. If insufficient water is left in a river for it to function as an ecosystem, it loses most of its intrinsic uses such as the capacity to supply and/or purify water. The "environment" is the resource, and maintenance of the integrity of the resource is a priority, after which the needs of users can be addressed. This concept has only very recently been recognised by DWAF and is not yet official policy (DWAF, 1995).

From this it is clear that the natural environment is fundamentally different from other user groups. The term itself needs careful definition. Too frequently the natural environment is equated with nature conservation - and then linked geographically to formally conserved areas. Aquatic ecosystems **are** the natural environment, whether they are wetlands, lakes, estuaries,

or rivers. In the case of rivers, which are the focus of this project, the whole length of the river from source to estuary is included regardless of the land-use of the catchment. What is needed is a management approach which formally recognises the aquatic ecosystem as the resource, so that both research and management can focus on the maintenance of these systems in a functional state. (Biodiversity, which can be measured using rapid biological assessment protocols, is an example of an index which could be used to assess "functional state".)

1.1.2 Recognition of the need for experimental research

Research on water quantity required for environmental flow for rivers is well advanced (King and Tharme, 1994). The need for experimental research on the water quality requirements of riverine fauna was highlighted in 1989 at a Kruger National Park Rivers Research Programme (KNPRRP) workshop which was held to try to establish environmental water quality guidelines for the KNP rivers (Moore, 1990). It became apparent that no data existed on the water quality requirements or tolerances of any indigenous riverine organisms.

Projects to assist in the development of environmental water quality guidelines have developed from a range of perspectives. Kempster *et al.* (1980) used the international literature to produce a general summary of water quality criteria, with some reference to the natural aquatic environment. Following the 1989 workshop, preliminary guidelines for KNP rivers were published using percentiles of the occurrence of variable concentrations from the historical water quality record (Moore, 1990; van Veelen, 1990; Moore *et al.*, 1991). Dallas and Day (1993) reviewed the literature on the effect of water quality variables on riverine ecosystems, and correlated water quality variables with the natural distribution patterns of South African riverine fauna (Dallas *et al.*, 1996).

The artificial stream project aims to use the experimental method of ecotoxicology (Rand and Petrocelli, 1985). In South Africa, riverine macroinvertebrates have most frequently been collected from riffle habitats, which are also the main habitat sampled in bioassessment studies (Chutter, 1972; 1994). In order to relate experimental data to field distributions and bioassessment data, the artificial stream system was designed for the use of riffle organisms as test animals. Since these current dwelling organisms have specific hydraulic habitat requirements (Statzner *et al.*, 1988), the major specification for an experimental system was for a flowing water system where the range of hydraulic conditions could be accurately described.

1.1.3 Environmental water quality guidelines

The development of the first environmental water quality guidelines for South Africa is a current project. They will incorporate the recognition that the environment cannot be viewed simply as a user, and consequently these guidelines will differ from those published for the other users (DWAF, 1993). The format for environmental guidelines is currently being finalised, and the basis for deriving guideline values is well advanced (Roux *et al.*, in press).

Roux *et al.* (in press) envisage that guidelines will be developed for the South African situation, rather than adopting or modifying existing international guidelines. The first set of guidelines will be substance-specific as single species tests remain the most common and reliable basis for the estimation of environmental damage from exposure to toxic substances (Cairns, 1983). Acute exposure values (AEV) and continuous exposure values (CEV) will be

derived (Stephan *et al.*, 1985) and used as the basis for guideline development. Data requirements for this derivation are considerable (Roux *et al.*, in press):

" Results of acute tests with at least one species of freshwater animal in at least eight families such that all of the following are included:

* cold water fish (indigenous or salmonid)
* warm water fish (cichlid, cyprinid, clarid)
* planktonic crustacean (cladoceran, copepod) e.g. *Daphnia* spp.
* benthic crustaceans (ostracod, isopod, amphipod, crayfish)
* insects (**mayfly**, dragonfly, damselfly, stonefly, **caddisfly**, mosquito, midge)
* a family in a phylum other than Arthropoda or Chordata (Rotifera, Annelida, **mollusca**)

Results of chronic tests or acute-chronic ratios of species of aquatic animals in at least three different families are used, provided that of the three species:

* at least one is a fish, and
* **at least one is an invertebrate**. "

Where experimental data on local species or locally tested standard laboratory organisms are not available, two international data bases will be used (ASTER, 1994 and ACQUIRE, 1994). The role of artificial stream research is to provide data which can be used to develop and refine environmental water quality guidelines. The organisms highlighted above are those for which the artificial stream system can provide results.

1.2 PROJECT AIMS

The long term aim of this project is:

To use an artificial stream system to measure the tolerances of selected stream macroinvertebrates to a range of environmental variables under controlled conditions, so as to use them as water quality indicators.

The immediate aims of this project are:

1. To synthesise information on the design, construction, operation and research planning of experimental stream systems world-wide.

   * Chapter 2 - Design and construction, and Chapter 5 - Collaboration.

2. To design, plan and build recirculating artificial stream systems at Rhodes University, together with smaller-scale, portable, mesocosm laboratory systems.

   * Chapter 2 - Design and construction.

   This aim was achieved only because of the committed support of the Department of Water Affairs and Forestry.

3. To establish procedures for the selection and maintenance of stream macroinvertebrates, and to design appropriate experimental procedures.

* This aim was transferred to the Standard Laboratory Organism project. Prof. Jay O'Keeffe and Dr. Carolyn Palmer are project leaders, and the research officer is Mrs. Lil Haigh. The project will end in December 1995, and the aims are:

    i. To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.

    ii. To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.

    iii. To attempt to establish methodologies for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macroinvertebrate tolerances to various conditions in the artificial stream project.

    Procedures for the transport and laboratory maintenance of selected species have been established. A reproducing population of the limpet *Burnupia stenochorias* has been maintained in the laboratory and limpets have been supplied to the artificial stream project. Optimal production conditions for the limpets are under investigation. The mayfly *Adenophlebia auriculata* has been identified as a second suitable candidate species. Field data on its population structure and life history have been collected, and some progress has been made with laboratory fertilisation. Rearing of laboratory fertilised eggs to nymphs of a size suitable for experimentation is underway.

    The continuation of this rearing research is absolutely essential for the success of the next phase of the artificial stream project.

4. To calibrate the experimental system by monitoring physico-chemical changes in channels containing only water, and water with substrate.

* Chapter 3 - Calibration of the stream system.

5. To conduct experiments on the tolerances of selected species to salinity, initially in the smaller laboratory recirculating systems, and then in the experimental stream.

* Chapter 4 - Salinity tolerances.

Due to delays associated with malfunction of the pumps in the large streams, they have not been used for tolerances testing. They have however been calibrated, and initial experiments have provided information for Chapter 6, where some of the pitfalls of artificial stream research are described.

6. To initiate a country-wide collaboration between researchers involved in programmes on water quality and assimilative capacity.

   * Chapter 5 - Collaboration.

The remaining chapters in the report are: Chapter 6, where there is a record of how this research was approached, which avenues were fruitful, and which pitfalls should be avoided; Chapter 7, in which conclusions are drawn; and Chapter 8, which outlines our recommendations both for future research directions and the management and application of the artificial stream laboratory. Each chapter has been written as a stand-alone document, with its own introduction and reference list. For this reason there is some repetition of the background and motivation for the project.

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CHAPTER 2

DESIGN AND CONSTRUCTION OF AN ARTIFICIAL STREAM SYSTEM AND LABORATORY

This chapter has been published:
Palmer C G, Rowlston W S, Jezewski W A and Skotnicky P I (1994) The design and research potential of an artificial stream system for the investigation of macroinvertebrate water quality tolerances. Water SA 20:247-258.

Summary An artificial stream laboratory has been developed at Rhodes University, in collaboration with the Water Research Commission and the Department of Water Affairs and Forestry. It provides a controlled environment, with a defined and replicated range of hydraulic conditions, so that experimental research can be conducted using rheophilous organisms. In the first instance, research will focus on the response of riverine macroinvertebrates to changes in water quality. The system is also ideal for the investigation of hydraulic requirements of riverine taxa. In this paper we describe the role artificial stream research can play in water quality management; briefly review artificial stream designs; and provide design details of the artificial stream system which has been built.

2.1 INTRODUCTION

Water quality management in South Africa has recently changed from the uniform effluent standard approach, to the receiving water quality objectives (RWQO) approach (Van der Merwe and Grobler, 1990). The implementation of the RWQO approach aims at the maintenance of water quality in a state "fit for use" by any or all of 5 designated water resource users: industry, agriculture, domestic supply, recreation and the environment (Department of Water Affairs and Forestry, 1993). Water is a limited resource which is unevenly geographically distributed, and users compete for water supply. However, the allocation of water, both in terms of quality and quantity, to the natural environment, needs to be considered separately from the other users, because the maintenance of river ecosystems and catchments in a state of ecological integrity, where biological processes remain functional, is a prerequisite for meeting most other user requirements. If water quantity and quality are inadequate for the maintenance of ecological function, then many other user requirements cannot be met either. It is therefore imperative that realistic environmental requirements are defined, and management objectives provided.

The Department of Water Affairs and Forestry (DWAF) has prioritised setting water quality guidelines for all the user sectors. Guidelines for 4 users, but not for the natural environment, have been published (DWAF, 1993). Setting environmental water quality guidelines that are appropriate to the South African situation has proved to be difficult. Various information sources are available: the historical patterns of chemical concentration can be used; published water quality guidelines set for other countries can be consulted; the natural distribution patterns of freshwater biota can be related to regional patterns of water quality; and

ecotoxicological studies can provide experimental results concerning the tolerance limits of specific taxa. These taxa can either be standard laboratory organisms, such as *Daphnia* (Roux *et al.*, 1993), or local South African riverine taxa.

An artificial stream system has been designed and built with the specific aim of providing a controlled flowing water environment, where riverine organisms from local rivers can be subjected experimentally to changes in water quality. In this paper we present:

- a brief review of artificial stream designs
- a description of the selected design
- the operation, and planned experimental use of the system.

2.2 ARTIFICIAL STREAMS - DESIGN ALTERNATIVES

One of the most obvious features of natural ecosystems is the complexity of interactive effects between abiotic and biotic variables. In an experimental approach, the general aim is to maintain certain variables constant while investigating the effects of others by altering them within controlled limits. Artificial stream research is based on the further premise that the provision of a flowing water environment is fundamental to any experimental research on riverine organisms (Frutiger, 1984).

At the more natural end of artificial stream design spectrum are outdoor systems which allow control of selected variables while leaving others, such as light, daylength and temperature to fluctuate with the natural environment. Such designs offer less experimental control and are favoured by those who believe that "conclusions and generalities from artificial stream systems which incorporate more of the characteristics of natural environments are more directly related to those environments" (Clark *et al.*, 1980). Outdoor systems vary in "naturalness" from channels placed in a stream and naturally colonised (Hildebrand, 1974; Kowanacki *et al.*, 1985; Poff and Ward, 1990); through channels next to a stream with stream water diverted through them (Clark *et al.*, 1980; Bothwell, 1988; Allard and Moreau, 1985; 1987; Eichenbacher *et al.*, 1985; Arthur, 1988); to large recirculating systems (Ladle, 1976, 1977; Reynolds *et al.*, 1990).

Ladle and Frutiger (1991) both regard artificial stream research in large outdoor systems as useful, but caution that it is capital intensive, and requires considerable manpower both for maintenance, and sample and data processing. Other difficulties include the cost and difficulty of providing replicate systems, and the recognition of considerable between-channel variation. A very large system at the Monticello Ecological Research Station, Minnesota (Arthur, 1988) has provided useful results, but is operated at a scale that is not economically viable in South Africa.

Smaller channels can be housed inside, and offer opportunities for more operational control: "Laboratory streams confer advantages of reduced complexity and increased control, and permit examination of the interaction between physico-chemical variables and biological systems" (Horne and Bennison, 1987a). Laboratory streams generally have channels which range in length from 1 m to 15 m. Channels are usually artificially colonised, water may be recirculated or through flow, and channels may be circular (Higler, 1975; Ciborowski, 1983; Adams *et al.*, 1987; Pontasch and Cairns, 1989) or straight (Whitford *et al.*, 1964; McIntire,

1968; Feldmeth, 1970; Kapoor, 1972; Bott *et al.*, 1977; Clifford *et al.*, 1979; Thirb and Benson-Evans, 1982; Burton *et al.*, 1985; DeNicola *et al.*, 1990).

The large-scale streams we have designed and built are based on a design developed in Australia by Horne and Bennison (1986, 1987a, 1987b). In justifying the development of an Australian artificial stream facility, they noted the limitations of using Northern Hemisphere toxicity data; the paucity of data on responses of local taxa to pollution; and argued that the facility provided an opportunity for collecting data which would be relevant to Australian conditions. These arguments apply equally in South Africa.

Three major criticisms may be levelled at artificial stream research: 1) the limited degree to which results are applicable to the natural environment; 2) the physical scale of many artificial streams is too small for natural processes to occur; and 3) they are usually inadequately replicated, so it becomes impossible to assess within-system variability, and to evaluate between-treatment experimental results in comparison.

The artificial stream laboratory in Grahamstown was designed with these criticisms in mind. We accept that no indoor system can mimic the natural world, but argue that the operational control gained allows valuable opportunities for consideration of both single variables, and complex effluent effects. The system aims to provide a range of hydraulic conditions which can be accurately described, since rheophilous stream organisms have specific hydraulic preferences and requirements (Statzner *et al.*, 1988). The system includes models at 2 scales: large-scale models which aim to adequately model natural physical conditions; and smaller portable models which can be used in the laboratory or in the field.

2.3 DESIGN OF THE LARGE THREE-CHANNEL SYSTEM

Figure 2.1 shows the structure and components of a single three-channel unit. Details of the experimental stream units, in the form of detailed drawings, perspective drawings and design sketches are available on request from the second and third authors at the address given.

2.3.1 Design specifications

In the first instance the streams will be used to investigate the tolerances of stream macroinvertebrates to selected water quality variables. However, in order to distinguish organism responses to water quality it is important to define the hydraulic conditions under which they are held, since flowing water fauna are particularly responsive to hydraulic variables (Statzner *et al.*, 1988). Therefore, in addition to water quality control, design criteria required controlled variability over the flow depth and velocity regime in the channels. A variable flow rate to the channels, together with the ability to vary the channel gradient, provides as wide a range as possible of depth and velocity combinations. Water quality control is addressed by the use of materials which will neither affect the quality of the recirculating water, nor be affected by the water.

Three identical three-channel units were built to accommodate a control and 2 experimental units. Each recirculating unit was designed with 3 replicate channels in which the flow regime for any given flow rate was essentially identical.

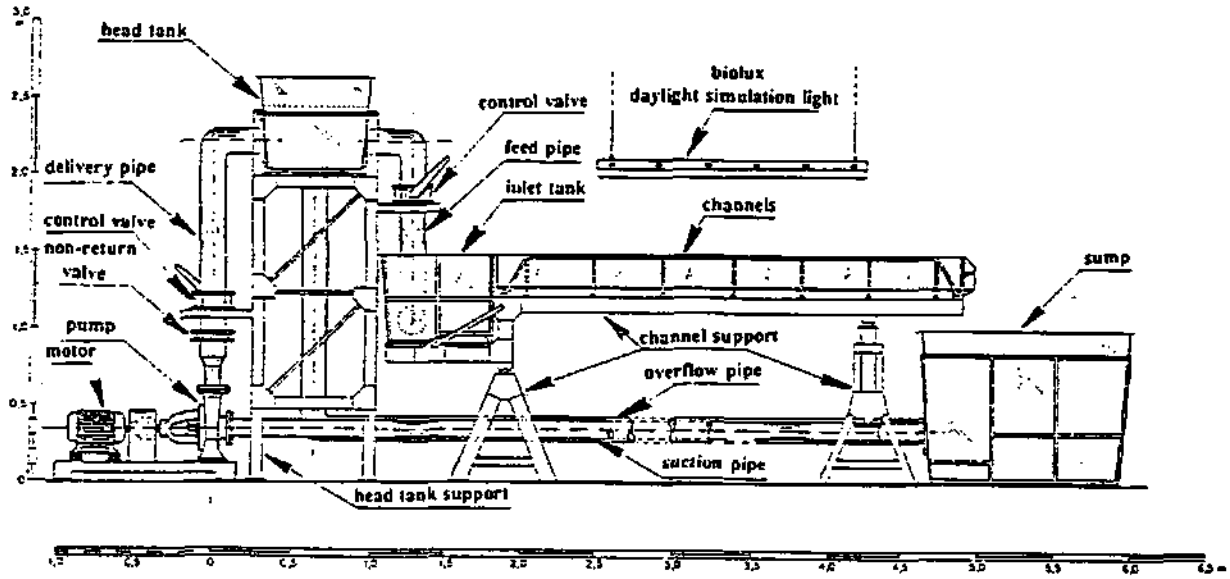

Figure 2.1 An elevation of one of three 3-channel artificial stream units: Water is pumped at a constant 20 l/s through a non-return valve (which prevents back-flow through the pump after switch-off or trip) and a control valve, up the delivery pipe into the head tank. Water flows to the perspex channels through a second control valve, via a perforated diffuser pipe in the inlet tank, which contains a shaped tank-to-channel transition to ensure equal delivery to each channel. If the second control valve is set to deliver less than 20 l/s the excess water overtops an internal weir in the head tank, and is returned direct to the sump via the overflow pipe. The suction pipe delivers water from the sump to the pump. The channel gradient can be varied from level to 4% via the adjustable channel support, and a wide range of hydraulic conditions can be achieved by varying discharge and slope.

2.3.2 Design approach

The hydraulic and structural design of each unit proceeded from the specification of the required flow regime in the channels. So as to be able to work with the whole range of rheophilous organisms, flows from low velocities (0.3 m/s) to flows in excess of 1 m/s were required: for example the pest species of blackfly *Simulium chutteri* is only found in velocities greater than 1 m/s. From this flow rate, appropriate pipe sizes, tank dimensions and the overall dimensions of the unit were progressively calculated.

The selection of an appropriate pumping unit was considerably complicated by the limitations of the power supply to the laboratory site. Although an adequate 380 volt 3-phase mains supply was available, Grahamstown's municipal electricity supply is subject to unpredictable outages, which can last for substantial periods. The experimental runs are scheduled to last for a minimum of 96 h, during which pump stoppage due to power failure would have catastrophic effects on the aquatic biota populating the channels, because the channels would drain down very quickly on cessation of flow. It was therefore vital to have an emergency backup power supply available, to come on line automatically and rapidly in the event of mains supply failure. A backup supply to a maximum of 3 kW was negotiated from a nearby source.

2.3.3 Materials specification

Because of the emphasis in the project on the control of water quality it was decided that all materials directly in contact with water were to be plastic-based: perspex, fibreglass, polyvinylchloride, etc., with documented high levels of chemical resistance. The exceptions were the pump volute casing and impeller, which were cast iron, and bolts on flanged pipework, which were high quality stainless steel. Economy dictated the use of mild steel and timber for the components of the support structures, for which protective coatings were specified.

2.3.4 Channels

Each of the 3 channels followed the basically trapezoidal cross-section of a stream, with a bottom width of 200 mm, depth of 200 mm and a side-wall angle of 70°, giving a top width of a little less than 350 mm. The total width of 3 parallel channels allowing for supporting ribs fixed the overall width of the unit at about 1 200 mm, and therefore influenced the size of tankage. A velocity range of 0.3 m/s to more than 1 m/s was required, together with a depth range of about 30 mm to 60 mm. Initially the use of fibreglass was favoured for the three-channel units, as the material is more robust than perspex, and the unit could have been completely jointless. The cost of this option was prohibitive. In addition, the provision of viewing windows complicated the design; construction in perspex has the advantage of providing full visibility into all parts of the channels. The channel length is therefore 3 200 mm, to suit the size of standard perspex sheets.

Preliminary analysis of the channel flow regime using the one-dimensional open channel flow package CFP (ECC, 1987) indicated that a maximum flow rate of 20 l/s, with the ability to vary the channel slope between 1% (0.57°) and 3% (1.72°) would achieve this velocity and depth range in a bare channel with no substrate. The hydraulic analysis of the channels is discussed later.

2.3.5 Pipework

The size of pipes was dictated by the necessity of keeping velocities as low as possible, both to reduce internal losses (pipe losses are proportional to the flow velocity squared), and to minimise turbulence on entry into tanks. The hydraulics of the piping system was analysed using the Darcy-Weisbach pipe friction equation (Institution of Water Engineers (IWE), 1969) (with an "f" value for plastic pipes, derived from the Moody Diagram (IWE, 1969), of between 0.016 and 0.017) to calculate pipe friction losses. "Minor" losses in bends, fittings and at pipe entries and exits were calculated using published loss coefficients (IWE, 1969). Pipe runs are short, and minor losses are generally much higher than losses due to pipe friction. Losses through the butterfly and non-return valves were estimated from data provided by the manufacturer/supplier, or from published data for similar valves.

As a result of this analysis a combination of pipes, valves and fittings of nominal internal diameters of 100, 125 and 150 mm was selected, which satisfied the hydraulic requirements of the system, and fitted into the space available in the laboratory. One of the uncertainties in the hydraulic analysis was the exact capabilities of the pump in terms of flow rate and delivery head, but it was recognised that control of the pump delivery could be exercised using the valve on the delivery line, for which purpose the butterfly valve selected is well-suited. The hydraulic analysis also indicated that the control valve on the line from head tank to feed tank would need to be throttled to about half-closed to limit the delivery to 20 l/s, and this proved to be the case. Although these control valves were very expensive they proved to be absolutely indispensable for flow control. The non-return (reflux) valve on the pump delivery line, also an expensive item, was necessary to prevent flow in this line reversing when the pump stops on power failure, which would cause serious damage to the pump when restarted on the backup power supply.

Unplasticised polyvinylchloride (uPVC) pipes are manufactured in South Africa and are relatively inexpensive. The fittings and valves were imported, however, and were very expensive. Costs of pipes, fittings, valves and bolts amounted to about 27% of the total materials costs of each stream unit.

2.3.6 Tankage

All the tanks were cast in fibreglass at DWAF's Pretoria hydraulics laboratory to specific dimensions dictated by hydraulic and space considerations: it was not possible to make use of proprietary tanks of standard sizes.

Sump: The sump is 1 200 mm square by 1 000 mm deep, with a total capacity of about 1 450 l, while the total volume of water in the system was calculated to be a little over 1 700 l. Should the pump fail, about 300 l is retained in the head and feed tanks, or stored in the low level pipework, and the capacity of the sump is sufficient for the balance, with a freeboard of about 30 mm.

Analysis, after a method proposed by Kulkarni and Shah (1987), indicated that in normal operation, the water level in the sump was sufficiently high above the pump suction pipe to preclude the formation and ingress to the pump of damaging air-entraining vortices. Under normal operation the water depth in the sump is 700 mm.

A major leak in the system during periods of unsupervised operation could result in the pump running dry, resulting in major damage to the motor. A float switch was installed in each sump, which will trip the pump should the sump water level fall by more than 200 mm.

Head tank: Variation of discharge into the channels is achieved by pumping into a head tank, and thence via a channel feed control valve into the feed tank and channels. A 400 mm high internal sharp-crested weir across the head tank is set so that the maximum flow rate of 20 l/s is delivered when the water level stands at the weir crest level. Partial closure of the feed control valve causes the water to overtop the weir, spilling into the reject pipe directly into the sump, with the balance of the flow continuing into the channels. Irrespective of the flow rate into the channels the pump continues to deliver its rated flow against a delivery head which varies by only about 70 mm between zero flow and 20 l/s into the channels - essentially a constant delivery head. This flow variation could also be achieved by the use of a variable speed motor, but the additional cost was prohibitive, and had the additional disadvantage of adding considerable complication to an otherwise relatively simple switchgear design.

Feed tank: The purpose of this tank (approximately 1 200 mm x 700 mm x 550 mm deep) is to distribute the flow from the head tank as evenly as possible among the 3 channels. A submerged horizontal diffuser pipe in the tank is perforated with a number of 20 mm diameter holes, which in total have the same area as the incoming pipe cross-section, thereby causing no increase in flow velocity, and arranged in a pattern so that the incoming flow is distributed across the full width of the tank. A shaped transition piece between the tank and the channels ensures that there are no abrupt discontinuities to cause turbulence in the channels. Although difficult both to construct and install, the transitions were observed to perform well.

2.3.7 Pumps

The hydraulic analysis of the pipework, channels and tankage indicated the need for a pump with a rating of 20 l/s against a total delivery head of at least 2 m, preferably 2.5 m. This is not a standard head/discharge combination: most proprietary off-the-shelf units capable of pumping 20 l/s do so against a delivery head of between 5 and 10 m, and require a motor of 2.2 kW. Delivery through smaller vertical lifts is achieved by destroying delivered head through a throttled delivery valve. This was not an option here, because the available backup power was limited to 3 kW, insufficient to run three 2.2 kW motors (a pump motor is rated for starting, and subsequently draws a little more than half its rated power in normal operation), still less to restart them.

It was necessary to have special units designed, with downrated (reduced speed) motors and oversized impellers, powered by a 1.1 kW motor, and which could therefore run on the available backup power. Nevertheless, to avoid overloading the backup supply, timers were included in the switchgear which, on restart under backup power, will stagger starting of the 3 pumps about 15 s apart.

Pump impeller shafts are fitted with mechanical seals, as standard greased glands can affect water quality. Because the pump is a special, there is no published performance data available. No proving tests were carried out in the manufacturer's works, so that even on delivery the exact duty point (discharge versus head) was known only theoretically. The

indications are that the pump delivers its claimed 20 l/s against a head in somewhat excess of 2 m: the excess head is destroyed by delivery through a half-closed control valve on the delivery line.

The special pump/motor units were about twice as expensive as a standard higher head unit, and the cost of the pumps and switchgear represented about 30% of the total materials costs of the units.

2.3.8 Structural design

The most important considerations in the design of the support structures were minimisation of deflection under maximum loading conditions, and rigidity.

Channel and feed tank support: Excessive deflection of the steel framework supporting the channels would not only result in variable and unpredictable hydraulic conditions in the channels, but could also over stress the perspex and cause cracking. The maximum deflection of the channel support is limited to 1 mm by the use of a primary frame out of 100 x 50 x 2 mm rectangular mild steel tubing, ledged with 50 x 50 x 2 mm square tubing. The feed tank support, in the larger sized tubing, is cantilevered from the channel support frame. In terms of strength this approach results in considerable over design: the maximum tensile stress in the main members was calculated to be only 12% of the permissible stress. Rigidity is provided by the use of substantial steel plate gussets at all joints.

Trestles: The rear (fixed) trestle, which carries about 75% of the total load of the channel/feed tank system, is considerably sturdier than the front (adjustable) trestle. Both are bolted to the laboratory floor. Two lifting mechanisms comprising 24 mm dia. square threaded rods in the front trestle facilitate adjustment of the channel gradient. The joint between the rear trestle and the channel support is essentially a hinge, whilst rollers at the head of the front trestle accommodate longitudinal movement of the channel support during gradient adjustment.

Head tank support: In addition to a working load, in excess of 300 kg, of the head tank and associated pipework and valves, this 2.4 m high structure was designed to carry the full weight of at least one person engaged in operational and maintenance activities. Its main members are 100 x 100 x 2 mm square tubing. It is firmly bolted to the laboratory floor.

2.3.9 Shortcomings in the design

In general the 3 large recirculating units perform as expected, and provide a flowing water environment in the channels with a considerable range of depth and velocity combinations. It is clear, however, after some months of operation, that some aspects of the design could be improved.

2.3.10 Tankage

Pipe joints: Jointing of pipes into tanks was effected by bolting through the tank walls using PVC stub flanges/backing rings and rubber gaskets. This gave rise to some difficulties in sealing the considerable number of possible leakage paths - extensive use was made of silicon sealant, which probably has a useful life of about 3 years. A better solution would have been to have the tanks fabricated in welded polypropylene sheet, into which flanged stub pipes can

be welded. This option was investigated at design stage. but the cost proved to be beyond the project resources.

Channel feed tank: The transition unit at the head of the channels was designed to facilitate a smooth transition from the feed tank into the 3 channels, avoiding abrupt cross-sectional changes and associated turbulence in the channel flow regime. Hydraulically it performs well. but with hindsight it was over-sized, extending too far down into the tank. and difficulty was experienced in sealing the unit into the tank.

2.3.11 Joints

Joint between channel feed tank and channels: The requirement for a degree of modularity in the stream unit precluded gluing of this joint, and the joint was sealed using quite large quantities of silicon sealant. The relatively brief life of this material indicates the need for regular maintenance on this important joint. The use of fibreglass was considered for the channels at design stage, which would have made a bolted/gasketed joint possible here, but the cost was prohibitive.

In general too much reliance was placed on the use of silicon sealant.

2.3.12 Channels

Initially fibreglass was the preferred material for the channel units, because the material is tougher than perspex, and the unit could have been completely jointless. Apart from cost considerations, the provision of viewing ports in the channels complicated the design. Hot moulding the perspex to form jointless channels was also considered, but required an oven larger than any available.

2.3.13 Overflow from head tank to sump

Water overtopping the head tank weir falls directly into the overflow pipe, entraining air which is carried into the sump, causing some turbulence and splashing. A perspex splash cover at the sump addresses the symptoms, but some form of baffle in the head tank could solve the problem at source.

2.3.14 Steelwork

All steelwork was etch-primed and painted with corrosion resistant paint, which according to the manufacturer's instructions did not need further coating. However. after only a few month's operation the steel-work shows some surface rusting, and will require attention. Application of a gloss finish would have been more effective - possibly a chlorinated rubber coating.

2.4 SMALL-SCALE MODEL OR RACEWAY DESIGN

Small-scale models were constructed on a raceway design (Ciborowski, 1983). Each raceway is constructed from 5 mm perspex, set into and glued to a perspex base which is reinforced by a 25 mm marine-ply wood base (Figure 2.2). The channel width is 125 mm, and the working length 470 mm. Water is recirculated by a paddle wheel driven by a Bosch CHP 12v (9 390 292 085) windscreen-wiper motor screwed to the wood base in the centre of the raceway well. The motor was selected so it would run in the field using a DC energy source,

such as a car battery, and can also be powered by the mains supply in the laboratory via a transformer/rectifier. The 2 motor speed settings provide the opportunity for 2 different speed settings for the paddle: at the higher motor speed, 60 r/min, the 330 mm diameter paddle has a peripheral speed of 1 m/s, while the lower speed of 45 r/min produces a paddle peripheral speed of 0.75 m/s. In the first model we used a variable resistor to alter speed, but at low speeds the motor overheated. The motors operate reliably for periods of several weeks at either of the 2 given settings.

The most serious problem with the raceways has been leaking. Despite reinforcement of the channel sides with perspex buttresses, transporting or moving the raceways stresses the joints sufficiently to cause leakages. To date this has not been solved, and it means there is time wasted in maintenance.

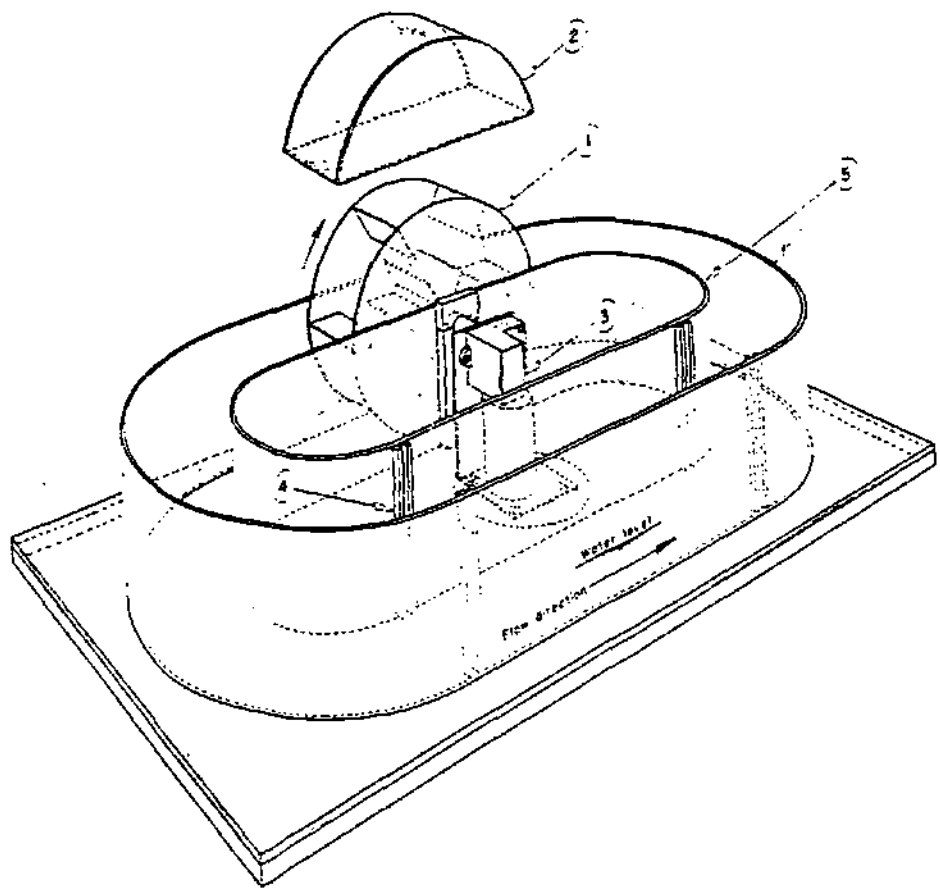

Figure 2.2 Diagram of a perspex, portable raceway which is used both as a small-scale model in the artificial stream laboratory, and in the field.

2.5 LABORATORY LAYOUT AND EXPERIMENTAL DESIGN

Three 3 channel units are accommodated lengthwise in the artificial stream laboratory, and 3 raceways are connected to each unit. This allows each experiment to run at 2 scales, with 3 replicates at each scale. Single variables can be selected and maintained at different experimental levels in each unit. Portable raceways can subsequently be taken into the field, and the experiment repeated under river-side conditions using river water and freshly collected

animals. In this way the small-scale raceways are used for "field verification" of the large-scale channel systems, with laboratory raceways providing a comparison of scale effects.

The laboratory systems require a ready supply of experimental animals and additional raceways are kept in the laboratory for rearing experiments.

In addition to the hydraulic control offered within each unit, other factors such as temperature, daylength and light can also be controlled in the laboratory. Light is provided by OSRAM "biolux" tubes which provide a full spectrum of wavelengths, similar to sunlight. Intensity is controlled by suspending the lights on chains, so their height above the channels can be adjusted. Lights are on a daylength adjustable timer. Air temperature control is provided by two Sanyo SA246SE (24000 btu) air conditioners which allow control within 1°C, between 21 and 24°C. Water circulated through the channels can be heated to 26°C, as the pumps heat the water, and cooled to 14°C by epoxy coated copper cooling coils in the sump through which the coolant R22 is circulated by a compressor. For any experimental period at a particular temperature setting, water temperature is maintained within a 2°C range.

In experiments where water quality variables are altered, the starting water quality must be specified. There is a range of alternatives. The laboratory is supplied with municipal tap water, there is a 10 000 l rain tank outside, and local river water could be brought in by water tanker.

2.6 DISCUSSION

Artificial stream research provides the opportunity of controlling variables, both physical and chemical, in a way that is impossible under field conditions. This means that the role of individual variables, and combinations of variables can be investigated. However, artificial stream research has limitations. The natural environment, with all its inherent variability is exchanged for operational control, which calls into question the applicability of results in the field. However, there are questions of pressing concern which are intractable to field research. These include questions about the tolerances of indigenous riverine fauna to changing water quality conditions.

Bioassessment and field distribution studies can provide correlative data concerning the environmental abiotic ranges within which natural populations survive. Such studies cannot, however, distinguish the effect of individual water quality variables; and other factors (such as biotic interactions) may restrict the distribution of a species to conditions well within its tolerance range. The absence of a species cannot necessarily be related to deteriorating water quality. Field distribution studies therefore cannot provide information on the tolerance limits of species to single water quality variables, or to complex effluent. This is the role of ecotoxicology (Metcalfe-Smith, 1991).

There are several toxicity testing options, including: the choice between the use of standard laboratory organisms or local riverine organisms; the choice between standing or flowing water test systems; in flowing systems the choice between through-flow and recirculating systems; the choice between lethal and sub-lethal tests (e.g. behaviour, growth, or reproduction); and in lethal tests the choice between acute (up to 96 h), sub-acute (4 to 7

days) or chronic (longer than 7 d) testing. In lethal testing the LC_{50}, or lethal concentration at which 50% of the population dies is the standard measure of response.

In South Africa, toxicity testing already has a role in water quality management: when DWAF receives requests to grant a permit for effluent disposal, the toxicity of the effluent to biotic components is tested using standard laboratory organisms, and a battery of acute and chronic tests. This could be extended in that toxicological objectives could be included in permit requirements. Compliance with such effluent standards would be monitored routinely by the polluter, using toxicity tests. This would usually involve the use of standard laboratory organisms.

The artificial stream laboratory provides flowing water recirculating systems in which indigenous riverine organisms can be subjected to lethal or sub-lethal tests. There are 3 main applications:

- If routine bioassessment indicates a water quality problem by the decline or disappearance of specific taxa, taxonomic composition can be correlated with a range of variables, using multivariate statistics, to indicate the possible causative factor/s. The responses of taxa which were in the stream can be tested at a range of concentrations to establish LC_{50}, and NOEL (no observed effects level) values, which could be used to provide the polluter with a range of limits for effluent concentrations.
- Environmental water quality guidelines can be refined at any chosen scale from river reach, to river, to ecoregion, using organisms typical of the chosen system. Specific ecosystems, and variables of concern can be selected, and a data base developed with information which links the indirect methods of setting guidelines to specific riverine conditions at a spatial scale selected by water quality managers.
- The toxicity of whole effluent can be tested in the field using river water and organisms from the proposed receiving water.

In conclusion, we suggest that the artificial stream laboratory provides the facilities to form a valuable part of a suite of available toxicity testing options, which in turn are part of the wider arena of water quality management. In addition to water quality research, the systems are ideal for investigating the precise hydraulic preferences of stream organisms. This has applications in providing water quantity guidelines. At changing discharges the proportional availability of various hydraulic condition changes, with information on hydraulic habitat preferences predictions could be made concerning the biological consequences of altered flow regimen. And in addition to freshwater research, the systems can be filled with water of any of a range of salinities, and with suitable modifications to reduce flow velocities, be used to simulate estuarine conditions.

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Appendix 2.1

Large multi-channel systems: Details of equipment and materials

Pumps, motors and switchgear

Pump/motor units: AZG 100-200/180 CI/CI/MS all-iron pumps, to deliver 20 l/s against ±2.5 m head, each mounted on a common mild steel baseplate and direct- coupled to a 1.1 kW 6 pole 380 volt 3 phase motor. The pump has a 180 mm dia. impeller, 125 mm dia. suction flange, 100 mm dia. delivery flange, and is fitted with mechanical seals.

Starter/control panel: Switchgear is contained in a mild steel cabinet fitted with door-interlocked circuit breaker and a voltmeter with a 3 position selector switch, and comprises for each of 3 pumps a starting contactor, overload relay, run ammeter, stop/run selector, power-on/run/trip indicators and a 3-pole circuit breaker. A mercury float switch in each sump trips the pump should the water level in the sump drop to unacceptably low levels. An adjustable timer is fitted to each starter to facilitate staggered restart after mains power failure, to avoid overloading the back-up power supply.

Pipework, fittings and valves

* All pipework is Class 4 (±4 bar pressure rating) uPVC Type 1 of 110, 125 and 160 mm outside dia.
* Fittings (bends and tees) are PVC with solvent welded joints to pipes.
* Joints to tanks and valves are by means of glued PVC stub flanges with bolted PVC backing rings and EPM (ethylene-propylene copolymer) gaskets. Bolts at tank joints are stainless steel; all other bolts are mild steel.
* Control/isolating valves are fitted on the delivery lines between the pumps and head tanks, and between the head tanks and channels: these are 150 mm dia. rapid action butterfly valves of all-plastic and stainless steel construction.
* Non-return (reflux) valves are fitted on the delivery lines immediately above the pumps: these are 150 mm diameter wafer-type valves with polypropylene bodies and discs, without spring disc reset.

Channels

Channels and head tank weirs are in 6 and 8 mm thick polymethylacrylic (acrylic sheeting or perspex) sheet, with solvent-glued joints.

Tankage

All tanks are cast in fibreglass, using Crystic 406PA low exotherm general purpose polyester resin with chopped strand glass mat: a minimum wall thickness of 6 mm was achieved, and the sumps were reinforced with cast-in 19 mm square steel tubing.

Support structure

The steel support structures are in welded mild steel square and rectangular tube and flat bar, etch-primed and painted with corrosion-resistant paint. Platforms for head and feed tanks, and channel support ribs are in heavily varnished marine ply 20 mm thick.

Appendix 2.2

Hydraulic analysis of large system channels

Flow in the 3 channels was analysed using the one-dimensional open channel flow software CFP (channel flow profiles) (ECC, 1987), for a range of flows up to 25 l/s, and for a range of channel gradients from 0% to 3%.

For a bare channel with no substrate a value of 0.009 was used for the Manning's "n" roughness coefficient (Chow, 1959). (Tables 2.1 to 2.5), giving calculated average velocities and depths of flow on the channel centre lines for flows of 10, 15, 20 and 25 l/s and channel gradients of 0, 0.5, 1, 2 and 3%. Results are given at 400 mm intervals, from a point 400 mm from the upstream end of the channel to a point 400 mm from the downstream end, ignoring the transitional lengths from the feed tank and into the free overfall into the sump. Figure 2.3 is a generalised plot of the depth and velocity results for the bare channel case which indicates the range of depths and velocities to be expected for the middle 1 600 mm section of the channel, the probable test section, for each flow rate and channel slope. The flow rate lines are envelopes of the individual plots of depth against velocity at each calculation point along the channel.

Analyses were also carried out for 2 possible substrate cases: two 50 mm size stones at 100 mm intervals along the channel and; two 40 mm size stones at 100 mm intervals along the channel, in a general pebble matrix 25 mm thick. The results are presented in summary as a comparison with the bare channel results (Table 2.6).

TABLE 2.1

DEPTH-VELOCITY CALCULATIONS, AT 400 mm INTERVALS DOWN THE LENGTH OF THE CHANNEL, FOR FLOW RATES OF 10, 15, 20 AND 25 l/s. SLOPE SETTING - 0%. FLOW IS SUBCRITICAL AT ALL FLOW RATES.

Distance along channel (mm)	10 l/s		15 l/s		20 l/s		25 l/s	
	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)
400	41	0.37	52	0.44	62	0.49	71	0.53
800	41	0.38	51	0.44	60	0.49	70	0.54
1 200	40	0.39	50	0.45	59	0.50	68	0.55
1 600	39	0.40	49	0.46	58	0.52	67	0.56
2 000	38	0.41	48	0.48	57	0.53	66	0.57
2 400	37	0.43	47	0.49	55	0.55	63	0.59
2 800	35	0.45	45	0.52	53	0.57	61	0.61

TABLE 2.2

DEPTH-VELOCITY CALCULATIONS, AT 400 mm INTERVALS DOWN THE LENGTH OF THE CHANNEL, FOR FLOW RATES OF 10, 15, 20 AND 25 l/s. SLOPE SETTING - 0.5% AT THE MIXED FLOW REGIME FOR 10 l/S. * INDICATES SUBCRITICAL FLOW. FLOW FOR HIGHER DISCHARGES IS SUPERCRITICAL, BUT WITH A FROUDE NUMBER CLOSE TO UNITY. WATER SURFACE IS LIKELY TO BE WAVY, AND GRADIENT IS NOT LIKELY TO PRODUCE STABLE FLOW CONDITIONS.

Distance along channel (mm)	10 l/s		15 l/s		20 l/s		25 l/s	
	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)
400	29	0.59	36	0.66	43	0.72	50	0.77
800	27	0.59	35	0.68	41	0.73	49	0.78
1 200	27	0.60	35	0.68	41	0.74	49	0.79
1 600	36	0.45*	35	0.68	40	0.75	46	0.80
2 000	38	0.41*	35	0.69	40	0.76	46	0.81
2 400	41	0.39*	35	0.70	40	0.77	47	0.81
2 800	31	0.52*	34	0.70	40	0.77	47	0.81

TABLE 2.3

DEPTH-VELOCITY CALCULATIONS, AT 400 mm INTERVALS DOWN THE LENGTH OF THE CHANNEL, FOR FLOW RATES OF 10, 15, 20 AND 25l/s. SLOPE SETTING - 1%. FLOW IS SUPERCRITICAL AT ALL FLOW RATES.

Distance along channel (mm)	10 l/s		15 l/s		20 l/s		25 l/s	
	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)
400	24	0.65	33	0.73	39	0.79	46	0.83
800	23	0.70	30	0.78	37	0.83	44	0.88
1 200	23	0.71	30	0.80	37	0.86	43	0.91
1 600	23	0.73	30	0.82	36	0.88	42	0.93
2 000	22	0.74	29	0.83	35	0.90	41	0.95
2 400	21	0.74	29	0.84	34	0.91	40	0.97
2 800	21	0.75	28	0.85	34	0.92	39	0.98

TABLE 2.4

DEPTH-VELOCITY CALCULATIONS, AT 400 mm INTERVALS DOWN THE LENGTH OF THE CHANNEL, FOR FLOW RATES OF 10, 15, 20 AND 25 l/s. SLOPE SETTING - 2%. FLOW IS SUPERCRITICAL AT ALL FLOW RATES.

Distance along channel (mm)	10 l/s		15 l/s		20 l/s		25 l/s	
	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)
400	22	0.74	30	0.81	36	0.87	42	0.92
800	20	0.81	26	0.89	32	0.95	40	1.00
1 200	18	0.85	25	0.94	31	1.01	37	1.06
1 600	18	0.88	24	0.98	30	1.05	36	1.10
2 000	18	0.91	24	1.01	29	1.08	34	1.14
2 400	17	0.92	23	1.03	28	1.11	34	1.17
2 800	17	0.93	23	1.05	28	1.13	32	1.19

TABLE 2.5

DEPTH-VELOCITY CALCULATIONS, AT 400 mm INTERVALS DOWN THE LENGTH OF THE CHANNEL, FOR FLOW RATES OF 10, 15, 20 AND 25 l/s. SLOPE SETTING - 3%. FLOW IS SUPERCRITICAL AT ALL FLOW RATES.

Distance along channel (mm)	10 l/s		15 l/s		20 l/s		25 l/s	
	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)	Depth (mm)	Vel. (m/s)
400	20	0.80	27	0.88	34	0.94	40	0.98
800	18	0.90	24	0.98	31	1.05	36	1.09
1 200	17	0.96	23	1.06	29	1.12	35	1.17
1 600	17	1.01	22	1.11	27	1.18	34	1.23
2 000	16	1.04	21	1.14	26	1.22	32	1.28
2 400	16	1.06	20	1.17	25	1.26	30	1.32
2 800	15	1.07	20	1.19	24	1.28	29	1.35

TABLE 2.6

DEPTH-VELOCITY CALCULATIONS, AND FLOW REGIME AT 4 SLOPES, AND 4 FLOW RATES, WITH 2 SUBSTRATE TYPES:

1. TWO 50 mm STONES, EVERY 100 mm DOWN THE LENGTH OF THE CHANNEL
2. A PAVEMENT OF 25 mm PEBBLES. WITH TWO 40 mm STONES EVERY 100 mm DOWN THE LENGTH OF THE CHANNEL.

THIS IS A SUMMARY OF RESULTS FOR THE MIDDLE 1 600 mm OF THE CHANNEL.

Substrate Condition		50 mm stones (n = 0.03)			40 mm stones in 25 mm thick matrix (n = 0.022)		
Slope %	Flow (l/s)	Velocity range	Depth range	Flow regime	Velocity range	Depth range	Flow regime
0	10	0.28-0.33	55-46	Sub	0.30-0.36	50-45	Sub
	15	0.33-0.40	68-57	Sub	0.36-0.43	63-53	Sub
	20	0.38-0.45	78-66	Sub	0.41-0.48	70-62	Sub
	25	0.42-0.49	87-75	Sub	0.45-0.52	80-70	Sub
1	10	0.39-0.40	40-39	Sub	0.47	33	Sub
	15	0.46-0.47	50-49	Sub	0.54	43	Sub
	20	0.51-0.53	59-57	Sub	0.60	50	Sub
	25	0.55-0.57	67-66	Sub	0.66	57	Sub
2	10	0.50	34	Sub	0.58	27	Super
	15	0.56	41	Sub	0.67	35	Super
	20	0.63	48	Sub	0.75	41	Super
	25	0.70	53	Crit	0.81-0.82	47-46	Super
3	10	0.55	30	Super	0.66	24	Super
	15	0.64	38	Super	0.76-0.77	31-30	Super
	20	0.71	44	Super	0.84-0.85	37-36	Super
	25	0.77	50	Super	0.90-0.93	43-41	Super

Appendix 2.3

Hydraulic analysis of large system pipework

Pipe friction losses calculated using the Darcy-Weisbach equation (IWE, 1969):

$$h_p = f.l.v^2 / 2.g.d$$

where:

h_p = friction headloss in pipe (m)
f = friction factor (0.016 to 0.017 for uPVC pipes of dia. 100 to 160 mm, assuming an absolute roughness of 0.01 mm)
l = length of pipe (m)
v = average velocity in pipe (m/s)
g = acceleration due to gravity (9.81 m^2/s)
d = pipe internal diameter (m)

Minor pipeline losses (bends, fittings, entry and exit losses) calculated using the expression

$$h_m = k.v^2 / 2.g$$

where:

h_m = headloss (m)
k = loss coefficient from IWE (1969)

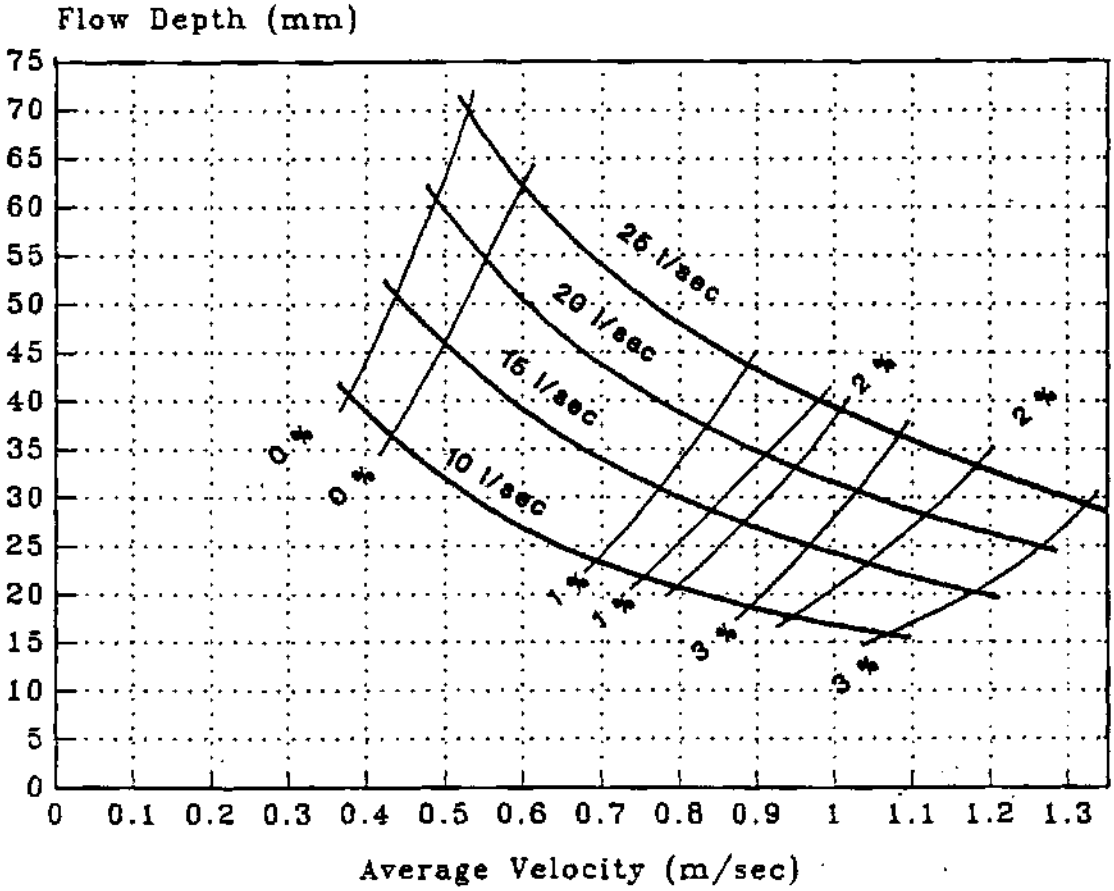

Figure 2.3 A generalised plot of calculated average velocities and centreline depths in the middle 1 600 mm of a channel with no substrate, showing the expected range of depth and velocity combinations at flow rates of 10, 15, 20 and 25 l/s, and at channel gradients of 0, 1, 2 and 3%.

CHAPTER 3

CALIBRATION OF THE STREAM SYSTEM

Summary The objective of calibrating the streams was to establish the physical and chemical conditions in the streams before organisms were introduced; then to monitor behaviour of organisms in the streams before water quality conditions were altered. This chapter deals with several aspects of calibration: hydraulic conditions in the channels, temperature conditions in the channels and in the laboratory, water quality (selected constituents such as pH, oxygenation, and nutrients) and behavioural responses of organisms to selected slopes, velocities and substrata.

3.1 INTRODUCTION

The term calibration is used here to describe the process of defining experimental conditions in the artificial stream laboratory.

The first step was hydraulic calibration. This involved confirming that hydraulic conditions in each of the channels, and in the three systems, were the same for any given discharge and slope setting. Hydraulic conditions were measured for a range of slope, discharge and substrate settings so experimental conditions could be selected and accurately described.

The system has a combination of temperature control options. The laboratory is air conditioned, and each set of channels has a water chiller. Temperature calibration involved checking the range of temperatures which could be maintained, and the degree of variation over time.

After installation, certain key water quality constituents were monitored in the three systems. This may prove to have additional significance in the context of the ultimate statistical analysis of experimental data. The three channels in each system were designed as replicates, but since they share the same water source they are not in fact independent replicates. Each 3-channel system is independent of the others, but channels can only be regarded as pseudoreplicates.

However, cost precluded nine separate systems, and the nine channels could not be routed randomly between the three pumps. It can be argued that three separate test populations are better than one, and it can be shown that all the water quality constituents and hydraulic conditions measured in the systems did not differ significantly over a test period of seven days. The pseudoreplicate status of the channels may therefore be less problematic. However, the problem remains that if conditions change unexpectedly in one system all three replicates will be affected. This is dangerous, especially as it places at risk the interpretation that any experimental difference is in fact a result of the experimental conditions. The conservative decision is therefore to use each system as a single system and the three systems as replicates,

which trebles the time period for experiments. Decisions regarding replication will have to be made depending on the circumstances of each experiment.

Since the ultimate aim was to assess macroinvertebrate responses to changes in water quality, it was important to monitor organism responses to the experimental system before water quality was changed at all. This was termed behavioural calibration, and had the potential to be extended infinitely if responses to all combinations of hydraulic, temperature and light conditions were measured. An exhaustive approach was not feasible, and a small selection of slope, discharge and substrate conditions were selected and organism responses observed. The results have been largely inconclusive - indicating we are still in the early stages of developing successful procedures for using the large systems. A similar "learning curve" was followed using the raceways, and the results presented here will ultimately belong with those in Chapter 6 where method development is recorded.

3.2 HYDRAULIC CALIBRATION

This section was written by Mr W.S.J. Rowlston, DWAF.

The first stage of hydraulic calibration was the theoretical calculation of flow conditions in each of the channels at different slope and discharge settings (Appendix 2.2, Palmer *et al.*, 1994).

3.2.1 Objectives of hydraulic calibration

Each of the three 3-channel stream units is designed so that the flow delivered from the feed tank is distributed equally among the three channels; that is to say that for any combination of discharge and channel gradient, the average velocity and depth of flow at any point in one channel should be the same at the equivalent points in the other two channels. Furthermore, the design of each of the three stream units is identical, so that the same discharge/gradient combination in all three units should produce the same hydraulic conditions in all nine channels.

However, fabrication and constructional tolerances, together with slight differences in pump, motor and valve characteristics among units, combine to make this ideal intra and inter-unit similarity of hydraulic conditions unlikely to be achieved in practice. One objective of the hydraulic calibration was therefore to assess, against accurately metered flow rates, the accuracy of the three-way distribution of flow among the three channels of a stream unit.

The suitability of the stream units for the purposes of the research project was based to a large extent on the ability of the channels to produce a specified range of velocity/depth conditions in the channels, as predicted by a 1-dimensional analysis of the channel hydraulics. A second objective was therefore to compare the hydraulic conditions obtaining in the units with those calculated, and to assess the success of the predictions.

Budget limitations precluded the incorporation of formal flow measuring devices into the stream units. The third objective was to establish control valve settings in each of the three units installed in the Grahamstown laboratory, so that the flow rate delivered to the channels was known as accurately as possible, and so that hydraulic conditions in each of the three streams could be set as nearly as possible the same.

3.2.2 Calibration and commissioning

Calibration at DWAF's Hydraulic Laboratory

The most comprehensive exercise took place at the Department of Water Affairs and Forestry's Pretoria West Hydraulics Laboratory, in respect of the first 3-channel unit constructed.

The feed tank (including its perforated diffuser pipe) and channels, mounted on the fixed and adjustable trestles, were connected to the laboratory reticulation system, in which flow rate is measured by Gaussian electro-magnetic flow meters with a claimed accuracy of +/- 0.25% of indicated flow rate. In a bare channel (ie. without substrate) water levels and average velocities were measured at five points along the centre-line of each channel, for the middle 1800mm reach, for flow rates of 10, 15, 20 and 25 litres/second, and for gradients of 0, 0.5, 1 and 2%. Sixteen tests were performed. Figure 3.1 shows the locations of the measuring points.

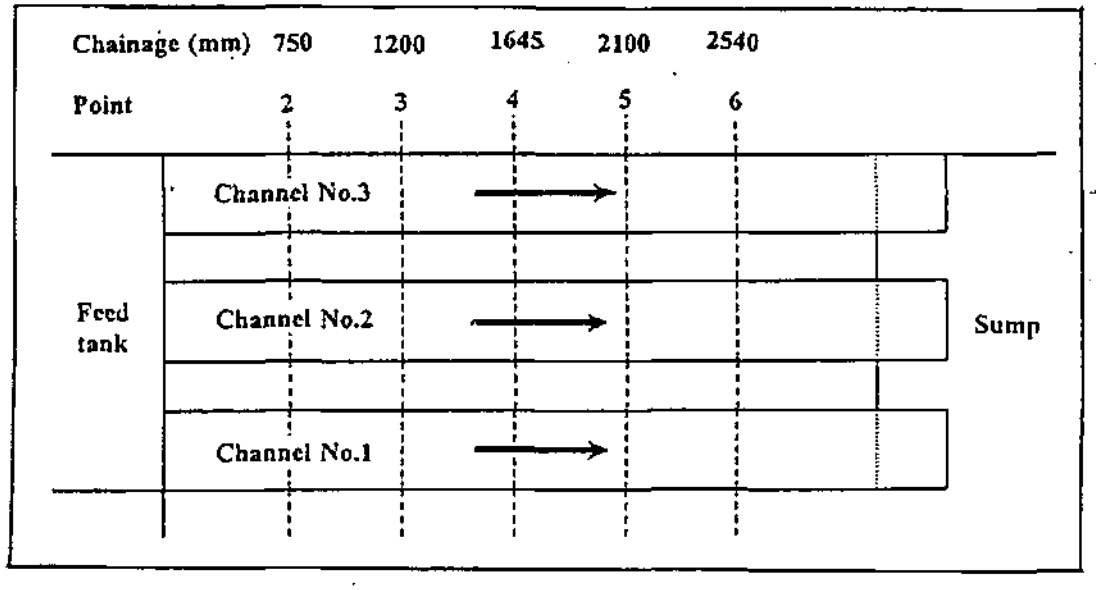

Figure 3.1 Schematic plan of experimental stream unit showing depth and velocity measuring points.

Depths of flow were measured using a simple point gauge, with a measurement accuracy of 0.5mm. Flow velocities were measured using an Ott propeller meter. The smallest propeller available was 30mm in diameter: where the flow depth was less than 30mm it was not possible to record a velocity (the whole propeller must be submerged); for flow depths between 30mm and 37.5mm velocity was measured 15mm above the channel floor (between 0.5 and 0.6 of the total depth); for flow depths exceeding 37.5mm velocity was measured at 0.6 of the depth. (In a logarithmic velocity profile the average velocity in the vertical water column occurs at 0.6 of the depth of flow). The velocity recorded at any point was the average of at least two readings of the meter.

Discharge in any channel was estimated by calculating the product of area of flow, calculated from the measured geometry of each channel, and average velocity. The distribution was compared with an equal three-way distribution among the channels.

Commissioning at Rhodes IWR Stream Laboratory, Grahamstown
Flow velocities and depths were recorded in this first stream unit (Blue) installed in the Grahamstown laboratory at the same points in the channels, in the same manner, and using the same measuring equipment as in DWAF's laboratory, for nominal flow rates of 10, 15 and 20 litres/second, at a gradient of 1%. Three tests were performed.

These commissioning tests were used to calibrate the head tank to feed tank control valve for flow rates of 10, 15 and 20 litres/second.

The second (Yellow) and third (Red) stream units were commissioned using only one flow rate - 20 litres/second, at a channel gradient of 1%. Two tests were performed.

Calibration at Rhodes IWR Stream Laboratory, Grahamstown
Three additional tests were carried out subsequent to the installation and commissioning of the stream units, all in respect of the Blue unit. Depths and velocities were recorded in one channel at an adverse (negative) gradient to assess the uniformity of hydraulic conditions under conditions for which no analysis was performed. Two limited preliminary tests were carried out with limited substrate in position, to assess the changes in hydraulic conditions from the bare channel case.

Results
The results are presented as follows:

Calibration at DWAF's Hydraulics laboratory: Blue Unit: Tables 3.1a - 3.1n.
Commissioning at Rhodes IWR Stream Laboratory: Blue Unit: Tables 3.1o - 3.1q, Yellow Unit: Table 3.2a, and Red Unit: Table 3.2b. All these were carried out in a bare channel. Tables 3.1q, 3.2a and 3.2b are the only results which can be used to compare the performance of the three stream units.

Calibration at Rhodes IWR Stream Laboratory: Blue Unit: Tables 3.1r - 3.1t. Table 3.1r gives results of the adverse gradient test. Tables 3.1s and 3.1t give results of the preliminary tests with substrate, as described in the tables.

(i) Test No: 1:0/10 (iii) Gradient: 0% (iv) Discharge (l/sec): 10 (vi) Substrate: None
(ii) Date: 13:1:93 (v) Indicated Range (l/sec):9.94 - 10.08

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1)]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.28]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [6.3/8]	9 Percent Error [8/(iv)]
2	750	41	0.0088	0.35	3.10	30.28	42	0.0090	0.39	3.55	34.71	41	0.0088	0.41	3.58	35.01	10.23	2.27
3	1200	41	0.0088	0.36	3.20	31.68	39	0.0083	0.42	3.47	34.30	39	0.0083	0.41	3.44	34.02	10.11	1.07
4	1645	39	0.0083	0.36	3.02	30.81	38	0.0081	0.42	3.43	34.95	37	0.0079	0.43	3.36	34.25	9.81	-1.91
5	2100	36	0.0077	0.40	3.03	30.08	39	0.0083	0.44	3.62	36.03	35	0.0074	0.46	3.41	33.90	10.06	0.61
6	2540	32	0.0068	0.45	3.06	31.31	35	0.0074	0.46	3.42	35.07	34	0.0072	0.46	3.26	33.63	9.76	-2.38
				Av Disch	3.08	30.83			Av Disch	3.50	35.01			Av Disch	3.41	34.17	9.99	-0.07
				Std.Devn	0.067				Std Devn	0.078				Std Devn	0.099			

Table 3.1a: Stream Unit No.1 (blue) - Calibration at DWAF Hydraulic Laboratory

(i) Test No: 2.0/15 (iii) Gradient: 0% (iv) Discharge (l/sec): 15 (vi) Substrate: None
(ii) Date: 14:1:93 (v) Indicated Range (l/sec):14.90 - 15.18

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1)]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.28]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [6.3/8]	9 Percent Error [8/(iv)]
2	750	53	0.0116	0.43	5.00	31.61	54	0.0118	0.46	5.47	34.58	52	0.0114	0.47	5.35	33.81	15.82	5.41
3	1200	52	0.0114	0.44	5.02	32.74	50	0.0109	0.47	5.14	33.58	49	0.0106	0.49	5.16	33.69	15.32	2.12
4	1645	51	0.0111	0.44	4.91	32.26	49	0.0106	0.50	5.33	34.99	47	0.0102	0.49	4.99	32.76	15.22	1.48
5	2100	46	0.0099	0.47	4.65	31.09	48	0.0104	0.51	5.32	35.55	44	0.0095	0.53	4.99	33.36	14.95	-0.31
6	2540	42	0.0090	0.52	4.65	31.44	45	0.0097	0.53	5.16	34.86	44	0.0095	0.53	4.99	33.70	14.80	-1.33
				Av Disch	4.85	31.83			Av Disch	5.28	34.70			Av Disch	5.09	33.47	15.22	1.48
				Std Devn	0.162				Std Devn	0.120				Std Devn	0.143			

Table 3.1b: Stream Unit No.1 (blue) - Calibration at DWAF Hydraulic Laboratory.

(i) Test No:3:0/20 (iii) Gradient: 0% (iv) Discharge (l/sec):20 (vi) Substrate: None
(ii) Date: 14:1:93 (v) Indicated Range (l/sec): 19.90 - 20.18

Location		Channel No. 1					Channel No. 2					Channel No. 3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.4 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	61	0.0140	0.51	7.10	33.60	62	0.0138	0.52	7.12	33.69	60	0.0133	0.52	6.91	32.71	21.13	5.64
3	1200	60	0.0133	0.52	6.89	32.51	59	0.0130	0.53	6.90	33.55	57	0.0125	0.54	6.77	32.94	20.56	2.80
4	1645	60	0.0133	0.52	6.89	32.51	59	0.0130	0.55	7.21	33.77	58	0.0128	0.57	7.28	34.08	21.35	6.77
5	2100	55	0.0121	0.55	6.66	32.02	57	0.0123	0.57	7.14	34.33	54	0.0118	0.59	7.00	33.64	20.80	3.98
6	2540	50	0.0109	0.58	6.27	31.38	53	0.0116	0.60	6.90	34.52	52	0.0114	0.60	6.81	34.11	19.98	-0.12
				Av Disch	6.76	32.54			Av Disch	7.05	33.97			Av Disch	6.95	33.49	20.36	3.81
				Std Devn	0.281				Std Devn	0.131				Std Devn	0.179			

Table 3.1c: Stream Unit No.1 (blue) - Calibration at DWAF Hydraulics Laboratory

(i) Test No: 4:0/25 (iii) Gradient: 0% (iv) Discharge (l/sec): 25 (vi) Substrate: None
(ii) Date: 14:1:93 (v) Indicated Range (l/sec): 24.90 - 25.18

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	69	0.0155	0.54	8.28	33.38	70	0.0157	0.52	8.25	33.26	67	0.0150	0.55	8.27	33.35	24.79	-0.83
3	1200	68	0.0152	0.55	8.41	33.59	67	0.0150	0.57	8.48	33.86	65	0.0145	0.56	8.15	32.55	25.03	0.11
4	1645	67	0.0150	0.56	8.43	33.12	66	0.0148	0.58	8.56	33.57	64	0.0142	0.60	8.50	33.31	25.51	2.05
5	2100	63	0.0140	0.60	8.35	33.05	64	0.0142	0.60	8.57	33.92	60	0.0133	0.61	8.35	33.04	25.28	1.11
6	2540	58	0.0128	0.64	8.22	32.60	61	0.0135	0.64	8.64	34.27	59	0.0130	0.64	8.36	33.13	25.22	0.89
				Av Disch	8.34	33.15			Av Disch	8.50	33.78			Av Disch	8.32	33.08	25.17	0.66
				Std Devn	0.083				Std Devn	0.138				Std Devn	0.116			

Table 3.1d: Stream Unit No.1 (blue) - Calibration at DWAF Hydraulics Laboratory.

(i) Test No:5:0.5/20 (iii) Gradient: 0.5% (iv) Discharge (1/sec): 20 (vi) Substrate: None
(ii) Date: 15:1:93 (v) Indicated Range (1/sec): 19.90 - 20.15

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av.Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	43	0.0093	0.60	5.55	33.43	43	0.0093	0.60	5.58	33.62	43	0.0093	0.59	5.47	32.95	16.61	-16.96
3	1200	43	0.0093	0.62	5.70	33.69	43	0.0093	0.64	5.93	35.11	42	0.0090	0.59	5.27	31.20	16.90	-15.48
4	1645	43	0.0093	0.61	5.68	33.22	42	0.0090	0.66	5.93	34.67	41	0.0088	0.62	5.49	32.11	17.09	-14.53
5	2100	48	0.0104	0.56	5.82	32.04	43	0.0093	0.66	6.14	33.82	43	0.0093	0.71	6.20	34.14	18.16	-9.21
6	2340	45	0.0097	0.59	5.70	30.78	43	0.0093	0.68	6.30	34.05	40	0.0086	0.76	6.51	35.18	18.51	-17.47
				Av Disch Std Devn	5.69 0.084	32.59			Av Disch Std Devn	5.98 0.241	34.25			Av Disch Std Devn	5.79 0.479	33.17	17.45	-12.73

Table 3.1e: Stream Unit No.1 (blue) - Calibration at DWAF Hydraulics Laboratory

(i) Test No: 6:0.5/25 (iii) Gradient: 0.5% (iv) Discharge (1/sec): 25 (vi) Substrate: None
(ii) Date: 15:1:93 (v) Indicated Range (1/sec): 24.83 - 25.11

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2 3 4 5 6	750 1200 1645 2100 2340					No	measurements	taken	standing	waves in	channels							
				Av Disch Std Devn					Av Disch Std Devn					Av Disch Std Devn				

Table 3.1f: Stream Unit No.1 (blue) - Calibration at DWAF Hydraulics Laboratory.

(i) Test No:7:1/10 (iii) Gradient: 1% (iv) Discharge (l/sec): 10 (vi) Substrate: None
(ii) Date: 14:1:93 (v) Indicated Range (l/sec): 9.95 - 10.07

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3 1 Depth (mm)	4 1 Area (m2)	5 1 Av Vel (m/sec)	6 1 Discharge (l/sec) [4 1x5 1]	7 1 Percent Discharge [6.1/8]	3 2 Depth (mm)	4 2 Area (m2)	5 2 Av Vel (m/sec)	6 2 Discharge (l/sec) [4 2x5 2]	7 2 Percent Discharge [6 2/8]	3 3 Depth (mm)	4 3 Area (m2)	5 3 Av Vel (m/sec)	6 3 Discharge (l/sec) [4.3x5 3]	7 3 Percent Discharge [6 3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	24	0 0050	-	-	-	25	0 0052	-	-	-	24	0 0050	-	-	-	-	-
3	1200	21	0 0048	-	-	-	23	0 0048	-	-	-	22	0 0046	-	-	-	-	-
4	1645	22	0 0046	-	-	-	22	0 0046	-	-	-	22	0 0046	-	-	-	-	-
5	2100	22	0 0046	-	-	-	22	0 0046	-	-	-	21	0 0044	-	-	-	-	-
6	2540	22	0 0046	-	-	-	22	0 0046	-	-	-	21	0 0044	-	-	-	-	-
				Av Disch Std Devn	- -	- -			Av Disch Std Devn	- -	- -			Av Disch Std Devn	- -	- -	-	-

Table 3.1g: Stream Unit No. 1 (blue) - Calibration at DWAF Hydraulic Laboratory
Note: Depths of flow too small to measure velocity

(i) Test No:8:1/15 (iii) Gradient: 1% (iv) Discharge (l/sec): 15 (vi) Substrate: None
(ii) Date: 14:1:93 (v) Indicated Range (l/sec): 14.91 - 15.08

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3 1 Depth (mm)	4 1 Area (m2)	5 1 Av Vel (m/sec)	6 1 Discharge (l/sec) [4 1x5 1]	7 1 Percent Discharge [6 1/8]	3 2 Depth (mm)	4 2 Area (m2)	5 2 Av Vel (m/sec)	6 2 Discharge (l/sec) [4 2x5 2]	7 2 Percent Discharge [6 2/8]	3 3 Depth (mm)	4 3 Area (m2)	5 3 Av Vel (m/sec)	6 3 Discharge (l/sec) [4.3x5.3]	7 3 Percent Discharge [6 3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	33	0 0070	0 72	5 02	32 76	33	0 0070	0 74	5 17	33.70	33	0 0070	0 74	5 14	33.54	15 33	2.175
3	1200	30	0 0063	-	-	-	31	0 0065	-	-	-	30	0 0061	-	-	-	-	-
4	1645	30	0 0063	-	-	-	29	0 0061	-	-	-	29	0 0061	-	-	-	-	-
5	2100	28	0 0059	-	-	-	29	0 0061	-	-	-	28	0 0059	-	-	-	-	-
6	2540	28	0 0059	-	-	-	29	0 0061	-	-	-	28	0 0059	-	-	-	-	-
				Av Disch Std Devn	- -	- -			Av Disch Std Devn	- -	- -			Av Disch Std Devn	- -	- -	-	-

Table 3.1h: Stream Unit No. 1(blue) - Calibration at DWAF Hydraulics Laboratory
Note: Depths of flow generally too small to measure velocity

(i) Test No:9:1/20 (iii) Gradient: 1% (iv) Discharge (1/sec): 20 (vi) Substrate: None

(ii) Date: 14:1:93 (v) Indicated Range (1/sec): 19.89 - 20.06

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av.Vel (m/sec)	6.1 Discharge (1/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av.Vel (m/sec)	6.2 Discharge (1/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (1/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	39	0.0083	0.80	6.62	33.37	40	0.0086	0.78	6.72	33.84	39	0.0083	0.78	6.51	32.79	19.85	-0.75
3	1200	37	0.0079	0.85	6.69	33.22	37	0.0079	0.86	6.75	33.56	37	0.0079	0.85	6.69	33.22	20.12	-0.61
4	1645	35	0.0074	0.88	6.53	33.20	35	0.0074	0.90	6.69	33.98	34	0.0072	0.90	6.46	32.83	19.68	-1.61
5	2100	36	0.0077	0.89	6.81	32.98	36	0.0077	0.94	7.22	34.96	34	0.0072	0.92	6.62	32.06	20.65	-3.24
6	2540	35	0.0074	0.93	6.94	33.16	35	0.0074	0.96	7.13	34.08	34	0.0072	0.95	6.86	32.76	20.93	-4.66
				Av Disch	6.72	33.18			Av Disch	6.90	34.09			Av Disch	6.63	32.73	20.25	-3.23
				Std Devn	0.143				Std Devn	0.227				Std Devn	0.140			

Table 3.1i: Stream Unit No. 1 (blue) - Calibration at DWAF Hydraulics Laboratory

(i) Test No:10:1/25 (iii) Gradient: 1% (iv) Discharge (1/sec): 25 (vi) Substrate: None

(ii) Date: 14:1:93 (v) Indicated Range (1/sec): 24.76 - 25.32

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (1/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (1/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (1/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	46	0.0099	0.85	8.43	33.74	47	0.0102	0.83	8.47	33.90	45	0.0097	0.83	8.09	32.35	25.00	-0.02
3	1200	46	0.0099	0.88	8.76	33.88	45	0.0097	0.91	8.82	34.12	43	0.0093	0.89	8.28	32.00	25.86	3.44
4	1645	42	0.0090	0.95	8.60	33.89	41	0.0088	0.96	8.47	33.38	41	0.0088	0.94	8.30	32.73	25.37	1.49
5	2100	41	0.0088	0.96	8.41	33.03	42	0.0090	0.98	8.88	34.81	39	0.0083	0.98	8.20	32.16	25.51	2.03
6	2540	41	0.0088	0.96	8.40	32.34	41	0.0088	1.03	9.02	34.73	39	0.0083	1.03	8.55	32.93	25.95	3.83
				Av Disch	8.52	33.57			Av Disch	8.73	34.19			Av Disch	8.28	32.44	25.54	2.15
				Std Devn	0.138				Std Devn	0.221				Std Devn	0.152			

Table 3.1j: Stream Unit No. 1 (blue) - Calibration at DWAF Hydraulics Laboratory

(i) Test No:11:2/10 (iii) Gradient: 2% (iv) Discharge (l/sec): 10 (vi) Substrate: None
(ii) Date: 15:1:93 (v) Indicated Range (l/sec): 9.92 - 10.08

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av.Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	18	0.0037	-	-	-	19	0.0039	-	-	-	19	0.0039	-	-	-	-	-
3	1200	18	0.0037	-	-	-	18	0.0037	-	-	-	18	0.0037	-	-	-	-	-
4	1645	17	0.0035	-	-	-	17	0.0035	-	-	-	17	0.0035	-	-	-	-	-
5	2100	17	0.0035	-	-	-	17	0.0033	-	-	-	16	0.0033	-	-	-	-	-
6	2540	17	0.0035	-	-	-	17	0.0033	-	-	-	16	0.0033	-	-	-	-	-
				Av Disch Std Devn	- -	-			Av Disch Std Devn	- -	-			Av Disch Std Devn	- -	-	-	-

Table 3.1k: Stream Unit No.1 (blue)- Calibration at DWAF Hydraulics Laboratory
Note: Depths of flow too small to measure velocities

(i) Test No:12:2/15 (iii) Gradient: 2% (iv) Discharge (l/sec): 15 (vi) Substrate: None
(ii) Date: 15:1:93 (v) Indicated Range (l/sec): 14.90 - 15.17

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	26	0.0054	-	-	-	27	0.0057	-	-	-	27	0.0057	-	-	-	-	-
3	1200	25	0.0052	-	-	-	24	0.0050	-	-	-	23	0.0048	-	-	-	-	-
4	1645	23	0.0048	-	-	-	23	0.0048	-	-	-	23	0.0048	-	-	-	-	-
5	2100	22	0.0046	-	-	-	24	0.0050	-	-	-	23	0.0048	-	-	-	-	-
6	2540	22	0.0046	-	-	-	23	0.0048	-	-	-	22	0.0046	-	-	-	-	-
				Av Disch Std Devn	- -	-			Av Disch Std Devn	- -	-			Av Disch Std Devn	- -	-	-	-

Table 3.1l: Stream Unit No.1 (blue)- Calibration at DWAF Hydraulics Laboratory
Note: Depths of flow too small to measure velocity

(i) Test No:13:2/20 (iii) Gradient: 2% (iv) Discharge (l/sec): 20 (vi) Substrate: None
(ii) Date: 15:1:93 (v) Indicated Range (l/sec): 19.88 - 20.13

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	32	0.0068	0.97	6.54	33.74	33	0.0070	0.93	6.48	33.42	32	0.0066	0.94	6.36	32.84	19.32	-3.38
3	1200	30	0.0063	1.04	6.58	33.02	31	0.0065	1.03	6.74	33.84	30	0.0063	1.04	6.60	33.13	19.91	-0.45
4	1645	28	0.0059	-	-	-	29	0.0061	-	-	-	28	0.0059	-	-	-	-	-
5	2100	27	0.0057	-	-	-	28	0.0059	-	-	-	27	0.0057	-	-	-	-	-
6	2540	27	0.0057	-	-	-	28	0.0059	-	-	-	27	0.0057	-	-	-	-	-
				Av Disch	6.56	33.38			Av Disch	6.61	33.64			Av Disch	6.48	32.99	19.64	-1.79
				Std Devn	0.019				Std Devn	0.131				Std Devn	0.118			

Table 3.1m: Stream Unit No. 1 (blue)- Calibration at DWAF Hydraulics Laboratory
Note: Depths of flow generally too small to measure velocities

(i) Test No:14:2/25 (iii) Gradient: 2% (iv) Discharge (l/sec): 25 (vi) Substrate: None
(ii) Date: 15:1:93 (v) Indicated Range (l/sec): 24.76 - 25.32

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent Error [8/(iv)]
2	750	39	0.0083	1.00	8.36	33.69	40	0.0086	0.97	8.32	33.54	39	0.0083	0.98	8.13	32.77	24.82	-0.73
3	1200	36	0.0077	1.09	8.31	33.95	36	0.0077	1.06	8.09	33.04	35	0.0074	1.09	8.08	33.09	24.48	-2.07
4	1645	34	0.0072	1.16	8.37	33.91	34	0.0072	1.15	8.32	33.71	33	0.0070	1.14	7.99	32.37	24.68	-1.27
5	2100	33	0.0070	1.20	8.38	33.00	34	0.0072	1.22	8.79	34.64	32	0.0068	1.21	8.21	32.36	25.38	+1.52
6	2540	33	0.0070	1.25	8.75	33.43	33	0.0070	1.27	8.84	33.80	32	0.0068	1.27	8.57	32.77	26.17	+4.67
				Av Disch	8.43	33.59			Av Disch	8.47	33.75			Av Disch	8.20	32.65	25.11	+0.42
				Std Devn	0.159				Std Devn	0.294				Std Devn	0.201			

Table 3.1n: Stream Unit No.1 (blue)- Calibration at DWAF Hydraulics Laboratory

(i) Test No:15:1/10 (iii) Gradient: 1% (iv) Discharge (1/sec): 10 (nominal) (vi) Substrate: None
(ii) Date: 29:1:93 (v) Indicated Range (1/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(iv)]
2	750	26	0.0055	-	-	-	24	0.0050	-	-	-	24	0.0050	-	-	-	-	-
3	1200	23	0.0048	-	-	-	23	0.0048	-	-	-	21	0.0044	-	-	-	-	-
4	1645	23	0.0048	-	-	-	22	0.0046	-	-	-	21	0.0044	-	-	-	-	-
5	2100	22	0.0046	-	-	-	23	0.0048	-	-	-	21	0.0044	-	-	-	-	-
6	2540	22	0.0046	-	-	-	22	0.0046	-	-	-	20	0.0041	-	-	-	-	-
				Av Disch Std Devn	- -	-			Av Disch Std Devn	- -	-			Av Disch Std Devn	- -	-	-	-

Table 3.1o:Stream Unit No. 1 (blue) - Commissioning at Rhodes IWR Stream Laboratory
Note: Depths of flow too small to measure velocities

(i) Test No:16:1/15 (iii) Gradient: 1% (iv) Discharge (1/sec): 15 (nominal) (vi) Substrate:None
(ii) Date: 29:1:93 (v) Indicated Range (1/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(iv)]
2	750	32	0.0068	0.78	5.27	34.63	31	0.0065	0.79	5.16	33.94	30	0.0063	0.76	4.78	31.43	15.21	1.40
3	1200	30	0.0063	0.83	5.27	34.05	30	0.0063	0.83	5.26	33.98	29	0.0061	0.81	4.95	31.98	15.48	3.19
4	1645	29	0.0061	0.88	5.18	34.71	29	0.0061	0.86	5.26	33.98	27	0.0057	0.86	4.86	31.33	15.50	3.33
5	2100	29	0.0061	-	-	-	29	0.0061	-	-	-	27	0.0057	-	-	-	-	-
6	2540	29	0.0061	-	-	-	29	0.0061	-	-	-	27	0.0057	-	-	-	-	-
				Av Disch Std Devn	5.31 0.052	34.46			Av Disch Std Devn	5.21 0.047	33.96			Av Disch Std Devn	4.86 0.069	31.58	15.40	2.64

Table 3.1p: Stream Unit No. 1 (blue) - Commissioning at Rhodes IWR Stream Laboratory
Note: Some depths of flow too small to measure velocities

(i) Test No:17:1/20 (iii) Gradient: 1% (iv) Discharge (1/sec): 20 (nominal) (vi) Substrate: None
(ii) Date: 29:1:93 (v) Indicated Range (1/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(i)]
2	750	38	0.0081	0.84	6.84	33.87	39	0.0083	0.84	7.01	34.75	37	0.0079	0.80	6.33	31.38	20.18	0.89
3	1200	39	0.0083	0.85	7.11	35.12	36	0.0077	0.89	6.84	33.76	35	0.0074	0.85	6.30	31.12	20.25	1.25
4	1645	35	0.0074	0.92	6.83	34.93	32	0.0068	0.94	6.39	32.67	33	0.0070	0.91	6.33	32.40	19.54	-2.28
5	2100	34	0.0072	0.93	6.70	32.75	35	0.0074	0.99	7.35	35.96	32	0.0068	0.95	6.40	31.29	20.44	2.22
6	2540	36	0.0077	0.94	7.19	34.16	35	0.0074	0.99	7.35	34.91	32	0.0068	0.96	6.51	30.93	21.06	5.28
				Av Disch	6.93	34.16			Av Disch	6.99	34.43			Av Disch	6.38	31.41	20.39	1.47
				Std Devn	0.188				Std Devn	0.361				Std Devn	0.076			

Table 3.1q: Stream Unit No. 1 (blue) - Commissioning at Rhodes IWR Stream Laboratory

(i) Test No: 20:-¼15 (iii) Gradient: -¼% (iv) Discharge (1/sec): 15 (vi) Substrate:None
(ii) Date: 3:11:93 (v) Indicated Range (1/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(i)]
2	750	-	-	-	-	-	-	-	-	-	-	61.0	0.0135	0.41	5.50	-	-	-
3	1200	-	-	-	-	-	-	-	-	-	-	59.0	0.0130	0.42	5.44	-	-	-
4	1645	-	-	-	-	-	-	-	-	-	-	57.0	0.0125	0.44	5.54	-	-	-
5	2100	-	-	-	-	-	-	-	-	-	-	53.5	0.0117	0.47	5.49	-	-	-
6	2540	-	-	-	-	-	-	-	-	-	-	51.0	0.0111	0.50	5.56	-	-	-
				Av Disch	-	-			Av Disch	-	-			Av Disch	5.51	-	-	-
				Std Devn	-				Std Devn	-				Std Devn	0.042			

Table 3.1r: Stream Unit No. 1 (blue) - Calibration at Rhodes IWR Stream Laboratory

(i) Test No:21:-¼/15 (iii) Gradient: 1% (iv) Discharge (l/sec): 15 (nominal) (vi) Substrate: See note
(ii) Date: 3:11:93 (v) Indicated Range (l/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av.Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av.Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(iv)]
2	750	-	-	-	-	-	-	-	-	-	-	62	0.0138	0.41	5.70	-	-	-
3	1200	-	-	-	-	-	-	-	-	-	-	58	0.0128	0.44	5.60	-	-	-
4	1645	-	-	-	-	-	-	-	-	-	-	56	0.0123	0.43	5.33	-	-	-
5	2100	-	-	-	-	-	-	-	-	-	-	53	0.0116	0.47	5.46	-	-	-
6	2540	-	-	-	-	-	-	-	-	-	-	52	0.0114	0.51	5.74	-	-	-
				Av Disch	-	-			Av Disch	-	-			Av Disch	-	-	-	-
				Std Devn	-				Std Devn	-				Std Devn	-			

Table 3.1s: Stream Unit No. 1 (blue) - Calibration at Rhodes IWR Stream Laboratory
Note: Substrate 1/3 of width of channel No. 3 paved with stones approximately 19 mm high between Points 2 & 3.

(i) Test No:22:-¼/15 (iii) Gradient: -¼ (iv) Discharge (l/sec): 15 (nominal) (vi) Substrate: See note
(ii) Date: 3:11:93 (v) Indicated Range (l/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av.Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av.Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(iv)]
2	750	-	-	-	-	-	-	-	-	-	-	64	0.0142	0.44	6.24	-	-	-
2a	975	-	-	-	-	-	-	-	-	-	-	62	0.0138	0.46	6.31	-	-	-
3	1200	-	-	-	-	-	-	-	-	-	-	45	0.0097	0.43	4.34	-	-	-
4	1645	-	-	-	-	-	-	-	-	-	-	47	0.0102	0.47	4.76	-	-	-
5	2100	-	-	-	-	-	-	-	-	-	-	50	0.0109	0.46	5.05	-	-	-
6	2540	-	-	-	-	-	-	-	-	-	-	50	0.0109	0.50	5.39	-	-	-
				Av Disch	-	-			Av Disch	-	-			Av Disch	-	-	-	-
				Std Devn	-				Std Devn	-				Std Devn	-			

Table 3.1t: Stream Unit No. 1 (blue) - Calibration at Rhodes IWR Stream Laboratory
Note: Substrate: three rows of 11 stones approximately 19 mm high between Points 2 & 3 in Channel No. 3.

(i) Test No:18:1/20 (iii) Gradient: 1% (iv) Discharge (1/sec): 20 (nominal) (vi) Substrate:None
(ii) Date: 30:6:93 (v) Indicated Range (1/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [3.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(iv)]
2	750	39.5	0.0085	0.71	6.21	32.81	40.5	0.0087	0.80	6.91	36.55	38.5	0.0082	0.70	5.80	30.65	18.92	-5.41
3	1200	39.5	0.0085	0.78	6.63	35.05	38	0.0081	0.86	7.02	37.11	38	0.0081	0.75	6.05	31.97	19.70	-1.49
4	1645	36.5	0.0078	0.83	6.43	33.97	37.5	0.0080	0.89	7.17	37.89	38	0.0081	0.80	6.52	34.46	20.11	0.57
5	2100	35.5	0.0076	0.89	6.71	35.48	37	0.0079	0.93	6.54	34.58	33	0.0070	0.87	6.10	32.24	19.35	-3.24
6	2540	34	0.0072	0.93	6.73	35.58	34	0.0072	0.99	7.12	37.63	32	0.0068	0.95	6.43	33.98	20.29	1.44
				Av Disch	6.54	34.58			Av Disch	6.95	36.74			Av Disch	6.18	32.66	19.67	-1.63
				Std Devn	0.199				Std Devn	0.225				Std Devn	0.263			

Table 3.2a: Stream Unit No. 2 (yellow) - Commissioning at Rhodes IWR Stream Laboratory

(i) Test No:19:1/20 (iii) Gradient: 1% (iv) Discharge (1/sec): 20 (nominal) (vi) Substrate: None
(ii) Date: 30:6:93 (v) Indicated Range (1/sec):-

Location		Channel No.1					Channel No.2					Channel No.3					Whole Unit	
1 Point	2 Chainage (mm)	3.1 Depth (mm)	4.1 Area (m2)	5.1 Av Vel (m/sec)	6.1 Discharge (l/sec) [4.1x5.1]	7.1 Percent Discharge [6.1/8]	3.2 Depth (mm)	4.2 Area (m2)	5.2 Av Vel (m/sec)	6.2 Discharge (l/sec) [4.2x5.2]	7.2 Percent Discharge [6.2/8]	3.3 Depth (mm)	4.3 Area (m2)	5.3 Av.Vel (m/sec)	6.3 Discharge (l/sec) [4.3x5.3]	7.3 Percent Discharge [6.3/8]	8 Total Discharge [Sum 6]	9 Percent error [8/(iv)]
2	750	41	0.0088	0.65	5.96	32.45	45	0.0097	0.68	6.66	36.32	37.5	0.0080	0.72	5.73	31.23	18.33	-8.25
3	1200	41.5	0.0089	0.71	6.37	34.65	39	0.0084	0.86	7.14	37.16	35.5	0.0076	0.76	5.74	29.69	19.22	-3.91
4	1645	40	0.0086	0.74	6.35	32.33	39.5	0.0085	0.87	7.37	37.54	35	0.0074	0.80	5.92	30.14	19.63	-1.84
5	2100	36	0.0077	0.82	6.32	33.00	37.5	0.0080	0.94	7.57	38.29	33	0.0070	0.84	5.87	29.72	19.76	-1.21
6	2540	33.5	0.0071	0.90	6.40	32.56	33.5	0.0071	1.00	7.09	36.12	32	0.0068	0.91	6.15	31.32	19.64	-1.82
				Av Disch	6.28	32.49			Av Disch	7.17	37.10			Av Disch	5.88	30.41	19.32	-3.41
				Std Devn	0.163				Std Devn	0.301				Std Devn	0.359			

Table 3.2b: Stream Unit No. 3 (red) - Commissioning at Rhodes IWR Stream Laboratory

3.3 DISCUSSION OF RESULTS

3.3.1 Flow distribution among the channels of individual stream units

In all tests where velocity measurements were possible, flow rate was derived from a combination of average velocity and area of flow (the latter calculated from depth and channel geometry). There is sufficient variation of flow rate calculated in this way within individual channels to doubt the validity of characterising the average velocity across the channel cross-section by one measured point velocity, sometimes not at the ideal depth. In addition, in four of the tests depths were too small to allow velocity measurements, and in a further two only a partial set of velocity readings could be taken: in these cases there is no, or only limited assessment of flow distribution. Flow depth was however recorded at all points in all channels in all tests and, because of its simplicity, there is less possibility of error in its measurement. If flow depths at equivalent points in separate channels are the same, it follows that flow areas are equal and, if the gradients are the same, discharges are also equal. Because of the steepness of the side walls of the channel section the depth/cross-sectional area relationship is to all intents and purposes linear, and depth can be used as a convenient surrogate for discharge in assessing the flow distribution among channels.

A summary of the results of the depth analysis is as follows:

Calibration tests at DWAF Hydraulics Laboratory, Pretoria (Blue Unit only)

Across individual measuring points the worst flow split was 31.8/32.7/35.5%, indicating a variation of flow rate of -4.5% about the ideal. The worst whole channel average flow split for any one test was 32.4/33.8/33.8%, indicating a variation of flow rate of -2.8%/+1.4% about the ideal. These variations will cause velocity variations among the three channels of a single unit of about +/-3%, and are considered to be acceptable. Note: The results of 12 tests were used in this analysis. The results of two tests (Tables 3.1e and 3.1f) for a gradient of 0.5%, were disregarded in the analysis: hydraulic conditions at this gradient were close to a critical regime, which is unstable, and produces a wavy water surface. This is very much as predicted in the 1-dimensional analysis. Use of this gradient in experimental work is not recommended.

Commissioning at Rhodes IWR Stream Laboratory, Grahamstown

Across individual measuring points the worst flow split was 30.4/33.2/35/4%, indicating a variation of flow rate of -8.9%/+9.3% about the ideal. The worst whole channel average flow split for any one test was 31.0/34.3/34.7%, indicating a variation of flow rate of -7.1%/+4.2% about the ideal. (Both of these results were in respect of the Red Unit. The results for the other two streams showed a better distribution, but less good than those achieved in the calibration tests in Pretoria). These variations will cause velocity variations among the three channels of a single unit of about +/-6%, which is considered less than acceptable, and warrants further investigation. Note: The results of five tests were used in this analysis, three on the Blue Unit, and one each on the other two streams.

3.3.2 Comparison of flow conditions among stream units

The results of only three tests (Tables 3.1q, 3.2a & 3.2b) can be used to compare the performance of the three units. In the worst case the depth (and therefore the area) of flow varied across the nine channels of the three units by -11.8/+13.8%, indicating that the velocity would vary by about +/-12%. This is an unacceptably high variation among the three stream

units. However, the assumption in this comparison was that the flow rates in the three units were the same, and the high variation of inter-unit flow depths indicates that this almost certainly was not the case. Flow rates were set using the calibrated control valves, and it is clear that this is too coarse a method. To ensure, as far as is possible within the constructional tolerances of the units, that velocities are equal at equivalent points in each stream for comparable gradients, it is recommended that depths of flow are set equal at selected points in each unit prior to commencing experimental work.

3.3.3 Comparison with predicted hydraulic conditions

Only the results of tests carried out at the Pretoria laboratory, where flow rates were accurately know, were used in this exercise. A (less than detailed) comparison of flow depths predicted by the 1-dimensional analysis of the channel hydraulics with those measured in the streams shows good, often excellent, similarity. It is concluded that the hydraulics of the streams in terms of depths and average velocities of flow can be adequately predicted by computer modelling.

3.4 CONCLUSIONS AND RECOMMENDATIONS

3.4.1 Flow distribution among the channels of individual stream units

In the calibration of the first (Blue) stream in Pretoria the flow distribution among the three channels was such that velocity at any point varied by a maximum of about 3% about the ideal mean: this is considered acceptable. In the commissioning tests in Grahamstown the flow distribution was less good, resulting in a maximum velocity variation among the three channels of any one unit of about 6% about the ideal mean: this is less acceptable, and some additional calibration works is recommended.

3.4.2 Flow conditions among stream units

The three similar tests which form the basis of this comparison indicate an unacceptably high velocity variation of about 12% about the ideal mean across the nine channels of the three units. Flow rates in the three units were however, probably not exactly the same. The flow control valves are not sufficiently precise to set exactly equal flow rates in the streams, and it is recommended that depths of flow are set as near as possible equal in the channels before commencing experimental work.

3.4.3 Prediction of hydraulic condition

The 1-dimensional computer program CFP (Channel Flow Profiles) adequately simulated depths and average velocities of flow in the channels.

3.4.4 Hydraulic conditions with substrate

The results of the tests with substrate have not been discussed here: the increase in velocity and reduction in depth were much as expected. Hydraulic variables need to be determined for each specific case, as the possible variations of substrate size and pattern of distribution on the channel floor make calibration for a general case impractical.

3.5 TEMPERATURE CALIBRATION

This section was written by Dr G. J. Youthed. IWR.

Air temperature in the stream laboratory is controlled by two Sanyo SA246SE(24000 btu) air conditioners. The temperature of the water in the channels can be raised above the laboratory temperature by the heating effect of the pumps, and cooled by a chiller consisting of epoxy coated copper cooling coils in the sump through which the coolant (R22) is circulated by a compressor.

Temperature calibration of the artificial stream system took place in several stages, namely calibration of the air conditioners, water temperature without the pump, the effect of the pump on water temperature and the effect of the chiller on water temperature.

3.5.1 Calibration of air conditioners

The range and efficiency of the air conditioners was evaluated by raising the thermostat setting one notch each day while monitoring the ambient and laboratory temperature using a hand-held mercury thermometer. All readings were taken at approximately 9 a.m. The temperature in the laboratory was measured at three different points and Figure 3.2a shows that the temperature at the bench end of the laboratory was generally 1-2°C lower than the temperature in the centre of the laboratory or at the sump end. This was due to poor insulation at the bench end and also to the exchange of air when the door was opened. The temperatures in the centre and at the sump end of the laboratory showed a maximum difference of 0.7°C. Variations in the ambient temperature of up to 7.8°C had little noticeable affect on the laboratory temperature. A slight drop in laboratory temperature occurred on day 4 when the ambient temperature was recorded at 10.4°C.

3.5.2 Channel water temperature without the pump running

Water temperature in the channels was generally 1-2°C lower than the laboratory temperature (Figure 3.2b), possibly due to evaporative cooling. Ambient temperature had little noticeable effect on either the laboratory or channel temperature even during prolonged power failures. A drop of 7°C in the ambient temperature on day 6 caused a 3°C drop in laboratory temperature and a 2°C drop in water temperature despite the fact that the air conditioners were off for a considerable period during days 4,5 and 7.

3.5.3 Effect of the pump on channel water temperature

After an initial two day period the pump raised the temperature of the channel water by an average of 2.8°C (Figure 3.2c) above the laboratory temperature. During the final 3 days of the experiment the water temperature remained constant.

3.5.4 Calibration of chiller

To evaluate the effectiveness of the chiller and to determine whether or not the stream water temperature could be maintained at a constantly low temperature, the laboratory air conditioners were set on their lowest setting (1) and the chiller set at 10°C. After 24 hours the water temperature had dropped to 14°C and maintained this temperature (± 2°C) for the duration of the experiment (Figure 3.2d). The water temperature was an average of 2.8°C lower than the laboratory temperature.

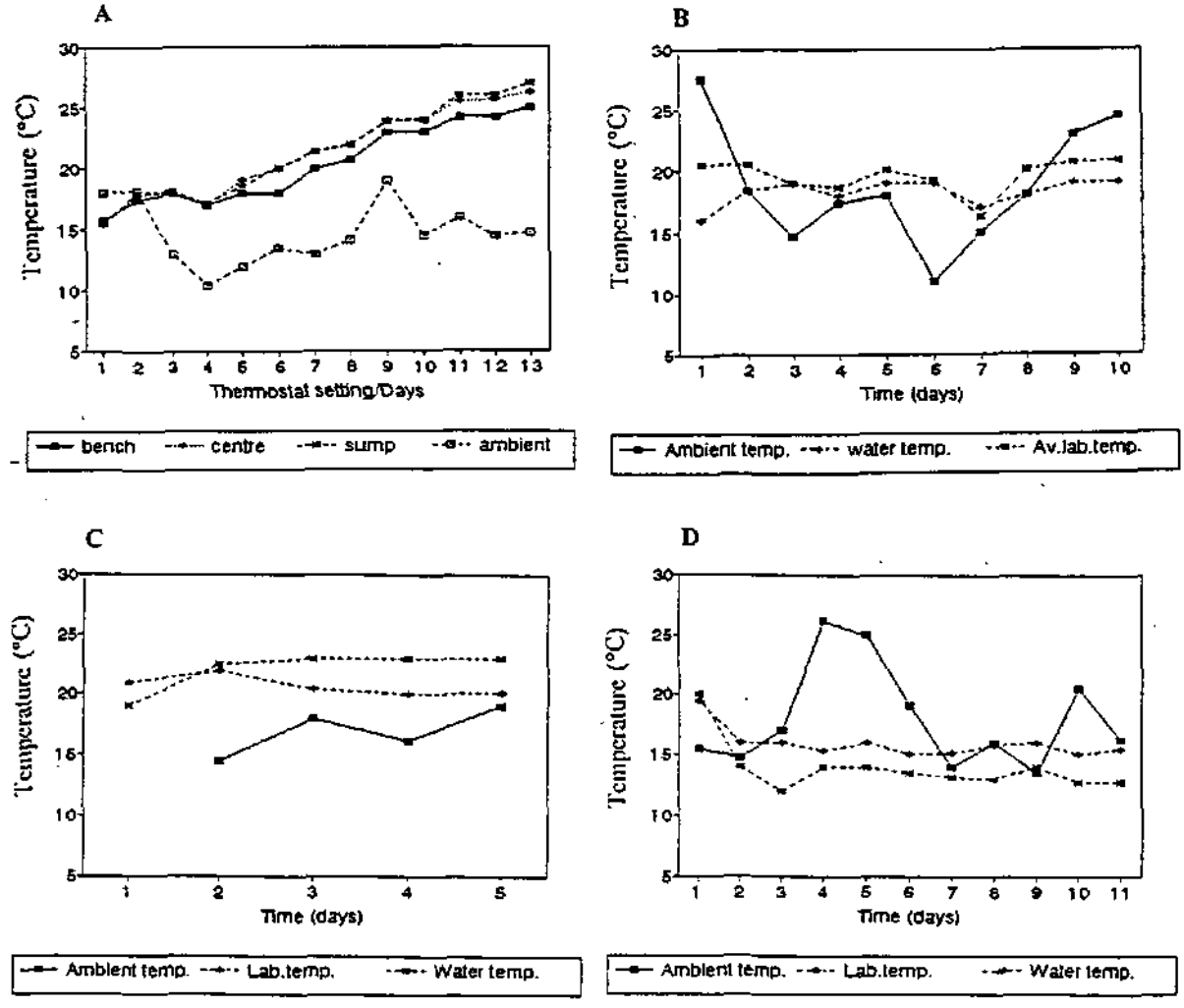

Figure 3.2 A. The temperature at three different positions in the stream laboratory when the air conditioner thermostat settings were raised by one notch a day. The ambient temperature is also shown.
B. Water temperature in the artificial stream channel when the pump was not running. The mean laboratory temperature and the ambient temperature are also shown. The air conditioners were switched off for varying lengths of time on days 4, 5 and 7 due to power failures.
C. Water temperature in the artificial stream channel when the pump was running. Laboratory and ambient temperatures are also shown.
D. The temperature of the water in the artificial stream channel when the chiller was set at 10 °C. Laboratory and ambient temperatures are also shown.

3.5.5 Summary

The air conditioners were capable of maintaining the laboratory at temperatures ranging from 16°C to 26°C ± 1°C. Water temperature in the channels, when the pumps were not running, was maintained at 1-2°C lower than the laboratory temperature over a period of 10 days. Running the pumps raised the water temperature by 2.8°C. The chillers reduced the water temperature by 2.8°C ± 2°C.

3.6 WATER QUALITY CALIBRATION

3.6.1 Introduction

Since the artificial streams were designed to conduct tolerance experiments where water quality would be altered by changing the concentration of a single constituent, or a complex effluent, it was important to establish the range of variation in the systems of selected variables when the streams were run over periods of time longer than the envisaged experimental times. Acute (96 hour) experiments were planned, so water quality calibration experiments were run over seven days. Systems were monitored with water, then with substrata added, and finally with substrata and organisms (to ascertain the effects of test populations on the selected constituents).

Bearing in mind the lack of within system-independence, the aim of the water quality calibration in the large systems was twofold: 1) to describe between-system variation; and 2) to describe within-system variation over 7 days. The raceways were independent of each other, so they were monitored for between-raceway variation, also for seven days.

3.6.2 Methods and results

Water quality calibration was conducted in both the large stream systems and in the small-scale raceways. When streams were filled with municipal water they were run for 48 hours to remove chlorine, as they would be in a tolerance experiment. Day 1 commenced after this dechlorination period, but when rain water was used monitoring commenced immediately. Temperature, pH, and conductivity, as well as nutrient concentrations (total ammonium, nitrite, nitrate and phosphate) in the sump of each stream system, were measured three times per day for seven days. The raceways were filled with municipal water (dechlorinated using a carbon filter), or rainwater, and the same variables were measured once per day for seven days. The stream system and raceway runs were not conducted simultaneously, but were both conducted in the artificial stream laboratory.

Constituents were measured with the following equipment: temperature - hand-held mercury thermometer; pH - Hannah field kit, hand-held probe; and nutrients - Merck spectroquant spectrophotometric system, with a lowest detection limit of 0.01 mg l^{-1} for each of the nutrients.

Calibration with municipal water at three temperatures

The three stream systems, and three raceways were filled with municipal water and monitored for seven days, repeated at 15°C, 20°C, and 25°C. At 15°C the streams were cooled using the chiller units, all other temperature were achieved using the air conditioners alone (Figures. 3.3 - 3.10).

Calibration of raceways with rainwater at 20°C
Three raceways were filled with rain water and monitored for a week (Figure 3.11).

Calibration with municipal water and rain water, with and without substrate and organisms
The raceways were run for a week each at 15°C, 20°C, and 25°C with municipal water, and each raceway with five periphyton-covered stones and a population of 20 limpets (*Burnupia stenochorias)*. The same set up was repeated with rainwater at 20°C. The streams were run over two seven day periods at 20°C. Water source (rain water and municipal water), substrate (10 periphyton covered stones), and the addition of test organisms (40 limpets) varied (Figures 3.12 - 3.19). Limpet mortalities in raceways were recorded (Figure 3.20).

Physico-chemical variation
There was minimal variation between the three stream systems, or between three raceways at any of the test temperatures, with or without substrata or test animals (Figures 3.3 - 3.19). Variability between streams 1, 2, and 3 was investigated using a two-way ANOVA without interaction, with the two factors: time and the three streams. There were four instances where one stream was significantly different ($p<0.01$) from the others (nitrate at 15°C and 20°C, conductivity at 25°C, and dissolved oxygen at 20°C). Of these only the pH varied in the first 96 hours, and pH varied in total between 7 and 7.2 (Fig. 3.3). The difference in oxygen concentration can be attributed to a single rise in concentration to 110% on day 7 in stream 3 (Fig. 3.6). The nitrite variation arose from differences of not more than 0.01 mg l^{-1} after day 6 (Fig. 3.9). In each case the difference was evaluated as being unlikely to affect biological responses. The stream systems are therefore described as providing consistently similar water quality environments.

Although there was no significant variability between the raceways, the variability over time was greater that in the streams. For example conductivity varied by more than 10% (Fig. 5.3B), which might influence test biota. When experiments are conducted in raceways it is important to monitor conditions, especially conductivity every 12 hours and stabilise the system (for example by the addition of extra "control" source water). Temperatures in the raceways were less stable than in the streams (Figure 3.2). In the streams pH (Fig. 3.3), temperature (Fig.3.4), and conductivity (Fig. 3.5) did not vary over time, but the nutrients did (Fig. 3.7-3.10). There was evidence of nitrogen accumulation, with increases in concentrations of ammonium, nitrite, and nitrate after day 5. This would probably have been due to microbial bio-film activity. Phosphate also reached detectable levels after day 5. During the time of a usual acute tolerance test (96h) nutrient levels were undetectable. At higher temperatures the rate of nutrient increase was greater. If the streams are used for chronic testing the build up of ammonium would have to be monitored, and % volume replacements, or charcoal and/or bio-filtration considered.

Of the nutrients, nitrite was the only one for which concentrations in one stream varied significantly from the others. Nitrite is a transient form of nitrogen in the nitrogen cycle which could account for its greater measured variability.

In the raceways, changes in nutrient concentrations were less consistent, and in some cases were detectable within the first 96 hours (Figures 3.7 -3.10 and 3.16 - 3.19). This is probably due to the relatively higher surface-area to volume ratio in the raceways compared with the streams.

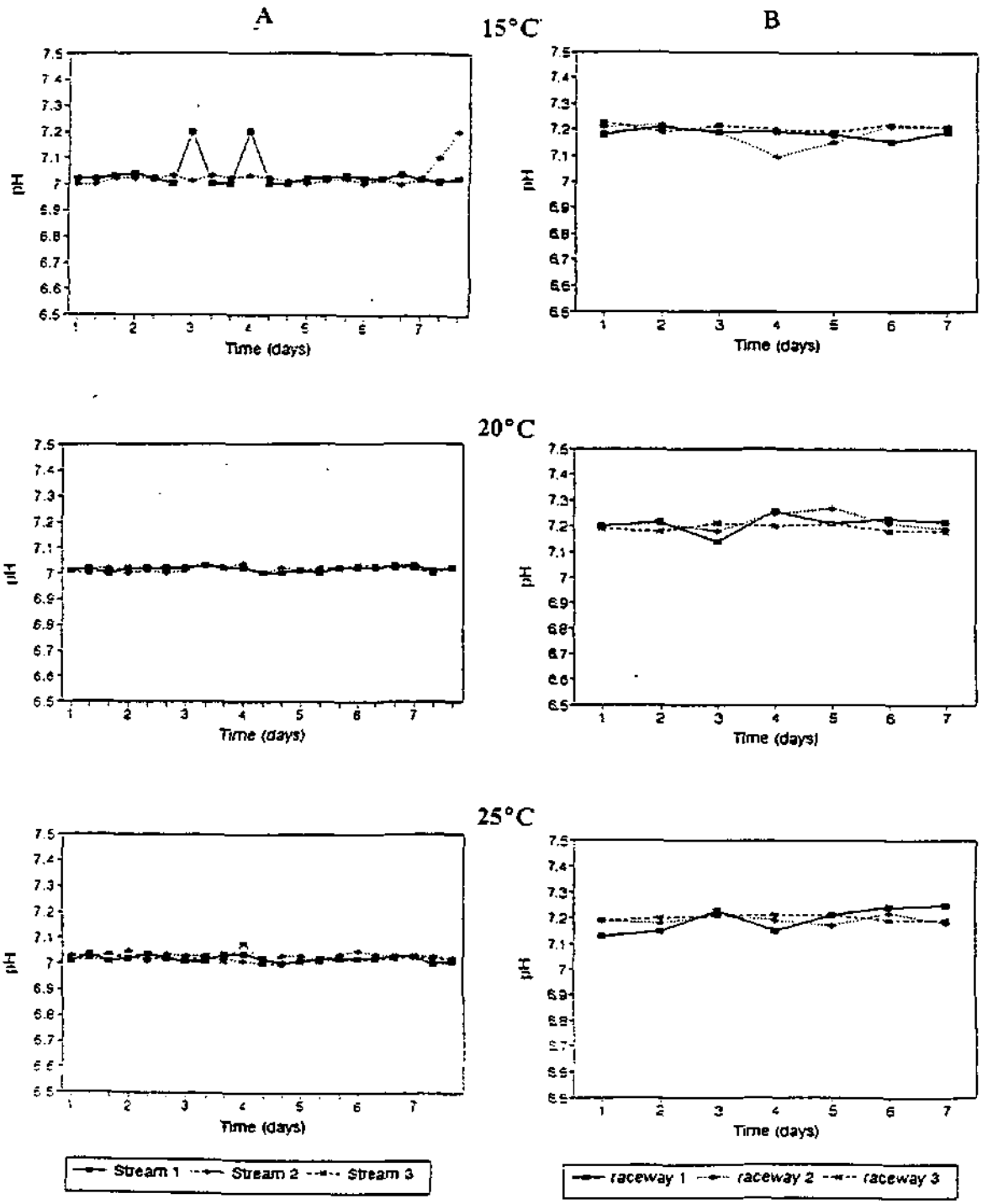

Figure 3.3 Changes in pH in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

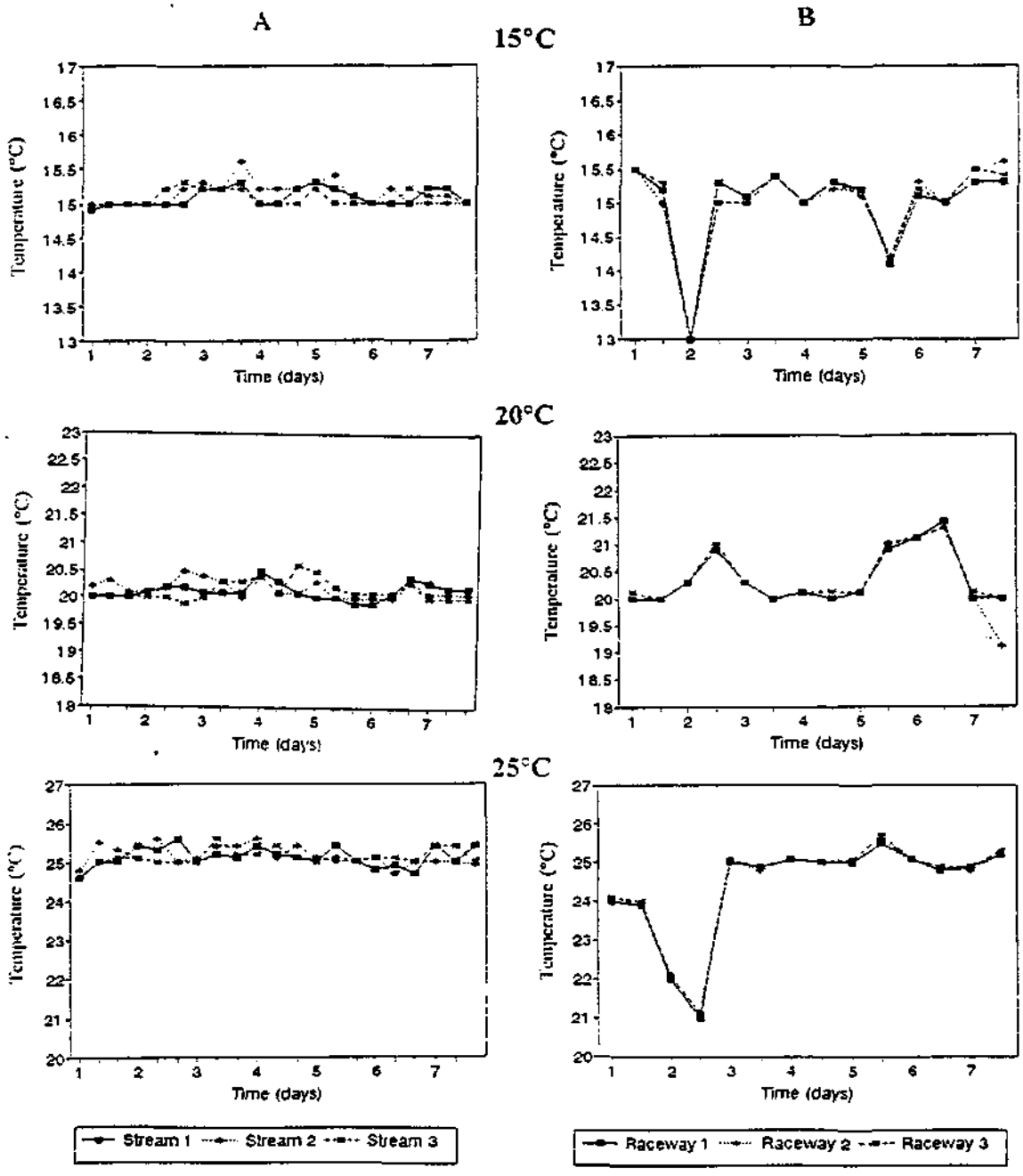

Figure 3.4 Changes in temperature (°C) in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

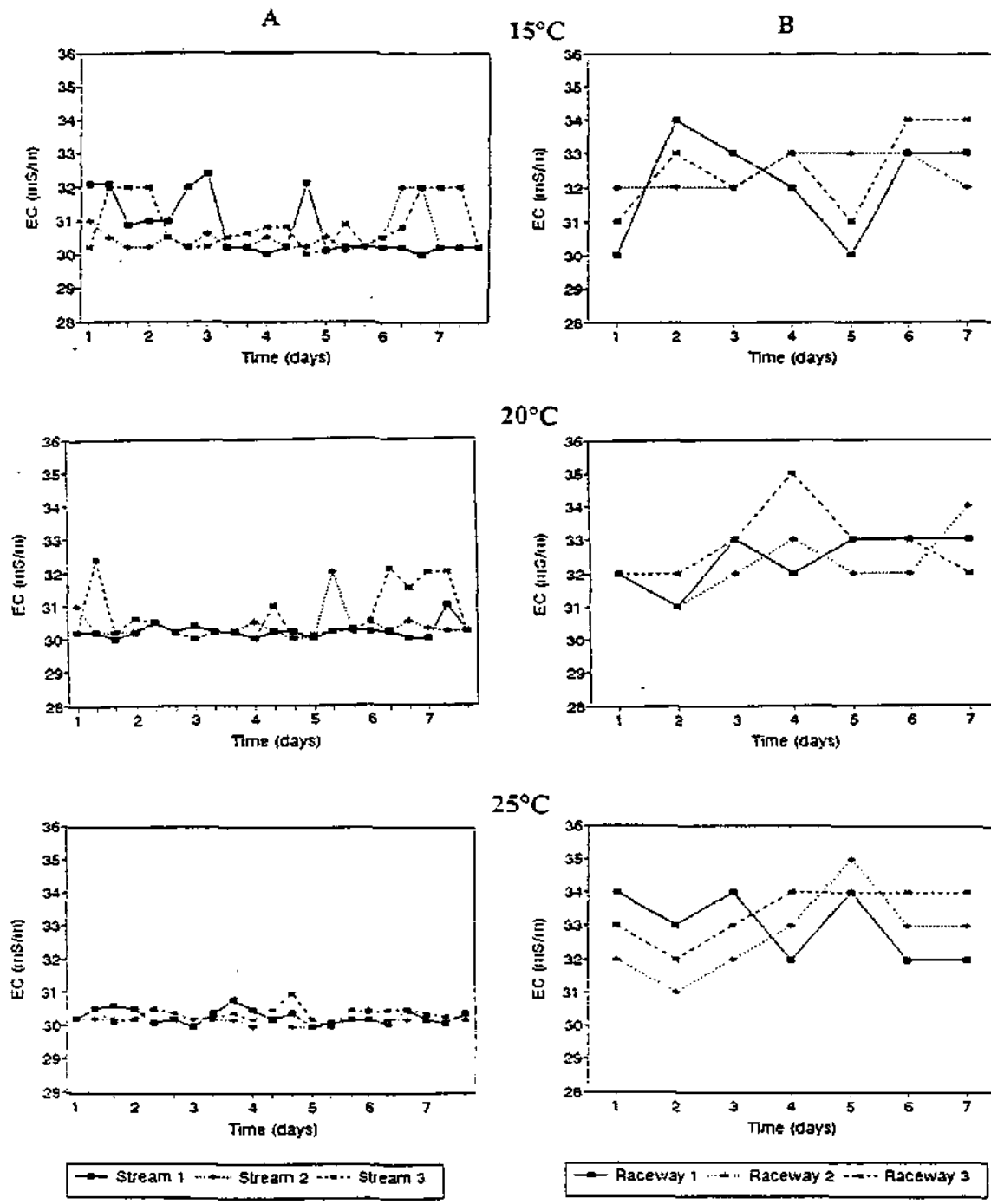

Figure 3.5 Changes in conductivity ($mS.m^{-1}$) in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

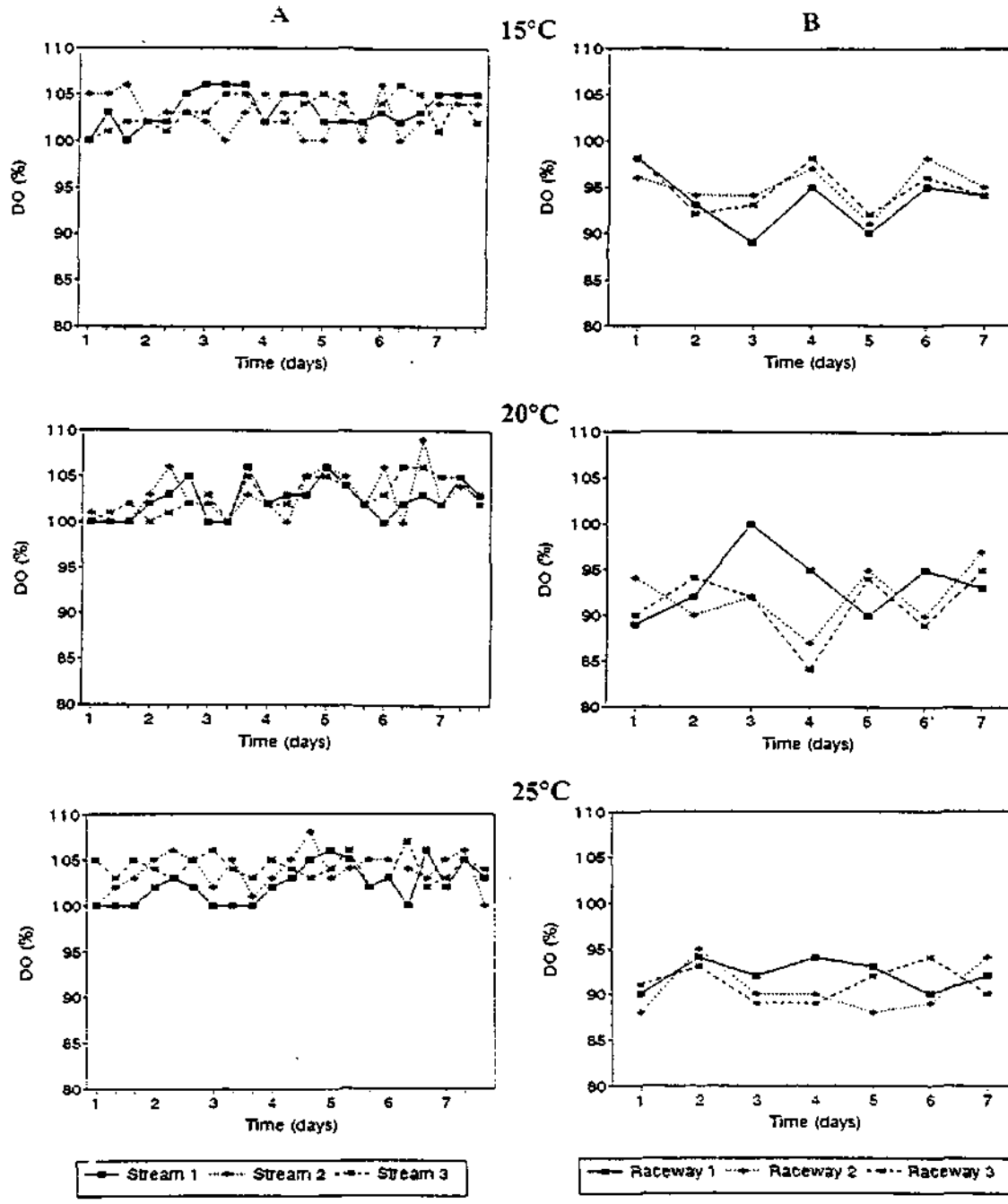

Figure 3.6 Changes in dissolved oxygen (%) in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

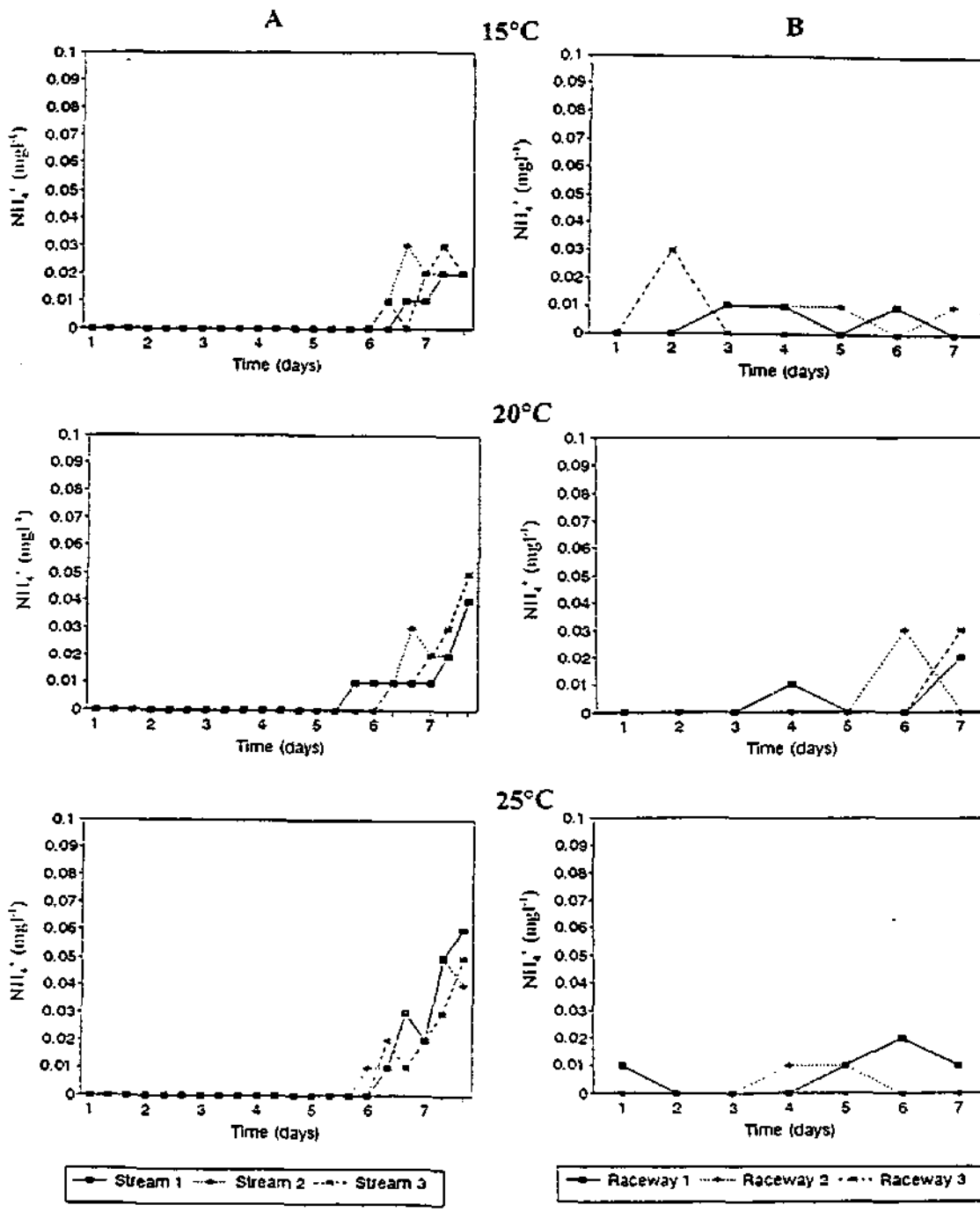

Figure 3.7 Changes in total ammonium concentration ($mg.l^{-1}$) in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

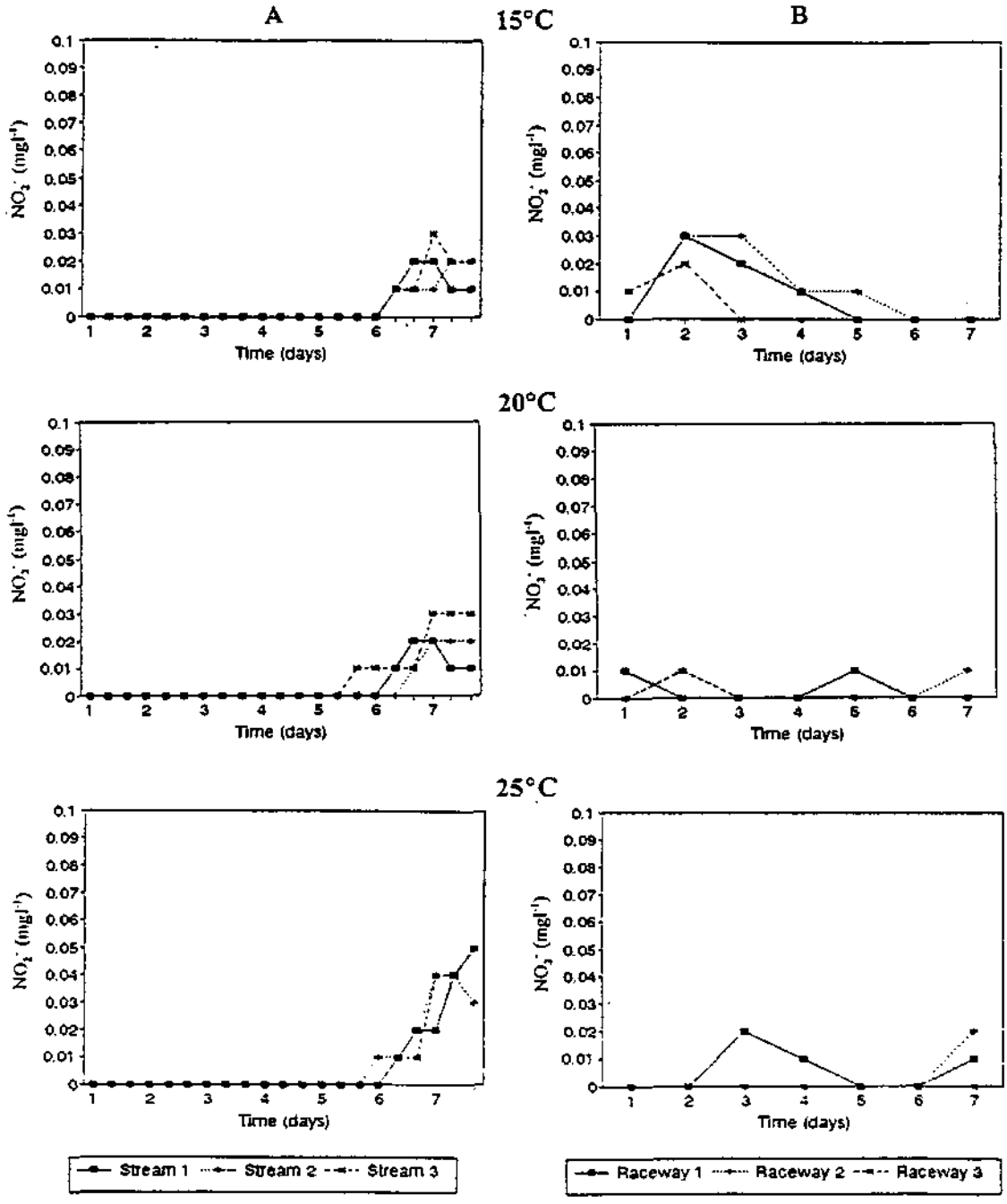

Figure 3.8 Changes in total nitrite concentration (mg.l^{-1}) in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

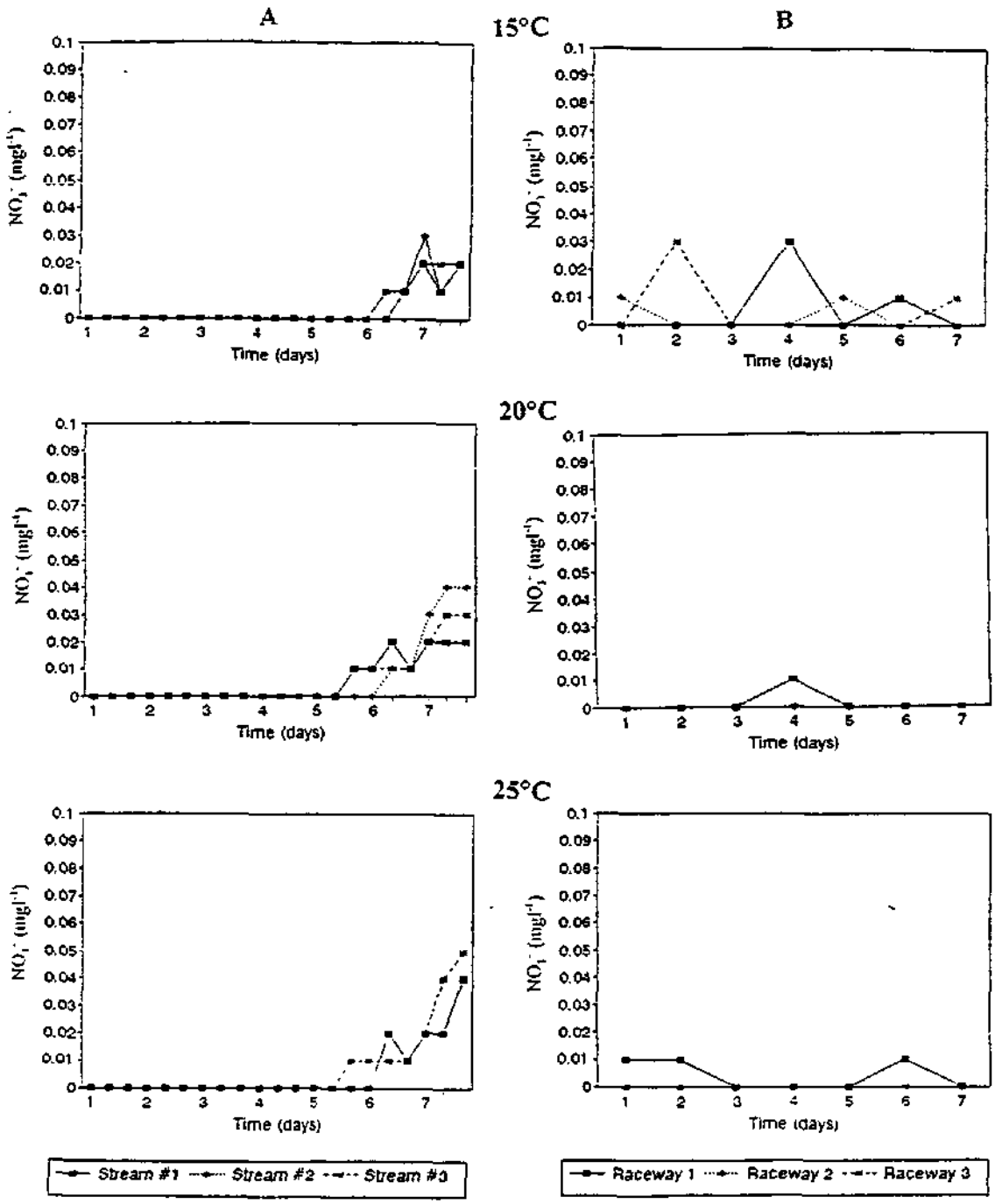

Figure 3.9 Changes in total nitrate concentration ($mg.l^{-1}$) in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

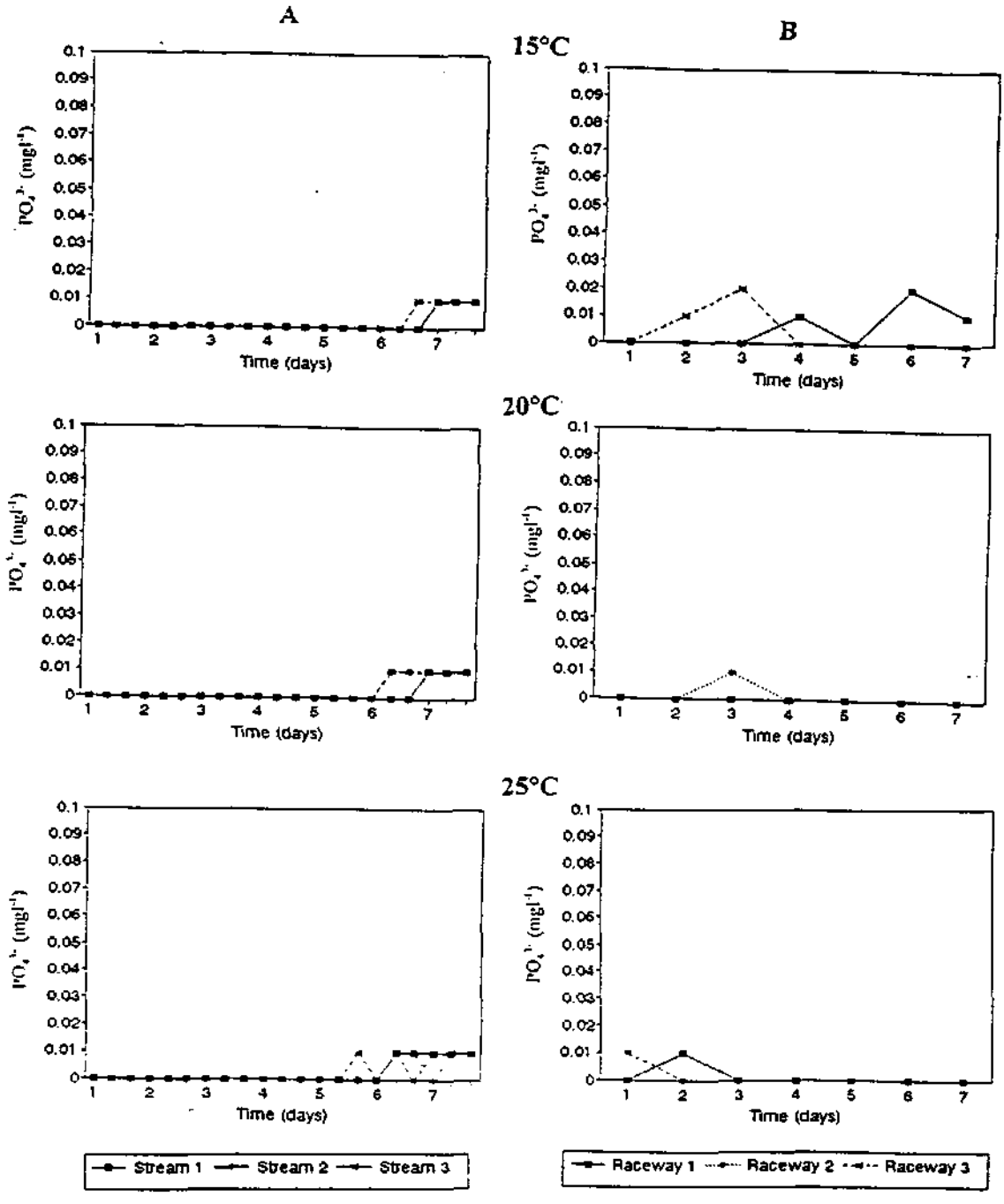

Figure 3.10 Changes in total phosphate concentration ($mg.l^{-1}$) in the sump of three artificial stream systems (A) and three raceways (B), recirculating municipal water, over a period of 7 days, at 15°C, 20°C, and 25°C.

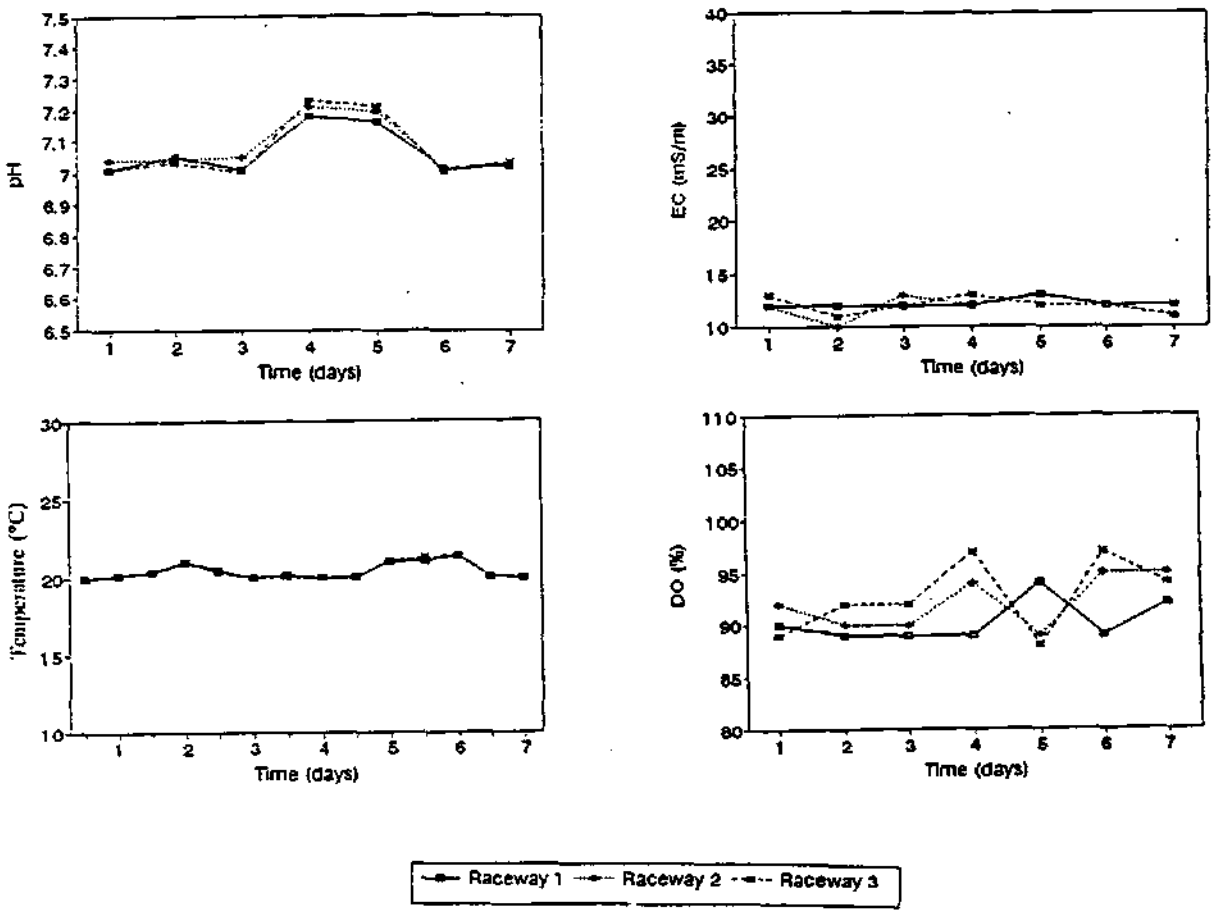

Figure 3.11a Changes in pH, temperature, and conductivity and dissolved oxygen in three raceways, recirculating rain water, over a period of 7 days, at 20°C.

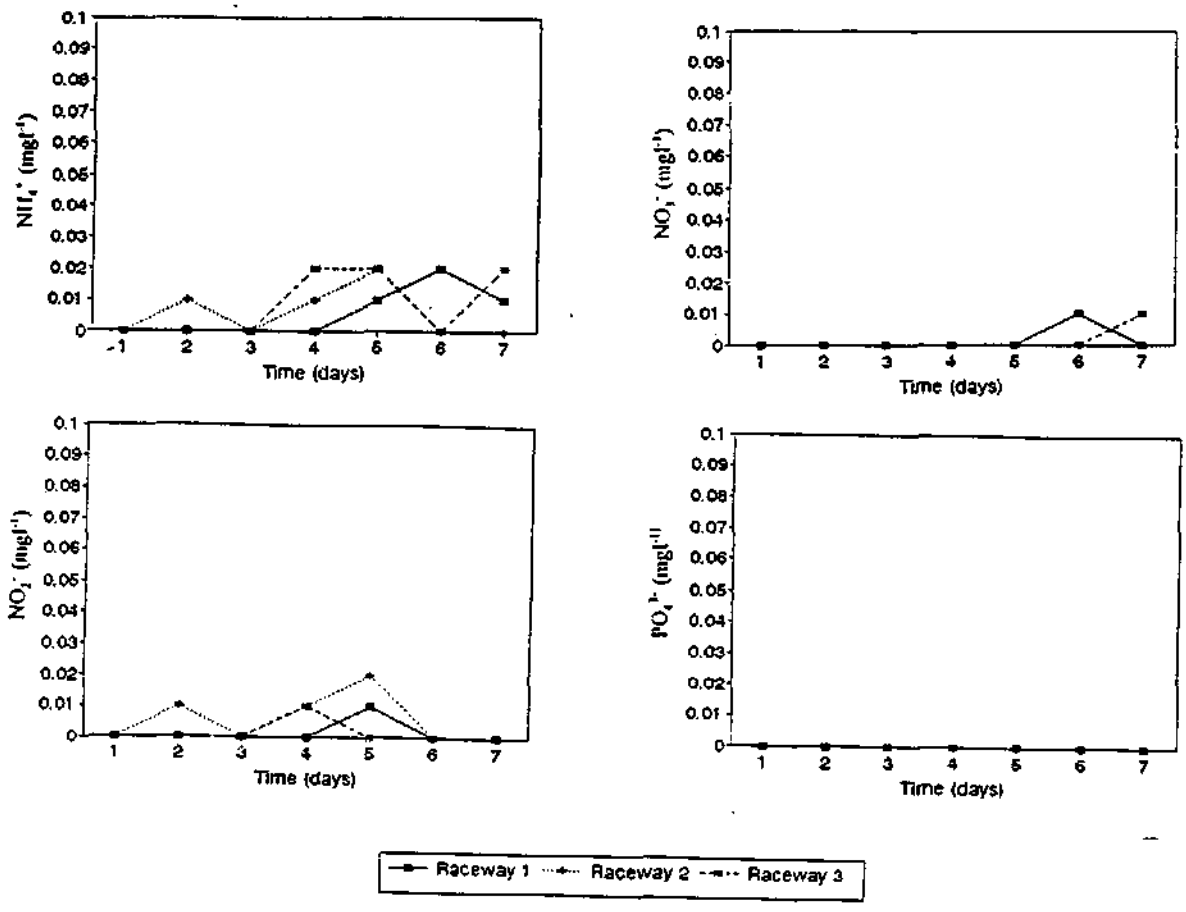

Figure 3.11b Changes in total ammoniun, nitrite, nitrate and phosphate in three raceways, recirculating rain water, over a period of 7 days, at 20°C.

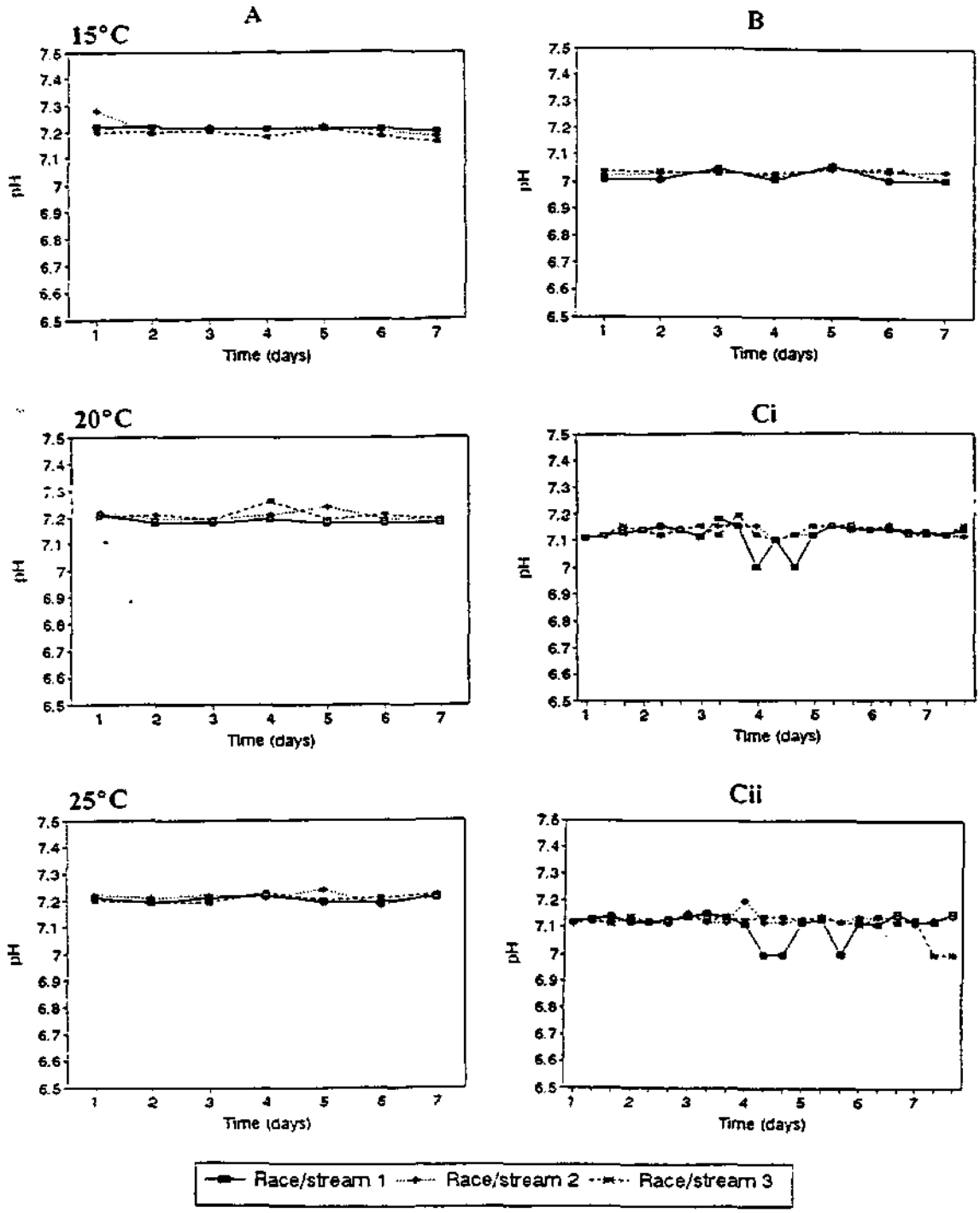

Figure 3.12 Changes in pH in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

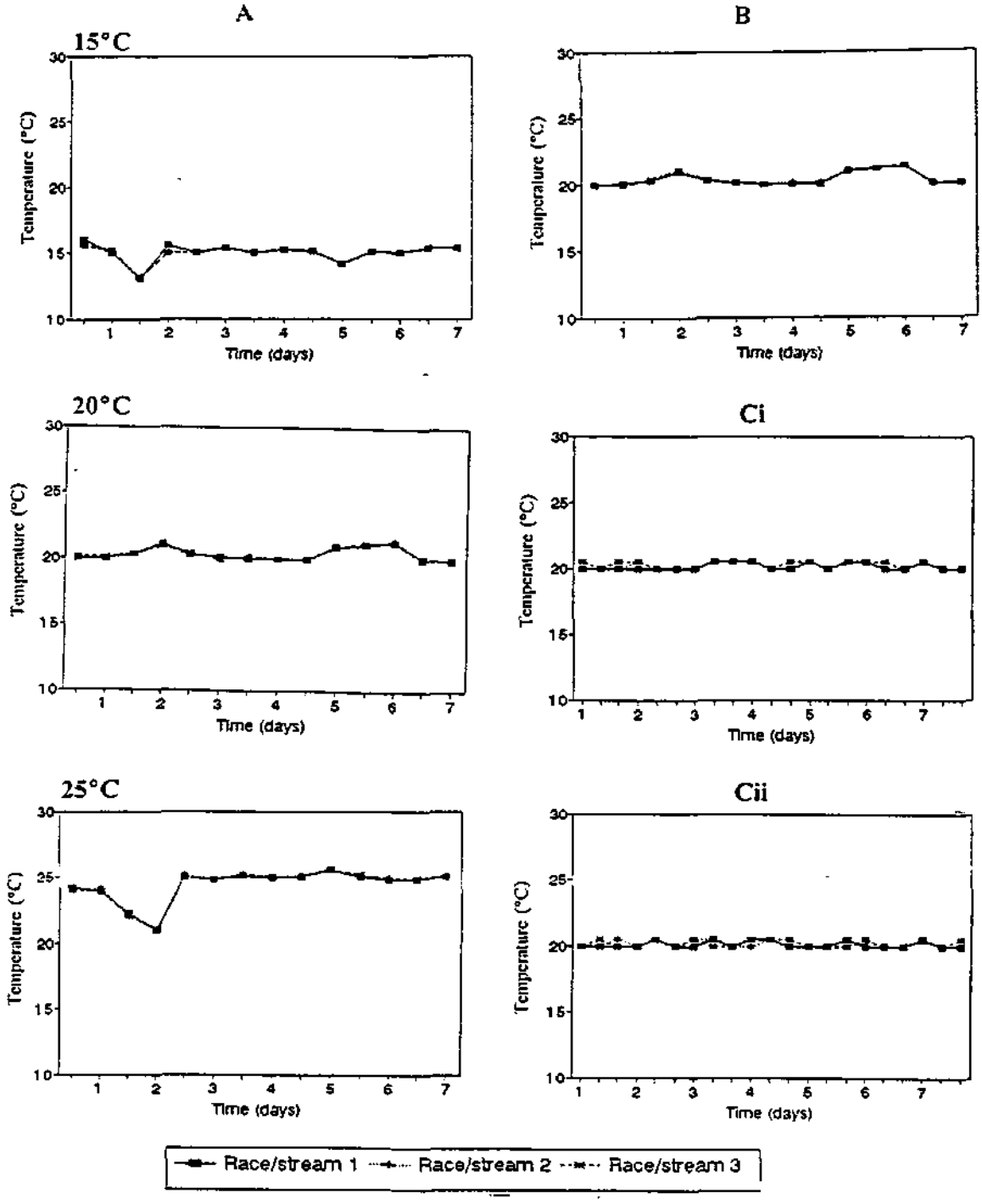

Figure 3.13 Changes in temperature (°C) in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

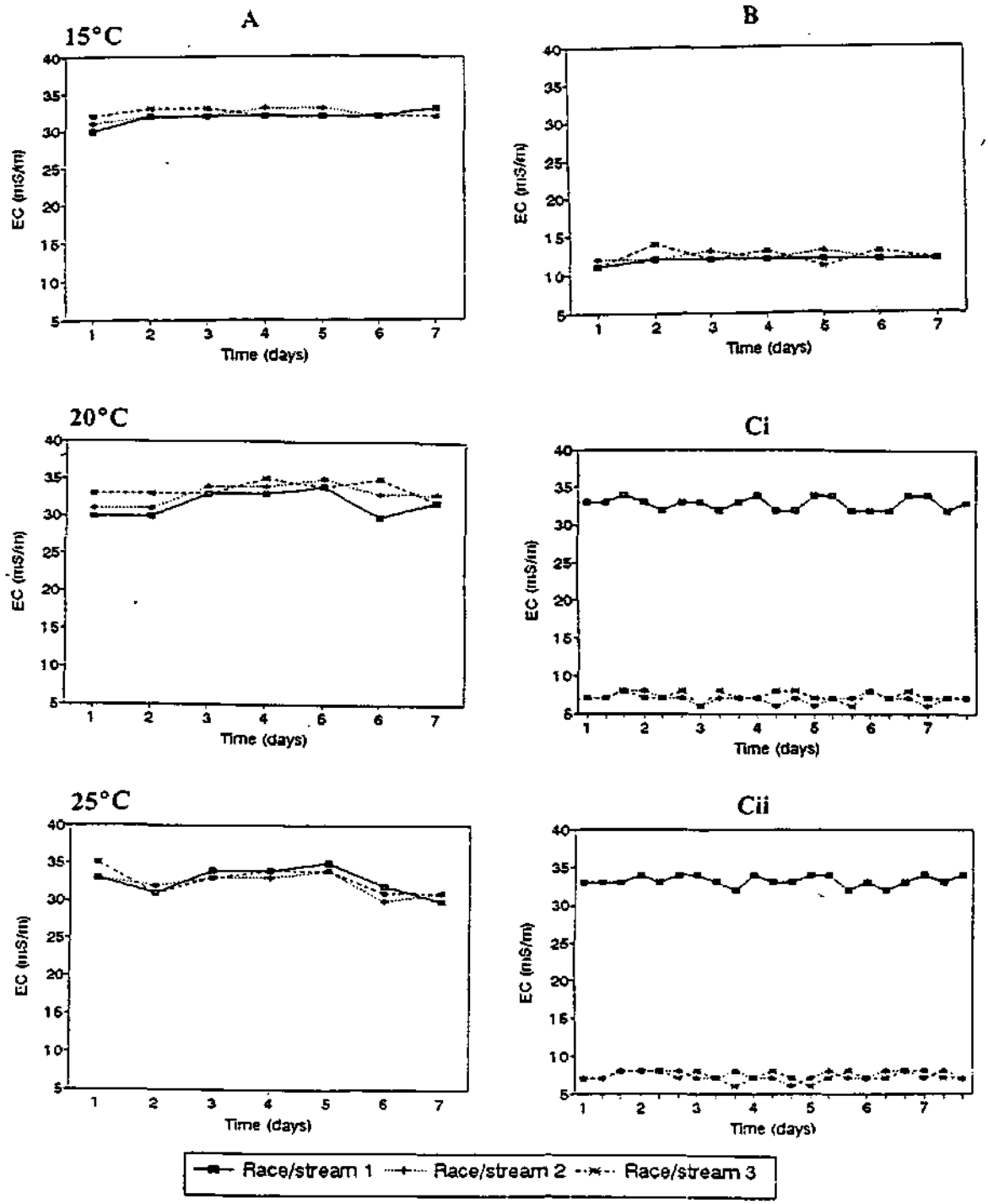

Figure 3.14 Changes in conductivity ($mS.m^{-1}$) in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

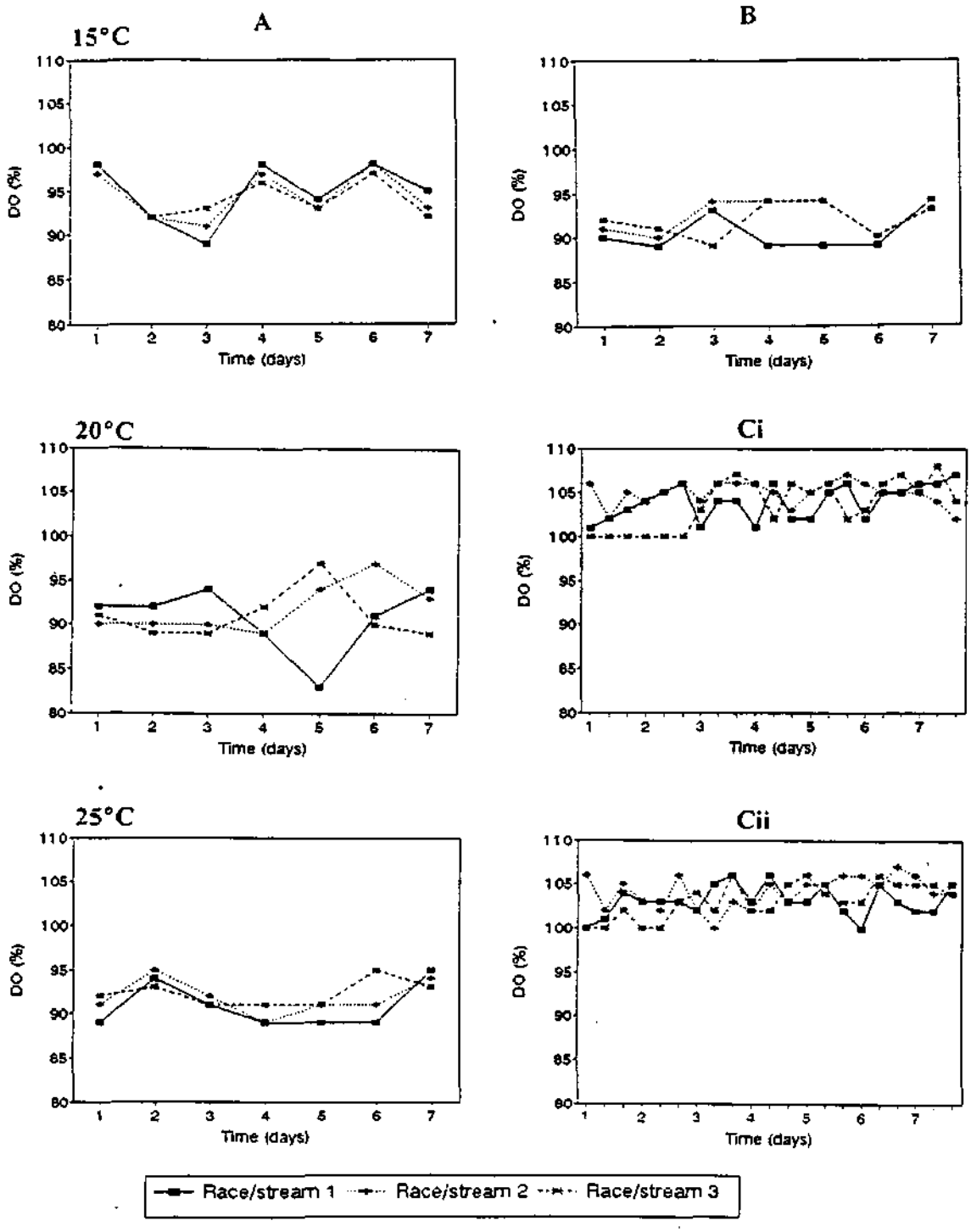

Figure 3.15 Changes in dissolved oxygen (%) in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

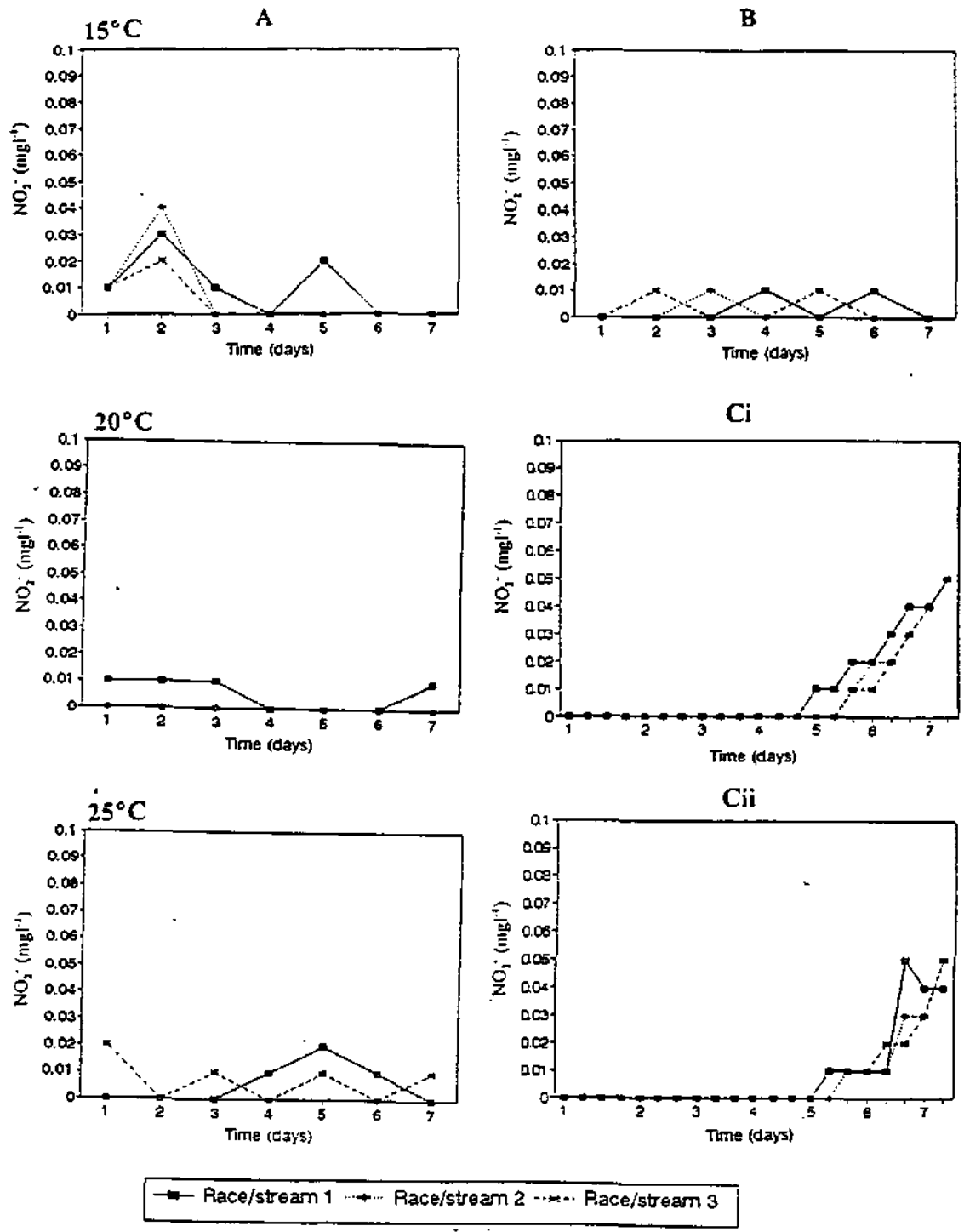

Figure 3.16 Changes in total nitrite concentration (mg.l^{-1}) in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

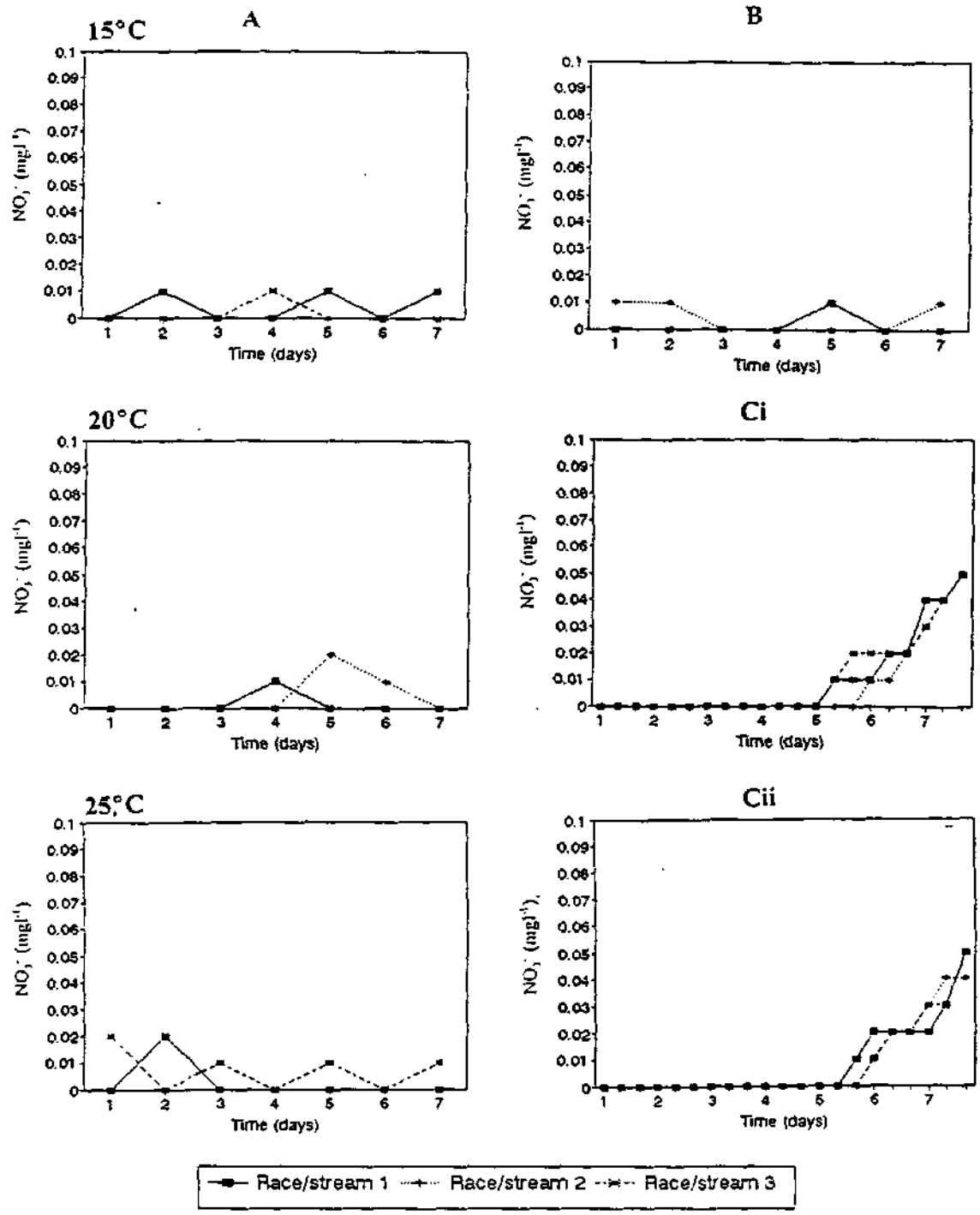

Figure 3.17 Changes in total nitrate concentration ($mg.l^{-1}$) in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

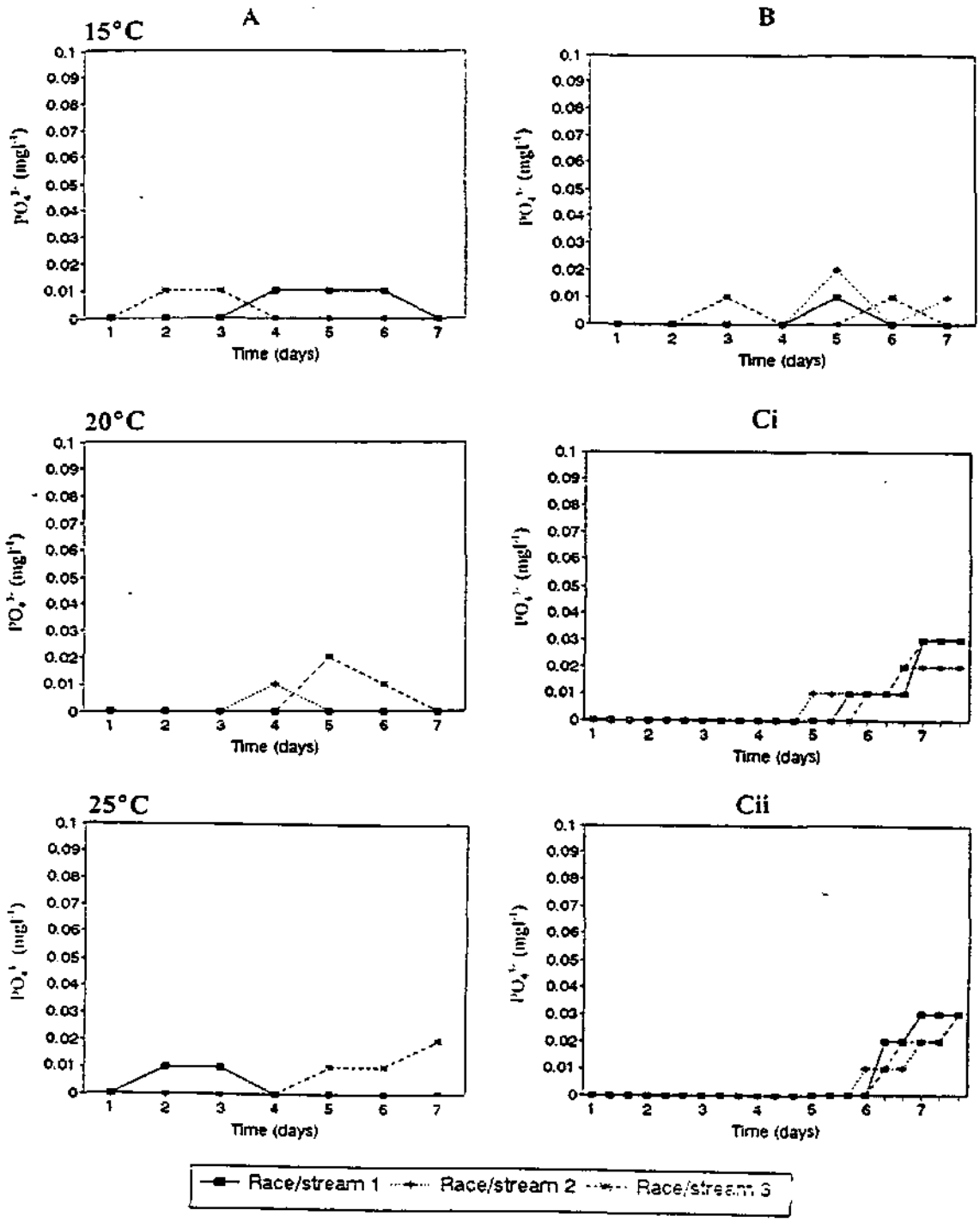

Figure 3.18 Changes in total phosphate concentration ($mg.l^{-1}$) in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

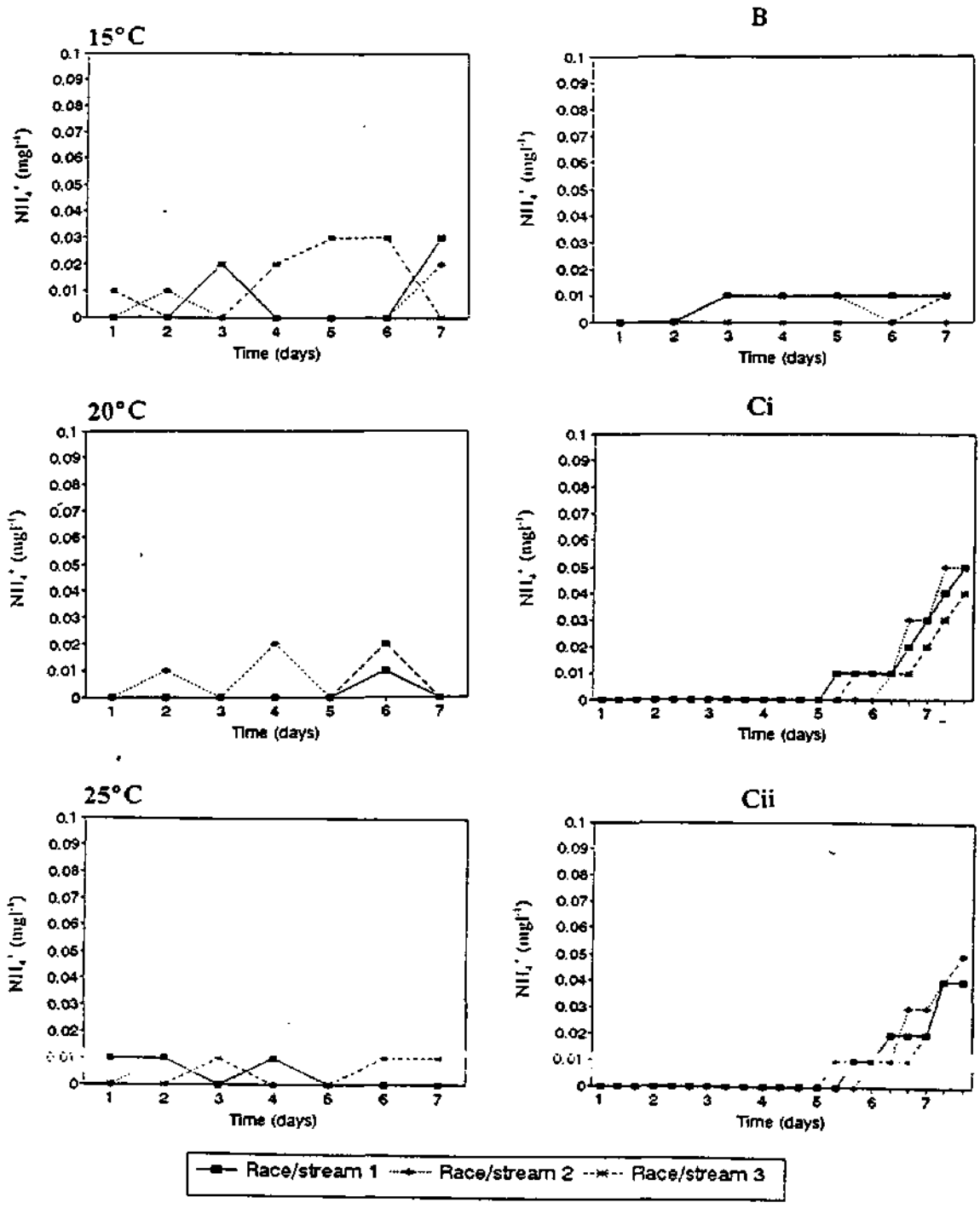

Figure 3.19 Changes in total ammonium concentration ($mg.l^{-1}$) in: (A) three raceways, recirculating municipal water, each with a population of 20 freshwater limpets, over a period of 7 days, at 15°C, 20°C and 25°C. (B) three raceways recirculating rainwater, each with a population of 20 freshwater limpets, over a period of 7 days, at 20°C. (C) three artificial stream systems at 20°C, monitored over 7 days, with differing source water, substrata, and in the presence and absence of test organisms. i: Stream 1 - municipal water; Stream 2 - rain water; Stream 3 - rain water and periphyton covered stones. ii: Stream 1 - municipal water and periphyton covered stones; Stream 2 - rain water, periphyton covered stones, and 40 limpets; Stream 3 - rain water, periphyton covered stones, and 40 limpets.

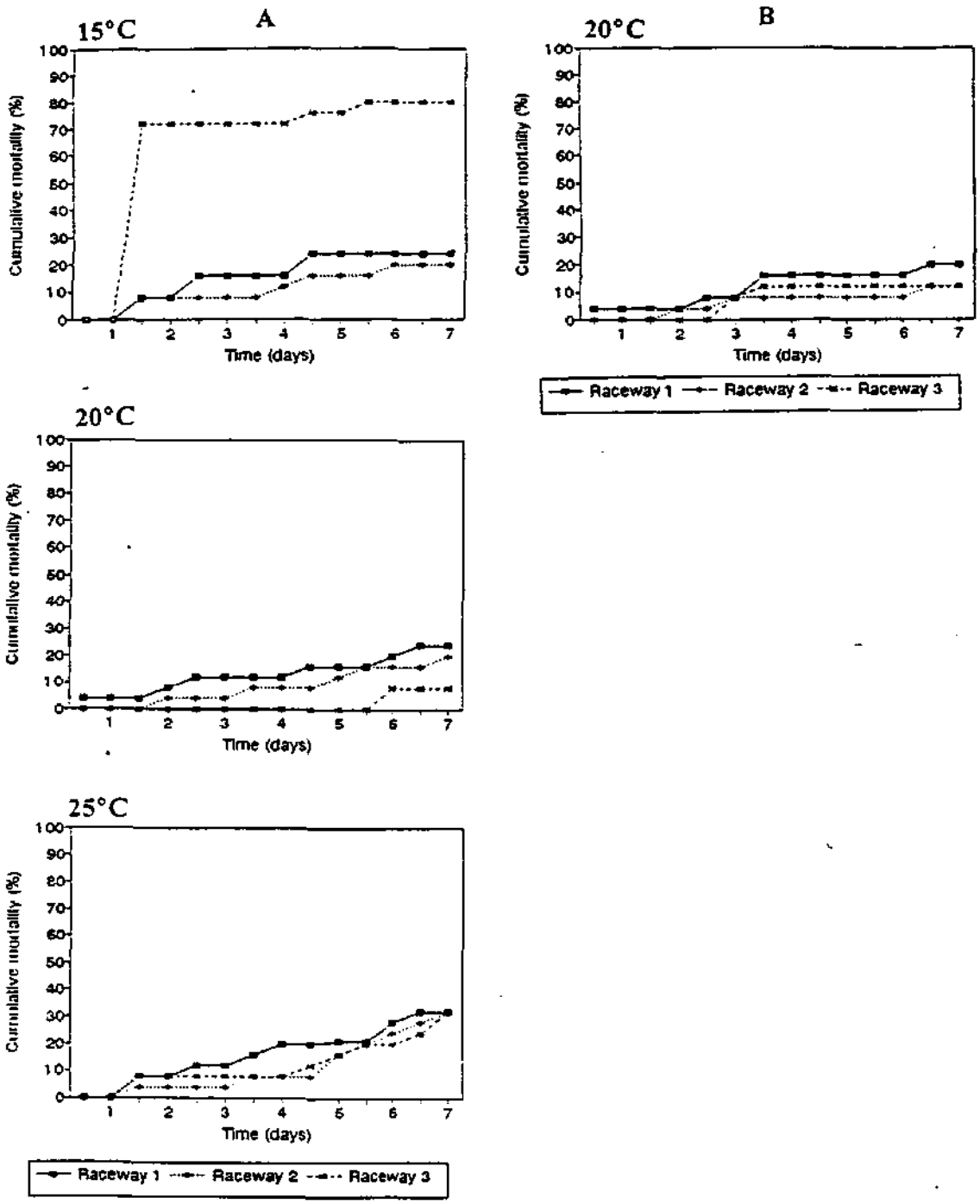

Figure 3.20 Cumulative mortalities of the limpet *Burnupia stenochorias* in three raceways recirculating (A) municipal water at 15°C, 20°C, and 25°C; and (B) rainwater at 20°C.

3.6.3 Conclusions

Water quality was stable in the three stream systems with regard to temperature, pH and conductivity, and what variability there was followed the same magnitude and pattern in the three systems. Nutrient concentrations increased over time, but were undetectable over at least a four-day period. The pattern and magnitude of this increase was the same in each of the systems and for all treatments. These variables will be monitored during the course of each tolerance test.

Since the volumes in the raceways were smaller and there was more variability in nutrient concentrations in the first 96 hour period than in the streams, tolerance tests conducted in raceways have followed a recirculating renewal method where a percentage (20%-50%) of the volume is replaced daily (APHA, 1992).

3.7 BIOLOGICAL CALIBRATION

3.7.1 Introduction

One of the requirements for ecotoxicity testing is less than 10% mortality in the control population (APHA, 1992). So far this has been impossible to achieve using wild populations of riverine macroinvertebrates. In the first sets of raceway experiments control mortality was 40-60%. It is now possible to keep control mortality between 10% and 20% in the raceways. Methods for achieving this differ for different test organisms, but one of the most important factors is handling. Many of the exploratory steps that were only partially successful in the use of the raceways for tolerance testing are reported in Chapter 6, and the limpet handling experiment is described in 3.5.2. Successful use of the raceways for tolerance testing is reported in Chapter 4.

Techniques for the use of the large stream systems for tolerance testing are still at the exploratory stage. Three experimental runs have been undertaken with the aim of establishing which substrate/flow combination gave the best animal retention and survival results. None of the results was sufficiently satisfactory to allow tolerance testing in the large streams to commence. Many of the test organisms either drift or are washed into the sump bag and rapidly die, but most are put into the stream and never seen again (a direct consequence of a larger scale system). However the ultimate success in using the raceways is encouraging, and perspex-frame net (0.05mm) "baskets" are being constructed to fit into each channel of the large systems, to retain test organisms.

3.7.2 Handling of the limpet *Burnupia stenochorias*

Ancylid limpets (*B. stenochorias)* were collected from the Bloukrans River at the railway bridge (10 km from Grahamstown). Limpets were gently pried off rocks (handled), or rocks with limpets attached to them were collected (not handled), placed in a cooler box with river water, and aerated until their return to the laboratory. In the laboratory limpets were sorted, and 40 were placed in each of six raceways, and 40 in each of six channels (2 stream systems). Limpets that had been removed from rocks (handled) were placed in 3 raceways; and in the 3 channels of one system; and groups of limpets on rocks (not handled) were placed in the other 3 raceways and three channels of a second stream system. The raceways were filled with river water, and the stream systems with dechlorinated municipal water. After a 48h acclimation period at 20°C, dead animals were removed. The maximum dead per experimental group was 5, and where less than 5 had died live ones were removed, so that all replicates started with 35 organisms.

Chemical variables were monitored. Nutrient concentrations were measured at the beginning of day one, end of day two and end of day four. (ammonium, nitrite, nitrate and phosphate). Temperature, dissolved oxygen (DO), pH and conductivity were checked in the morning (am) and evening (pm) each day (Tables 3.3 and 3.4)

Table 3.3 Water quality variables in raceways during the limpet handling experiment.

RACEWAYS		1		2		3	
DAY 1		am reading	pm reading	am reading	pm reading	am reading	pm reading
	pH	7.4	7.4	7.4	7.4	7.4	7.4
	DO(%)	100	104	105	103	103	104
	Temp(°C)	20.1	20.2	20.1	20.2	20.1	20.2
	NH_4^+ (mg l^{-1})	0.04		0.04		0.03	
	NO_2^- (mg l^{-1})	0.08		0.03		0.05	
	NO_3^- (mg l^{-1})	0.07		0.05		0.09	
	PO_4^{3-} (mg l^{-1})	0.14		0.15		0.14	
DAY 2	pH	7.4	7.4	7.4	7.34	7.4	7.36
	DO(%)	100	103	103	104	104	103
	Temp(°C)	20	20.2	20	20.2	20	20.2
DAY 3	pH	7.4	7.5	7.6	7.5	7.4	7.4
	DO (%)	103	101	101	100	104	104
	Temp(°C)	20.1	20.3	20.1	20.3	20.1	20.3
DAY 4	pH	7.5	7.4	7.4	7.4	7.3	7.4
	DO(%)	103	103	104	104	104	103
	Temp(°C)	20	20.2	20	20.2	20	20.2
	NH_4^+ (mg l^{-1})	0.03		0.04		0.03	
	NO_2^- (mg l^{-1})	0.05		0.07		0.07	
	NO_3^- (mg l^{-1})	0.05		0.06		0.07	
	PO_4^{3-} (mg l^{-1})	0.15		0.16		0.18	
DAY 5	pH	7.4	7.5	7.6	7.5	7.4	7.4
	DO (%)	103	101	101	100	104	104
	Temp (°C)	20.1	20.3	20.1	20.3	20.1	20.3

Table 3.4 Water quality data from the large stream systems from the limpet handling experiment (Section 3.7.2).

		pH	DO (%)	EC (mS/m)	TEMP (°C)	NH_4^+ (mg l^{-1})	NO_2^- (mg l^{-1})	NO_3^- (mg l^{-1})	PO_4^{3-} (mg l^{-1})
STREAM 1	Day 1	7.2	100	25.6	20	0.01	0.01	<0.01	0.05
STREAM 2		7.2	102	25.6	20	0.01	0.01	0.01	0.05
STREAM 1	Day 2	7.2	102	25.4	20				
STREAM 2		7.2	103	25.7	20				
STREAM 1	Day 3	7.2	101	25.6	20.1				
STREAM 2		7.3	102	25.4	20.1				
STREAM 1	Day 4	7.3	103	25.3	20.2	0.02	0.04	0.04	0.14
STREAM 2		7.3	102	25.4	20	0.03	0.03	0.05	0.1
STREAM 1	Day 5	7.3	104	25.7	20				
STREAM 2		7.2	100	25.1	20.1				

In all the raceways there was a daily 50% water change. This was done by reducing the water level in the raceway by half, and then adding river water.

Cumulative mortalities (%) were plotted against time (days) (Figure 3.21). Multiple regression analysis was used to compare the responses of handled and not handled limpets within groups, and between groups (Table 3.5). In most cases there was no significant difference within each group of three replicates. If there was a significant difference between the set of three replicates then the two more similar replicates were compared and in each case there was no significant difference between them. There was a significant difference between the response of handled and not handled limpets, with handled limpets dying much more rapidly.

It was concluded that limpets should be placed in experimental channels attached to a substrate. Since these animals are now breeding successfully in the laboratory, they will frequently be used as test organisms.

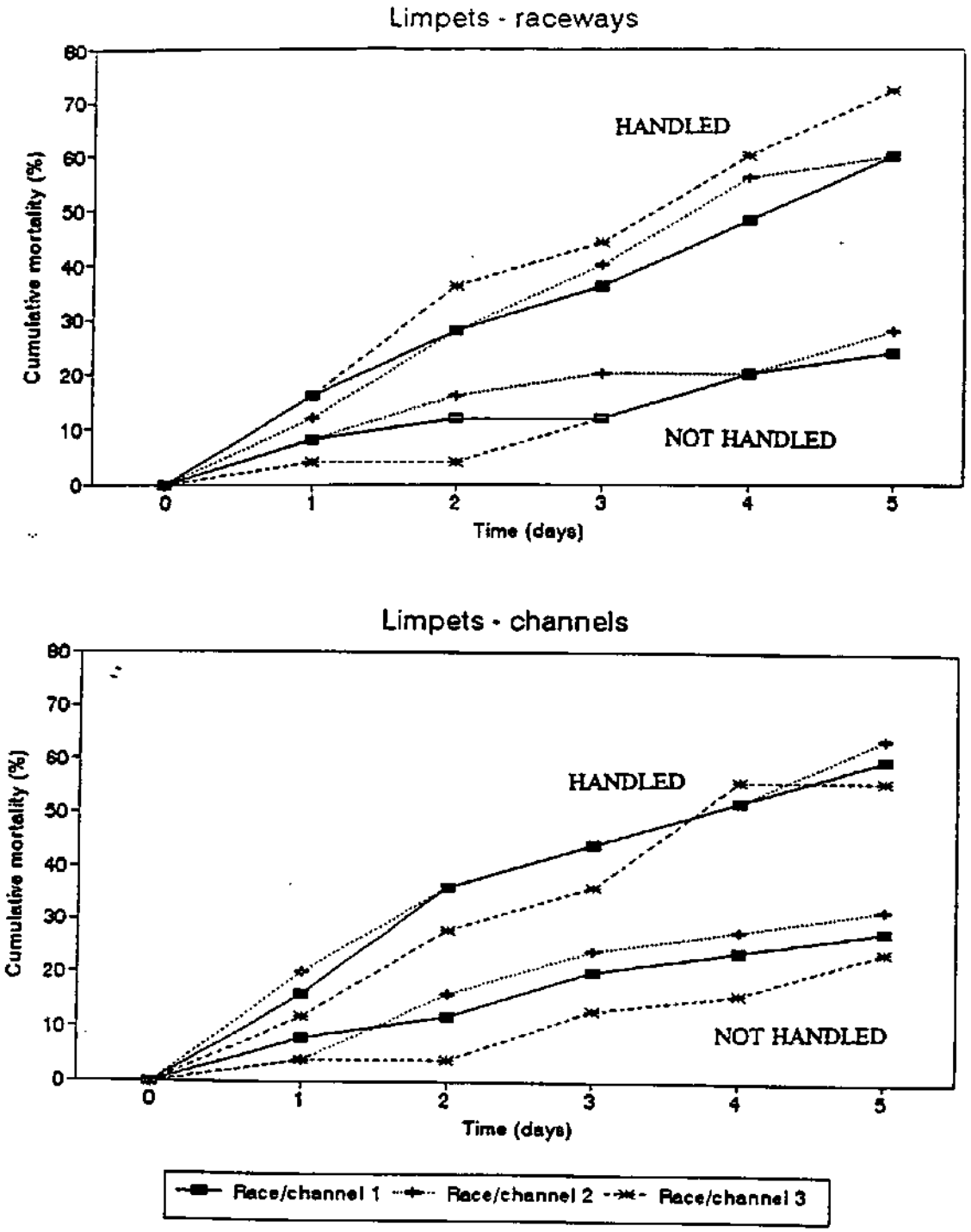

Figure 3.21 Effects of handling (removal from river stones - the natural substratum) and not handling (placed in test systems attached to river stones) on ancylid limpets held in raceways and channels.

Table 3.5 Comparison of slopes of the rate of percentage cumulative limpet mortality using regression analysis (BMDP).

			DF	F ratio	P
Channels	1-3	handled	4	0.742	0.5814
	1-3	not handled	4	9.761	0.0009 **
	1-2	not handled	2	2.648	0.1310
		3 handled vs 3 not	2	117.099	0.0001 **
		3 handled vs 2 not	2	91.899	0.0001 **
Raceways	1-3	handled	4	6.515	0.0050 **
	1-2	handled	2	1.068	0.3880
	1-3	not handled	4	2.821	0.0732
		3 handled vs 3 not	2	197.141	0.0001 **
		2 handled vs 3 not	2	215.489	0.0001 **

** : statistically significant at the 99% confidence level.

3.7.3 Responses of baetid mayflies in large stream systems

Introduction

One of the challenges of using indigenous riverine animals as ecotoxicological test organisms is obtaining a sufficient supply. The Standard Laboratory Organism project (Haigh *et al.*, 1994) aims to identify and breed populations of two test species. After two and a half years the project has succeeded in breeding ancylid limpets, and in identifying the mayfly *Adenophlebia auriculata* (Leptophlebiidae) as a potential second test organism, but the field populations will not sustain repeated collections for experimental purposes. In the mean time, the streams closest to Grahamstown have seasonally abundant populations of baetid mayfly nymphs. We have therefore used baetids as test organisms in initial tolerance testing and in three sets of behavioural calibration experiments. They are not ideal both because of seasonal variations in abundance, and because they are a multi-species population. Living nymphs cannot be accurately identified to species when alive, and identification is only confirmed at the end of an experiment once all the test organisms are preserved.

The aim of all three experiments was to observe the location in the stream of experimental populations over a 5 day period, and to see which of a variety of substrate and flow conditions were best suited for tolerance testing.

Methods

The stream systems were set at a selected hydraulic regime governed by a discharge of 15 l sec-1 and a channel gradient or slope of 0.25%. (Examples of the relationship between slope and discharge are given in Tables 3.1 and 3.2. The stream are designed so that the channels can be raised by 1% - a negative slope, or lowered by up 4% - a positive slope). The systems

were run for 48h to remove chlorine. after which 30 baetid nymphs were introduced to the top end of each channel. (The nymphs were placed in a petri dish covered by a piece of mesh. This was submerged, and the nymphs moved onto the mesh which was lodged at the top end each channel until all had moved in the channel.) Water from the channels fell into large 0.5mm mesh bags in the sump, so animals dislodged from each channel were isolated, and prevented from moving freely in the sump, or being sucked through the pump. Substrate and channel ends were varied. Channels were either fitted with end-nets (1mm mesh) or left open. Substrate variations included: bare channels; a 1m strip of 1mm mesh plastic net glued with silicon onto the channel floor, beginning 1m from the inlet tank end of each channel; three rows of ovoid kiln-baked kaolin "stones" placed in each channel, beginning 1m from the inlet tank end, with another two rows of stones placed 1m down stream of the first set of stones; a combination of floor-net and stones, with three rows at the top end, and two at the bottom end of the floor-net; or black plastic sheeting stuck underneath each channel to prevent under-lighting.

The number of animals on the channel floor and in the sump-bag were counted; mortalities were recorded; and numbers of missing nymphs inferred. The mean and standard error for three channels in each system are given in Figures 3.22 - 3.24.

Results

Experiment 1 (Figure 3.22)
Temperature: 20°C; Discharge: 15 l sec^{-1}; Slope: 0.25%
Number of nymphs per channel: 40.

Stream 1: Floor-net, open ends:
In these channels there was nothing on the perspex channel floor to cling onto. After an hour almost 50% of the nymphs had disappeared and of those that could be found, most were in the sump bags. All the mayflies that could be found were dead after 56 hours.

Stream 2: Stones, end-net:
In these channels there were stones in the channel and a net at the end. There was still only a 15% retention of mayflies in the channels after eight hours. After 24 hours 60% could not be found, but 10% survived in the sump bags for six days.

Stream 3: Bare channels, black plastic, end net:
Fewer mayflies were not found in this system, but there was still high mortality, and very low retention in the channels.

These results indicate that some substrate is necessary and that both stones and netting on the channel floor are useful, but that end nets do not contribute to retention in the channels.

Experiment 2 (Figure 3.23)
Temperature: 20°C; Discharge: 15 l sec^{-1}; Slope: 0.-25%
Number of nymphs per channel: 30.

Stream 1: Stones and end net.
Stream 2: Stones, open channels, black plastic.
Stream 3: Stones, floor-net, open ends.

This slope gave better results than Experiment 1. Stream 3, with stones and a floor-net had the best retention and survival. However, after 96h, only 50% of the test population remained alive in the channels, a proportion which is inadequate for tolerance testing.

Experiment 3 (Figure 3.24)
Temperature: 20°C; Discharge: 15 l $sec._1$; Slope: -0.25%
Number of nymphs per channel: 30

Stream 1: Stones, floor-net, open ends.

Careful repetition of the best conditions in previous runs, with stones and net on the channel floor, gave slightly better, but still unsatisfactory, results. Half the nymphs could not be located by the end of the experiment, and only 5 of the original 30 remained alive in the channel. Clearly another approach was necessary.

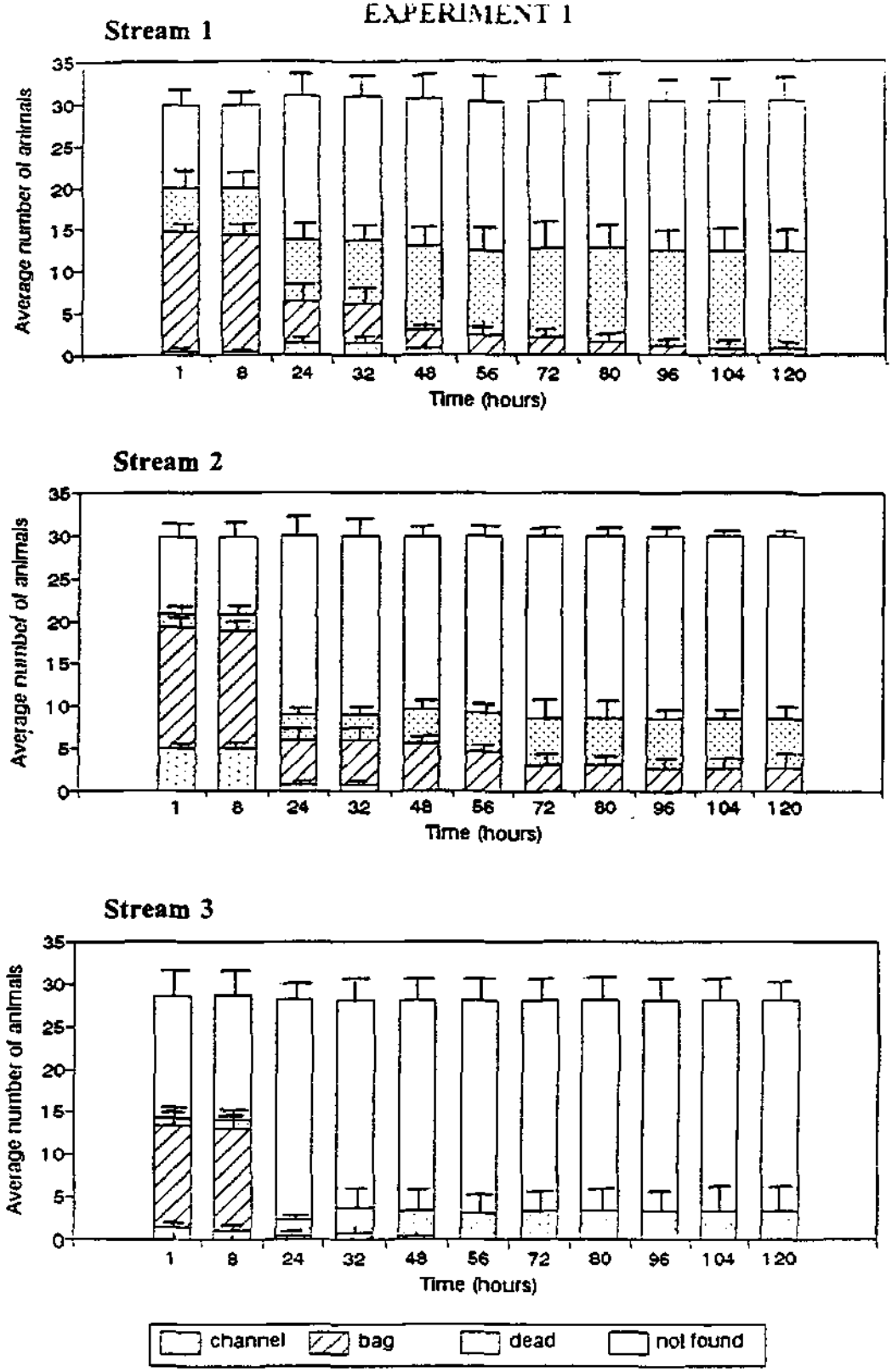

Figure 3.22 Baetid nymphs were counted in the channel and sump bags, dead animals were removed, and missing nymphs were calculated. Mean numbers for the three channels in each stream are presented. Standard errors are indicated by vertical bars which are only shown extending above the bar- graph line for clarity, but extend equally below the bar-graph line. (Temperature: 20°C; discharge: 15 l sec^{-1}; slope: 0.25%, starting number: 30 nymphs). Stream 1 - Floor net, and open ends. Stream 2 - Stones, and end net. Stream 3 - Bare channels, black plastic, and end net.

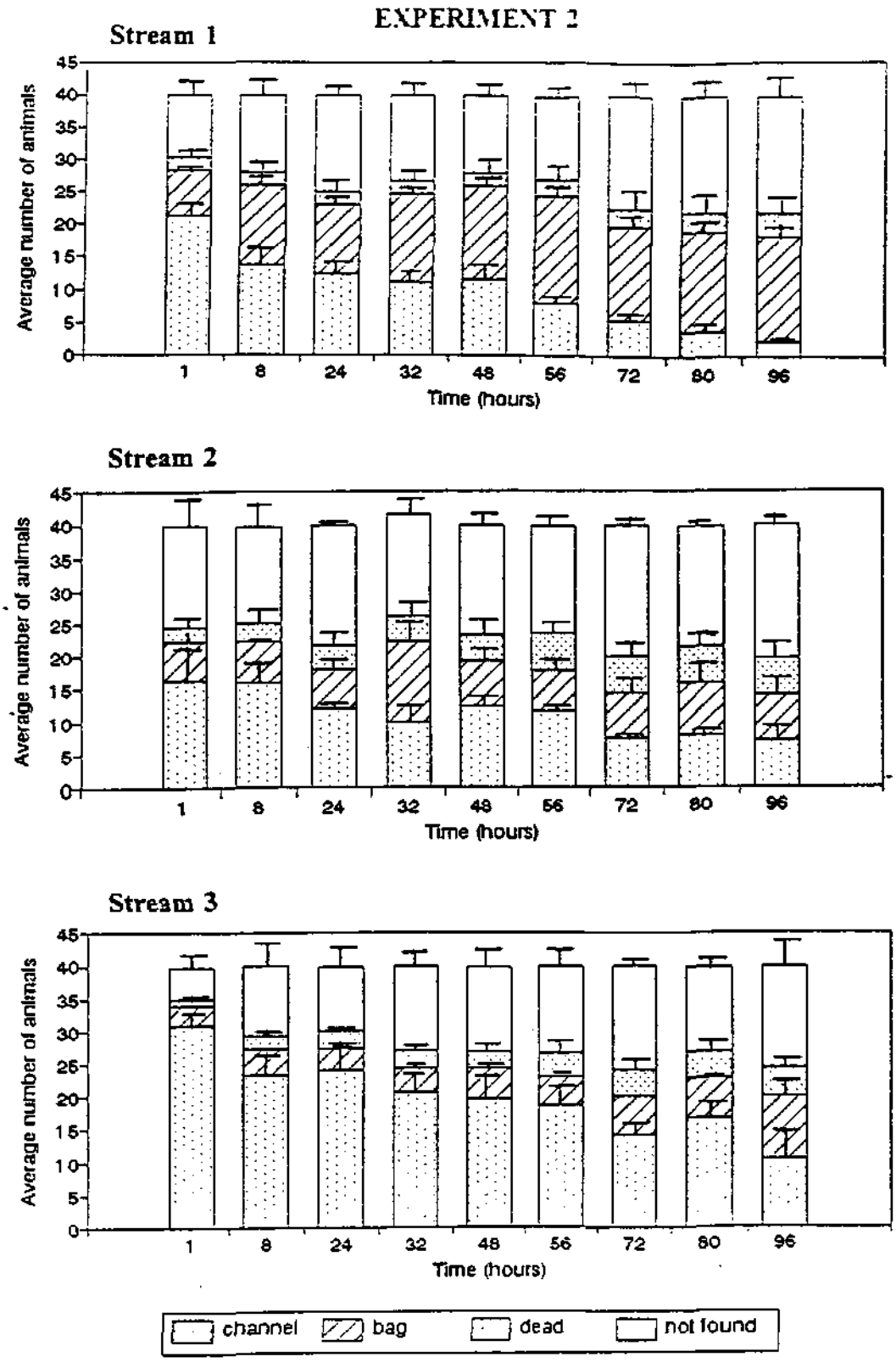

Figure 3.23 Baetid nymphs were counted in the channel and sump bags; dead animals were removed; and missing nymphs were calculated. Mean numbers for the three channels in each stream are presented. Standard errors are indicated by vertical bars which are only shown extending above the bar- graph line for clarity, but extend equally below the bar-graph line. (Temperature: 20°C; discharge: 15 l sec^{-1}; slope: -0.25%; starting number: 40 nymphs). Stream 1 - Stones and end net. Stream 2 - Stones, open channels and black plastic. Stream 3 - Stones, floor net, and open ends.

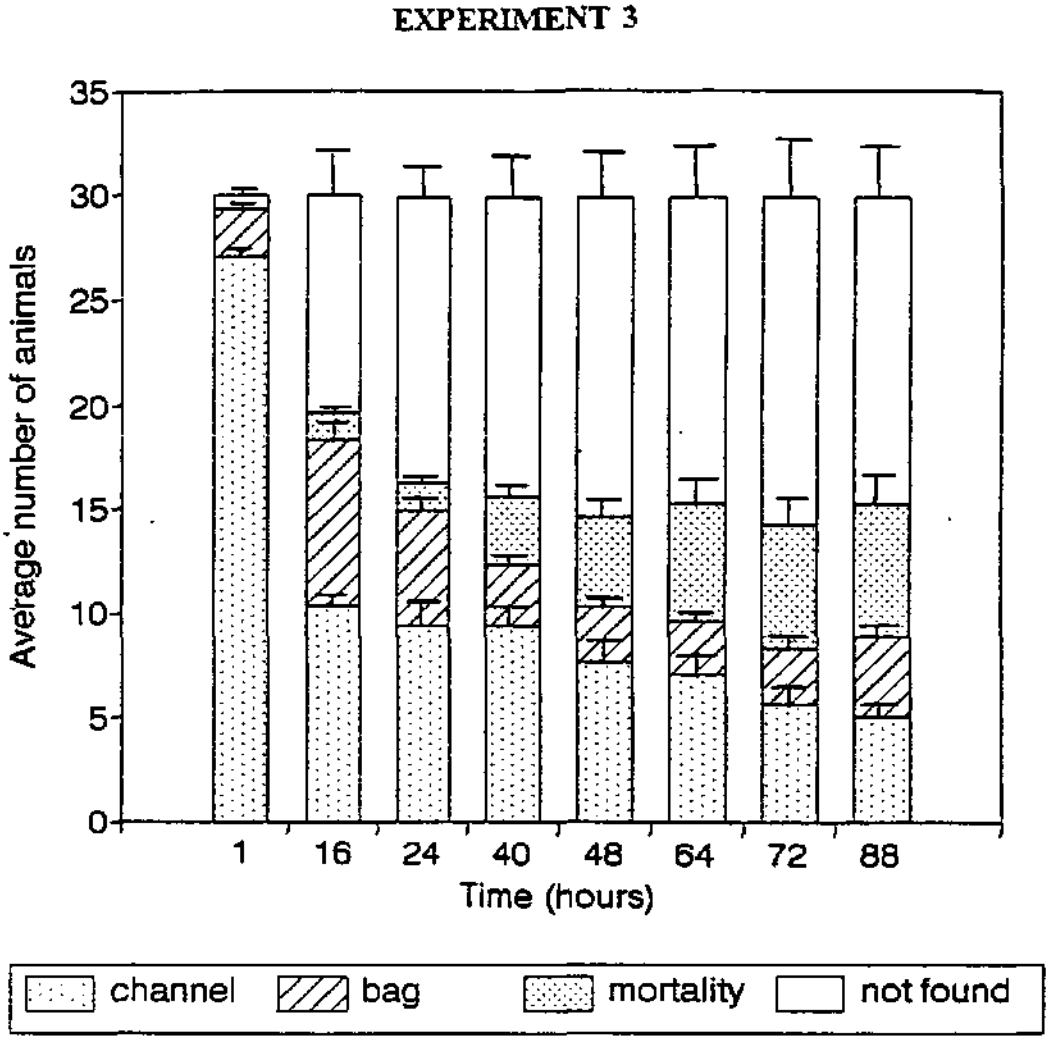

Figure 3.24 Baetid nymphs were counted in the channel and sump bags; dead animals were removed; and missing nymphs were calculated. Mean numbers for the three channels in the stream are presented. Standard errors are indicated by vertical bars which are only shown extending above the bar- graph line for clarity, but extend equally below the bar-graph line. (Temperature: 20°C; discharge: 15 l sec^{-1}; slope: -0.25%; starting number: 30 nymphs). Stream 1 - Stones, floor net and open ends.

Discussion

These, and the raceway tolerance experiments conducted in Grahamstown (Chapter 4), point to several important conclusions:

1. The baetid mayfly nymphs from the Bloukrans River are inappropriate test organisms for three reasons: the river is too polluted to be an adequate site for the collection of tolerance test organisms, as experimental responses could be influenced by previous environmental stresses; the baetids are a multi-species population, and there is no way of selecting one species alone for experiments; the baetids are too small, they are difficult to contain, and difficult to find during experiments; it has proved impossible to achieve adequate survival in the controls of experiments, either in the raceways or in the large streams.
2. The work of the Standard Laboratory Organism project proves to be essential for the success of the artificial stream work. We are dependent on adequate test populations and it is not feasible to depend on riverine populations.
3. Until the breeding programme is underway at an adequate rate, tolerance testing must be timed to coincide with the highest populations of the leptophlebiid mayfly *Adenophlebia auriculata*, in the Palmiet River. The nymphs of this mayfly are an ideal size for a test organism, they respond well to artificial holding conditions, and the Palmiet River is unpolluted. Populations from the upper reaches of the Buffalo River could also be used.
4. Other rivers in a reasonable radius around Grahamstown must be reconnoitred for populations of *A. auriculata*.
5. Perspex frames covered in 0.5mm mesh net to form closed "baskets" (1m in length) will be fitted snugly into the large stream channels to contain test populations.

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CHAPTER 4

EXPERIMENTAL TOLERANCE TESTING

Summary The effect of increased salinity on Bloukrans (Eastern Cape) and Sabie River (Mpumalanga) macro-invertebrates was investigated in experimental flowing-water systems using sodium chloride and sodium sulphate as toxicants. Tolerances of Sabie River invertebrates to Selati River water were also investigated.

4.1 INTRODUCTION

4.1.1 Ecotoxicology and ecotoxicity testing

Implementation of the receiving water quality objectives (RWQO) approach to water quality management was rapidly followed by the publication of water quality guidelines for four user sectors: agriculture, domestic users, industry and recreation (DWAF, 1993). The delay in producing environmental water quality objectives arises from the recognition that the format used in the other guideline documents is inappropriate for the environment.

In the four published guideline documents specific ranges are given for each variable, together with a description of consequences or effects. In the case of the natural environment this is not appropriate as specific consequences are generally unknown. Descriptions would also not be relevant as the ecosystem acts as an interactive whole. It is therefore more sensible to move towards risk evaluation. Van Veelen and Swart (1992) suggested four ranges of water quality condition: ideal, acceptable, tolerable and unacceptable conditions. Water quality objectives should also be two-tiered - one level that should ideally be achieved and maintained i.e. the no observed effect level (NOEL), and a second one that should not be exceeded i.e. the maximum level of acceptability (MLA) or maximum acceptable tolerance concentration (MATC). A third level, the critical level (CL), can be identified. Conditions reaching this level require immediate action.

The most recent discussions of environmental water quality guidelines suggested that since the goal of providing guidelines was the conservation of aquatic ecosystems in a functional state, there should be a single guideline that would be protective of environmental function, based on chronic toxicity testing data. Criteria for dealing with short-term pollution events such as spills would be provided by acute toxicity data, with the application of safety factors depending on the completeness of the data set (Roux and Jooste, in prep.). Regardless of the exact nature of environmental guideline development in South Africa, the central role of toxicity data, both from chronic and acute toxicity testing, is certain. The role of the artificial stream laboratory therefore is to provide data on indigenous riverine invertebrates, and to contribute to the validity of the guideline values.

The development of guidelines is only the first step in water quality management. The six-step water quality-based approach as proposed by Jaworski and Mount in 1985, is as follows:

1. Establishment of water quality standards;
2. Derivation of waste load allocations;

3. Development of effluent limitations;
4. Design of wastewater treatment facilities;
5. Operation and maintenance of these facilities, and;
6. Compliance monitoring of effluents and receiving waters.

The first critical step is that of establishing water quality standards, and serves as the basis for implementing all subsequent steps. Setting water quality guidelines therefore consists of two important components:

1. designated user(s) - in this case the environment; and
2. scientifically-derived criteria to protect the user(s) i.e. toxicity tests.

The measurement of toxicity is essential in the formulation of quality standards for receiving waters. Toxicity tests determine the concentration of a chemical which causes either death or some altered physiological or behavioural process showing some level of interference with the normal life cycle of a test organism. Toxicity data therefore provides information about the mechanisms of toxicity, synergistic or antagonistic interactions with various environmental parameters, and the overall impact of stresses (Coler and Rockwood, 1989).

As pollutants can exert a variety of toxic effects either directly or indirectly at a range of ecological levels, a number of investigative methods have been developed to determine organism tolerances. A range of acute (48 to 96 hour tests), sub-chronic (4-7 days) and chronic or life-cycle (LC) tests (7 days to several weeks), can be used for toxicity testing, which can be applied either to single or multiple species. Testing can either be sub-lethal i.e. monitoring behavioural changes, or lethal i.e. monitoring mortality. In lethal testing, the LC_{50} or lethal concentration at which 50% of the population dies, is the standard measure of response. There is no overall constant ratio between acute and chronic toxicity levels as the shape of toxicity curves (Figure 4.1) and the distances between specific points of effect (threshold, intoxication, mortality) are both species- and toxicant-dependent (Persoone *et al.*, 1990).

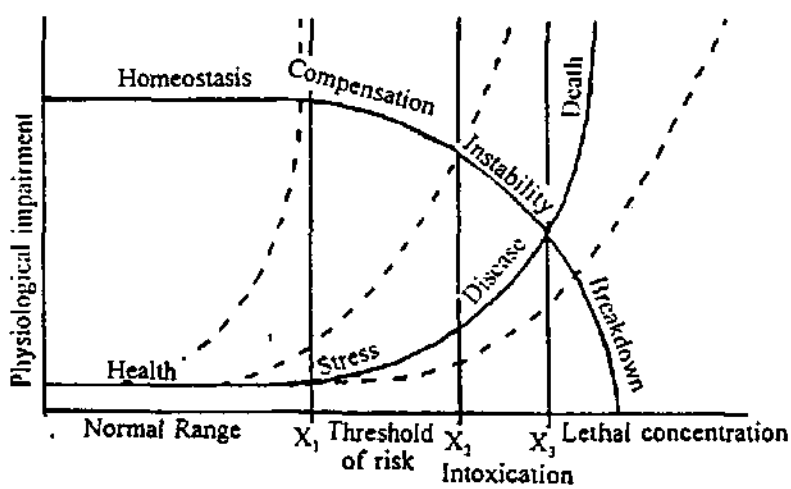

Figure 4.1 Diagram of effects of a chemical at increasing concentrations on the physiological processes of an organism. X_1, X_2, X_3 represent different chemicals with different toxicity curves and threshold concentrations (Persoone *et al.*, 1990).

The basic requirements for toxicity tests are an abundant supply of water of desired quality, an adequate flowing water system constructed of non-polluting and non-absorbing materials, adequate space and good testing equipment, and a source of healthy experimental organisms (APHA, 1992). When selecting organisms for toxicity testing the following criteria should be considered: their sensitivity to the pollutant under consideration, their general physical condition and adaptability to laboratory conditions, their ecological importance, knowledge of their environmental requirements, and their geographical distribution, abundance and availability for ecotoxicological studies (APHA, 1992). Ideally test organisms should also be endemic to the region of study (Coler and Rockwood, 1989). Fish and the water flea, *Daphnia*, have been widely used for ecotoxicological studies. In addition, algae and bacteria such as *Salmonella typhimurium* have also been used for toxicity testing (APHA, 1992). Although macroinvertebrates occupy a key position as intermediate consumers in the pelagic and benthic food chains of aquatic ecosystems (Persoone and Janssen, 1993), little information is available on their tolerances to any water quality variables. Indigenous riverine fauna have been particularly poorly studied, and riffle-dwelling invertebrates were therefore selected as the subject of this study. Macroinvertebrates are also considered useful test animals due to their abundance in unpolluted streams and their sensitivity to low concentrations of pollutants (APHA, 1992). Benthic macroinvertebrates are widely considered to be indicators of environmental pollution (Chapman *et al.*, 1982). Riffle-dwelling organisms are particularly useful as they are in constant close contact to any water-borne pollutant due to their movement within the water column. As *Daphnia* is routinely used as a test organism in aquatic toxicology in South Africa, one of the future aims of the artificial stream project is to compare tolerances of riffle-dwelling riverine taxa with those of laboratory-reared *Daphnia pulex*. *Daphnia* tolerance experiments are currently being carried out at the Institute for Water Quality Studies (IWQS) in Pretoria (Roux, pers. comm.).

According to the Department of Water Affairs (DWA) (1986) salinisation, eutrophication and pollution by micro-pollutants are the three main factors affecting water quality in South Africa. Of these, salinisation, or the accumulation of dissolved inorganic salts, has been identified as the most important problem facing the managers of South African freshwaters (Commission report, 1970; Davies and Day, 1986; Stander, 1987). Salinity was therefore selected as the first water quality variable under investigation by the artificial stream project.

4.1.2 Salinisation

Salinisation of South African rivers was reviewed by Maart (1992) (Appendix 4.1). The three main causes of increasing salinity in South African freshwaters are natural salinisation, agricultural activities, and urban and industrial activities (Davies and Day, 1986).

There are many factors affecting natural salinisation, including atmospheric precipitation, evapo-transpiration and catchment lithology. The accumulation of salts is also controlled by factors such as low relief and shallow groundwater inflows, low run-off : rainfall ratios, high evaporation : precipitation ratios and evaporation-crystallization processes (Maart, 1992). During high flows, precipitation chemistry plays a greater role in salinisation through run-off. At low flows, rock dominance is more important as flow consists primarily of interflow and baseflows (Cornish, 1987). The geomorphology or geological formations of an area may also contribute to salinisation e.g. easily-degraded rocks with naturally high mineral levels such as the Malmesbury shales of the Western Cape which affect the Berg River system, or salt-

impregnated formations which leach salt after heavy rains e.g. the Karoo shale formations (Davies and Day, 1986).

The impact of salinisation due to irrigation is considerable. Spray irrigation in even slightly arid areas can lead to a build-up of naturally-occurring salts in the soil as the water evaporates on, or before reaching the soil. This increased salt load is then washed down into the groundwater and finally into the rivers. The process is exacerbated in areas where irrigation water already has high salt loads due to the region's geomorphology. Evaporation rates and soil types also influence salt accumulation in the soil (Davies and Day, 1986). The clearing of natural vegetation can lead to secondary salinisation due to groundwater responses. The shallower roots of the crop replacing the natural vegetation leads to an increased downward flux of water. Salt leached from the soil is displaced downward, raising the salinity level of the groundwater. Excess water, as a result of deforestation, now causes the water table and salt to rise to the surface, leading to further salinisation of the soil and subsequently the rivers (Allison *et al.*, 1990). Evapotranspiration from irrigated land also increases salt concentrations in soil water, which eventually moves into the rivers. As storage dams present a large surface area for evaporation, impoundments are also a source of rising salinity levels e.g. increased mineralization of the Buffalo River after the erection of Laing and Bridle Drift Dams (Selkirk and Hart, 1983). Impounding a river, such as the Pongolapoort Dam on the Pongola River, also prevents the natural flushing out of accumulated salts in floodplain areas of the river. This influence is mostly related to the discharge of saline industrial and sewage effluents, and the wash-off of large quantities of dissolved solids into rivers. Unfortunately, the high salinity of some effluents limits the industrial recycling of water as salt accumulation in recycling plants necessitate replacement with fresh water (DWA, 1986). Seepage from mine dumps is also a source of high salinity in streams.

Organic and inorganic ions in fresh water result from complex processes such as the production and degradation of terrestrial and aquatic biomass, the weathering of rocks and leaching of soil by rainfall, adsorption reactions on suspended particles, particle coagulation and sedimentation in water bodies, and chemical and biological activity in sediments (Ure and Davidson, 1995). Material dissolved in water is commonly measured as total dissolved solids (TDS), electrical conductivity (EC), or salinity. There is a strong correlation between these variables in most waters. TDS represents the total quantity of dissolved material, organic and inorganic, ionized and un-ionized, in a water sample. Conductivity is a measure of the ability of a sample of water to conduct an electrical current. Since most dissolved material is ionic, TDS and EC correlate closely. This does not apply in waters rich in dissolved organic carbon (DOC), as most DOC compounds are non-ionic. Since molar conductivity varies from ion to ion, slight variations will also occur depending on the proportions of the major ions in solution (Dallas and Day, 1993). For South African waters therefore, it is widely accepted that the relationship between TDS and EC exists as follows (D. Grobler, 1989, pers. comm. - cited by Dallas and Day, 1993):

$$TDS\ (mg.l^{-1}) = EC\ (mS.m^{-1}) \times 6.6$$

In this document, increasing salinity is used to describe increasing saltiness of experimental water due to the addition of specific salts. This increase in saltiness or salinity is measured as conductivity in this instance, and the terms conductivity or EC may therefore be used when discussing salinity.

It has been increasingly realized that the distribution, mobility and biological availability of chemical elements depend not only on their concentrations but, critically, on the forms in which they occur in natural systems (Ure and Davidson, 1995). Toxicity therefore depends on chemical speciation, which is in turn influenced by general water chemistry conditions such as pH and hardness (Wade *et al.*, 1995). The concentration, proportion and identity of ions contributing to conductivity or TDS should therefore be known. The ions most commonly found in natural waters are the cations calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}), and the anions, bicarbonate (HCO_3^{-}), carbonate (CO_3^{2-}), chloride (Cl^{1-}) and sulphate (SO_4^{2-}). In South Africa, the natural waters of the Highveld are dominated by calcium, magnesium and bicarbonate ions, while the coastal and arid west regions are dominated by sodium and chloride ions (Dallas and Day, 1993).

Very little information is available on the salinity tolerances of riverine organisms, particularly macroinvertebrates (Noble, 1970). Many freshwater species are however able to survive at high salinities, e.g. the green alga *Monoraphidium circinale* (Prinsloo and Pieterse (1992), cited in Dallas and Day, 1993). Reice and Herbst (1982) showed that salinity affects decomposition rates of *Phragmites australis* in springs of different salinities in the Dead Sea basin in Israel. Salinity was therefore a significant factor in controlling this ecosystem-level process.

Tolerance experiments carried out on four Australian fish species (Williams and Williams, 1991) indicated LC_{50} tolerances to high TDS concentrations (20 800 - 43 700 $mg.l^{-1}$) provided TDS levels were increased slowly over a number of days. This suggests that it may be the rate of change of TDS rather than the final salinity that is the most critical. However, Tyson (1993) found no significant difference between mortality data for acclimated and non-acclimated *Paramelita nigroculus* amphipods during acute and chronic salinity bioassays. Tolerance to TDS or salinity therefore appears to be species-specific (Dallas and Day, 1993), with organisms having osmo-regulatory organs being able to adjust to slow changes in salinity by physiological acclimation. Results of studies on the effect of salinity upon metal toxicity have been variable (Herbert and Wakeford (1964), Herbert and Shurben (1965), Brown *et al.* (1967)).

As conductivity is a measure of the number of charged particles or ions in solution, it is also a measure of the total quantity of salts. Fluctuations in conductivity can therefore cause changes in osmotic, ionic and/or the water balance of an organism. The extent of the change relies on the nature of the salts and its medium, as the bioavailability and subsequent toxicity of a pollutant is influenced by general water quality conditions.

4.1.3 Specific aims of this research programme

Salinity tolerance experiments

In addition to ascertaining macroinvertebrate tolerances to low, medium and high conductivity (the parameter used to measure salinity) water, two different salts were selected to assess how different agents of elevated conductivity may contribute to organism mortality. Sodium chloride was chosen as it is a common salt, and sodium sulphate as it is an important pollutant resulting from mining in the Mpumalanga Highveld and the Phalaborwa Lowveld regions adjacent to the Kruger National Park (KNP). Sulphate pollution can mainly be attributed to saline mine water contamination due to the oxidation of pyrite and marcasite, which

contaminates natural surface water systems. Sodium, chloride and sulphate ions have all been shown to contribute significantly to high TDS levels recorded in the Olifants River system (Mpumalanga), particularly during times of drought (van Vuren *et al.*, 1994).

Selati River tolerance experiment (simulated "whole effluent" testing)
Water quality guidelines to be developed for the environment will include both guidelines specific to particular variables, being concentrations of one variable such as salinity, and its consequences; and whole effluent guidelines, that is the dilution factor required for a particular effluent (Roux *et al.*, in press). To briefly investigate "whole effluent" testing, Sabie River invertebrates were exposed to saline polluted Selati River water to assess the response of clean-water organisms to polluted conditions.

4.2 GENERAL METHODS

The first step in toxicity testing and developing water quality standards is normally acute toxicity studies, as these studies indicate individual species sensitivity to a pollutant as well as lethal concentrations (Parkerton *et al.*, 1989). This information can also be used as a basis for long-term studies, thus establishing concentration limits to preserve aquatic life. Acute studies are easier to conduct as maintaining animals under experimental conditions for long periods of time may be impractical and problematic. This study employed 96-hour acute tests which has been reported as the maximum time during which invertebrates do not require feeding (Cairns *et al.*, 1976; Arthur *et al.*, 1987; Hickey and Vickers, 1992). APHA (1992) and Rosenberg and Resh (1993) also state that animals should not be fed during short-term tests. The test criterion was mortality, and LC_{50} levels determined i.e. the toxicant concentration causing 50% mortality. Mortality was defined and observed as immobility of the organism, and lack of movement upon touching.

Acute LC_{50} experiments were carried out in two phases. Firstly, unreplicated range-finding (RF) tests using two species of indigenous invertebrates were carried out, and secondly, definitive tests which were carried out in triplicate using only one macroinvertebrate species. To compensate for positional effects, replicate raceways were arranged randomly around the laboratory. Although unreplicated, range-finding experiments provide some indication of where LC_{50}s lie, and therefore render information useful for definitive testing (APHA, 1992).

Ecotoxicity studies were carried out in experimental stream systems known as raceways (Figure 4.2). Raceway components in contact with the water were sealed with silicone sealant (recommended by APHA, 1992) and constructed of perspex (polymethylmetacrylic), which is relatively inert, non-absorbing and has high levels of chemical resistance (Maizey Plastics - Physical properties and chemical resistance chart). As macroinvertebrates are riffle-dwelling animals in constant contact with flowing water, raceways had to be flowing water systems. The current is generated by a paddle wheel which is driven by a small electric motor. The constant recirculation of water also aids oxygenation. To facilitate easy observation of the animals they were confined to a specific area of the raceway by placing screens at each end of a channel. Four kaolinite stones were placed within this channel to serve as a substrate. Screens also serve as another substrate, particularly for emerging animals. Emerging animals were prevented from leaving the raceways by screens placed over each raceway. Raceways were kept under controlled light and temperature conditions where possible.

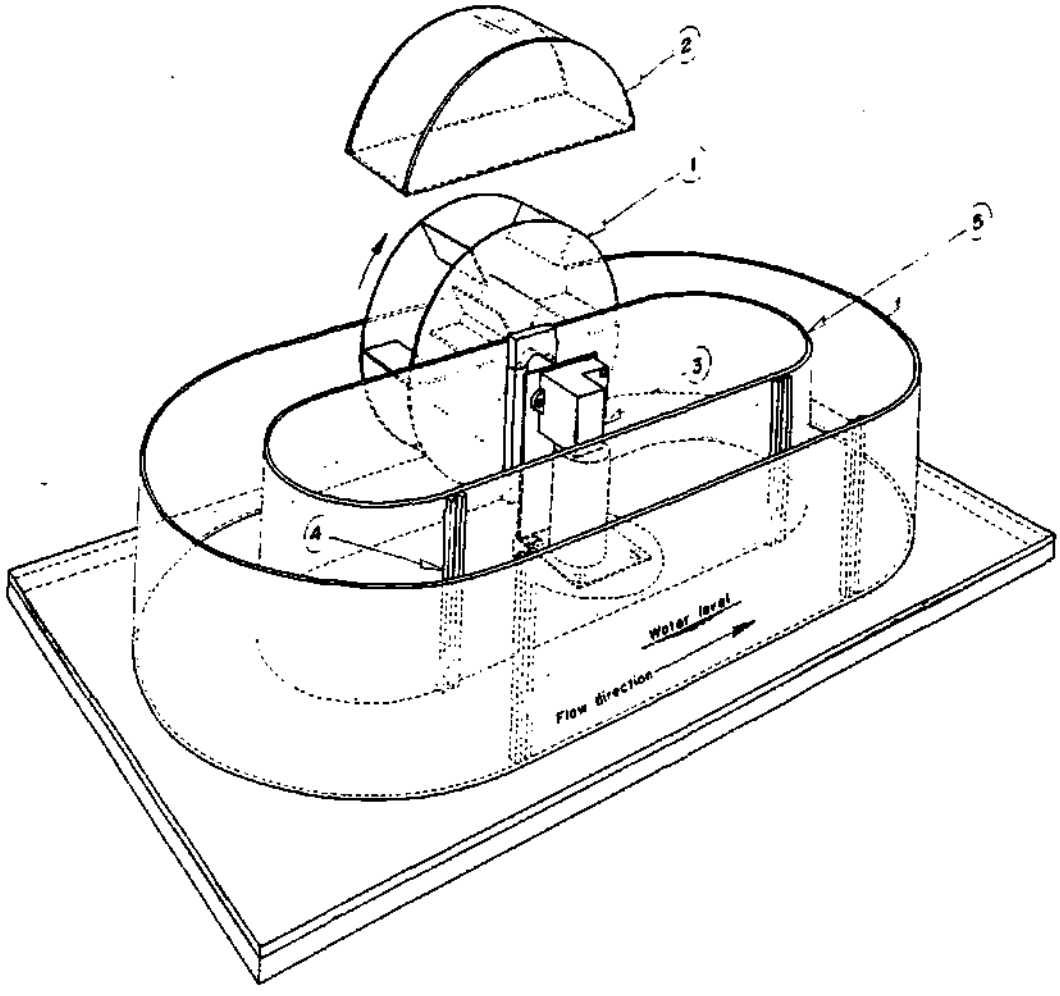

Figure 4.2 Diagram of a portable raceway used for ecotoxicity studies.

Each raceway was filled with 12.5 litres of river water which was circulated for a few hours prior to animals being placed on kaolinite stones in the raceways. Macroinvertebrates were then acclimated unfed for 36 - 48 hours. APHA (1992) recommend a testing chamber of minimum 8 litre capacity and an acclimation period of at least 48 hours. Mortality during acclimation should not be more than 5%. The length of test organisms selected for testing should not vary in size by more than 50% between the smallest and largest (Coler and Rockwood, 1989). After the acclimation period all dead animals were removed and numbers standardized before the addition of the salt solution. Each raceway therefore had the same number of organisms before the onset of toxicity testing. Twenty percent (2.5 litres) of the total water volume in each raceway was replaced daily with fresh salt solution. Water was

therefore removed from each raceway and replaced with 2.5 litres pre-prepared salt solution up to the required conductivity. This method is known as a (recirculating) renewal system and reduces the build-up of toxins and metabolites such as ammonia in the water (Coler and Rockwood, 1989). Toxicant concentrations also vary during the experiment due to evaporation, absorption and metabolism by the animals, and chemical and microbiological breakdown (Abel, 1989). Replacement of 100% of test solution would be preferable, but is not practical in these large experimental systems.

As the toxicity of any pollutant is influenced by environmental conditions such as pH and dissolved oxygen (Abel, 1989), physico-chemical conditions (temperature, pH, dissolved oxygen (DO) and conductivity (EC)) in each raceway were monitored daily. A Hanna HI 193 meter was used for oxygen measurements, a Checkmate CCA475627 kit for pH and oxygen readings, and an Amel digital conductivity meter (model 160, electrode model 192, or graphite electrode model 193 for high salt solutions) for EC measurements. Mortalities were noted twice daily and nutrient concentrations i.e. ammonium, nitrate, nitrite and phosphate, monitored spectrophotometrically every second day using either a Hach DR/2000 or Spectroquant 118 photometer. Water samples were taken from each raceway and analyzed within a few hours. Ammonium was analyzed by the indophenol blue method, nitrate by reacting samples with nitrospectral in concentrated H_2SO_4 to produce a dark red-coloured nitro compound, nitrite by reacting samples with sulphanilic acid and N-1-naphthylethylenediamine dihydrochloride to produce a magenta azo dye (Griess' Reaction), and phosphate was measured by the phosphomolybdenum blue (PMB) method (Merck Manual Photometer SQ118). Levels of detection, or measuring ranges, were determined by the dilution (river) water used for each experiment. Nutrient cycles and guideline values are described in Appendix 4.2.

It is essential that the results of nutrient analyses are interpreted with methods clearly in mind. Although different methods appear to give relatively consistent results if sample treatment is the same (P. Kempster, pers. comm.), the pre-treatment of samples greatly influences final results. Samples analyzed spectrophotometrically in the laboratory at Skukuza or Grahamstown were not filtered or preserved in any way. Although phosphate results appear to be very high, results in this chapter are for total reactive orthophosphate, i.e. the orthophosphate content of samples which have not been filtered, hydrolysed or digested (APHA, 1992), and not dissolved orthophosphate as often used by analytical laboratories e.g. IWQS in Pretoria.

Monitoring physico-chemical constituents and nutrient concentrations is essential to ecotoxicity studies as metabolites such as ammonia may increase to unacceptably high concentrations resulting in stress or death of test organisms (APHA, 1992). Mortality can then not necessarily be linked to the toxicant. The use of control systems is therefore also essential to defining causes of mortality.

To determine tolerances of riverine organisms to sodium sulphate and sodium chloride, LC_{50} values were statistically determined using the EPA probit analysis programme, version 1.4. Probit analysis is the most widely used LC_{50} calculation procedure and uses the probit transformation of mortality data in combination with a standard curve-fitting technique. As this is a parametric procedure requiring a normal distribution of data, data must yield symmetric dose-response curves for analysis to be carried out (APHA, 1992).

4.3 EASTERN CAPE

4.3.1 Study area

These studies were carried out using water and macroinvertebrates from the Bloukrans River near Grahamstown in the Eastern Cape (Figure 4.3). The river is situated in the Kowie River catchment, and flows down the Belmont Valley where it receives large volumes of run-off from urban and township areas in the immediate vicinity of Grahamstown. The river serves as a reservoir for pollutants in the absence of adequate sewage and sanitation facilities. Further downstream the river runs through agricultural lands where contamination by fertilizers may contribute to the high nitrate and phosphate levels recorded at the collecting site. The output from the maturation ponds of the Grahamstown Sewage Disposal Works is located just above the collecting site (Scott, 1986) (indicated on map), and contributes to the high nutrient levels recorded.

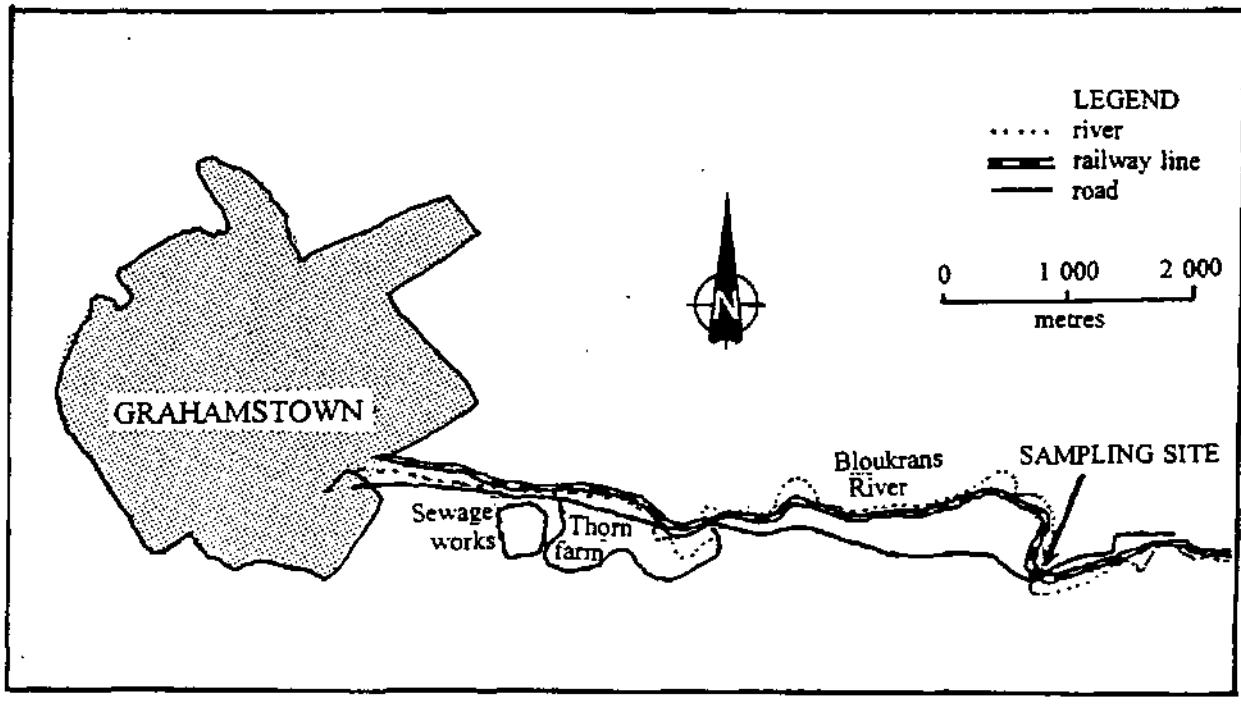

Figure 4.3 Map of the Belmont Valley showing the position of the Bloukrans River.

Table 4.1 represents water quality conditions at this site.

Table 4.1 Physico-chemical constituents and nutrient concentrations (expressed in $mg.l^{-1}$) at the collecting site on the Bloukrans River, Eastern Cape.

pH	Temp. (°C)	EC ($mS.m^{-1}$)	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}
7.25	16	130.3	0.14	77.4	0.99	9.6

4.3.2 Specific methods

Baetid mayflies (Order Ephemeroptera, Family Baetidae) from the Bloukrans River were selected for both range-finding and definitive studies due to abundance, as it was essential to have a large enough pool of organisms for continuous sampling. Although it was unlikely that all organisms were at the same life-stage, early mayfly nymphs were selected. As it was not possible to taxonomically classify live organisms, mixed baetid populations were used for ecotoxicity testing. Table 4.2 represents the percentage frequency of different baetid species in the populations used per experiment. Species include *Baetis harrisoni*, *Centroptilum parvum*, *Centroptilum excisum* and *Demoulinia* complex. Mixed species, such as *Baetis* spp., have been successfully used for toxicity testing by both the United States Environmental Protection Agency (US EPA) and the American Society for Testing and Materials (ASTM) (Persoone and Janssen, 1993).

Table 4.2 Percentage frequency of baetid species used in each of the range-finding and definitive ecotoxicity studies.

Tests - sodium sulphate	***B. harrisoni***	***C. parvum***	***C. excisum***	***D.* complex**
Range-finding	17.1	77.6	-	5.3
400 $mS.m^{-1}$	8.5	91.5	-	-
800 $mS.m^{-1}$	27.6	72.4	-	-
900 $mS.m^{-1}$	-	100.0	-	-
1 000 $mS.m^{-1}$	15.6	81.3	-	3.1
Tests - sodium chloride				
Range-finding	94.1	5.9	-	-
250 $mS.m^{-1}$	75.0	25.0	-	-
450 $mS.m^{-1}$	55.0	42.5	2.5	-
550 $mS.m^{-1}$	65.5	34.5	-	-
1 000 $mS.m^{-1}$	93.7	6.3	-	-

In addition, the ancylid *Burnupia stenochorias* (Phyllum Mollusca, Family Ancylidae) was selected for range-finding experiments as ancylids are widely distributed in most freshwater habitats. *B. stenochorias* is found particularly in the riffle areas of well-oxygenated streams (Brown, 1980). *B. stenochorias* were collected from the Bloukrans River and held in a rearing laboratory for 2 weeks before sub-adults were selected for ecotoxicity trials. Twenty *B. stenochorias* were collected on a tile from rearing channels and placed into raceways for acclimation. *B. stenochorias* acclimation mortality was clearly correlated to transport and handling methods. No mortality was recorded during acclimation when animals were carefully transported and acclimated to laboratory conditions before being placed into

raceways. However, baetids were used for definitive testing as there was a limited number of *B. stenochorias* available. High mortalities were also recorded in all experimental systems during the sodium chloride RF experiment (Figure 4.5b). Between 40 and 50 baetids were collected in the field. Baetid acclimation mortality ranged between 2 and 8%.

Eastern Cape studies were carried out at the artificial stream laboratory in Grahamstown. Laboratory temperatures were maintained between 18° and 20°C (as recommended by Sloof, 1983) and illumination controlled by OSRAM biolux lights set at a 12:12 hour light:dark cycle. These lights provide a spectrum of wavelengths similar to sunlight. The following conductivities / concentrations of industrial grade sodium sulphate ($Na_2SO_4.10\ H_2O$) and sodium chloride (NaCl) (Table 4.3) were selected for range-finding and definitive tests. Solutions were prepared in Bloukrans River water of approximately 132 $mS.m^{-1}$ during sodium sulphate testing and 71 $mS.m^{-1}$ during sodium chloride testing. These conductivities are those of the natural river water. It is probable that the fluctuations in EC could be due to the dilutory effects of rainwater. In each case control systems were of river water only.

Table 4.3 Conductivity ranges and concentrations of sodium sulphate and sodium chloride selected for range-finding and definitive tests.

Range-finding tests		**Definitive tests**	
Sodium sulphate	**Sodium chloride**	**Sodium sulphate**	**Sodium chloride**
250 $mS.m^{-1}$ 2.6 $g.l^{-1}$	250 $mS.m^{-1}$ 1.3 $g.l^{-1}$	400 $mS.m^{-1}$ 6.0 $g.l^{-1}$	250 $mS.m^{-1}$ 1.3 $g.l^{-1}$
400 $mS.m^{-1}$ 6.0 $g.l^{-1}$	400 $mS.m^{-1}$ 2.5 $g.l^{-1}$	800 $mS.m^{-1}$ 14.6 $g.l^{-1}$	450 $mS.m^{-1}$ 2.9 $g.l^{-1}$
500 $mS.m^{-1}$ 8.0 $g.l^{-1}$	500 $mS.m^{-1}$ 3.4 $g.l^{-1}$	900 $mS.m^{-1}$ 16.6 $g.l^{-1}$	550 $mS.m^{-1}$ 3.8 $g.l^{-1}$
750 $mS.m^{-1}$ 13.5 $g.l^{-1}$	1 000 $mS.m^{-1}$ 8.0 $g.l^{-1}$	1 000 $mS.m^{-1}$ 18.8 $g.l^{-1}$	1 000 $mS.m^{-1}$ 8.0 $g.l^{-1}$
1 000 $mS.m^{-1}$ 18.8 $g.l^{-1}$	1 200 $mS.m^{-1}$ 9.8 $g.l^{-1}$		

Conductivity / concentration ranges selected for definitive testing were on the basis of unreplicated range-finding mortality results. The highest and lowest concentrations were repeated to determine upper and lower limits of mortality; two more conductivities were selected around the apparent LC_{50} value. Mortalities recorded during the sodium sulphate RF experiment were low, except at 1 000 and 1 200 $mS.m^{-1}$. Conductivities selected for definitive tests were therefore 800 and 900 $mS.m^{-1}$. As mortalities recorded during the sodium chloride RF experiment were 28.6% and 71.4% for 400 and 500 $mS.m^{-1}$ respectively, 450 and 550 $mS.m^{-1}$ were selected for definitive testing.

During the sodium sulphate experiments nutrient analyses were carried out using a Hach DR/2000 spectrophotometer, while oxygen levels were measured using the oxygen probe of

the Checkmate CCA475627 kit. During the sodium chloride experiments a Spectroquant 118 photometer was used for nutrient analyses, and a Hanna HI 193 DO meter for oxygen measurements. This meter was calibrated to compensate for altitude and salinity effects as required by its design.

To test for the statistical significance of mortalities recorded between replicates of definitive experiments, and between mortalities at different salt concentrations, % cumulative mortality data were arc sin-transformed and one-way analysis of variance tests (ANOVA) carried out at a 95% confidence level. Statgraphics version 5.0 was used for the statistical testing (Statgraphics Corporation, 1990).

4.3.3 Results

Physico-chemical constituents and nutrient concentrations
Tables 4.4 and 4.5 present the range of values for these variables recorded during the range-finding and definitive experiments for sodium sulphate and sodium chloride experiments respectively.

Table 4.4 Ranges of physico-chemical constituents and nutrient concentrations (expressed in $mg.l^{-1}$) monitored during sodium sulphate experiments.

Test	pH	Temp. (°C)	DO (%)	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}
Range-finding	7.19-7.28	16.0-17.5	70.0-89.0	<0.00-0.09	55.4-82.7	0.50-1.52	10.1-27.4
400 $mS.m^{-1}$	7.17-7.26	15.0-17.0	64.0-92.4	0.03-0.30	16.0-142.0	0.20-1.20	18.8-24.0
800 $mS.m^{-1}$	7.19-7.28	14.0-16.5	67.0-82.0	<0.00-0.12	2.0-127.0	0.20-38.00	16.4-29.8
900 $mS.m^{-1}$	7.19-7.27	14.0-16.5	69.0-85.0	<0.00-0.12	26.0-117.0	0.60-29	12.2-22.3
1 000 $mS.m^{-1}$	7.17-7.26	15.0-17.0	63.0-92.3	0.04-1.80	35.0-145.0	0.30-1.30	20.8-23.7

Table 4.5 Ranges of physico-chemical constituents and nutrient concentrations (expressed in $mg.l^{-1}$) monitored during sodium chloride experiments.

Test	pH	Temp. (°C)	DO (%)	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}
Range-finding	6.76-7.65	13.5-18.0	88.4-101.3	<0.00-0.13	45.8-211.1	0.04-0.80	6.7-9.0
250 $mS.m^{-1}$	7.27-7.73	15.0-17.0	95.1-99.9	0.02-0.13	81.0-166.3	0.22-1.00	8.1-10.6
450 $mS.m^{-1}$	7.25-7.92	16.0-22.0	92.9-100.0	<0.00-0.16	44.0-169.8	0.06-0.18	7.3-8.7
550 $mS.m^{-1}$	7.13-7.78	16.0-22.0	92.6-96.8	<0.00-0.18	24.6-176.9	0.02-0.22	6.3-9.6
1 000 $mS.m^{-1}$	7.18-7.70	16.0	88.1-104.1	<0.00-0.04	86.2-200.6	0.20-0.35	8.1-9.1

Although a wide fluctuation in DO levels was seen during the sodium sulphate experiment, levels seldom dropped below the guideline value for the protection of aquatic life, i.e. 66.0 -

68.9% at 16° and 21°C respectively (Hart and Fuller, 1974). As these systems are recirculating it seems unlikely that oxygen levels should drop so low. The increase in oxygen levels during the sodium chloride experiment was due to the use of a different oxygen meter (see Section 4.3.2).

Little variation was seen in other physico-chemical factors during experiments, but nitrate, nitrite and phosphate levels ranged widely. Despite the levels recorded being natural to the nutrient-enriched Bloukrans River, nitrite often exceeded the guideline value for aquatic toxicity (see Appendix 4.2). Although phosphate results represent total reactive orthophosphate rather than only the dissolved fraction, high levels recorded may be due to the widespread application of fertilizers in this area. The organisms present in this river are probably acclimated to these nutrient-enriched conditions, but it is not unlikely that increasing nutrient levels during toxicity studies may have influenced experimental results.

Mortality results

Cumulative mortalities of baetid spp. and *B. stenochorias* during range-finding experiments, and of baetid spp. during sodium sulphate and sodium chloride definitive experiments, are shown in Figures 4.4 - 4.7 respectively.

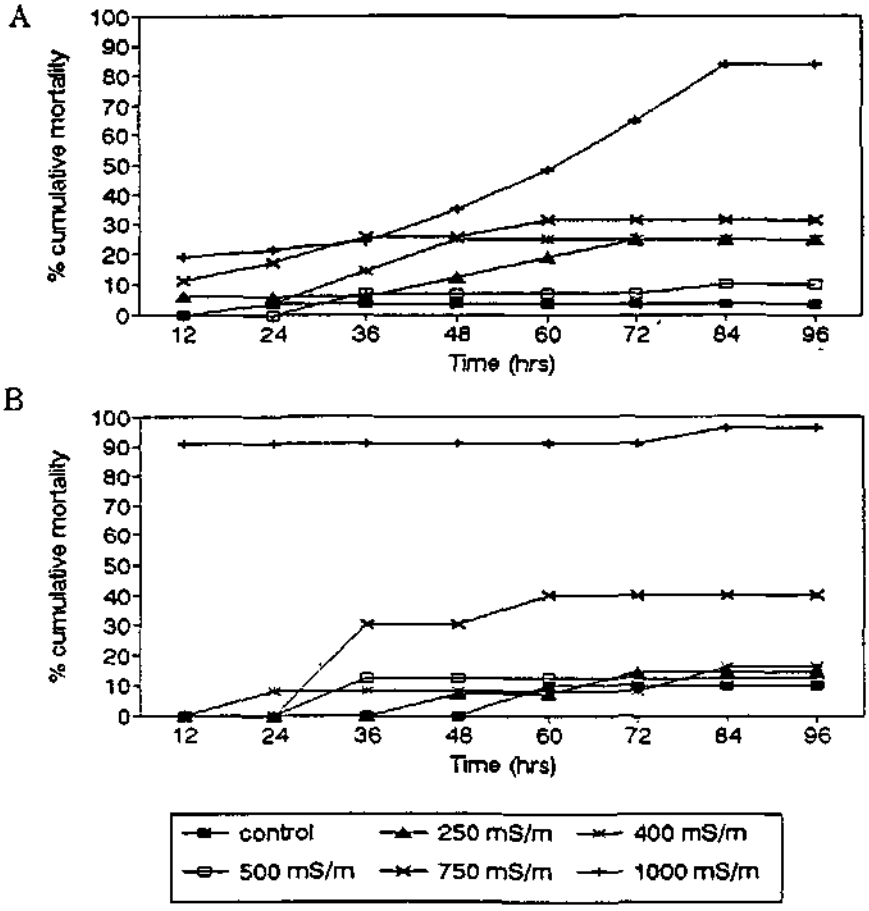

Figure 4.4 Cumulative mortality of test organisms during the sodium sulphate RF experiment.

(A) - Baetid spp.
(B) - *B. stenochorias.*

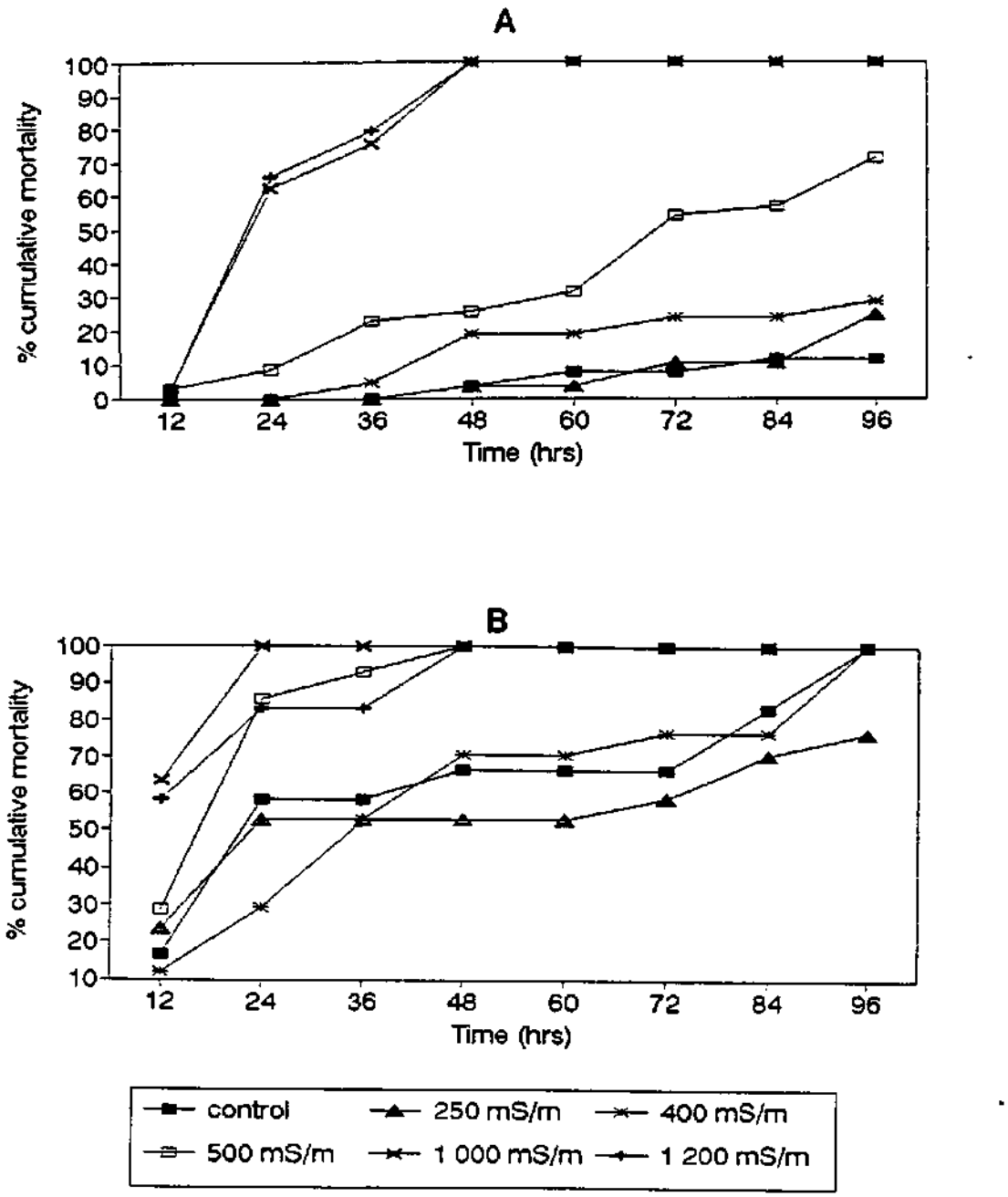

Figure 4.5 Cumulative mortality of test organisms during the sodium chloride RF experiment.

(A) - Baetid spp.
(B) - *B. stenochorias.*

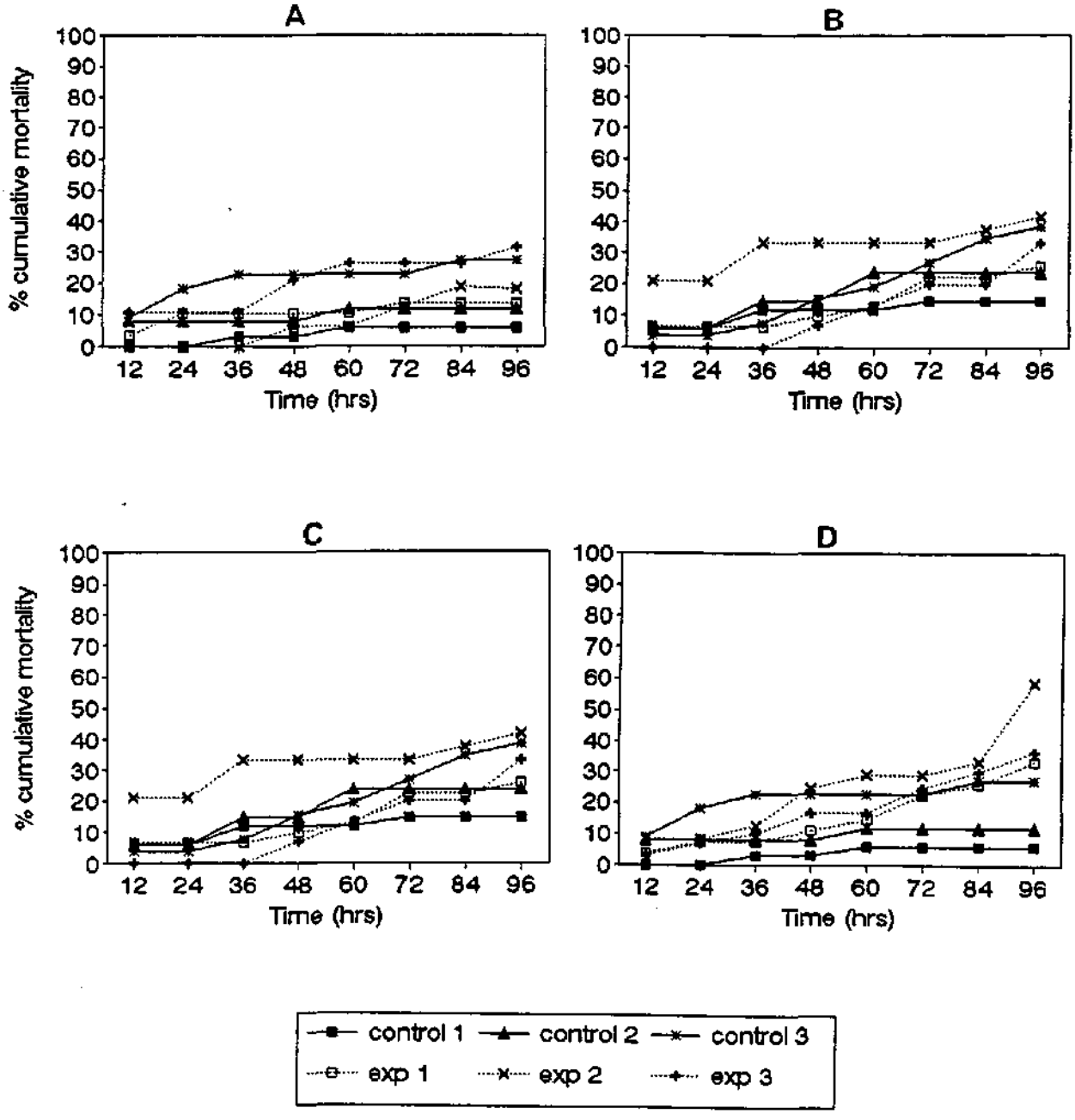

Figure 4.6 Cumulative mortality of baetid spp. during sodium sulphate definitive experiments.

(A) - 400 $mS.m^{-1}$ / 6.0 $g.l^{-1}$
(B) - 800 $mS.m^{-1}$ / 14.6 $g.l^{-1}$
(C) - 900 $mS.m^{-1}$ / 16.6 $g.l^{-1}$
(D) - 1 000 $mS.m^{-1}$ / 18.8 $g.l^{-1}$

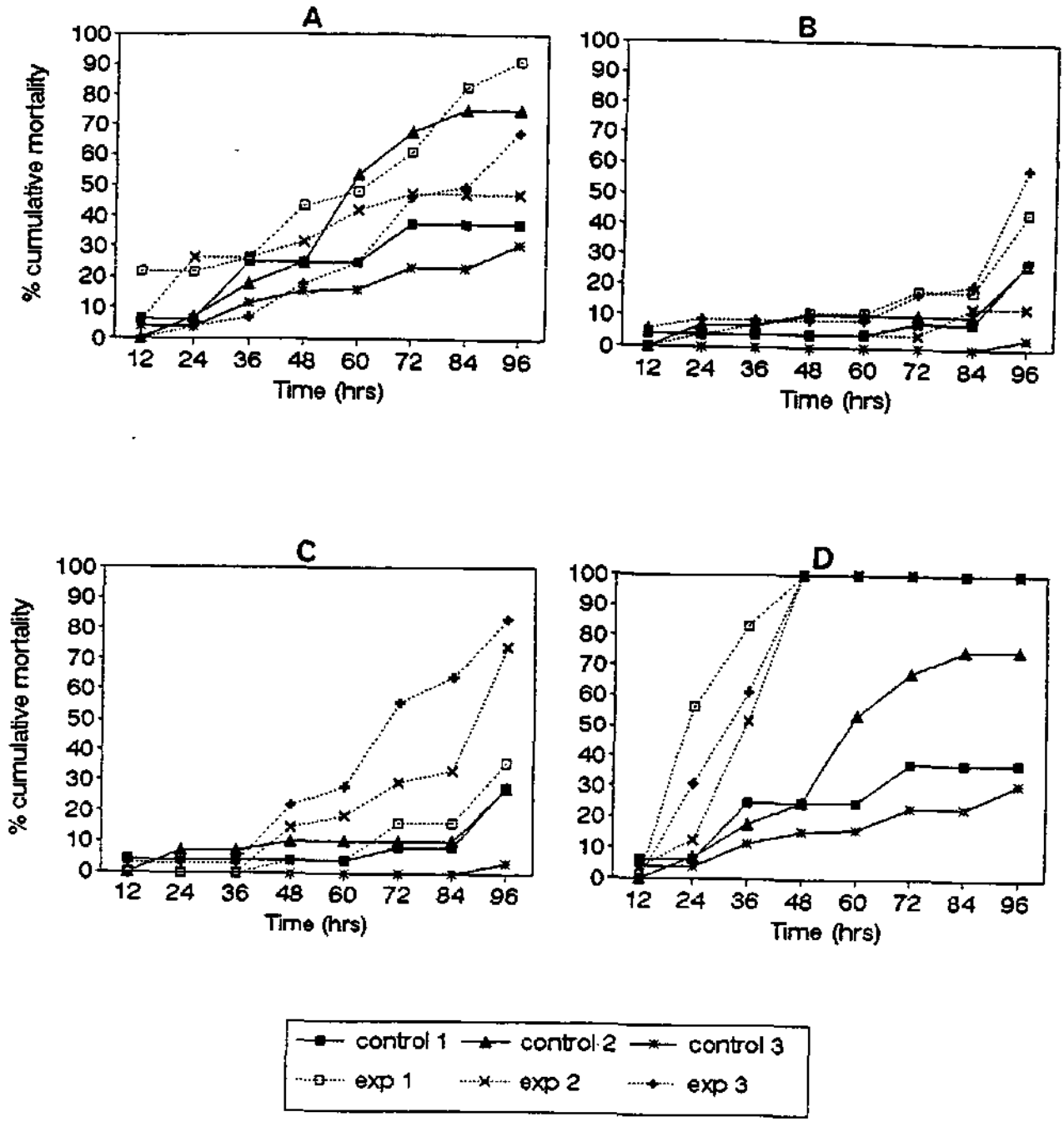

Figure 4.7 Cumulative mortality of baetid spp. during sodium chloride definitive experiments.

(A) - 250 $mS.m^{-1}$ / 1.3 $g.l^{-1}$
(B) - 450 $mS.m^{-1}$ / 2.9 $g.l^{-1}$
(C) - 550 $mS.m^{-1}$ / 3.8 $g.l^{-1}$
(D) - 1 000 $mS.m^{-1}$ / 8.0 $g.l^{-1}$

Sodium sulphate
Note that when results are referred to as being replicable, it indicates that they are not significantly different from each other, and are therefore true replicates.

Although unreplicated, range-finding (RF) tests indicated that mortality for both baetid spp. and *B. stenochorias* was linked to high conductivities only. Definitive test results were inconclusive, with an inadequate degree of replication. Although some control mortalities of experiment 1, i.e. 400 $mS.m^{-1}$, were significantly different from results of experimental systems, controls were not true replicates ($f = 6.281$ and $P<0.00001<0.05$). The controls of experiment 2 (800 $mS.m^{-1}$) were replicable, but experimental systems were not, and controls were not significantly different from the experiments ($f = 2.168$ and $P<0.05$). Despite the control and experimental mortalities of experiment 3 (900 $mS.m^{-1}$) being true replicates, they were not significantly different from each other ($f = 2.168$ and $P<0.05$). The results of experiment 4 (1 000 $mS.m^{-1}$) were again inconclusive, although experimental systems were replicated ($f = 6.281$ and $P<0.00001<0.05$). A significant difference was apparent between mortalities recorded at 400 $mS.m^{-1}$ and those at both 900 $mS.m^{-1}$ and 1 000 $mS.m^{-1}$. The results for experiments run at 900 $mS.m^{-1}$ and 1 000 $mS.m^{-1}$ were not significantly different ($f = 2.834$ and $P<0.05$).

Due to the inconsistency of these results, an LC_{50} value could not be determined for sodium sulphate toxicity.

Sodium chloride
As detected during the sodium sulphate RF experiment, baetid mortality was clearly linked to higher conductivities, i.e. 1 000 $mS.m^{-1}$ and 1 200 $mS.m^{-1}$, or above 8.0 $g.l^{-1}$. *B. stenochorias* results were inconclusive. Although acclimation mortality was low, mortalities increased in all systems after 12 hours.

As suggested by RF results, mortalities of control systems and those held at 250 $mS.m^{-1}$ were not significantly different. Both control and experimental system results were replicable ($f = 5.135$ and $P<0.05$). Although mortalities recorded in control and experimental systems of experiments 2 and 3, i.e. 450 and 550 $mS.m^{-1}$, were duplicated, no significant difference was apparent between control and experimental results of either experiment ($f = 3.264$ and $P<0.05$). For Experiment 4, (1 000 $mS.m^{-1}$) experimental and control results were perfectly replicable and significantly different ($f = 5.135$ and $P<0.05$). Statistical results clearly show that mortalities linked to 250, 450 and 550 $mS.m^{-1}$ are not significantly different from each other, but are all significantly different from the results recorded at 1 000 $mS.m^{-1}$ ($f = 7.521$ and $P<0.00001<0.05$).

As mortality data did not meet the distribution properties required by the probit analysis programme, an LC_{50} value could not be determined. Mortality results were not distributed over the range of conductivities tested. Within 36 hours 100% mortality had been reached at 1 000 $mS.m^{-1}$, while all other conductivities were not significantly different from each other or from control systems.

4.3.4 Discussion and conclusion
These experiments clearly demonstrate the difficulties associated with using baetids for toxicity studies in these particular experimental systems. Control mortalities were as high as

30%, considerably above the 10% control mortality required by Coler and Rockwood (1989) for reliable testing. The quality of Bloukrans River water probably contributed to the unreliable results. Although *Burnupia* spp. and baetids such as *Baetis harrisoni* and some *Centroptilum* species are tolerant to organic pollution (Dallas and Day, 1993), further declining water quality over the time of the experiments may have contributed to mortality. Although control mortalities of sodium chloride experiments were often high, the analyses of mortality results showed a clear link between significant mortality and conductivities around 1 000 $mS.m^{-1}$. It is not known how the sulphate ion may physiologically affect invertebrates, whereas sodium chloride is a ubiquitous salt, so that linking mortalities of baetid populations to sodium sulphate concentrations may require more refined methodology.

A major difficulty experienced during this research was the selection and availability of experimental organisms. Ideally, toxicity studies should be carried out using riverine test specimens from clean and undisturbed waters (APHA, 1992), such as the Palmiet River outside Grahamstown. However, the Palmiet is a small stream with a limited population production, and harvesting should ideally not affect the natural regenerative capacity of a river. Organisms were therefore unavailable from this river when required for method development prior to the KNP research trip, and Bloukrans River invertebrates consequently had to be used. This highlights the requirement for a supply of laboratory-reared riffle-dwelling organisms for toxicity studies. A supply of organisms of known origin would also reduce the variability associated with using a population of organisms, such as the baetids used during this study. As shown in Table 4.2, it is not possible to select the same species during collection in the field. It is also difficult to select invertebrates of a similar life-cycle stage. In addition, it was on occasion necessary to use smaller-sized invertebrates due to a low abundance of more mature, larger animals. These smaller animals may also have contributed to high mortality rates due to their vulnerability. Large losses were often recorded during experiments, possibly due to smaller-sized animals getting caught in the paddle-wheels of the raceways. Control mortalities for both sodium sulphate and sodium chloride experiments were often high, and replication inadequate, further emphasizing the problems associated with using baetid populations for ecotoxicity studies in these experimental systems. Although range-finding experiments indicated some tolerance to sodium sulphate by both experimental organisms, these results could not be validated by definitive testing and therefore remain inconclusive. For future experiments, it is essential to use either laboratory-reared organisms, or to identify a clean water source of a single species which could be successfully used for toxicity testing. This would require some information on the life-cycle of a selected organism, e.g. the leptophlebiid *Adenophlebia auriculata* found in the Palmiet River, so as to correlate experiments with emergence, and ensure a supply of appropriately-sized invertebrates. These investigations are currently being carried out at the IWR by Mrs. L. Haigh and colleagues.

4.4 MPUMALANGA

The KNP has been identified as an important contributor to South Africa's tourist industry, and the water quality and quantity requirements of the rivers have been identified as priorities by the KNP Rivers Research Programme (KNPRRP). The KNPRRP is a cooperative undertaking by resource-use managers, funding agencies and researchers, which addresses the water quality and water quantity requirements of the rivers flowing through the KNP. The programme was initiated in December 1988 jointly by the Department of Water Affairs,

Department of Environment Affairs, Foundation for Research Development, Transvaal Provincial Administration, National Parks Board, Water Research Commission and various research institutions. The aim of the KNPRRP is to contribute to the conservation of the natural environment of rivers and to develop an understanding of the ecological functioning of these systems so as to provide some predictive and advisory function to water managers (Breen *et al.*, 1994).

4.4.1 Study area

There are six major rivers flowing through the KNP (Figure 4.8), of which the Sabie and Olifants Rivers present contrasting water quality conditions. The Sabie River has been found to be the least mineralized of the KNP rivers (van Veelen, 1990). It may therefore also be the most vulnerable to any changes in the catchment which may affect water quality. The major threats to this river are perceived to be rapid population development, increased agricultural activity and afforestation (van Veelen and Swart, 1992). All these activities lead to increased water abstraction from the river. Besides the obvious effects on water quantity, abstraction also serves to aggravate water quality problems by concentrating pollutants and reducing the dilution effect of the water.

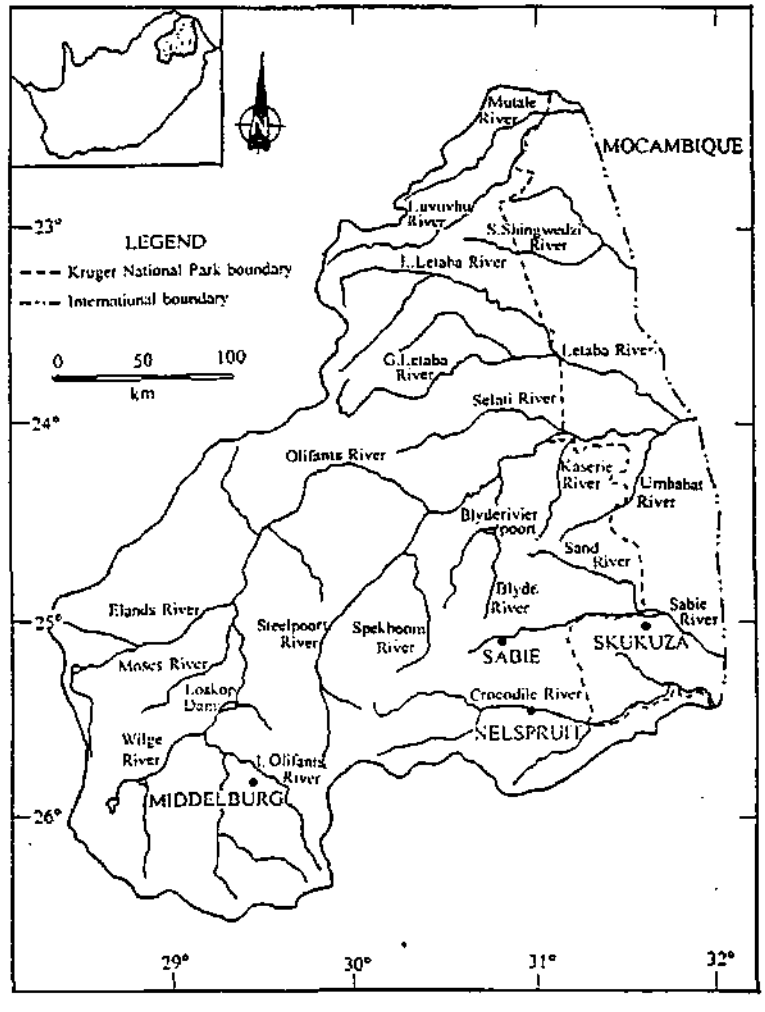

Figure 4.8 The major river systems of the Kruger National Park (Breen *et al.*, 1994).

The Olifants River is the second largest river in Mpumalanga. The major tributaries of this river system include the Wilge, Klein Olifants, Moses, Elands, Steelpoort, Blyde, Selati and Timbavati Rivers. In contrast to the Sabie River, the Olifants River System has suffered severe degradation due to factors such as poor agricultural and land management practices, and urban, mining and industrial development in the upper catchment of the Mpumalanga Highveld and the Phalaborwa Lowveld regions. Chemical weathering of the underlying granite of the Basement Complex and the shales and mudstone of the Karoo Sequence have also contributed to the mineralization of the river (van Vuren *et al.*, 1994). These factors have culminated in increased levels of chemical and organic pollution and poor water quality. The Olifants River has therefore been identified as a river with high silt loads, salinity and pollutant levels (Venter and Deacon, 1992).

Before initiating laboratory studies, the evaluation of selected Mpumalanga rivers was undertaken to identify a near-pristine source of invertebrates for ecotoxicity studies, which would provide a reference baseline of invertebrate tolerances. According to the APHA (1992), riverine test specimens should be collected from clean, natural waters.

4.4.2 Specific methods

4.4.2.1 Biomonitoring

Recently water quality management strategy has moved toward a user-based philosophy, comprehensive monitoring and assessment programmes have been established to provide information which would assist the effective regulation of rivers. One such programme is aquatic biomonitoring e.g. SASS3 (South African Scoring System version 3), a modified version of the SASS2 rapid bioassessment protocol which was developed for South African conditions and proposed by Chutter in 1991. The SASS2 biotic index is based on the BMWP (Biological Monitoring Working Party) system, widely used in Britain. SASS uses biological indicators to gain an impression of the biotic health of aquatic ecosystems (Roux, 1993). It denotes a score to each river based on the identity and abundance of the invertebrates present and provides an index of the relative biological health or integrity of the river. The distribution of benthic macroinvertebrates is widely considered to reflect the chemical quality of surface waters (Sloof, 1993). The SASS3 methodology is described on the sampling sheet in Appendix 4.3a. As habitat is the principal determinant of attainable biological potential, loss of habitat may have a major impact on ecosystem integrity. It is therefore important to evaluate habitat as part of the aquatic environment. Habitat assessment was carried out using a habitat quality index (HQI) which was compiled by the Hydrological Research Institute (now the Institute for Water Quality Studies) in Pretoria (Roux, 1993) (Appendix 4.3b). This HQI is a modification of an existing habitat assessment index, compiled and used by the US EPA (Plafkin *et al.*, 1989).

Index values and ASPT scores relate to biotic health in the following way (Table 4.6):

Table 4.6 The proposed relationship between scores and biotic integrity (Roux. 1993).

Index value	ASPT score	Biotic health
0 - 50	< 3	Poor
50 - 80	3 - 5	Fair
80 - 120	5 - 7	Good
> 120	> 7	Excellent

A SASS3 evaluation of river conditions was carried out on a selected site of the following Mpumalanga rivers:

1. Blyde River - below the road bridge at Exeter (lower reaches and outside the KNP);
2. Olifants River - approximately 1 km upstream of Mamba within the KNP;
3. Sabie River - below the weir near the Kruger gate (within the KNP);
4. Selati River - below the road bridge and downstream of Foskor (near Phalaborwa and outside the KNP).

The Olifants River system was selected for evaluation as its tributaries, the impacted Selati and relatively unpolluted Blyde Rivers, represent comparative conditions for investigating the effect of degraded water quality conditions on aquatic communities. These tributaries drain the Lowveld and join the mainstream of the Olifants River before its confluence with the Letaba River and entry into the KNP.

4.4.2.2 Salinity tolerance experiments

The following two indigenous Sabie River invertebrate species were selected for range-finding studies:

Order	Ephemeroptera	Trichoptera
Family	Tricorythidae	Philopotamidae
Genus	*Tricorythus* sp.	*Chimarra* sp.

Ephemeropterans are wide-spread, occurring in unpolluted waters and have long been regarded as sensitive to pollution (Matthew, 1968). Ephemeropterans were therefore identified as good indicators of water quality. *Tricorythus* sp. was selected as it is found at high densities along much of the Sabie River and generally showed low mortalities under laboratory conditions. To evaluate possible differences in sensitivity between species, caddisflies (trichopterans) were also selected for ecotoxicity studies. Definitive tests were carried out using *Tricorythus* sp. because of low mortalities recorded under experimental conditions. Twenty-five *Tricorythus* sp. and 20 *Chimarra* sp. individuals per raceway were collected in the field, with acclimation mortalities ranging between 4-8% for *Tricorythus* sp. and 12% for *Chimarra* sp..

Sabie River studies were carried out at the KNPRRP laboratory at Skukuza. Due to the design of the laboratory, temperature and light conditions could not be strictly controlled. Laboratory temperatures ranged between 14° and 23°C, and biolux tubes provided

wavelengths of light similar to sunlight. Lighting was generally maintained at a 12:12 hour light:dark cycle. The following conductivities and concentrations of industrial grade sodium sulphate ($Na_2SO_4.10\ H_2O$) and sodium chloride (NaCl) were selected for range-finding and definitive tests. Solutions were prepared in Sabie River water of approximately 11.5 - 12.5 $mS.m^{-1}$. These conductivities are those of the natural river water. In each case control systems were of river water only.

Table 4.7 Conductivity ranges and concentrations of sodium sulphate and sodium chloride selected for range-finding and definitive tests.

Range-finding tests		**Definitive tests**	
Sodium sulphate	**Sodium chloride**	**Sodium sulphate**	**Sodium chloride**
100 $mS.m^{-1}$ 0.66 $g.l^{-1}$	100 $mS.m^{-1}$ 0.52 $g.l^{-1}$	50 $mS.m^{-1}$ 0.20 $g.l^{-1}$	100 $mS.m^{-1}$ 0.52 $g.l^{-1}$
250 $mS.m^{-1}$ 1.83 $g.l^{-1}$	250 $mS.m^{-1}$ 1.35 $g.l^{-1}$	100 $mS.m^{-1}$ 0.66 $g.l^{-1}$	250 $mS.m^{-1}$ 1.35 $g.l^{-1}$
500 $mS.m^{-1}$ 3.65 $g.l^{-1}$	500 $mS.m^{-1}$ 2.80 $g.l^{-1}$	200 $mS.m^{-1}$ 1.46 $g.l^{-1}$	400 $mS.m^{-1}$ 2.10 $g.l^{-1}$
750 $mS.m^{-1}$ 5.50 $g.l^{-1}$	750 $mS.m^{-1}$ 4.23 $g.l^{-1}$	600 $mS.m^{-1}$ 4.40 $g.l^{-1}$	600 $mS.m^{-1}$ 3.38 $g.l^{-1}$
1 000 $mS.m^{-1}$ 7.34 $g.l^{-1}$	1 000 $mS.m^{-1}$ 5.65 $g.l^{-1}$		

Conductivity / concentration ranges selected for definitive testing were on the basis of unreplicated range-finding mortality results. Definitive testing was carried out in triplicate. The highest and lowest concentrations were repeated to determine upper and lower limits of mortality; two more conductivities were selected around the apparent LC_{50} value. Sodium sulphate RF experiment mortalities were 60.0 and 83.3% for 100 (0.66 $g.l^{-1}$) and 250 $mS.m^{-1}$ (1.83 $g.l^{-1}$) respectively; 100 and 200 $mS.m^{-1}$ were therefore selected for definitive tests. Sodium chloride RF experiments showed 31.8 and 78.3% mortality at 250 (1.35 $g.l^{-1}$) and 500 $mS.m^{-1}$ (2.80 $g.l^{-1}$) respectively. Conductivities selected for definitive tests were therefore 250 and 400 $mS.m^{-1}$.

Nutrient analyses were carried out using a Spectroquant 118 photometer (see section 4.2 for methods), and oxygen measurements determined using a Hanna HI 193 DO meter calibrated to compensate for altitude and salinity effects. At the onset and termination of each definitive experiment, microbial populations were monitored using a pour plate method (Appendix 4.4) as a check on water quality. Two unfiltered water samples (1 x 600ml, 1 x 200ml) were also taken from each raceway for full chemical analyses carried out at IWQS in Pretoria. One sample was preserved with pre-prepared mercuric chloride ampoules for the determination of the nutrients NH_4-N, NO_3+NO_2-N and PO_4, dissolved organic carbon (DOC), TDS and macro-elements, and the other sample analysed for both dissolved and acid-extractable trace metals. Trace metals were analyzed using multichannel inductively coupled plasma (ICP) emission spectrometry, Ca and Mg by atomic absorption, K and Na by flame emission spectrometry,

and other constituents - F, Si, SO_4, Cl - by using an automated flow system (auto analyzer). Methods of analysis are described in DWAF Analytical Methods Manual (1992). Total alkalinity (TAL expressed as mg/L $CaCO_3$) (titrimetric method) and hardness were determined in the laboratory for samples taken at the beginning and end of each definitive experiment (Appendices 4.5a and b).

Kaolinite stones were also analysed at IWQS for acid-extractable metals bound to the surface which may affect water quality. Each stone was placed in 100ml 10% (v/v) nitric acid solution in an ultrasonic bath for 2 hours. The stones were then left in the nitric acid solution for 48 hours, after which the solution was heated for approximately 2 hours and analysed for acid extractable metals (P. Kempster, pers. comm.).

To statistically test for significance of mortalities recorded between replicates of definitive experiments, and between mortalities at different salt concentrations, % cumulative mortality data were arc sin-transformed and one-way analysis of variance tests (ANOVA) carried out at a 95% confidence level. Statgraphics version 5.0 was used for the statistical testing (Statgraphics Corporation, 1990).

4.4.2.3 Selati River tolerance experiment

To assess the response of unpolluted riverine organisms to polluted river conditions, macroinvertebrates from the Sabie River were exposed to Selati River water for an acute 96-hour period and mortality monitored. The following two mayfly species were selected for this study:

Order	Ephemeroptera	Ephemeroptera
Family	Tricorythidae	Leptophlebiidae
Genus	*Tricorythus* sp.	*Choroterpes* sp.

Invertebrates were collected in the field and 25 of each species acclimated in each of six raceways for 48 hours. A two-stage acclimation period was instituted to reduce the effect of changing water quality conditions during acclimation. The first stage was a 24 hour period during which animals were held in Sabie River water to ensure the customary acclimation to the artificial conditions in the laboratory. A mortality count was carried out after this stage and invertebrate numbers equalized to ensure that each raceway had the same number of organisms. Acclimation mortality was 4% for *Tricorythus* sp. and 8% for *Choroterpes* sp.. Fifty percent of the water was then removed from each raceway and replaced with Selati River water (i.e. 1:1 Sabie:Selati River waters). Organisms were acclimated for a further 24 hours. Mortalities were recorded and numbers equalized again between all raceways. Mortality during the second phase was 8% for *Tricorythus* sp. and 4.3% for *Choroterpes* sp.. The experiment was initiated with the replacement of water in each raceway by 100% Selati River water. The study was carried out in triplicate, with three Sabie River systems acting as the river water controls, and three Selati River raceways being the experimental systems. As in salinity tolerance experiments, 20% of the 12.5 litres river water was renewed daily i.e. a recirculating renewal system was employed.

This experiment was also carried out in the KNPRRP laboratory at Skukuza. Laboratory temperatures were read at mid-day and fluctuated between 25° and 27°C. Nutrient analyses were carried out using a Spectroquant 118 photometer, and a Hanna HI 193 meter, calibrated to compensate for altitude effects, used for oxygen measurements. Microbial populations were

monitored at the onset and end of each experiment using a pour plate method (Appendix 4.4), while water samples were sent to IWQS for analysis. Total alkalinity (TAL expressed as $mg.l^{-1}$ $CaCO_3$) and hardness were determined on site in the laboratory at Skukuza (Appendices 4.5a and b).

4.4.3 Results

4.4.3.1 Biomonitoring and selection of a study area

As the integrity of an ecosystem is reliant on physico-chemical, nutrient, biological and habitat characteristics, some aspects of these components were included in the survey and are reported in Tables 4.8 and 4.9.

Table 4.8 SASS3 and habitat index scores for selected river sites (August 1994).

River	Sample score	Number of taxa	ASPT score (sample score/no. of taxa)	Habitat score
Blyde	188	23	8.2	98
Olifants	153	21	7.3	102
Sabie	188	27	7.0	110
Selati	67	13	5.2	104

Table 4.9 Physico-chemical constituents and nutrient concentrations (expressed in $mg.l^{-1}$) for selected river sites (August 1994).

River	pH	Temp. (°C)	EC ($mS.m^{-1}$)	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}
Blyde	7.18	15.0	23.4	0.01	<0.2	0.05	2.21
Olifants	7.28	19.0	145.2	0.19	3.3	0.31	0.38
Sabie	7.23	15.0	10.4	0.05	<0.0	0.02	2.12
Selati	7.19	18.5	184.7	0.08	4.9	0.31	1.04

As the biotopes of the rivers concerned were comparatively similar, SASS3 scores could be directly compared. Both the Blyde and Sabie Rivers scored well on sample and ASPT scores, reflecting good biotic health for this sampling event, while the Selati River appeared to be quite impaired. Low ASPT but high habitat scores, as demonstrated by the Selati River result, is often indicative of biological impairment. Sample scores below 80 generally indicate damage. Although the Olifants River has suffered severe degradation due to agricultural, urban, mining and industrial development in the upper catchment of the Mpumalanga Highveld and the Phalaborwa Lowveld regions, the habitat score was high, probably accounting for the high invertebrate diversity recorded. Appendix 4.6 lists the species found at each river site during the SASS3 survey.

Although phosphate results represent total reactive orthophosphate, and not dissolved orthophosphate (see section 4.2 for methods), the higher Blyde and Sabie results versus those of the Olifants and Selati Rivers cannot be explained. Besides phosphate results, both the Sabie and Blyde Rivers showed similar near-pristine water quality conditions. The Blyde River was discarded as a study site as its invertebrate densities were too low to ensure an adequate supply of test organisms. The Sabie River was therefore finally selected as the study area due to its high abundance of invertebrates. It was also logistically much easier to work from the KNPRRP laboratory.

4.4.3.2 Salinity tolerance experiments

Physico-chemical constituents and nutrient concentrations

Tables 4.10 and 4.11 represent the range of values for these variables recorded during both range-finding and definitive tests for sodium sulphate and sodium chloride experiments respectively.

Table 4.10 Ranges of physico-chemical constituents and nutrient concentrations (expressed in $mg.l^{-1}$) monitored during sodium sulphate experiments.

Test	pH	Temp. (°C)	DO (%)	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}
Range-finding	6.95-7.20	10-14	65.0-96.0	<0.01-2.1	<0.2-2.3	<0.02-0.17	<0.06-2.1
50 $mS.m^{-1}$	6.96-7.07	10-13	87.6-97.0	<0.01-0.1	<0.2-3.3	<0.02-0.24	<0.06-1.69
100 $mS.m^{-1}$	6.94-7.04	10-14	87.5-105.0	<0.01-0.07	<0.2-8.7	<0.02-0.14	<0.06-0.46
200 $mS.m^{-1}$	6.93-7.04	10-16	78.1-95.6	<0.01-0.21	<0.2-5.1	<0.02-0.19	<0.06-0.15
600 $mS.m^{-1}$	6.94-7.04	9-15	86.6-97.2	<0.01-0.43	<0.2-4.6	<0.02-0.10	<0.06-0.55

Table 4.11 Ranges of physico-chemical constituents and nutrient concentrations (expressed in $mg.l^{-1}$) monitored during sodium chloride experiments.

Test	pH	Temp. (°C)	DO (%)	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}
Range-finding	6.96-7.08	10-15	81.8-97.0	<0.01-0.07	<0.2-4.8	<0.02-0.17	<0.06-1.38
100 $mS.m^{-1}$	6.99-7.11	11-16	82.5-101.8	<0.01-0.07	<0.2-6.3	0.03-0.21	<0.06-0.18
250 $mS.m^{-1}$	6.98-7.17	13-16	90.0-100.0	<0.01-0.07	<0.2-2.4	<0.02-0.20	<0.06-1.11
400 $mS.m^{-1}$	6.94-7.11	13-16	91.2-100.0	<0.01-0.04	<0.2-5.4	0.03-0.14	<0.06-1.03
600 $mS.m^{-1}$	6.94-7.11	10-13	89.1-101.6	<0.01-0.08	<0.2-3.2	0.03-0.20	<0.06-0.81

The only parameter which ever appeared to exceed the relevant water quality guideline for aquatic toxicity (see Appendix 4.2) was phosphate. However, these levels could not be directly compared with guideline values due to the use of different analytical methods (see Section 4.2). However, IWQS analyses of phosphate levels in the Sabie River sometimes exceeded the 0.3 $mg.l^{-1}$ guideline value for the protection of aquatic life.

Microbial populations

The common water bacterium, *Klebsiella rhinosderomatis*, was found in all water samples. Concentrations increased from approximately 1 500 to 3 200 colonies.ml^{-1} during experiments in both control and experimental systems. A second pseudomonad bacterium was also identified at very low numbers. Little increase in its numbers were evident during the experiments.

Water chemistry

The analysis of samples by IWQS showed little fluctuation of most constituents during the duration of experiments. Total alkalinity fluctuated between 62 and 101 mg.l^{-1} during both sodium sulphate and sodium chloride experiments, while hardness fluctuated around 69.4 mg.l^{-1}. US EPA guidelines (1986) state that alkalinity should not be reduced below 20 mg.l^{-1} $CaCO_3$. Shifts in dissolved organic carbon (DOC) concentrations were noted from day 1 to day 4 of each experiment, the extent of change appears to be related to the salt being tested and its conductivity. The amount of DOC present initially is dependent on the river water collected, but fluctuations during experiments are ascribed to DOC acting as a ligand and binding site for free ionic species (Ure and Davidson, 1995). Concentrations would therefore fluctuate with the general water quality conditions and the amount of free ions available for binding. TDS levels obviously increased with salt addition, as well as the relevant ionic species, i.e. sodium, chloride and sulphate ions. Table 4.12 represents the concentrations of these ions at the various conductivities, as well as TDS levels. Results are for day 4 samples and represent a mean for samples of the relevant three raceways.

Table 4.12 Concentrations of ionic species and TDS levels (expressed as mg.l^{-1}) as monitored during definitive experiments.

Test	TDS	Na^+	SO_4^{2-}	Cl^-
Sodium sulphate				
50 mS.m^{-1} - 0.20 g.l^{-1}	432	108	212	
100 mS.m^{-1} - 0.66 g.l^{-1}	907	256	548	
200 mS.m^{-1} - 1.46 g.l^{-1}	1 816	563	1 139	
600 mS.m^{-1} - 4.40 g.l^{-1}	6 334	1 968	4 193	
Sodium chloride				
100 mS.m^{-1} - 0.52 g.l^{-1}	684	220		350
250 mS.m^{-1} - 1.35 g.l^{-1}	1 694	614		915
400 mS.m^{-1} - 2.10 g.l^{-1}	2 714	986		1 568
600 mS.m^{-1} - 3.18 g.l^{-1}	4 417	1 656		2 473

The analysis of kaolinite stones did not show any detectable metals bound to the surfaces. As kaolin is a 1:1 layer, structured aluminium-silicate, the clay has a low surface area and low cation and anion exchange capacity (P. Kempster, pers. comm.). Its contribution to changing water quality is therefore minimal.

Mortality results

Tables 4.13 and 4.14 represent mortalities after 96 hours, and Figures 4.10 to 4.12 cumulative mortalities recorded during definitive experiments for both sodium sulphate and sodium chloride experiments.

Table 4.13 Mortalities of *Tricorythus* sp. after 96 hours at a range of conductivities reached using sodium sulphate.

Raceway	Sodium sulphate			
	Experiment 1	Experiment 2	Experiment 3	Experiment 4
control 1	4.17%	13.01%	12.5%	15.0%
control 2	0%	9.09%	20.0%	0%
control 3	9.5%	4.16%	17.4%	0%
exp 1	0%	52.17%	85.7%	100%
exp 2	0%	50.0%	50.0%	100%
exp 3	0%	59.34%	65.2%	100%

Conductivities in replicate experimental systems (exp 1, 2 and 3):
Experiment 1: 50 $mS.m^{-1}$ / 0.20 $g.l^{-1}$
Experiment 2: 100 $mS.m^{-1}$ / 0.66 $g.l^{-1}$
Experiment 3: 200 $mS.m^{-1}$ / 1.46 $g.l^{-1}$
Experiment 4: 600 $mS.m^{-1}$ / 4.40 $g.l^{-1}$

Table 4.14 Mortalities of *Tricorythus* sp. after 96 hours at a range of conductivities reached using sodium chloride.

Raceway	Sodium chloride			
	Experiment 1	Experiment 2	Experiment 3	Experiment 4
control 1	0%	0%	4.3%	0%
control 2	9.5%	0%	4.2%	0.1%
control 3	0%	0%	0%	0%
exp 1	8.7%	26.1%	27.3%	52.2%
exp 2	0%	16.7%	56.5%	79.2%
exp 3	13.6%	21.7%	54.5%	61.1%

Conductivities in replicate experimental systems (exp 1, 2 and 3):
Experiment 1: 100 $mS.m^{-1}$ / 0.52 $g.l^{-1}$
Experiment 2: 250 $mS.m^{-1}$ / 1.35 $g.l^{-1}$
Experiment 3: 400 $mS.m^{-1}$ / 2.10 $g.l^{-1}$
Experiment 4: 600 $mS.m^{-1}$ / 3.18 $g.l^{-1}$

The LC_{50} for sodium sulphate was observed at approximately 100 $mS.m^{-1}$ or 0.66 $g.l^{-1}$, while the value for sodium chloride was not so clearly evident.

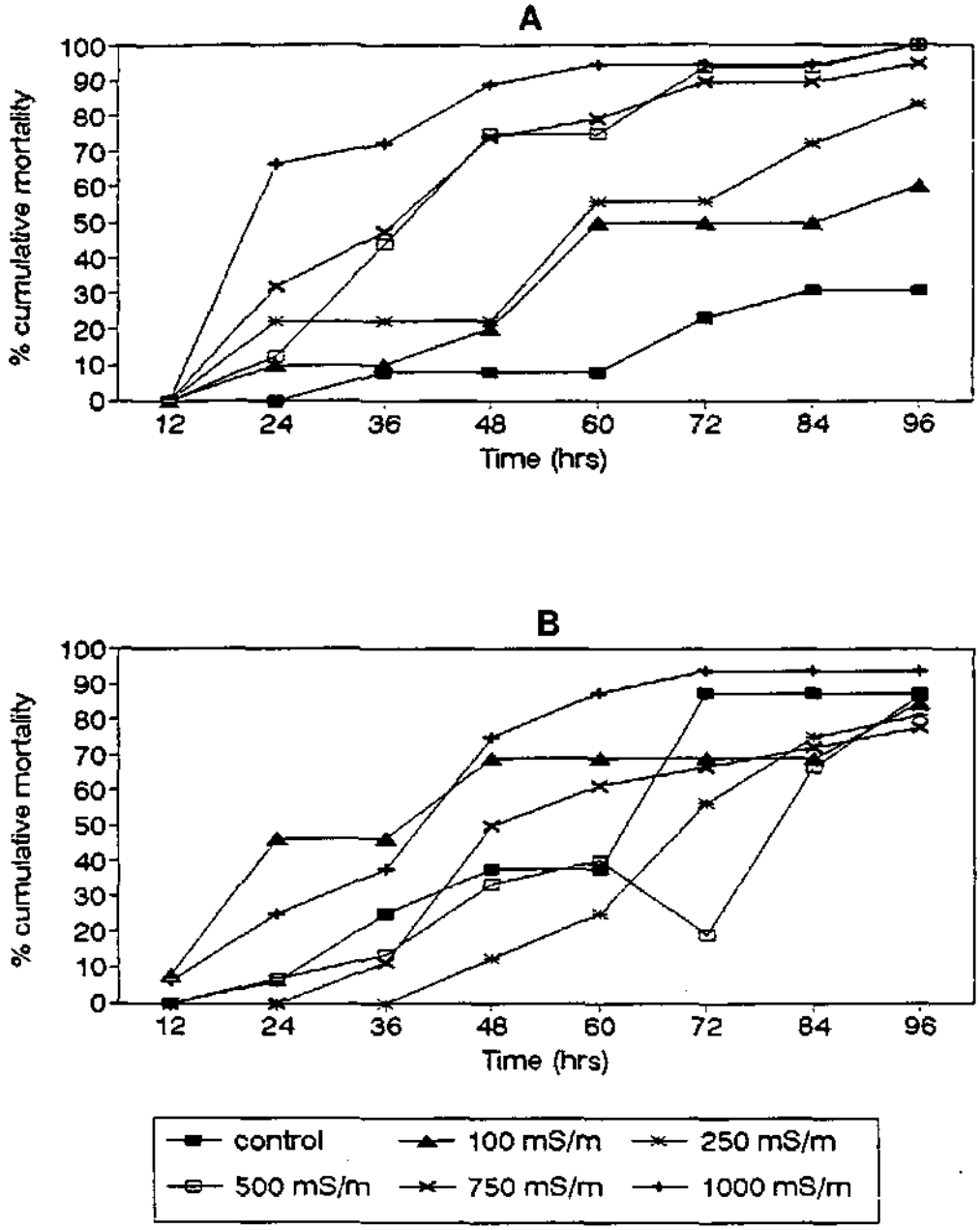

Figure 4.9 Cumulative mortality of test organisms during the sodium sulphate RF experiment.

(A) - *Tricorythus* sp.
(B) - *Chimarra* sp.

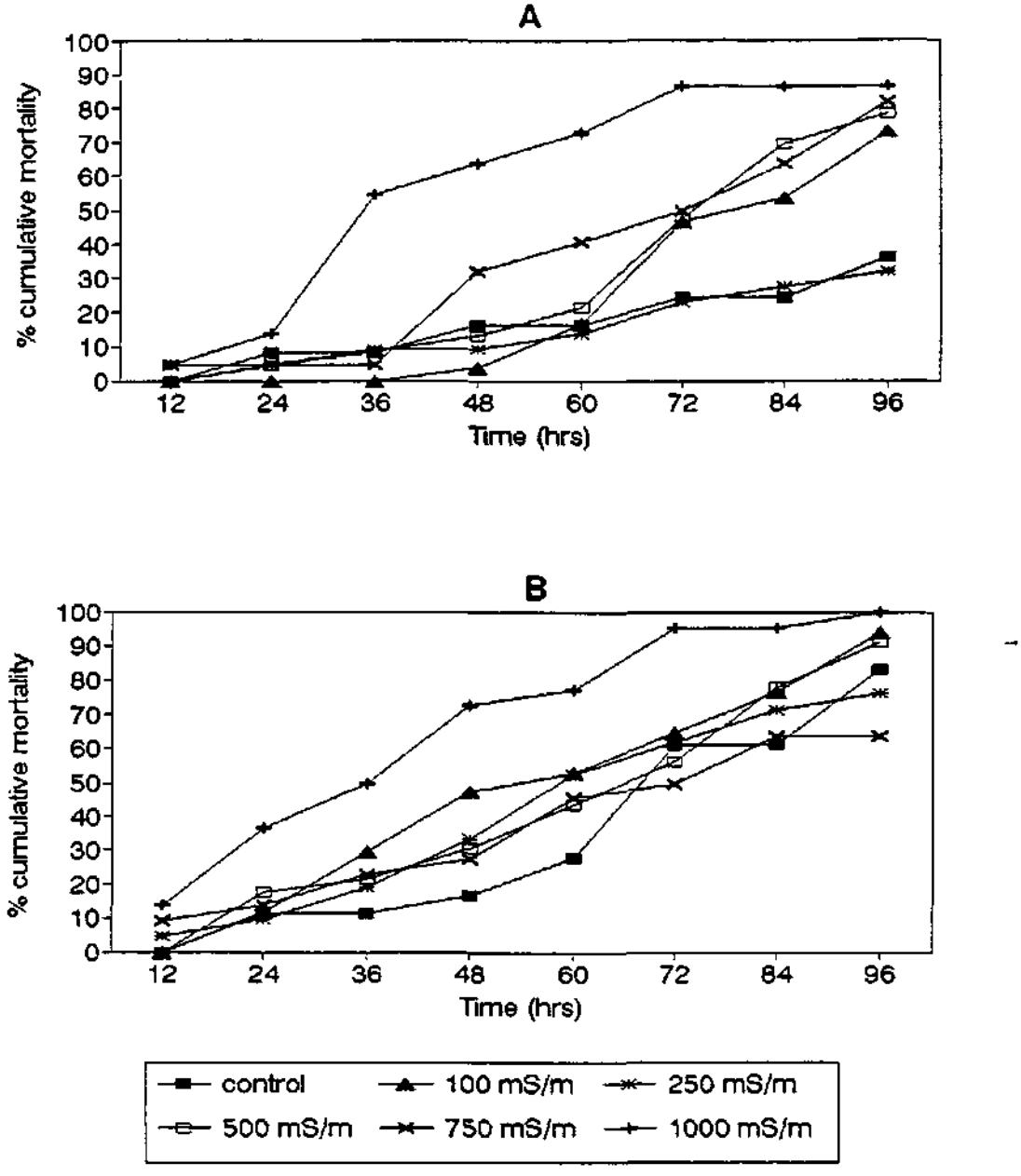

Figure 4.10 Cumulative mortality of test organisms during the sodium chloride RF experiment.

(A) - *Tricorythus* sp.
(B) - *Chimarra* sp.

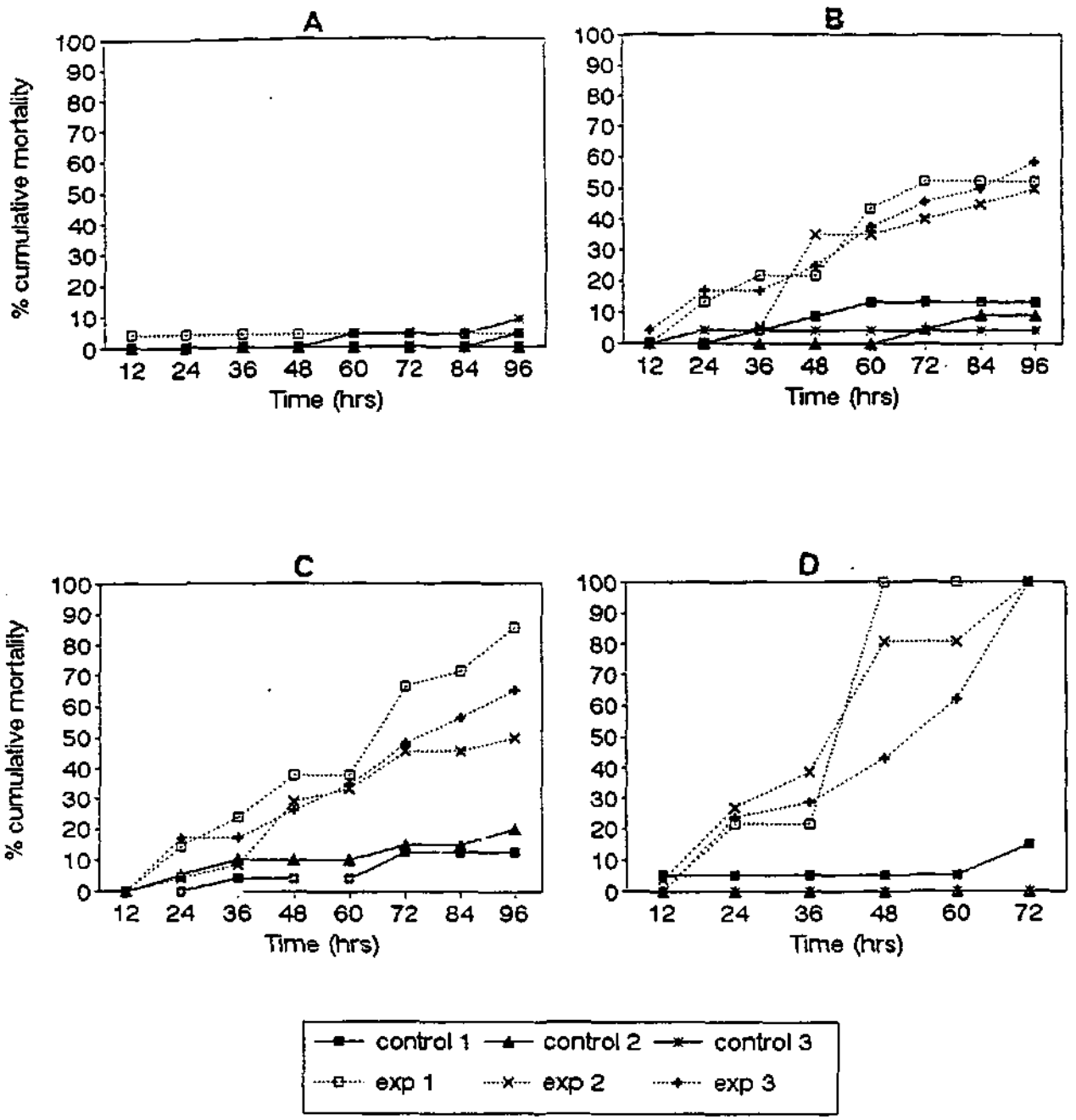

Figure 4.11 Cumulative mortality of *Tricorythus* sp. during sodium sulphate definitive experiments.

(A) - 50 $mS.m^{-1}$ / 0.20 $g.l^{-1}$
(B) - 100 $mS.m^{-1}$ / 0.66 $g.l^{-1}$
(C) - 200 $mS.m^{-1}$ / 1.46 $g.l^{-1}$
(D) - 600 $mS.m^{-1}$ / 4.40 $g.l^{-1}$

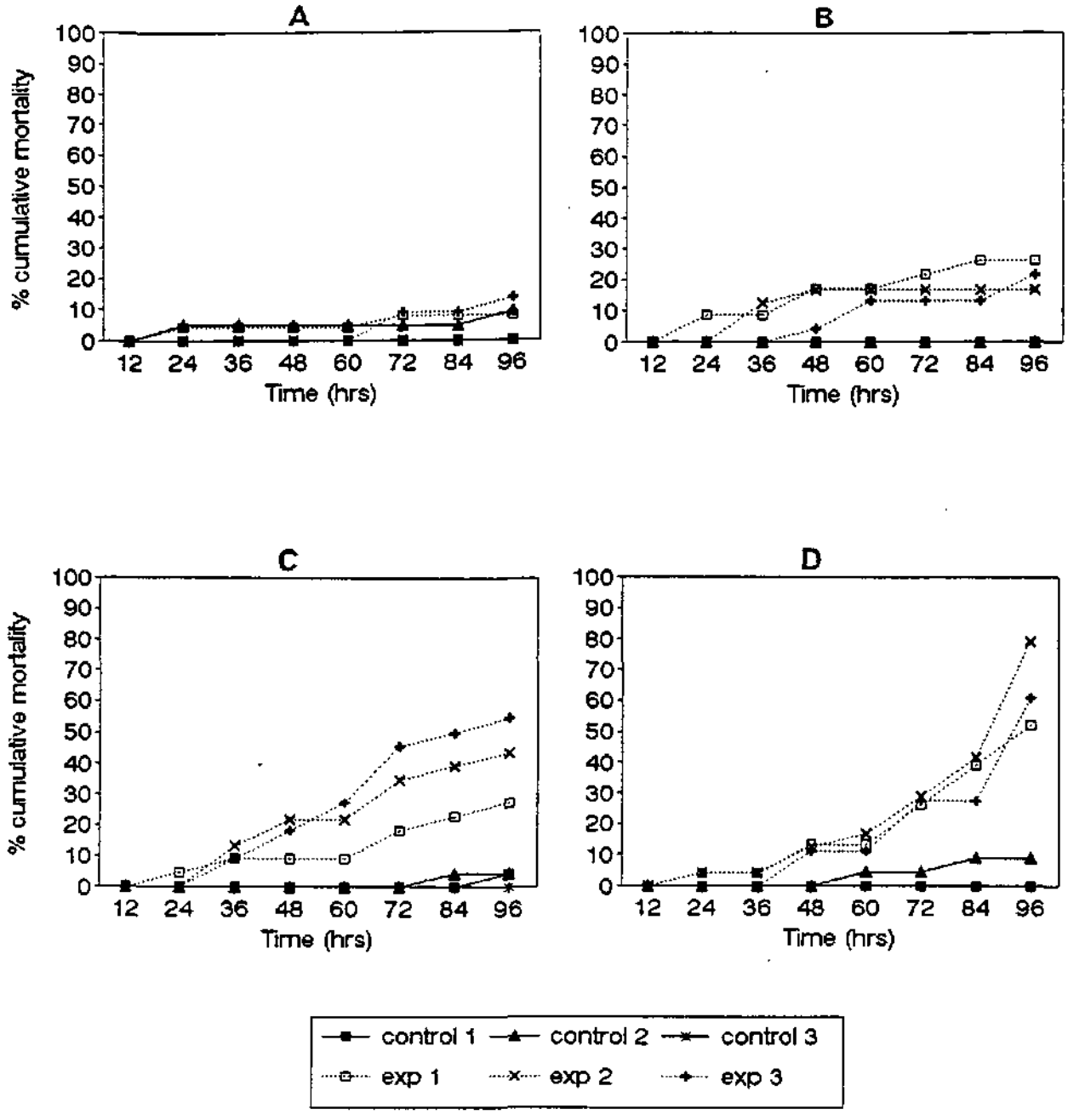

Figure 4.12 Cumulative mortality of *Tricorythus* sp. during sodium chloride definitive experiments.

(A) - 100 $mS.m^{-1}$ / 0.52 $g.l^{-1}$
(B) - 250 $mS.m^{-1}$ / 1.35 $g.l^{-1}$
(C) - 400 $mS.m^{-1}$ / 2.10 $g.l^{-1}$
(D) - 600 $mS.m^{-1}$ / 3.18 $g.l^{-1}$

Sodium sulphate

The statistical analysis of cumulative mortalities recorded during definitive experiments showed that at 50 $mS.m^{-1}$ no significant difference could be detected between mortalities in control and experimental systems ($f = 16.654$ and $P<0.00001<0.05$). At 100 $mS.m^{-1}$, both control and experimental mortalities were replicated, and a significant difference evident between them ($f = 6.799$ and $P<0.05$). The same result was seen at 200 $mS.m^{-1}$ ($f= 4.919$ and $P<0.05$), and at 600 $mS.m^{-1}$ ($f = 6.619$ and $P<0.05$). Mortalities recorded at 50 $mS.m^{-1}$ were significantly different from mortalities at all other conductivities, while there was no statistical difference between results of 100 $mS.m^{-1}$ and 200 $mS.m^{-1}$. The difference between these and results noted at 600 $mS.m^{-1}$ was significant ($f = 5.769$ and $P<0.00001<0.05$).

The LC_{50} value for sodium sulphate was experimentally observed at 100 $mS.m^{-1}$ or 0.66 $g.l^{-1}$. The data could not be analyzed using the probit programme as the probit model requires a normal distribution of concentration response data.

Sodium chloride

Mortalities recorded for controls and experimental systems at 100 $mS.m^{-1}$ were not replicable or significantly different ($f = 6.566$ and $P<0.05$). Control and experimental mortalities were replicable at 250 $mS.m^{-1}$, and significantly different from each other ($f = 13.749$ and $P<0.00001<0.05$). These results were also observed at 400 $mS.m^{-1}$ ($f = 9.017$ and $P<0.00001<0.05$), and at 600 $mS.m^{-1}$ ($f = 6.076$ and $P<0.05$). Results recorded at 100 $mS.m^{-1}$ were significantly different from those recorded at all other conductivities, while the results of 250 $mS.m^{-1}$ were not significantly different from those of 400 and 600 $mS.m^{-1}$. The results noted at 400 and 600 $mS.m^{-1}$ were also not significantly different from each other ($f = 3.656$ and $P<0.05$).

As the LC_{50} for sodium chloride was not clearly evident from experimental observations, data were statistically analyzed using the probit programme. Chi-square heterogeneity for the two duplicate sets run was 0.73 and 1.790 respectively. Due to mortality data not being significantly different at 250, 400 and 600 $mS.m^{-1}$, the LC_{50} could only be allocated to a range between 400 and 800 $mS.m^{-1}$ *i.e.* above 2.10 $g.l^{-1}$.

4.4.3.3 Selati River tolerance experiment

Physico-chemical constituents and nutrient concentrations

General water quality conditions remained constant throughout the experiment for both Sabie and Selati River waters. Physico-chemical constituents ranged as follows: pH 7.04 - 7.40, temperature 18° - 21°C, DO 83.4% - 100%, EC 14 - 19 $mS.m^{-1}$ for the Sabie River controls and 190 - 238 $mS.m^{-1}$ for the Selati River systems.

Nutrient concentrations also remained relatively constant throughout this study. The percentage of toxic un-ionized ammonia ranged between 0.4 and 1.2% of total ammonia for the conditions of this study (pH 7.0-7.5 and 20°C), with the ammonium ion (NH_4^+) contributing 99.6 - 98.8% of total ammonia. Since ammonia concentrations decrease with increasing salinity (Train, 1979), the contribution of toxic ammonia would have been even lower in the more saline Selati River raceways. The highest ammonium concentration recorded during the Selati River study was 0.09 $mg.l^{-1}$, suggesting it unlikely that toxic ammonia levels were reached during this study. Nitrite concentrations never exceeded 0.2 $mg.l^{-1}$, which falls within the USA (1988) and Canadian guidelines (1987) (cited in Dallas and

Day, 1993). The highest nitrate level recorded during this research was 5.5 $mg.l^{-1}$ for the Sabie River, well below the Special Effluent Standard for South Africa and the Canadian guidelines (see Appendix 4.2). Total reactive orthophosphate levels in the Selati River systems were initially high (around 0.68 $mg.l^{-1}$), but rapidly declined to between 0.3 and 0.35 $mg.l^{-1}$.

Microbial populations

Two common water bacteria were identified at low concentrations, i.e. *Klebsiella rhinosderomatis* and a pseudomonad bacterium. *K. rhinosderomatis* concentrations remained relatively constant in Selati River systems (about 2 400 $colonies.ml^{-1}$), while an increase from approximately 550 to 1 300 $colonies.ml^{-1}$ was evident in the Sabie River systems. As a similar scale of increase was seen in both control and experimental systems during salinity tolerance experiments, it was not thought to affect invertebrate mortality during this experiment.

Water chemistry

The analysis of samples by IWQS showed the following comparative water chemistry conditions for the Sabie and Selati River waters (Table 4.15). The degree of degradation of the Selati River is clearly evident from this data. Water quality remained consistent over the duration of the experiment.

Table 4.15 Comparative water chemistry information for the Sabie and Selati Rivers (September 1994).

Variable ($mg.l^{-1}$)	Sabie River	Selati River
TAL as $CaCO_3$	68.0	247.0
Hardness	69.4	905.8
TDS	134.0	1 702.0
Fluoride	0.9	3.8
Sodium	8.0	156.0
Magnesium	9.0	174.0
Sulphate	11.0	742.0
Chloride	7.0	178.0
Potassium	2.2	67.8
Calcium	13.0	76.0
Boron	0.008	0.140
Chromium	0.003	0.003
Iron	0.024	0.003
Copper	0.004	0.004
Zinc	0.003	0.016
Strontium	0.064	3.514
Molybdenum	0.006	0.102

Results are for metals dissolved in the water column.

Mortality results

Cumulative mortalities of *Tricorythus* sp. and *Choroterpes* sp. in Sabie and Selati River water are shown in Figure 4.13. Mortality of *Tricorythus* sp. was clearly linked to experimental water, while *Choroterpes* sp. appears to survive well in either system.

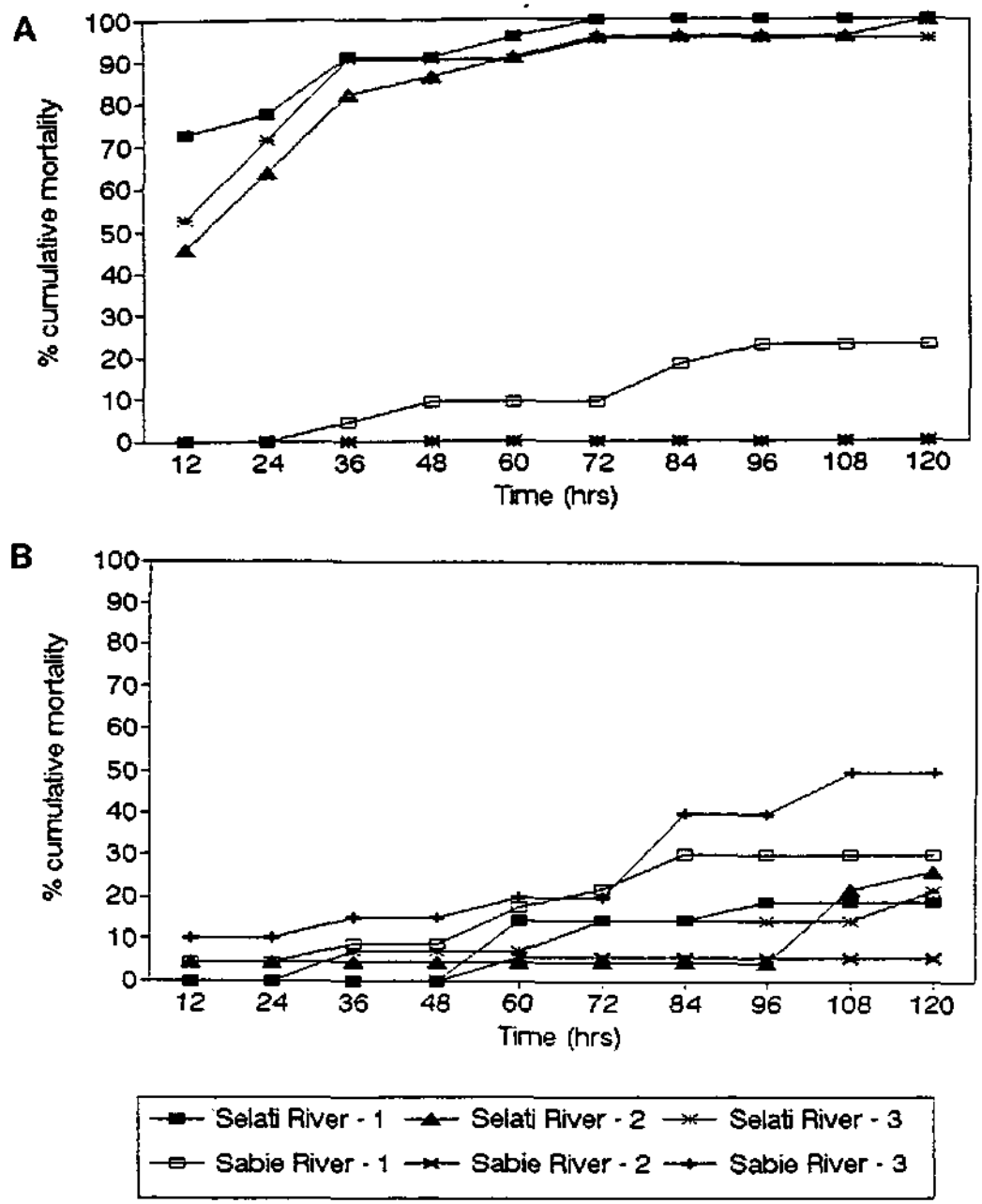

Figure 4.13 Cumulative mortality of experimental organisms in Sabie and Selati River water.

(A) - *Tricorythus* sp.
(B) - *Choroterpes* sp.

The reproducibility of replicates, and mortality results in Sabie and Selati River waters, was assessed for *Tricorythus* sp. and *Choroterpes* sp. using multiple regression analysis. Statgraphics version 6.0 was used for the statistical analysis (Statgraphics Corporation, 1990).

***Tricorythus* sp.**
No significant difference was apparent between the replicate populations in Selati River water ($f = 1.748$ and $P = 0.172$), indicating that survival in this system is replicable. However, in Sabie River waters, one replicate was significantly different from the other two ($f = 173,057$ and $P = 0.00001$). The survival rates between populations in Selati River water compared with those in Sabie River water was however significantly different ($f = 832.982$ and $P = 0.00001$). Survival therefore appears to be related to the test water.

***Choroterpes* sp.**
No significant difference was evident between the replicate populations in Selati River water ($f = 0.581$ and $P = 0.679$), again indicating the reproducibility of the system. However, a significant difference was evident between the replicate populations in Sabie River systems ($f = 64.341$ and $P = 0.00001$), and reproducibility could not be assumed. As in the case of *Tricorythus* sp., there was a significant difference between survival rates in Selati River water, than in Sabie River water ($f = 5.136$ and $P = 0.008$).

Choroterpes sp. results were therefore inconclusive, and mortality could not be linked to the composition of test waters. Observations indicated that many *Choroterpes* sp. individuals were in their final instar preparing to emerge and may therefore have been more susceptible to laboratory conditions. Although survival between the Sabie and Selati River systems is less pronounced for *Choroterpes* sp., *Tricorythus* sp. clearly survive better in Sabie versus Selati River water.

When comparing the survival of *Tricorythus* sp. and *Choroterpes* sp. in Selati River water ($f = 976.170$ and $P = 0.00001$), it was seen that *Choroterpes* sp. showed higher survival rates than *Tricorythus* sp..

4.4.4 Discussion and conclusion

4.4.4.1 Biomonitoring
The basis of biological surveillance is that the adverse effects of a pollutant on a receiving water would be manifested as modifications of density, distribution, or presence and absence of resident aquatic organisms. Monitoring these populations over a period of time will therefore give some indication of their tolerance of, or susceptibility to the pollutant(s) in question (Abel, 1989). However, field data may be difficult to interpret, and changes may only tentatively be ascribed to the pollution event. Interpretation is particularly difficult if a mixture of pollutants is present simultaneously. The tolerance of the aquatic community to pollutants or to each individual component of a pollutant cannot be accurately assessed without the assistance of ecotoxicological studies. Field observations may also be costly and labour intensive, while inefficient sampling may lead to inaccurate results. Toxicity testing can be replicated and more carefully controlled. Although predictions based on toxicity tests do not always concur with field observations, more reliable results are obtained. Field surveys may provide the most realistic and relevant evidence that ecological integrity has been

disturbed, but they cannot establish a causal link to a contaminant. Chemical surveys can confirm the presence and identity of a pollutant, but despite the compromise on realism only toxicity testing can show that a link exists between the contaminant and any adverse effects *monitored* (Cairns *et al.*, 1992). Ideally, both ecological field observations and toxicological studies should be used to provide a more accurate assessment of the impact of pollution on riverine organisms.

4.4.4.2 Salinity tolerance experiments

Although Sabie River water is relatively pristine and unpolluted, water chemistry data can assist in defining the contribution of selected chemical parameters to water quality. There is an interaction of factors such as pH, dissolved organic carbon, total alkalinity and TDS in determining the fate of any ionic species. The Sabie River system is ideal for ecotoxicological studies as the water chemistry is uncomplicated by fluctuating metal concentrations, which would further contribute to the complexing of sodium, chloride and sulphate ionic species. The binding of these ions removes them from the water column and reduces their bioavailability to experimental organisms. It then becomes necessary to model the system and attempt to predict the movement of these ions in water of a particular quality.

The clarity of results achieved from the Sabie River system is in direct contrast to the results associated with the Bloukrans River in the Eastern Cape (Section 4.3). As well as the problems associated with using baetid populations instead of more uniform and larger tricorythids, the water of the Bloukrans River is highly nutrient-enriched and receives large volumes of pollution from urban areas. Although the pH remained constant during Bloukrans River experiments, the influences of other water quality parameters were not evaluated. High levels of dissolved organic matter (measured as DOC) may be found in polluted waters (Dallas and Day, 1993), which would result in increased complexation of ionic species with a subsequent reduction in bioavailability. As the chemical analyses of water samples is costly and not always possible, it is necessary to carry out toxicity tests in water of good quality and therefore limit factors which may contribute to mortality.

One of the aims of this research and of the artificial stream project is to assign a guideline value to salinity for the protection of aquatic ecosystems, and subsequently the natural environment. Published guidelines include the Australian TDS guideline for the natural environment (Hart *et al.*, 1992) i.e. 1 000 $mg.l^{-1}$, which is equivalent to approximately 150 $mS.m^{-1}$ in waters low in organic material. An important finding of this research was the difficulty associated with assigning a single guideline value for conductivity. It has become apparent that mortality linked to conductivity or salinity is clearly dependent on the salt used to elevate conductivity. *Tricorythus* sp. was shown to be highly susceptible to increased sodium sulphate levels, with an LC_{50} of 100 $mS.m^{-1}$ or 0.66 $g.l^{-1}$, while no significant mortality was noted at this EC when using sodium chloride. Sodium chloride was associated with much higher tolerance levels. The analysis of range-finding results indicated a LC_{50} at approximately 550 $mS.m^{-1}$, while the statistical analysis of definitive test results could only identify a LC_{50} range of 400 to 800 $mS.m^{-1}$ i.e. above 2.10 $g.l^{-1}$. These results also appear to be species-specific for sodium sulphate. The LC_{50} value for *Tricorythus* sp. and sodium sulphate was 100 $mS.m^{-1}$ (0.66 $g.l^{-1}$), while that of the Bloukrans River baetid population was around 900 - 1 000 $mS.m^{-1}$ (about 16 to 18 $g.l^{-1}$). The apparent LC_{50} values for *Tricorythus* sp. and for the baetid population were similar when using sodium chloride. This apparent species specificity could however be linked to river water quality. The Sabie River has very

low sulphate concentrations, around 11 $mg.l^{-1}$, while organisms in the Bloukrans River may be acclimated and therefore more able to withstand and tolerate polluted conditions.

As it appears difficult to assign a single guideline value for conductivity, it may be necessary to determine the cause of mortality and assign guideline values for this parameter. At first glance it seems apparent that mortality must be linked to the total quantity of salts present i.e. TDS, as most of the TDS levels are above the DWA (1986) recommended limit of 350 - 550 $mg.l^{-1}$. Although these high TDS levels would affect the osmoregulatory mechanisms of the organism, it has been reported that within certain limits it is the rate of change rather than the absolute concentration which causes mortality (van Vuren *et al.*, 1994). Table 4.12 suggests that mortality may not only be linked to TDS concentrations. The LC_{50} value for *Tricorythus* sp. in sodium sulphate is equivalent to a TDS of about 900 $mg.l^{-1}$, while the range for sodium chloride is 2 000 - 4 000 $mg.l^{-1}$. The same evidence stands for the concentration of the Na^{+} ion. This then suggests that the SO_4^{2-} or Cl^{-1} anion may be related to toxicity. The organisms appear susceptible to much lower concentrations of the sulphate than the chloride ion, i.e. 500 $mg.l^{-1}$ SO_4^{2-} versus 1 500 to 2 500 $mg.l^{-1}$ Cl^{-1}. This concentration of sulphate exceeds the 250 $mg.l^{-1}$ guideline value suggested by Kühn (1991). Sulphate has been shown to have a definite effect on fish. Burnham and Peterka (1975) attributed decreased survival of fathead minnows to water of high sulphate concentration. Sulphates also give rise to gastro-intestinal irritation at a concentration of 600 $mg.l^{-1}$, if water is used for human consumption (DWA, 1986).

In an attempt to identify the causes of mortalities observed during these experiments, it is essential to investigate the physiology of these organisms. Once this knowledge is available, it can be determined whether mortality is linked to a loss of osmoregulatory functioning, or whether ion(s) may be disrupting essential physiological functioning. This disruption may be linked to the inhibition of important enzymatic processes.

4.4.4.3 Selati River tolerance experiment

Although ephemeropterans are widely considered to be good indicators of water quality (Matthew, 1968), tolerances to water quality are variable throughout this family. This variability is clearly evident from Appendix 4.6 which represents the range of ephemeropterans found in four Mpumalanga rivers. It is particularly interesting to note the tolerances of *Choroterpes* sp. and *Tricorythus* sp. in experimental systems, as representatives of both genera are found in the Sabie and the Olifants Rivers. This indicates that although *Tricorythus* sp. has some tolerance to more polluted waters such as the Olifants River, water quality in the Selati River may be above its tolerance limits. Table 4.15 clearly demonstrates the vast difference in water quality between the Sabie and Selati Rivers. Alternately, *Choroterpes* sp. is highly adaptable and although not found in the Selati River during this survey, good short-term survival was evident in Selati River water for the duration of this experiment. These results therefore represent a situation under which a more sensitive species, such as *Tricorythus* sp., would be lost to an impacted river such as the Selati River.

4.5 GENERAL DISCUSSION AND CONCLUSION

There are two main approaches to the evaluation of the deterioration of water quality at the ecosystem level. These are biomonitoring and the simulation of effects on simple systems to model changes in the more complex natural ecosystem. The latter approach was selected by

this study and results will ultimately be integrated with biomonitoring results. Although the experimental method is limited in its ability to predict general impacts on natural ecosystems, experimental ecotoxicology will provide data on the tolerances of riverine macroinvertebrates to selected pollutants.

Single-species testing was selected for toxicity studies as these tests are routinely employed to establish water quality criteria and to predict the environmental impact of hazardous materials on aquatic communities. However, several researchers have questioned the adequacy of single-species testing for predicting the effects of toxicants on natural communities in the field. Multi-species tests and field research are often considered to reflect conditions in the field more adequately, but these tests suffer from greater costs, loss of replicability and potential for environmental damage in the case of field studies (Clements *et al.*, 1988). Single-species tests are generally more simple, with a higher degree of replicability and ease of statistical analysis. However, the study reported here has demonstrated the species specificity of salinity tolerances. Single-species testing may therefore be more rigorous, but may be less applicable to natural systems. Some studies have however shown good agreement between single species tests and field data (Hansen and Garten, 1982; Adams *et al.*, 1983), while others have shown effects of toxicants in field experiments at considerably lower concentrations than indicated by single species bioassays (Dewey, 1986) (cited in Clements *et al.*, 1988). Generally, however, it has been shown that the bioavailability of many chemicals under field conditions is less than that observed under laboratory conditions (Parkerton *et al.*, 1989).

Short-term toxicity tests are particularly useful for estimating toxicity of materials for which toxicity data does not exist. The results of these tests can then be used to calculate acceptable concentrations for short exposures, such as spills into receiving waters (APHA, 1992). In some quarters short-term toxicity testing is thought not to have much predictive value. This claim is however contested by Persoone *et al.* (1990) who reported a number of cases where field results correlate well with acute toxicity values. They describe data obtained in short-term tests as an essential component used for establishing water quality guidelines as information is provided on direct effects of pollution. Indirect effects which may be due to factors such as altered interspecies interactions, are more difficult to predict.

Although single-species, short-term testing is the basis for determining harmful effects to more complex systems, results should be carefully interpreted. Results generated by these studies give a first impression of tolerances to various pollutants, and should always be used in combination with biomonitoring and field studies, as well as longer term toxicity tests. Short-term tests do not take into account any secondary effects that occur in the field and that influence the density and distribution of macroinvertebrate populations. These responses, such as competition, avoidance responses and downstream drift, may not be correlated to susceptibility to toxicants (Abel, 1989). It is therefore apparent that the response of fauna in natural situations will not necessarily be the same as implied by the laboratory data obtained.

There are a number of ecological effects of a pollutant on an invertebrate species that cannot be predicted by ecotoxicity studies or by monitoring water quality. These factors include interactions with other species (e.g. prey, predators, competitors) which share its habitat. The effect of these relationships may either accentuate or attenuate the toxicity of the pollutant (Abel, 1989). Animals, particularly invertebrates, may show interspecific and intraspecific

variation in susceptibility to pollutants. Several studies (e.g. Bell, 1971) using representative macroinvertebrate species from several orders, classes or phyla, have shown wide variation during acute LC_{50} tests. Interspecific variation may be attributed to size, age, life-cycle stages, health of the organism, as well as genetic variation between different strains or populations of a species. Acclimation, or long-term exposure to low levels of a pollutant under natural conditions, may also result in increased resistance (Abel, 1989).

The above-mentioned factors are difficult to control, particularly when using indigenous riverine fauna for toxicity testing. It is therefore difficult to draw any generalized conclusions regarding invertebrate tolerances, and each river system or catchment may have to be assessed separately. The guidelines already generated by DWAF (1993) for other designated water users, interpret salinity guidelines in terms of geomorphological areas. This is essential as the degree of mineralization of rivers will affect the acclimation of its resident fauna. This will in turn affect the salinity tolerance levels of the riverine fauna. Alternately, known pollution-sensitive species such as certain ephemeropteran species could be used for chronic toxicity testing and guidelines for the environment set at their tolerance levels. These levels should ensure the protection of the majority of aquatic invertebrate species.

Ecotoxicological tests run in controlled experimental systems therefore give an indication of the inherent tolerance of the organism under study, as results are not altered by stresses due to interrelationships between populations. Chemical stress induced by the interaction of the pollutant and general water quality conditions is frequently assessed, while other stresses are minimized by acclimation of organisms to laboratory conditions before the onset of toxicity testing. Control systems also provide a constant check on the effect of background factors on organism mortality.

Although the results presented in this study have demonstrated many of the difficulties associated with ecotoxicity testing using macroinvertebrates, it also presents an alternative to regulatory testing using laboratory-reared *Daphnia*. Information on *Daphnia* tolerances is also of little use to the development of water quality guidelines if their tolerances cannot be related to the natural biota. The experimental systems used here are at a larger scale than those traditionally used for regulatory testing, which provides a unique opportunity to observe indigenous riverine fauna in flowing-water conditions. If environmental toxicology is coming of age in the 1990s, it must start to ask more searching questions and test the limits of toxicity testing. Although the research presented here relies on many of the existing paradigms of traditional ecotoxicology, it is attempting to explore the link between laboratory studies and the use of indigenous riverine organisms. Despite the difficulties encountered during this research, the use of indigenous fauna to determine guidelines for the natural environment remains a plausible option. From the results it appears that guidelines may need to be linked to catchments and their particular land-use patterns. The intrinsic nature of each water body appears to have a distinct influence on the tolerance of its fauna, and their subsequent behaviour during toxicity studies. As there is no possibility of modelling the complexity of the natural environment, "ecology must combine holism with reductionism" (Odum, 1977).

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Appendix 4.1

The problem of salinity in South Africa: A literature review

This section was written by Mr B. A. Maart, previously of IWR.

1. INTRODUCTION

South Africa lies in the drought belt of the globe, the major semi-arid regions being in the west of the Drakensberg. The meteorological phenomenon of lower reliability with a decrease in the annual rainfall compounds this problem. Because of unavoidable losses by spillage and evaporation from storage, and the fact that not all the run-off can be diverted for use before reaching the sea, only 40% of the run-off can be made available for use through the provision of storage. It is estimated that natural water resources (including estimations of groundwater) amount to 75 010 x 10^3 $m^3.day^{-1}$. Total surface run-off is estimated at 53 500 million $m^3.annum^{-1}$, with 62% of this being utilisable (DWA, 1986).

The future consumption of water will be influenced by increasing population, the growing demands for irrigation water, and by the steady industrialisation and urbanisation of the South African economy. Unless steps are taken to plan the exploitation and augmentation of SA's water resources, shortages may be suffered later. The DWA (1986) lists the various instituted and promulgated steps taken to insure against this:

1. Efficient water storage
2. Reduction of evaporative losses
3. Underground storage
4. Catchment preservation
5. Control of flooding
6. Desalination of sea-water
7. Water recycling
8. Urban water reuse
9. Prevention of pollution
10. Water savings in power generation
11. Improvement of irrigation techniques
12. Climatological forecasting and planning
13. Extraction of atmospheric moisture
14. Water transport
15. Water resource development

The unfavourable geographical distribution and limited availability of water are only part of South Africa's problem. Water quality is also deteriorating in many regions, particularly in urban and industrial areas. Three main factors affect water quality, namely salinisation, eutrophication and pollution by micro-pollutants.

The pollution of steams is usually easily detectable by effective biological, chemical or physical monitoring. Slow, cumulative pollution is difficult to identify, and therefore difficult to cope with. In order to timeously detect this form of pollution (of which salinisation is a

typical example), the analysis of the interaction between all sources of pollution and hydrological systems is essential (DWA, 1986).

Salinisation is the process whereby the concentration of total dissolved solids (TDS) increases in inland waters (Allanson *et al.*, 1990). It is seen as the single most serious threat facing the public water system of South Africa (Commission Report, 1970; Stander, 1987).

An approximate relationship exists between TDS in $mg.l^{-1}$ and the electrical conductivity:

$$TDS\ (mg.l^{-1}) = EC\ (mS.m^{-1}) \times 6.6$$

The Water Act of 1956 (DWA, 1986) stipulates the purification of all dischargeable effluent and its return to the stream of origin. This, however, has led to increased return flows of saline effluent from urban, industrial and agricultural areas, making quality a more important factor than quantity, especially in the arid/semi-arid regions of the country (DWA, 1986).

Schofield & Ruprecht (1989) have identified four classes of water salinity:

1. Fresh
2. Marginal
3. Brackish
4. Saline

2. ORIGINS OF SALINISATION

There are three main individual sources of rising TDS levels in South African fresh water:

1. Natural salinisation (due to the prevailing climate, geomorphology, etc.)
2. Agricultural activities (due to irrigation practices, overgrazing, interruption/regulation of water flow) (Allanson *et al.*, 1990), and
3. Urban and industrial (including mining) activities. The main sources of TDS due to this category of activities are:
   - Underground water pumped by mines
   - Run-off and seepage from urban and industrial areas
   - Direct municipal and industrial effluents (Stander, 1987)

Studies are now being undertaken in areas where the salinity has risen to unacceptable levels. These include the Vaal River, Fish-Sundays River system, and the Berg and Breede Rivers in the Western Cape. The increased salinity of stored water and ground water in the Cape Midlands, the Karoo regions and parts of the SE Cape are also being investigated (DWA, 1986).

2.1 Natural salinisation

Gibbs (1970) proposed that three main natural mechanisms control world surface chemistry. These are atmospheric precipitation, rock dominance, and evapo-transpiration. It was found that the major mechanism controlling Australian mainland streamwaters was catchment lithology, not precipitation chemistry. Inland Australian rivers drain large arid/semi-arid regions of low relief. Water chemistry is therefore likely to be influenced by shallow

groundwater inflows that reflect the relatively high Na levels in the basin subsoils. Although the Na in the soils may be of oceanic origin carried by precipitation, the accumulation is due to the low relief, the low run-off:rainfall ratio, and the high evaporation:precipitation ratio. Due to the common element of aridity, this seems to be directly applicable to some areas of South Africa.

A study of the Murray River in Australia shows an increase in salinity with Na being the dominant ion, being derived from precipitation and saline irrigation drainage waters. The ionic concentration is thus dependent on the relative proportions of surface run-off, interflow and groundwater. The addition of all of these factors constitutes the total flow. Ionic concentration therefore varies with flow, and some Tasmanian rivers become rich in NaCl at higher flows as the NaCl is washed out of the soil and groundwater. Cornish (1987) summarises the interplay of the factors controlling the ionic concentration of surface waters as follows: At higher flows, precipitation chemistry plays a greater role through run-off. At low flows, rock dominance is more important, as flow consists primarily of interflow and baseflow. In arid regions, the evaporation-crystallization process will increase ionic concentration and change the relative compositions.

In South Africa, salt-impregnated geological formations also lead to salinisation. The Karoo shale formations, which leach salts after periodic heavy rains, give rise to highly saline base flows. This is compounded by the elevated water table, evaporative losses and flow reduction in rivers due to upstream diversions or impoundments, and drought conditions (Allanson *et al.*, 1990). The Malmesbury shales are mainly responsible for the salinisation of the Berg River system. The flow of the water in the fractured slates and shales are also important in the future development of the Atlantis aquifer. A delicate balance might exist between the freshwater in the sands of the Atlantis aquifer and the underlying saline water in the fractured Malmesbury rock. This balance could be shifted if the aquifer is developed further (Anon, 1987b).

2.2 Salinisation due to agricultural practices

2.2.1 Clearing of natural vegetation

The clearing of natural vegetation leads to secondary salinisation, where the salinity of the land, and hence, the streams, increases. This has been noted by Allison *et al.* (1990), where salinisation in S.Australia has been linked to areas where groundwater was near the land surface. In these areas, the response of groundwater levels to plant clearing can be rapid, with land and stream salinisation developing in 10-50 years due to the increased rate of groundwater recharge. In low-rainfall areas with a deep water table, the recharge rate is low following clearing. Plant clearing leads to an increase in the downward flux of water due to the shallow roots of the crop/pasture which replaces the natural vegetation. Salt is leached from the soil, and is displaced downward, raising the salinity level of the groundwater. Although deforestation changes the distribution of the salt, the problem seems to be excess water causing the existing salt to rise to the land surface, leading to salinisation of the soil (Allison *et al.*, 1990). The phenomenon of increased salinisation following deforestation has also been noted by Rippingale (1981) in the Swan-Avon river system in Western Australia.

Schofield and Ruprecht (1989) have also noted the increase in stream salinity in Western Australia following deforestation, and the degree of increase depends on annual rainfall, salt

storage, groundwater hydrology, the proportion cleared and clearing history of the catchment area. They found that in high rainfall areas, stream salinities increased for 8 years post clearing, before decreasing to a new equilibrium in approximately 60 years. In areas with lower rainfall, these periods are substantially greater.

Research of the Upper Colorado catchment aims at minimising the deep percolation of irrigation water which is responsible for the mobilisation of large quantities of salt in the gypsum-rich subsoil horizons. On-farm control measures essentially aim to improve the efficiency of surface irrigation, which is by far the most widely practised irrigation practice. The Grand Valley contributes 580 000 tons of salt to the Colorado River annually. The current ameliorative programme aims to reduce this by 130 000 tons, although this target is threatened by the inviolable and generally excessive water rights of the farmer, and the widespread tendency to over irrigate (Forster & Green, 1987).

Evapotranspiration from irrigated land also increases salt concentrations in the soil water. Leaching of the salts result in the return of saline flows to streams. In addition, leaching from weathered geological formations accumulate in impoundments in conjunction with natural salts whose release from the soil is accelerated by dryland agricultural practices. During soil erosion, dissolved salts are transported downstream and concentrate in soils where the water is used. If these salts are not leached or flushed (by even more fresh water), the salinity levels in the soil rise to saturation level (DWA, 1986).

A promulgated model for the study of salinisation of water in rural agricultural environments surrounding the Breede River Catchment has been initiated, the proximate aim of which was to develop a irrigation return flow sub-model. The approach adopted is aimed at the study of 1000 ha of irrigated land served by the Robertson canal (Anon, 1986). A subsequent study has shown that the major concern is the mobilisation of salts resulting from irrigation farming in the catchment. The Bokkeveld series of aquitards are the main contributors of salts, but the spatial distribution of these contributions is not well known. Part of the salt originates in (especially) newly ploughed soils, and partially through direct discharge of artesian saline water from fractures into the saturated zone and from there into the river channel. Much of the complexity of the flow, and thus the salinisation system through the Bokkeveld and soils has been elucidated with the help of the determination of ^{18}O values plotted against TDS (Anon, 1987b). However, Palmer (1991) concluded from his study of the Buffalo River that only a small proportion of the salinisation could be ascribed to agricultural practices in the surrounding catchment area.

Leaching associated with high salinity increases the demand of freshwater for irrigation, necessitating the release of water from upstream impoundments to counteract the rising downstream salinity levels (DWA, 1986). Irrigation with high TDS water is only acceptable if the soil has sufficient depth and has a high cation exchange capacity (Buckley *et al.*, 1987). The weight of these cause and effect relationships on the water quality and quantity has led to the intensive investigation of alternative or ameliorative measures, including large-scale desalination of irrigation water.

2.2.2 Impoundments

Mineral levels also increase as a result of evaporation from storage dams. The growing water demand and the need to counter sedimentation has necessitated the increase in surface area,

resulting in even further evaporative losses and a gradual rise in the mean salinity levels of dams (DWA, 1986).

Selkirk & Hart (1983) report increased mineralisation of the Buffalo River waters due to the erected impoundments of the Laing and Bridle Drift Dams (Selkirk & Hart, 1983). This was also noted by Palmer (1991).

This has also been noted by Suschka (1986) in his study of Mozambican rivers. The mineralisation of the Umbeluzi River has increased by a factor of three following the building of a dam. The construction of the Massingir Dam led to an increase in the salinity of the Elefantes River as a result of evaporative loss during water storage and infiltration through base soils in the dam. Evaporative loss, however, is the main cause of the salinity increase. In general, Suschka (1986) noted that the mineralisation of Mozambican rivers is low (< 200 $mg.l^{-1}$ for large rivers like the Zambezi). In arid regions, however, the mineralisation level is much higher (e.g. the Changane River with an average salt content of 3000 $mg.l^{-1}$).

2.3 Salinisation due to urban and industrial activities

The mineral content of some South African freshwater amount to 300 $mg.l^{-1}$ TDS, and can be projected to an average value of 800 $mg.l^{-1}$ by the end of the century . If remedial measures are applied timeously however, the rise might be contained to approximately 500 $mg.l^{-1}$ (Heynike, 1981).

The high salinity of some effluents limits the industrial recycling of water. Salt accumulation in recycling plants necessitates the frequent bleeding of water and replacement with fresh water, increasing the industrial use of freshwater even further (DWA, 1986).

The DWA has adopted a series of policies, measures and strategies to deal with rising pollution levels:

1. The polluter pays for the abatement of his own pollution.
2. Present pollution control measures are concerned with the dilution of liquid wastes. However, the increasing scarcity of freshwater for dilution and the high costs of aquaducts (canals) requires that research be undertaken into alternatives such as the removal of salts at the source. Desalination is a process that has received considerable interest. The procedure is discussed elsewhere in this review.
3. The measures implemented for the control of saline wastes also apply to the treatment and handling of intractable wastes that cannot be degraded. Disposal methods include encapsulation, co-disposal with certain other wastes, soak-aways, neutralisation, chemical treatment, and dumping at sea.
4. Control and management of eutrophication-inducing nutrients such as phosphates. As with salinity, control measures are implemented at point sources. This has been suggested by a number of authors (e.g. Hart *et al.*, 1987).
5. Monitoring and control of trace metals and micro-pollutants.
6. The application of technical innovation has led to the implementation of novel desalination procedures, applicable to inland industries producing highly saline effluents.

7. Disposal by reuse instead of return to the stream of origin. There are three areas where reuse of polluted water may be applicable:
    1. General reuse by local authorities
    2. Reuse by irrigation
    3. Reuse in industry
8. Improved desalination techniques and the reduced cost of desalination may lead to this procedure being implemented on a wide scale. Desalination research in South Africa centres on a variety of techniques, and these are discussed elsewhere in this review.

2.3.1 Salinity in the Rand Water Board area

The Rand Water Board (RWB) supply area is the largest user of South Africa's freshwater for urban, industrial and mining purposes. The bulk of the water supplied by the board, 98.6%, is abstracted from the Vaal Dam and the Vaal Barrage Basin (Heynike, 1981). Readily exploited resources within the catchment are fully committed, and water has had to be imported from neighbouring catchments, for example from the Komati in 1962, the Usutu in 1967, and the Tugela in 1976 (van Robbroeck, 1989).

A forecasted demand increase from 2848 million m^3 (1989) to 8370 m^3 (2025) is expected in this area, which by far exceeds the available supply (van Robbroeck, 1989).

Because of the concentration of mining and industrial activities, this area generates large volumes of domestic, industrial and mining effluent, which is discharged into local streams, mainly via the Klip and Zuikerbosch Rivers (Heynike, 1981). Most of this effluent reaches the Vaal Barrage, is abstracted with the natural run-off, processed, and returned to the water supply system. The combination of the large volumes of effluent and the wash-off of large quantities of dissolved solids has led to increasing salinity and eutrophication (DWA, 1986; Herold *et al.*, 1990). Concern regarding this increased salinisation has led to the establishment of an advisory committee for controlling the amount of salinity from the Vaal River system Founder members of the committee include the Department of Water Affairs (DWA), the RWB, the Johannesburg Municipality, the National Institute for Water Research (NIWR) and the Water Research Commission (WRC).

Rising salinity of the Vaal River became apparent in 1949, and, since then the TDS concentration in Vaal Barrage has more than doubled. It is estimated that, if the projected rise were allowed to continue, costs to be borne by users would amount to R76 - R139 million per annum (DWA, 1986).

A study of the Vaal Barrage by Jones *et al.* (1989) showed that there was a marked deterioration in water quality in the vicinity of sand dumps. Storm events resulted in direct run-off reducing the salt concentration in the streamflow. Thus direct run-off from the mine dumps is not the major source of the high salt concentration in the streamflow. These high levels exist in the baseflow. Seepage from the mine dumps into the streams themselves is the probable source of the high salinity noted in the streams. It is estimated that approximately 50 000 tonnes of salt originated from the mine dumps (Jones *et al.*, 1989).

A number of deductions have been drawn from the study initiated by the WRC:

1. 64% of the water supplied to the PWV area is returned as effluent.
2. Mine dewatering produces 25% of the total salt load reaching the Vaal Barrage. This is discharged in only 25% of the effluent volume. Industries and sewage works discharge 35% of the total load in 32% of the effluent volume.
3. Other sources contribute 40% of the salt load generated within the PWV area.
4. The TDS concentration of the Vaal Dam has risen at a rate of 2.5 $mg.l^{-1}.a^{-1}$.
5. TDS values of water supplied to the Johannesburg area exceeded 400 $mg.l^{-1}$ for 25% of the time. Peak values in the Johannesburg area exceeded 500 $mg.l^{-1}$, and in the Vereeniging area peak values exceeded 800 $mg.l^{-1}$.

Quality of mine service water is also an area of concern. This water is usually of a poor quality because of various salinisation mechanisms operative in mines, i.e. the oxidation of pyrite, intrusion of saline fissure water, salts derived from explosives, and salt dissolution during the processes of rock washing and dust suppression. Reasons for wanting to desalinate include severe corrosion of and scale formation in the service water circuit, reclamation of service water for re-use or compliance with effluent discharge requirements (Chamber of Mines Research Organisation, 1987).

In addition to gold and coal mining activities, the Vaal catchment is also subject to influences of several power stations, and three SASOL works. It is also thought that air pollution and acid rain may have an influence on the deteriorating quality in the Vaal Dam. In addition, illegal and undetected discharge of saline effluent into the stormwater drainage systems compound the salinisation problem (DWA, 1986).

Heynike (1981), in his consultancy study of mineralisation of the RWB's water supply, has drawn the following conclusions:

1. The hardness of the water increases concomitantly with a rise in the TDS, changing the character of the water from temporary to permanent hardness. This is due to increasing amounts of chlorides and sulphates, and less bicarbonates of calcium and magnesium. This could lead to corrosion and fouling of pipelines.
2. The high TDS content of potable water could lead to health problems, and the RWB tries to adhere to the WHO's standard of 500 $mg.l^{-1}$ TDS.
3. The RWB is not in favour of softening the water supply as a solution, and are researching the possibility of alternative preventative measures. The cost benefits associated with management of water quality in South Africa have been reviewed by Steffen, Robertson & Kirsten (1989) from the original report by Heynike (1981). They reach the same conclusions, although their costing survey seems much more severe than does that of Heynike (1981).

Model analyses have shown that, hypothetically, the introduction of a new industry that doubles the diffuse salt generation within the catchment would increase the TDS concentration in the run-off by only a few % in the first year, thereafter increasing substantially in subsequent years (DWA, 1986). Operational models have been of tremendous aid in selecting appropriate release volumes and rates which would help to predict the water quality following different treatments, and ensure minimum wastage (Anon, 1987a). An operational model has

also been used in the Orange-Fish/Sundays River Transfer system to provide computer-aided decision making as regards flow attenuation and water quality. The model currently under development simulates flow and salt load using a simple mass-balance approach. River salinisation is simulated using salt loading coefficients which are specific to both a particular stretch of river channel, and the wetted channel perimeter. This model was able to predict the severity of salt loading in certain impoundments (Tylcoat & Forster, 1987).

The quality of the water supplied by the RWB would deteriorate if attempts were not made to isolate and desalinate brines and salts from the water environment. The recovery of potentially valuable by-products from concentrated effluent would provide desalination incentive (DWA, 1986).

The best solution to the rising salinity was found to be to blend appropriate quantities of Vaal Dam water (having low TDS) with Vaal Barrage water (high TDS), thus maintaining acceptable TDS levels. Blending has been found to be inexpensive, to provide a benefit/cost ratio of 25:1, and very little additional water is required (DWA, 1986). The advantages of blending have been explained by the development of a suite of hydrosalinity models for the evaluation of a wide range of ameliorative measures for improving the mineral quality of the water supplied to the PWV area. The PWV blending option proved to be the most cost-effective, and implementation of this scheme commenced at the beginning of 1988. It is expected that PWV consumers will derive an economic benefit running into hundreds of millions of rands per annum (Herold *et al.*, 1990).

2.3.2 Salinity in the Buffalo River catchment

The two major impoundments on the Buffalo River are the Laing and Bridle Drift Dams, both situated downstream of the urban and industrial area of King Williamstown-Zwelitsha, and the deterioration of the water quality is a cause for concern. It is estimated that treated effluent from da Gama textiles and the King Tanning Company increases the TDS concentration in the river by an average of 455 $mg.l^{-1}$, which amounts to 88% of the total non-natural load. There is limited control over the disposal of highly saline effluent by irrigation, and run-off from these irrigated areas and seepage and overflows from evaporation ponds constitute the major threat to the Laing Dam. Contingency plans to counteract this include the building of a pipeline to convey effluent for disposal to the sea. However, the ecological impacts of this measure will have to be carefully considered. Nevertheless, the DWA (1986) recommends that industries producing large volumes of saline effluent be sited near the coast. Where this not possible, such industries should be required to isolate this effluent from less saline effluent and treat it separately.

In a study by Selkirk & Hart (1983), the Buffalo River catchment was divided into 4 major regions. In the upper catchment areas, due to afforestation in the Amatola State Forest, there is little evidence of erosion above Maden Dam, the first major impoundment. Water quality in this area is thus upstream of any major anthropogenic influence. Downstream of the Maden Dam is the Rooikrantz Dam. Inputs from the Tyusha River, and overspills from the Maden Dam serve as water source for this impoundment. Feeder streams from the Rooikrantz River drain the surrounding pasture land, and some evidence of catchment erosion is present. There is very little evidence of human impact in this section of the catchment area.

The second study site stretched from the Rooikrantz Dam wall to the headwaters of the Laing Dam. This is a highly industrialised area includes the metropolitan areas of King Williamstown and Zwelitsha. Return flows of upstream irrigation water, pipe laying operations, urban run-off and industrial effluent discharge, and sewage-works effluent all manifest themselves in this part of the catchment. Physico-chemical manifestation included an increase in alkalinity. Conductivity ranged from 35.5 to 315 $mS.m^{-1}$ i.e. a TDS of approximately 230 to 2 080 $mg.l^{-1}$. The general trend was that of an increase in conductivity with distance from the upper catchment.

The Laing Dam is the first major impoundment on the Buffalo River. Major feeders include the Yellowwoods and Tshabo Rivers. The catchment area services large urban settlements, limited agricultural practices, and intensive goat farming. pH remained alkaline, dropping after rains. Conductivity increased gradually during the year, but dropped following the rains. In general, as with the previous study site, a gradient exists with increasing conductivity in the downstream reaches. This gradient reflects the downstream displacement of more mineralised waters by run-off entering the upper reaches of the impoundment.

Bridle Drift is the largest impoundment of the Buffalo River, receiving major inflows by overspill of the Laing Dam. The immediate catchment is well wooded, and the NE sector contains the Mdantsane residential area. Three perennial streams transfer run-off from Mdantsane to the impoundment. Again, there seemed to be a gradual increase in conductivity with an increase in distance from the source due to downstream mineral displacement.

Selkirk & Hart (1983) thus reached the following conclusions regarding impoundment of the Buffalo River:

1. There is a drastic change in water quality in the lower reaches of the Buffalo River due to agricultural perturbation of the catchment.
2. As the river enters King Williamstown, there is a significant increase in TDS above the desirable limit for potable water of 500 $mg.l^{-1}$ set by the WHO. These TDS concentrations continue to rise as the industrial and other discharges reach the river.
3. By the time the water enters the Laing Dam, the water quality has deteriorated below acceptable levels. In the dam, the water is self-purified, followed by chemical treatment. The water is then returned to the town for reuse.
4. It is expected that the water quality of the Laing Dam will deteriorate with time. If the retention time of Laing Dam is decreased then water will flow into the Bridle Drift. The authors witnessed such a discharge, when the conductivity more than doubled in the lower impoundment after water was displaced from the Laing.
5. Closed loop operation of Laing Dam offers certain immediate advantages to Bridle Drift Dam, although it results in progressive mineralisation of the Laing Dam waters.
6. Increased sodium concentrations observed in the waters are probably the result of elution from the soils of the catchment.

The increase in conductivity noted by Selkirk & Hart (1983) has been confirmed by Palmer (1991), who found that impoundment by the Laing Dam resulted in increased downstream conductivities. This is a reflection of the poor water quality flowing into the Laing Dam, as well as the residence time within the reservoir. Palmer (1991) summarises the conductivity fluxes observed by a number of authors. Conductivities recorded in the middle and lower

reaches of the Buffalo River have increased steadily from 20 $mS.m^{-1}$ in 1950 to the present median of 55 $mS.m^{-1}$. In 1970, a flood reduced conductivities in Laing from 50 $mS.m^{-1}$ to 21 $mS.m^{-1}$, indicating that a large proportion (over 50%) of increased salinity in the Buffalo River can be attributed to human activities rather than natural salinisation.

2.3.3 Salinisation of the Fish/Sundays River system

There has been an observed increase in natural salinisation of the Fish/Sundays River system, mainly due to irrigation run-off, salt leaching and saline return flow, and the geomorphological status of the surrounding rock. The levels in this river system are approaching, and, at certain times, exceed acceptable levels. This problem has been partly alleviated by the introduction of water from the Orange River, which has resulted in dilution and thus a lowering of the dissolved mineral content of the water. The importing of Orange River water could increase the water supply of the Sundays River by 207 million $m^3.a^{-1}$, which is estimated to satisfy the water demand in this area until the year 2028. This imported water may in future be replaced by water from the Transkei if the Orange River water is required for increasing demands in the central area of the country. Due to the increasing shortage of freshwater and the high costs of aquaducts, research needs to be conducted into the removal of salts at the point source. Of prime importance seems to be the development of a hydrosalinity model for the evaluation of the water resource planning in this area (DWA, 1986).

Unfortunately, more detailed work as regards the monitoring and troubleshooting of the rising mineral content of the Sundays River (specifically the lower reaches) needs to be undertaken before conclusive proposals can be made as regards the amelioration of the problem in these waters (Tylcoat, 1985).

2.3.4 Treatment of saline effluents

There are a number of advanced technologies for the treatment of industrial brine effluents. These are reviewed by Hart *et al.* (1987) and Neytzell-de Wilde *et al.* (1987a, 1987b) and include:

1. Membrane desalination is being used more and more extensively, and includes reverse osmosis (RO) or hyperfiltration (HF) (producing a high quality water stream and a concentrate containing organic and inorganic constituents), ultrafiltration (UF) (producing a saline water stream and an organic concentrate), electrodialysis (ED) (producing an organic water stream and a saline concentrate), and cross-flow microfiltration (CFMF). These processes differ in the size of molecules to be separated from the aqueous medium. Various degrees and intensities of application are dictated by the origin and chemical make-up of the effluent to be treated.

   For the augmentation of natural resources, long-term research is also being directed into the feasibility of large-scale sea-water desalination and iceberg utilisation in some coastal areas. Pretreatment of sea-water prior to RO has also been highlighted as a potential area of investigation (DWA, 1986). Distillation remains the most widely applied process for seawater desalination, and is reasonably economical if low grade heat is available (Botha, 1984).

2. Chemical oxygen processes are implemented for high strength organic wastes or effluents containing significant amounts of non-biodegradable material for which biological oxidation is inadequate (Stewart, Sviridov & Oliver, 1986). Most recently, an aerobic reactor has been used at the Potsdam wastewater treatment plant for a dual digestion process. Pretreatment of the sludge with oxygen results in a significant reduction in the retention time in the anaerobic part of the process (Anon, 1989).

3. Adsorption technologies are used for the removal of dissolved contaminants by phase transfer. Ion exchange falls under this category, and because of the limited capacity of available resins, the process is limited to relatively low salinity brackish waters (<1000 $mg.l^{-1}$) (Botha, 1984).

4. Thermal separation processes are used for the evaporative concentration of various pollutants from water. Reject heat from power generation has also been identified as a potential means of desalination of saline mine water (Stewart, Sviridov & Oliver, 1986). Four basic types of evaporators are used commercially, and all have the inherent problem of corrosion and scaling. Steps including pH control by acid addition and the use of scale inhibitors have to be taken to overcome them (Botha, 1984).

5. Freezing Process have also been used with limited success (Botha, 1984).

Taking the economics of desalination into account, present technology does not appear to provide a solution to the problem of providing an acceptable quality water for irrigation or even stock watering. It seems, however, to be technically viable for domestic and industrial applications, thus providing access to an unconventional source of water (Botha, 1984).

2.3.5 Brine disposal

The drawback of desalination is the production of highly saline, small-volume brine solutions, creating further problems with final effluent disposal (DWA, 1986). Three disposal methods for brine concentrates have been considered, namely disposal to inland brine dams, discharge in deep disused gold mines, and coastal discharge through and overland pipe over a 600 km distance. The prerequisite is that the brine be in as small a volume as possible. The chemical species of the brine can be disposed of in the following manners (in order of increasing environmental impact):

1. Engineering out the source of the brine
2. Converting the brine into a saleable product
3. Indirect or diffuse discharge to the environment
4. Deactivation or conversion to an inert substance
5. Immobilisation or passive storage
6. Direct discharge to the environment (Buckley *et al.*, 1987).

More research emphasis is being placed on the marine disposal option for discharging the domestic and industrial effluent that result from increased coastal development. This area of interest has been kindled by the limited space available for effluent treatment, and the economic benefits associated with a marine discharge system. A prerequisite for marine disposal is the sub-level disposal with efficient diffusers (Fijen, 1989)

The large-scale implementation of desalination technology could therefore create serious brine disposal problems. This may require initial State or Regional Services council involvement in the establishment and operation of regional facilities, which could be privatised as soon as it becomes economically viable (Stander, 1987).

3. LEGISLATION REGARDING SOUTH AFRICAN WATER QUALITY

The South African Bureau of Standards (SABS) gives a recommended conductivity limit of 70 $mS.m^{-1}$ for water used for domestic purposes, which is equivalent to TDS values of 350-550 $mg.l^{-1}$. The maximum allowable conductivity limit for domestic use under extreme circumstances is 300 $mS.m^{-1}$, equivalent to a TDS concentration of 1950 $mg.l^{-1}$. Section 21 of the Water Act, 1956 promulgates effluent standards applicable to direct discharges into fresh water sources. It sets a limit on the conductivity of discharged effluent at 75 $mS.m^{-1}$ above that of the intake water. In respect to mining effluent, conductivity limit is set at 250 $mS.^{-1}$. Although much of the potable water in South Africa exceeds the allowable limit suggested by the SABS, this is of particular concern in the PWV area where pollutants are generated by man. This provides grounds for concern due to the possible synergistic effects of combinations of contaminants, as well as the effects of individual and combinations of organic compounds that may be present (DWA, 1986).

Due to the general decline in the water quality, the Water Act of 1956 was amended during 1984. In terms of section 21, a maximum increase of 75 $mS.m^{-1}$ above that of the intake water is acceptable. A special standard of no more than 15% increase in conductivity above that of the intake water is applicable in a number of selected rivers. In both General and Special Standard rivers, the upper limit to seepage from industrial or mining concerns is 250 $mS.^{-1}$. However, in the case of industrial effluent discharge to municipal sewers, exemption from the above standards is implied. An opportunity thus exists for dilution and discharge of highly saline effluents. In such cases, section 21 provides for the withdrawal of exemption. Thus the legislative act would override the municipal acceptance of such effluents (Stander, 1987).

Section 22 is aimed at control over diffuse sources of pollution such as contamination of urban and industrial run-off. Bad management of point sources often leads to diffuse pollution. The most important part of the proposed regulations is the prevention of diffuse pollution due to bad effluent handling and disposal practices (Stander, 1987).

Section 24 enhances the requirements and provisions of Sections 21 and 22 (Stander, 1987).

4. ECOLOGICAL RESPONSES TO RIVER SALINISATION

Secondary salinisation refers to the increases in salinity that are caused by human disturbance of the natural hydrological cycle. These are often low-level changes, resulting in increases in the salinity of the aquatic system from < 300-500 $mg.l^{-1}$ to between 300 and 10 000 $mg.l^{-1}$. The change in salinity in the receiving river depends on four factors, namely:

1. The salinity and ionic composition of the wastewater
2. The manner in which the wastewater is discharged (continuous or pulse releases)

3. The volume discharged, and
4. The flow conditions in the stream at the time of discharge (Hart *et al.*, 1990).

4.1 General lotic ecology

In order to understand the responses of rivers to increasing salinity, it is necessary to first grasp the essentials of lotic ecology. The effects of salinity on the different lotic communities are also discussed. This is done with a great deal of speculation due to the lack of scientific data needed to assess the biological effects of saline discharges (Hart *et al.*, 1991).

Different processes control different areas of the lotic system. Headwaters are characteristically well-shaded, and thus little primary production occurs in the streams. The primary food source is particulate organic matter (leaves, bark, twigs, etc.) and dissolved organic matter derived from the surrounding forest. The particulate organic matter is either directly consumed by certain macroinvertebrates (shredders and collectors), or decomposed by the microbial population (bacteria, fungi, protozoa). The resultant surface microbial films are, in turn consumed by other macroinvertebrates (scrapers, e.g. caddisfly larvae, snails). The macroinvertebrates are the primary food source for the secondary consumers (Hart *et al.*, 1990).

The highly shaded upper reaches are classified as heterotrophic, and are often characterised by low P/R ratios. They are thus net importers of energy (Hart *et al.*, 1990).

The lower reaches of the stream are wider and less shaded, and primary production is thus greater, making the stream an autotrophic system characterised by macrophytes, epiphytes and algal films. The P/R ratios are usually high, and the streams have a net export of energy. Lowland waters develop phyto- and zooplankton communities (Hart *et al.*, 1990).

Hart *et al.* (1990) has summarised the six main communities identified in streams:

1. Autotrophic communities are made up of two divisions: microalgal and macrophytes. Microalgae form both the periphyton and the phytoplankton communities. Macrophyte distribution is limited by current speed. As salinity increases the diversity of macrophyte species declines. Individuals of a particular species decreases in vigour depending on the salt sensitivity of the species. At salinities around 4000 $mg.l^{-1}$ most freshwater macrophytes disappear, being replaced by halophytic species which persist over a wider range of salinities. It is expected that microalgal species will exhibit the same response.

2. Riparian vegetation communities are associated with lowland rivers, and represent riverbank plant communities. The majority of macrophyte species are salt sensitive, inhibition setting in around salinity levels of 1000 $mg.l^{-1}$. Upper tolerance level seems to be around 4000 $mg.l^{-1}$.

3. Saprophytic communities include bacteria, fungi and protozoans. They colonise and break down particulate organic matter, which, in turn, may be consumed by detritivores. There is no evidence to indicate that small changes in salinity will have a deleterious effect on microbial populations. Bacteria are adept at salt adaptation, or the freshwater species will be replaced by similar salt-tolerant species.

4. Detritivore communities consist of micro- and macroinvertebrates that ingest particulate detritus, either in particle form, or from film layers. Macroinvertebrate detritivores can be divided on the basis of their food ingestion and selection. The three functional feeding groups include collectors, shredders and scrapers. The macroinvertebrates seem to be the most sensitive to water quality changes, toxic effects occurring in excess of 1000 $mg.l^{-1}$. The salt-sensitive species include simple multi-cellular species, insects and molluscs. Crustacea are the most tolerant. Simple multicellular organisms are salt sensitive due to their small size and simple osmoregulatory mechanisms, and include *Hydra* spp., flatworms and segmented worms. Amongst the insects, dragonflies, mayflies, stoneflies, caddisflies and certain waterbug species are the most salt sensitive species. In the molluscs, the gastropods are probably the most salt sensitive.

5. Herbivore communities consists of animals that eat living plant matter. Specialised animals called piercers pierce algal cells and ingest their contents, and include species mentioned under the previous community . These have been grouped together due to the limited information available on the structure and functioning of the macroinvertebrates in lowland streams and rivers.

6. Predator communities include invertebrates, fish and some higher vertebrates which feed predominantly on detritivores (Hart *et al.*, 1990). The main predators in lowland river systems that could be affected by increasing salinity include invertebrates and fish. Direct effects due to salinity occurs at salinity in excess of 1000 $mg.l^{-1}$. Adult fish appear quite tolerant of salinity, with adverse effects only setting in around 10 000 $mg.l^{-1}$. Members of the predator community feed mainly on the detritivore community, and therefore salinity indirectly affects predators.

4.2 Physiological adaptations to salinity fluxes

It seems that pulsed releases of saline wastewater, where the salinity of the river is increased rapidly and then falls, will have a greater effect on the biological communities than continuous release. Biota can tolerate higher salinity increases if they are given time to acclimate, although not much substantiation of this theory has been put forward. The range of tolerance is obviously dependent on the natural environment of the organism e.g. marine, estuarine or freshwater animals, and the salinity range normally encountered by the animal. There is a general lack of data on many potentially salt-sensitive species, and a lack of studies on the long term and sub-lethal effects of salt (Hart *et al.*, 1990).

Nevertheless, it is known that plants and animals have developed a wide range of physiological mechanisms and adaptations to maintain the necessary balance of water and dissolved ions in cells and tissues. Osmoregulation of salt and water content is important for processes involved in (de)hydration, metabolism, permeability and active transport, and cell excitability (Dorgelo, 1981). Thus, high salinity is lethal to freshwater organisms because the cells of the organism have either a lack of water or an excess of ions that can result in a range of toxic effects (Hart *et al.*, 1991).

The organisms ability to regulate the optimal internal osmotic concentration against external gradients determines the salt tolerance of the species. Good regulators are euryhaline, poor ones are stenohaline (Schmidt-Nielsen, 1975). The concentration of ions in the body fluids

of most invertebrates is above that of the surrounding medium. These animals are hyperosmotic. Water is gained by osmosis, and ions lost by diffusion. The animal counteracts this by excreting dilute urine and actively taking up ions. Freshwater organisms are therefore hyper-osmotic. If an animal has a lower osmotic concentration than the medium, it is said to be hypo-osmotic (Schmidt-Nielsen, 1975). Organisms inhabiting saline environments are hypo-osmotic regulators, excreting only small amounts of concentrated urine and possessing a range of mechanisms to exclude salt (Hart *et al.*, 1991).

Many invertebrates found in inland waters are of marine ancestry, having colonised from the sea via brackish estuarine waters. Recent colonists have a lower salt tolerance reflected in their high permeability to salts and water, and the greater expenditure of energy to maintain their ionic balance. Freshwater invertebrates are hyperosmotic regulators and cannot maintain their body solute concentration below that of the water they live in. As the salinity of the external medium increases they take up more and more and more ions. As a consequence, water is lost from the cells until the animal dies. The salinity at which death occurs varies according to the normal blood ionic concentration of the species. Generally, osmoregulatory mechanisms of most freshwater invertebrates fail when animals are exposed to solutions containing salts in excess of 9000 $mg.l^{-1}$, although most experience deleterious physiological, biochemical and behavioral effects at far lower salt concentrations (Hart *et al.*, 1991).

Organisms that are subject to changes in the medium ionic concentration possess one or more of the following mechanisms:

1. Detection and avoidance of changes in salinity;
2. Tolerance of extreme conditions on a short-term basis;
3. Physiological and morphological adaptation to medium changes;
4. Reduction in length of larval life, or suppression of the larval phase;
5. Responses of the larvae to salinity (Lockwood, 1976).

Physiological adaptations to a fluctuating environment are often supplemented by behavioral responses which enable animals to delay, avoid or moderate exposure to extreme conditions. This response is normally evident in estuarine organisms. Bivalves are known to close their shell valves, certain snails withdraw into their shells, and mud-dwelling forms retreat into their burrows or pull in their siphons. Euryhaline species adopt one of the following mechanisms to counteract increasing medium solute concentrations:

1. Volume regulation and flexibility in the rate of urine production,
2. Regulation of intracellular free amino acid and inorganic ion levels so as to maintain cell volume within narrow limits,
3. Maintenance of the body fluids hypotonic to highly saline media by:
    1. Active transport of inorganic ions out of the animal,
    2. Production of urine hypertonic to the body fluids,
    3. Reduction of surface permeability to water and salt.

In short-term experiments, the metabolic fluxes are dependent on the salinity to which the animal was adapted, and the salinity of the new environment. Three main categories of response follow increases in salinity:

1. Increased metabolic rate (e.g. *Palaemonetes varians* and *Metapenaeus monoceros*),
2. Reduction in oxygen uptake (e.g. *Asterias*)
3. Metabolic rate more or less unaffected by salinity variation (e.g. *Artemia salina* and *Theodoxus fluviatilis*).

The ability to vary oxygen consumption in response to changes in the medium necessitates that the organisms possess a measure of independence of oxygen levels in the water (Lockwood, 1976).

A variety of factors affect, or are hypothesised to affect the sensitivity to salt. These include:

1. The condition of the organism, i.e. reproductive state, stage within the moult cycle, sex, body size, nutritional state, all affect the ability to regulate cell solute concentrations in response to external salinities.
2. The period of acclimation also affects its salt tolerance. The longer an animal is exposed to a particular regime, the more tolerant it becomes. This is important in the consideration of release mechanisms of saline wastewater into freshwater environments.
3. Temperature affects the rate of acclimation and the general physiological condition of the animal. Hart *et al.* (1991) quotes a study of the prawn, *Macrobrachium holthuisi*, which exhibited a depression of oxygen consumption with increasing salinity beyond a critical point. The critical salinity level decreased with increasing temperature. At the higher salinities, behavioral observations suggested that the metabolic rate depression indicated severe environmental stress. The metabolic rate depression at higher salinities was also found to be much more pronounced at higher temperatures. This was also noted in *M. rosenbergii*, where metabolic rates were found to be influenced by temperature, salinity, and temperature-salinity interaction (Nelson *et al.*, 1977).

4.3 Insects

The most important feature of insects that enable them to successfully colonise freshwater is the cuticle, which retards the diffusion of water and salts between the blood and the external medium (Hart *et al.*, 1991).

In a study of saline lakes in western Australia, the less saline waters contained a range of insects, the species richness being determined by the degree of salinity. The abundance of all insect species was low in all waters tested, except in a permanent/semi-permanent flowing saline stream (Geddes *et al.*, 1981).

Mayflies (Ephemeroptera) and stoneflies (Plecoptera) are generally restricted to well-oxygenated, fast-flowing streams and rivers. They are not generally found in waters above 1000 $mg.l^{-1}$, thought certain species of Ephemeroptera can occur in areas with salinities of 1000 - 2240 $mg.l^{-1}$, although their abundance is much lower than in water with lower salinities. Highest numbers are found in the range 28 - 460 $mg.l^{-1}$. It seems likely that pulses of highly saline wastewater would have deleterious effects on these groups (Hart *et al.*, 1991).

Most dragonflies (Odonata) are found at salinities < 500 $mg.l^{-1}$, although some species have been found in waters up to 2240 $mg.l^{-1}$ and as low as < 50 $mg.l^{-1}$ (Hart *et al.*, 1991).

Water bugs (Hemiptera) have colonised inland saline waters. certain species surviving up to 12 400 $mg.l^{-1}$. It is unlikely that changes in salinity would adversely affect these species (Hart *et al.*, 1991).

Most beetles (Coleoptera) are restricted to freshwater (100 - 1000 $mg.l^{-1}$), although a few forms have colonised saline waters. Certain species can tolerate salinities as high as 93 000 $mg.l^{-1}$ (Hart *et al.*, 1991).

Dipterans appear to be the only insect order that has successfully colonised highly saline water, as some species are capable of hypo-osmotic regulation. Certain species have been found at salinities of 70 000 $mg.l^{-1}$ (Hart *et al.*, 1991).

The low richness of species, enormous densities of a single species and resulting low values of diversity may be indicative of highly degraded water (Bunn & Davies, 1992).

4.4 Molluscs

Rippingale (1981) notes that there have been some changes in the mollusc populations of some Australian rivers, where freshwater species have been replaced by those typical of estuarine conditions.

Geddes *et al.* (1981) observed the occurrence of the halobiont gastropod *Coxiella* in saline lakes in western Australia, its distribution indicating considerable tolerance to temperature change and aridity.

The aquatic snail *Coxiella striata* has been recorded at salinities ranging from 500 - 112 000 $mg.l^{-1}$. Although Australian freshwater molluscs occur at salinities ranging from 400 - 2240 $mg.l^{-1}$, abundance is much lower at lower salinities (< 1000 $mg.l^{-1}$). Pulmonate gastropods appear to be the most salt sensitive species (Hart *et al.*, 1991).

Hart *et al.* (1991) report a study of the Australian freshwater mussels *Velesunio ambiguus* and *Alathyria jacksoni*, which belong to the family Hyriidae. It was found that as salinity increased, osmoregulatory mechanisms were lost, and specimens osmoconformed (the transition point being approximately 2000 $mg.l^{-1}$). Upon transfer to hyperosmotic conditions (4000 $mg.l^{-1}$) without acclimation the animals suffered metabolic stress.

4.5 Crustacea

The crustaceans are undoubtedly the most salt tolerant of the macroinvertebrates (Hart *et al.*, 1991), and severe salinisation of rivers may favour a shift from an insect to a crustacean-dominated system (Bunn & Davies, 1992). In a study of Western Australian salt lakes by Geddes *et al.* (1981), it was found that crustaceans were by far the most dominant group, and were represented by 21 species of ostracods, 10 of anostracans, 10 of copepods, 3 of cladocerans, and 1 species of Conchostraca and Amphipoda. At salinities below 50 ppt there were often 6 or more species. At salinities from 50-100 ppt there were 1-5 species, while at salinities above 100 ppt there were 3 or fewer. Except in the most saline environments where

Parartemia occurred alone, usually more than half the species were ostracods. Many of the species observed were halobionts.

Anostracans observed included 2 species of the freshwater genus *Brachinella* and 8 of the halobiont genus *Parartemia*. Cladocerans observed included mainly *Daphnia* and *Moina*. Three dominant genera of copepods were noted, namely *Boeckella* (normally found in fresh and slightly saline waters), *Calamoecia* (found in all saline waters), and *Microcyclops*. Twenty species of ostracods were collected from 12 genera in the freshwater family Cyprididae, making this the most diverse group encountered. Five species and genera were restricted to low salinities, the remaining 6 being halophilic or halobiontic (Geddes *et al.*, 1981). Timms (1981) comments that observations of saline lakes in Victoria, Australia, show there is always a decrease in number of species with increasing salinity.

Geddes (1975a) demonstrated that *P.zietziana* has a very broad salinity-temperature survival range, and that the species can survive direct transfer over wide salinity and temperature differences. There is very little correlation between the lower salinity tolerances observed in the laboratory and in the field. It seems that other factors aside from salinity are responsible for limiting the field distribution to highly saline waters. It was also shown (Geddes, 1975b) that *P.zietziana* possess extremely strong powers of hypo-osmotic regulation, the osmoregulative capacity being greatest in the mid-temperature range (10 - 20°C), being impaired at high and low temperatures. Geddes (1975b) also demonstrated that populations acclimated to more saline media proved to be better hypo-osmotic regulators than those from less saline waters and hence are better adapted to high salinity. A comparative study has shown that sodium and chloride are the dominant ions in the haemolymphs of all crustaceans, with Na^+ usually accounting for more than 90% of the total cations. A series of enrichment experiments showed that the ionic composition of the haemolymph is maintained independently of the ionic composition of the medium

Studies in the 1970's have shown that summer plankton in saline pools in Western Australia is dominated by *Sulcanus conflictus*, a copepod typical of brackish water. As *S.conflictus* is never collected from freshwater, it seems likely that its occurrence in the saline pools is as a result of the increase in salinity in the past century (Rippingale, 1981).

Brand & Bayly (1971) investigated the osmoregulation of 4 species of calanoid copepods. They found that the estuarine species *Gladioferens pectinatus*, *G. spinosis* and *S. conflictus* all regulate both hypo- and hyperosmotically at high and low salinities, respectively. At high salinities, the inland water species *Boeckella triarticulata* has a regulation curve converging on the isosmotic line, which eventually follows it exactly. Although it can withstand saline water, it cannot regulate hypo-osmotically.

Denne (1968) investigated the osmotic and ionic regulation in the prawns *Macrobrachium australiense* and *M. equidens*. It was found that the brackish-water *M. equidens* is a hyper-hypo-osmotic regulator. Its blood freezing point depression remains constant except at high and low salinities, where regulation breaks down, indicated by a sudden rise or drop respectively in blood osmotic concentrations at these salinities. *M. australiense*, a freshwater species, is a hyperosmotic regulator, maintaining its blood concentration at a higher level than that of the medium. Normal blood freezing point depression is maintained at lower salinities,

and the ability to regulate is lost at higher salinities. Both species were found to regulate their Na and Cl blood concentrations by means of urine, under certain conditions of ionic medium concentrations.

Branchinella australiensis, a freshwater anostracan, is a hyperosmotic regulator, and dies in salinities approaching its isosmotic point. *B. compacta*, occurring in slightly saline pools, is a hyperosmotic regulator in waters with a lower osmotic pressure, and an osmoconformer in water with a greater osmotic pressure (Geddes, 1973).

Born (1968) investigated the osmoregulatory capacities of two caridean shrimps. *Syncaris pacifica* is restricted to freshwater, and the most striking adaptation is urine markedly hypo-osmotic to blood. *Palaemon macrodactylus*, on the other hand, inhabits a wide range of saline estuarine waters, and blood and urine are isosmotic throughout the tested range of salinities.

In a field study of two New Zealand freshwater crayfish, Wong & Freeman (1976) found that both species under study (*Paranephrops planifrons* and *P. zealandicus*) maintain constant haemolymph concentrations which does not differ from species collected from inland streams of low ionic concentration from that of animals from coastal areas of higher concentration. *P. zealandicus*, however, maintains a significantly higher haemolymph concentration, and it is thought that this difference is related to the prevailing environmental temperatures. Dorgelo (1981) reviews a number of works on the relation between temperature and osmoregulation. He concludes that temperature can affect the different physiological processes involved in osmoregulatory adjustment, such as water and ion efflux and influx through the body surface, blood-cell interchange of water and ions, urine production rate and urination frequency, and the role of non-electrolytes. In most crustacea, temperature has no effect at and around iso-osmoticity (as in osmoconformers).

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Appendix 4.2

Nutrient cycling and guideline values

Ammonification is the process by which decaying plant and animal matter is decomposed by bacterial populations, with the subsequent release of ammonia (NH_3). Ammonia is also excreted by aquatic organisms as a product of metabolism (Cole, 1979). Total ammonia is the sum of un-ionized ammonia (NH_3) and the ammonium ion (NH_4^+). The toxicity of ammonia is directly related to the concentration of the un-ionized form (NH_3), while the ammonium ion has little or no toxicity (Williams *et al.*, 1986). The quantity of ammonia in the water is reliant on a number of factors such as pH, temperature, dissolved oxygen levels and the chemical composition or ionic strength of the aqueous medium. At low to medium pH values the ammonium ion dominates, but as pH increases ammonia is formed. However, the concentration of ammonia decreases with increasing ionic strength and increasing salinity. Levels of un-ionized ammonia in the range of 0.2 to 2 $mg.l^{-1}$ have been shown to be toxic to some forms of freshwater aquatic life. The ammonia guideline for aquatic life is therefore set at 1/10 of the lower range i.e. 0.02 $mg.l^{-1}$ NH_3. At 20°C and pH 7.0 and 7.5, concentrations of total ammonia ($NH_3 + NH_4^+$) which contain 0.02 $mg.l^{-1}$ NH_3 are 5.1 and 1.6 $mg.l^{-1}$ respectively (Train, 1979).

The ammonium ion (NH_4^+) is microbially converted to nitrate by nitrification in the following two stages:

$$NH_4^+ + 1\frac{1}{2}\ O_2 \longrightarrow NO_2^- + H_2O + 2H^+ + \text{energy}$$

$$NO_2^- + \frac{1}{2}\ O_2 \longrightarrow NO_3^- + \text{energy}$$

Nitrite

As nitrite is rapidly oxidized to nitrate, it is normally only present in trace amounts. Anthropogenic activities which may increase nitrite levels include industry, sewage effluents and certain types of aquaculture (Lewis and Morris, 1986). Little information is available on nitrite toxicity, but it is the etiologic agent of the illness known as methemoglobinemia.

Fish appear to display species-specific toxicity e.g. the 96 hour LC_{50} value for rainbow trout is 0.33 to 1.32 $mg.l^{-1}$ NO_2, and 7.6 to 9.9 $mg.l^{-1}$ NO_2 for fathead minnow. It is obvious that the toxicity of nitrite is highly dependent on the chemical composition of the water (Rand and Petrocelli, 1985). Current available standards are 0.2 $mg.l^{-1}$ (USA guidelines, 1988, and Canadian guidelines, 1987) and 0.1 $mg.l^{-1}$ NO_2 (UK guidelines, 1989) (Dallas and Day, 1993).

Nitrate

Nitrates are the end products of the aerobic stabilization of organic nitrogen, but may also enter water via sewage effluent, fertilizers and agricultural run-off. Current standards are 6.6 $mg.l^{-1}$ NO_3 (Special effluent standard for South Africa), and 398 $mg.l^{-1}$ NO_3 (USA, 1989 and Canadian guidelines, 1987) (cited in Dallas and Day, 1993).

Phosphate

Orthophosphates (PO_4^{3-}) occur in solution, in particles or detritus, and in the bodies of aquatic organisms (APHA,1992). They are released on the decomposition of detritus by bacteria, but may also be indicative of contamination by agricultural fertilizers. The recommended South African dissolved orthophosphate guideline for the protection of aquatic organisms is 0.3 $mg.l^{-1}$ PO_4^{3-} (Kempster *et al.*, 1980).

SASS3 method and analysis sheet

SASS3

River. . . . . Date . . . . . Time . .

Sampling point . . . . . .

Temp °C . . . . . pH . . . . . Cond.(mSm^{-1}) . . . .

Biotopes sampled:

SIC . . . (Type/time . . . . .)

Marg veg . . . Dom. sp . . . . . .

Aq veg . . . Species . . . . . .

SOOC . . .%, Sand . . . . Mud . . . . gravel . . . .

Other . . . . . .

Procedure Protocols:

1. If stones in current (SIC) all kickable, sample for 2 min., otherwise for maximum of 5 min.
2. Gravel - 1/2 min.
2. Marg/Aq veg, back & forward sweep 2 m.
3. Stones out of current (SOOC) kick +/- 1 m^2.
4. Sand/mud stir with feet & sweep net over disturbed area for 1/2 minute.
5. Any other biotopes - 1/2 min.
6. Complete top of form.
7. Tip net contents into tray. Remove leaves, twigs & trash.
8. Check taxa present on above list FOR THE LESSER of 15 minutes or 5 minutes since the last taxon was found.
9. Estimate abundances on scale:
   A- 1 to 10; B- 10 to 100;
   C - 100 to 1000; D > 1000
10. BEFORE LEAVING THE SAMPLING POINT CHECK THAT THIS FORM HAS BEEN FULLY COMPLETED!

** pH < 6.5, score 13; pH > 6.8, score 6.
* air breathing families.

	Score	Presence/ Abundance
Porifera	5	
Coelenterata		
Hydra sp.	1	
Turbellaria		
Planaria	5	
Annelida		
Oligochaeta	1	
Hirudinea		
Leeches	3	
Crustacea		
Amphipoda	15	
Crabs*	3	
Shrimps	8	
Hydracarina		
Hydrachnellae	8	
Plecoptera		
Nemouridae	12	
Perlidae	12	
Ephemeroptera		
Polymitarcyidae	10	
Ephemeridae	15	
Baetidae 1 spp or	4	
2 spp or	8	
>2 spp	12	
Oligoneuriidae	15	
Heptageniidae	10	
Leptophlebiidae	**	
Ephemerellidae	15	
Tricorythidae	9	
Prosopistomatidae	15	
Caenidae	6	
Odonata		
Chlorolestidae	10	
Lestidae	10	
Protoneuridae	10	
Platycnemidae	10	
Coenagriidae	4	
Agriidae	10	
Chlorocyphidae	10	
Zygoptera juvs.	6	
Gomphidae	6	
Aeshnidae	8	
Corduliidae	8	
Libellulidae	4	

				Score	Presence/ Abundance
Hemiptera					
Notonectidae*				3	
Pleidae*				4	
Naucoridae*				10	
Nepidae*				3	
Belostomatidae*				3	
Corixidae*				3	
Gerridae*				5	
Veliidae*				5	
Megaloptera					
Corydalidae				12	
Trichoptera					
Hydropsychidae	1 sp or			4	
	2 sp or			6	
	>2 sp			12	
Philopotamidae				10	
Polycentropodidae				12	
Psychomyiidae				8	
Ecnomiidae				8	
Hydroptilidae				6	
Other movable case larvae:					
case types	score	fam.			
1	8	1		8	
2	15	1		15	
3	20	1		20	
4	30	2		30	
5	40	2		40	
>5	50	3		50	
Lepidoptera					
Nymphulidae				15	
Coleoptera					
Dytiscidae (adults*)				5	
Elmidae (adults*)				7	
Gyrinidae (adults*)				5	
Haliplidae (adults*)				5	
Helodidae				12	
Hydrophilidae (adults*)				5	
Limnichidae				8	
Psephenidae				15	
Diptera					
Blepharoceridae				15	
Tipulidae				5	
Psychodidae				1	
Culicidae*				1	
Dixidae*				13	
Simuliidae				5	
Chironomidae				2	
Ceratopogonidae				5	

			Score	Presence/ Abundance
Diptera				
Tabanidae			5	
Syrphidae*			1	
Athericidae			13	
Empididae			6	
Ephydridae			3	
Muscidae			1	
Gastropoda				
Lymnaeidae*			3	
Melaniidae*			3	
Planorbidae*			3	
Physidae*			3	
Ancylidae			6	
Hydrobiidae*			3	
Pelecypoda				
Sphaeriidae			3	
Unionidae			6	
Sample score				
No. of families				
score/taxon (ASPT)				
Air breathers fam.				
Air breathers score				

Other families present

Appendix 4.3b

Habitat quality index sheet

HABITAT QUALITY INDEX

River: _____ Date: _____

Station name: _____ Time: _____

HABITAT PARAMETER	EXCELLENT	GOOD	FAIR	POOR
1. Bottom substrate/ available cover	Greater than 50% rubble, gravel, submerged logs, undercut banks, or other stable habitat. 16-20	30-50% rubble, gravel or other stable habitat. Adequate habitat. 11-15	10-30% rubble, gravel or other stable habitat. Habitat availability less than desirable. 6-10	Less than 10% rubble, gravel or other stable habitat. Lack of habitat is obvious. 0-5
2. Embeddedness	Gravel, cobble and boulder particles are between 0-25% surrounded by fine sediment. 16-20	Gravel, cobble and boulder particles are between 25-50% surrounded by fine sediment. 11-15	Gravel, cobble and boulder particles are between 50-75% surrounded by fine sediment. 6-10	Gravel, cobble and boulder particles are over 75% surrounded by fine sediment. 0-5
3. Biotope diversity categories	Stones in current; fringing vegetation; stones out of current; sand, mud or gravel all present. 16-20	Only 3 of the 4 categories present. Missing stones in current receive lower score. 11-15	Only 2 of the 4 categories present. Missing stones in current receive lower score. 6-10	Only 1 of the 4 categories present. If no stones in current, score 0. 0-5
4. Velocity/depth categories	Slow (<0.3 m.s^{-1}), deep (>0.5 m); slow, shallow (<0.5 m); fast (>0.3 m.s^{-1}), deep, fast, shallow habitats all present. 17-15	Only 3 of the 4 categories present (missing riffles or runs receive lower score than missing pools). 8-11	Only 2 of the 4 categories present (missing riffles/runs receive lower score). 1-7	Dominated by one velocity/depth category (usually pool). 0-3
5. Bottom scouring and deposition	Less than 5% of the bottom affected by scouring and deposition 12-15	5-30% affected. Scour at constrictions and where grades steepen. Some deposition in pools. 8-11	30-50% affected. Deposits and scour at obstructions, constrictions and bends. Some filling of pools. 4-7	More than 50% of the bottom changing nearly year long. Pools almost absent due to deposition. Only large rocks in riffle exposed 0-3
6. Pool/riffle and run/bend ratios	Variety of habitat. Deep riffles and pools. 12-15	Adequate depth in pools and riffles. Bends provide habitat. 8-11	Occasional riffle or bend. Bottom contours provide some habitat 4-7	Essentially a straight stream. Generally all flat water. Poor habitat. 0-3
7. Bank erosion potential (signs of erosion and gradient of side slopes)	Stable. No evidence of erosion or bank failure. Side slopes generally <30%. Little potential for future problems. 9-10	Moderately stable. Infrequent, small areas of erosion mostly healed over. Side slopes up to 40% on one bank. Slight potential in extreme floods. 6-8	Moderately unstable. Moderate frequency and size of erosional areas. Side slopes up to 60% on some banks. High erosion potential during extreme high flow. 3-5	Unstable. Many eroded areas. Side slopes >60% common. Raw areas frequent along straight sections and bends. 0-2
8. Bank vegetative stability	Over 90% of the streambank surfaces covered by vegetation or boulders and cobble. 9-10	50-70% of the streambank surfaces covered by vegetation, gravel or larger material. 6-8	25-49% of the streambank surfaces covered by vegetation, gravel or larger material. 3-5	Less than 25% of the streambank surfaces covered by vegetation, gravel or larger material. 0-2
9. Streamside cover (Dominant vegetation)	Dominant vegetation is shrub. 9-10	Dominant vegetation is of tree form. 6-8	Dominant vegetation is grass, forbes or reeds. 3-5	Over 50% of the streambank has no vegetation; dominant material is soil, rock bridge materials, culverts, or mine tailings. 0-2

Appendix 4.4

Microbial determinations

The bacterial inhabitants of water are not uniform and vary widely depending on variables such as salts and organic matter content, pH, turbidity and temperature. Aquatic bacteria are not a homogeneous group and their representatives are found in almost all orders of bacteria (Rheinheimer, 1992). For this reason, it is advisable to either target a group of bacteria for monitoring purposes, or to use a non-selective nutrient-enriched medium which gives an indication of the general bacteriological quality of the water. Fluctuations in the microbial community can then be monitored over the duration of the experiment. This method will therefore give a standard plate count of viable aerobic and facultative anaerobic bacteria in water samples (DWAF Analytical Methods Manual, 1992).

The test medium to be used for this purpose is Yeast Extract Agar (YEA), which can be obtained commercially and reconstituted according to the manufacturer's instructions.

Method

1. Dissolve YEA (quantity as directed) in 1 litre distilled water. Dispense 20 ml aliquots into screw-cap bottles and sterilize by autoclaving. This medium may be stored at 4°C, but must be used within 3 months.
2. Dispense 9.9 ml aliquots of Ringer's solution (quarter strength) (obtained commercially) into screw-cap bottles and sterilize by autoclaving.
3. Prepare 10 ml 1:100 and 1:10 000 dilutions of each water sample by adding 0.1 ml sample to 9.9 ml autoclaved Ringer's solution.
4. Aseptically pipette 1 ml of sample, or dilution thereof, into a YEA aliquot (agar must be molten to 45°C before addition), swirl and pour into a petri-dish. Each sample must be carried out in duplicate. When set, incubate plates at 37°C for 24 - 36 hours.
5. For counting, select plates containing 30-300 colonies.

Method after Biolab culture media catalogue and DWAF Analytical Methods Manual (1992).

Appendix 4.5a

Total alkalinity (TAL) determination

(titrimetric method suitable for all alkalinity concentrations)

The alkalinity of water is its acid-neutralizing ability. The equivalent quantity of strong acid required to neutralize these ions is therefore equal to the total alkalinity.

TAL is determined by the titration of the sample with a standard solution of a strong acid. This visual method makes use of an acid-base indicator. Phenolphthalein and total alkalinity can therefore be determined by titrating to an end point pH of 8.3 and 4.5 respectively. The end point pH must be between 4 and 5 and is dependent on the alkalinity and free carbon dioxide concentration (DWAF Analytical Methods Manual, 1992).

Reagents

1. 0.1 mol.l^{-1} hydrochloric acid solution. Dilute 10 ml concentrated HCl to 1 litre with deionised or distilled water.
2. Phenolphthalein indicator. Dissolve 0.5g phenolphthalein in 50 ml absolute ethanol and add 50 ml deionised water.
4. Mixed indicator solution. Dissolve 1g methyl orange and 2.5g indigo carmine in deionised water and dilute to 1 litre.

Method

1. Add 2-3 drops of the phenolphthalein indicator to 25 ml of sample in a conical flask. If the sample remains colourless, the phenolphthalein alkalinity is nil. If the sample turns pink, the alkalinity can be determined by titrating with the standard acid till the pink colour disappears.
2. Add a few drops of the mixed indicator to the same solution. If the colour of the sample changes to purple, the total alkalinity is nil. If the colour changes to green, then the total alkalinity is determined by titrating with the standard acid till the colour changes noticeably to grey.

Calculations

1. Phenolphthalein alkalinity (mg.l^{-1} $CaCO_3$)

$$= \frac{5 \times A \times M \times 10^4}{V}$$

2. Total alkalinity (mg.l^{-1} $CaCO_3$)

$$= \frac{5 \times B \times M \times 10^4}{V}$$

where,
A = ml standard acid solution required for the phenolphthalein end point of pH 8.3.
B = ml standard acid solution required for the mixed acid indicator end point of pH 4.5.
M = the acid concentration in $mol.l^{-1}$.
V = ml sample.

Method adapted from DWAF Analytical Methods Manual (1992) and Standard Methods for the Examination of Water and Wastewater (APHA, 1992).

Interpretation

1. Carbonate (CO_3^{2-}) alkalinity is present when phenolphthalein alkalinity is not zero but is less than total alkalinity.
2. Hydroxide (OH^{-1}) alkalinity is present if phenolphthalein alkalinity is more than half the total alkalinity.
3. Bicarbonate (HCO_3^-) ions are present if phenolphthalein alkalinity is less than half the total alkalinity.

Appendix 4.5b
Hardness determination ($mg.l^{-1}$ $CaCO_3$)

The preferred method for determining hardness is to compute it from separate determinations of calcium and magnesium (carried out by Institute for Water Quality Studies) as follows:

Hardness ($mg.l^{-1}$ $CaCO_3$) = 2.497 [Ca ($mg.l^{-1}$)] + 4.118 [Mg ($mg.l^{-1}$)]

Method after Standard Methods for the Examination of Water and Wastewater (APHA, 1992).

Appendix 4.6

Species lists for Mpumalanga rivers investigated during the SASS3 survey. Habitats surveyed include stones-in-current (SIC), marginal vegetation (MV) and stones-out-of-current (SOOC).

		Blyde River	Olifants River	Sabie River	Selati River
Phylum: ARTHROPODA					
Class: INSECTA					
EPHEMEROPTERA					
Heptageniidae	*Afronurus harrisoni*	*		*	
Tricorythidae	*Tricorythus* spp.	*	*	*	
Leptophlebiidae	*Choroterpes elegans*		*	*	
	Choroterpes nigrescens	*			
Baetidae	*Demoulinia* complex	*	*	*	*
	Centroptiloides			*	
	Acentrella spp.			*	
	Afroptilum excisum	*			
Caenidae	*Caenis* sp. A	*	*	*	*
TRICHOPTERA					
Hydropsychidae	*Cheumatopsyche thomasseti*		*	*	*
	Cheumatopsyche afra	*			
	Aethaloptera maxima		*		
	Macrostemum capense		*		*
Philopotamidae	*Chimarra* spp.	*		*,	
Leptoceridae	*Leptocerus* spp.			*	
Ecnomidae	*Ecnomus* spp.			*	
Hydroptilidae	*Hydroptila* spp.	*			*
HEMIPTERA					
Geriidae			*		*
Naucoridae		*			
Veliidae					*

		Blyde River	Olifants River	Sabie River	Selati River
DIPTERA					
Chironomidae	Tanytarsini	*			
	Orthocladiinae	*	*	*	
	Chironominae	*	*	*	*
	Tanypodinae	*	*		
Ceratopogonidae		*			
Tabanidae		*	*	*	*
Tipulidae			*		
Atherixidae		*		*	
Simuliidae	*Simulium damnosum*			*	
	Simulium rotundum			*	
	Simulium unicornutum			*	
	Simulium adersi		*		*
	Simulium vorax		*		
	Simulium ruficorne				*
COLEOPTERA					
Gyrinidae				*	
Elimidae larvae		*	*	*	
Helodidae		*			
PLECOPTERA				*	
ODONATA					
Libellulidae			*	*	*
Gomphidae		*		*	*
Coenagriidae		*	*		*
Class: CRUSTACEA					
Shrimps		*		*	
Potamonidae	*Potamonautes* spp.			*	*
Phylum: ANNELIDA					
OLIGOCHAETA		*	*	*	
HIRUDINEA		*		*	

		Blyde River	Olifants River	Sabie River	Selati River
Phylum: MOLLUSCA					
PELECYPODA					
Unionidae	*Corbicula* spp.	*	*	*	
Phylum: TURBELLARIA					
Planariidae		*			
Phylum: CHORDATA					
Superclass: PISCES		*			
	Chiloglanis pretoriae		*		
	Glossogobius callidus			*	
	Pseudocrenilabrus philander				*

CHAPTER 5

COLLABORATION AND PROJECT TEAM ACTIVITIES

Summary This chapter is a synthesis of collaboration with other organisations, and is a record of activities of the team responsible for this research project.

5.1 COLLABORATION

One of the most exciting aspects of this project has been contact, workshops and collaborative research with researchers and managers in the fields of water quality and riverine ecology.

Project team as referred to in this chapter:
Prof Jay O'Keeffe JO'K
Dr Carolyn Palmer CGP
Dr Patsy Goetsch P-AG
Mr Qurban Rouhani QAR
Mr Brenton Maart BM
Mr Kekere Soxujwa KS

5.1.1 Department of Water Affairs and Forestry (DWAF)

The artificial stream laboratory has only been completed in its present form because of enthusiastic support from DWAF. Mr Wouter van der Merwe (Manager, Scientific Services) has provided encouragement and direction since the project's inception. Design and construction was supported by Mr Hendrik Best (Managing Engineer, Development) and Dr Jan Jordaan (Chief Engineer, Design Services). Engineers from Hydraulics Studies designed and built the streams, and conducted the hydraulic calibration: Mr Bill Rowlston (Deputy Chief Engineer, Strategic Planning), Mr Witek Jezewski (Principal Engineer, Hydraulic Services) and Mr Peter Skotnicky (Control Industrial Technician, Hydraulics Laboratory).

Once the streams were commissioned the project team were challenged as to the selection of field sites, and variables for testing. After correspondence with Mr. van der Merwe and Mr Sakkie van der Westhuizen (Director, Water quality Management) a Technical Advisory Committee from the Institute for Water Quality Studies (IWQS) was established to ensure the results of the project, and plans for future directions would provide the results most useful to DWAF. Members of the committee are: Dr. Henk van Vliet (Director, IWQS), Dr. Philip Kempster, Mr. Dirk Roux, Mr. Sebastian Jooste, and Mr. Dana Grobler.

From this developed the second phase of the artificial stream project which commences in July 1995. The second phase involves a comparison of results of riverine macroinvertebrate and *Daphnia* toxicity tests. The *Daphnia* testing will be done at IWQS, and the results will published jointly (IWQS collaboration highlighted below). A summary of the aims of the second phase are:

1. To continue the investigation of salinity tolerances:
   a. To conduct acute laboratory tolerance tests (in the large and small artificial streams) on the freshwater limpet *Burnupia stenochorias*, and at least one

mayfly species.

b. To compare the results of the laboratory tolerance experiments with tolerances of riverine organisms in the field.

c. To repeat the acute tolerance experiments in the laboratory, on *Daphnia* spp.

2. To investigate tolerances to a metal ion with relatively well understood speciation chemistry (e.g. copper):

   a. To conduct acute laboratory tolerance tests (only in the small artificial streams) on the freshwater limpet *Burnupia stenochorias*, and at least one mayfly species, to increased concentrations of $CuS0_4$, and to model copper species present using MINTEQA2.

   b. To compare these results to existing copper tolerance data on *Daphnia* spp.; and to extend such testing if necessary.

3. To compare the tolerances of *Daphnia* spp. with the stream organisms tested, in order to assess and compare the scale (e.g. regional or site specific) at which stream macroinvertebrates and *Daphnia* spp. tolerance data can be used to develop and refine water quality guidelines for the natural environment.

4. To apply the tolerance data to refine water quality guidelines for the natural environment with regards to salinity; and to contribute to guidelines for copper.

5.1.2 Kruger National Park Rivers Research Programme (KNPRRP)

One of the directives from DWAF in regard to the artificial stream project was ensure that field research was conducted in the KNP, and that results were linked to the KNPRRP.

At the April 1992 artificial stream workshop it was recommended that an experimental facility be built at the KNP. The feasibility of this was assessed by CGP in August 1992 (Appendix 5.1), when the first raceway was tested using battery power in the banks of the Sabie river.

A paper on the development of the artificial stream system to investigate the use of macroinvertebrates as water quality indicators was presented by CGP at the KNPRRP Second Annual Conference in Pretoria in June 1992.

Tolerance experiments have been conducted in the KNP on two occasions during this method development project (Chapters 4 and 8). In the second phase of artificial stream research, collaboration with the KNPRRP will remain a priority.

The water quality sub-programme of the KNPRRP has identified the investigation of the tolerances of organisms from the KNP rivers as one of the priority research needs (JO'K is water quality sub-programme leader, and CGP has attended meetings.)

In July 1994 CGP JO'K attended a workshop run under the auspices of the KNPRRP on a catchment process model HSPF. The workshop was presented by Dr Rob Johanson (Pacific University, California). The model differs from other hydrological models currently in use in South Africa in that it has a chemical modelling capacity. The link between HSPF and tolerance research is that HSPF can be used to describe the likely duration of particular conditions in a specific river. If we can relate this to tolerances of organisms it makes a

possible link between laboratory tolerance testing, conditions in the river, and catchment processes. CGP has arranged to work with Dr Mark Dent (Computer Centre for Water Research, University of Natal) on using tolerance data from Sabie River invertebrates in an initial modelling exercise for the planned Sabie river IFR workshop in 1995.

5.1.3 Water quality guidelines

The results of tolerance testing are directly applicable to the development and refinement of environmental water quality guidelines. CGP and P-AG have been sub-contracted on the guidelines project. As tolerance results become available they will be incorporated into the development and refinement of environmental water quality guidelines.

5.1.4 Chemical speciation modelling

Metals have consistently been identified as an area which should be considered for tolerance testing. Any attempt to establish the toxicity of metal ions to South African macroinvertebrates will require that metal speciation be taken into account. Simplistically, metals occur in aquatic environments in a range of forms: soluble free ions, molecules, and ion pairs; and insoluble precipitates. Usually only the free ions are toxic. The concentration of the free ion in solution will not relate directly to the amount of metal added experimentally, but will change depending on the chemistry of the source water, and factors such as pH.

Chemical speciation modelling is a tool which enables a researcher to describe the concentrations of probable chemical species in water. The input data are from detailed chemical analysis of the source water. The first stage of modelling is the description of the possible species present. After that it is possible to model what would happen if components were added.

Metal ions are not the only chemical components with complex behaviour in solution. So, for example are sulphates. In preparation for work on the toxicity of metals in the second phase, we have made contact with the Pollution Research Group, Natal University, led by Prof Chris Buckley. Prof Buckley has completed a WRC-funded project to make the chemical speciation model MINTEQA2 accessible to researchers, and visited Rhodes first in February 1994, and then in May 1994 to present a MINTEQA2 course. This was organised by the IWR and attended by: CGP, JO'K, P-AG, BM, and QAR (IWR); Mr B Wilhelmi (Biochemistry, Rhodes); and Mr R Lalloo, Mr W Edwards, Ms A Stoll, Mr W Lewis, and Ms G Boschoff (Biotechnology, Rhodes).
Prof Buckley and Dr Carol Kerr are available to assist with the interpretation of the model output.

Speciation modelling depends on detailed chemical analysis which is expensive. Analyses of water from the KNP tolerance testing was completed by the IWQS. After exploratory modelling of sulphate ion chemistry, Mr Roux, Mr Jooste (IWQS) and Dr Peter Wade (Watertek, CSIR) recommended that this approach is best left until work with metal ions has been initiated. Dr Wade is a speciation specialist, and uses the CSIR speciation model JESS, which has a greater modelling range and capacity than MINTEQA2. He is available as an advisor and critic. Closer to home, Dr Ansie de Kok (PE Technikon) is also available for consultation on chemical matters.

As a result of these initiatives researchers on the artificial stream project are in a position to tackle metal ion toxicity research and speciation chemistry in phase 2.

5.1.5 Artificial stream for microbiological research

The International Centre for Waste Technology (Natal University) has a WRC-funded project on the development of an artificial stream system for microbiological-scale research on the breakdown of xenobiotic compounds. The project researcher, Mr Charles Hunter, visited the Rhodes Artificial stream in 1993, and there has been a useful exchange of ideas.

5.1.6 Water quality interest group

Within the South African Society of Aquatic Scientists (SASAQS), CGP has initiated the water quality interest group. Through this group Dr Steve Mitchell (WRC) organised a workshop on toxicity methods, this initiative was furthered at a workshop on aquatic toxicity at the SASAQS conference in July 1994, and in 1995 an e-mail interest group - ECOTOXA was established. In June 1994 CGP co-ordinated a one-day session at the Afriwater 94 conference on riverine water quality, which served as a useful forum for contact and communication.

5.1.7 Blackfly growth

Dr Robert Palmer (Agricultural Research Council) has a WRC- funded project to investigate the control of problem blackfly in the Orange River. The artificial stream system can provide the high velocities *Simulium chutteri* requires. Dr Palmer spent 10 days in July 1994 familiarising himself with the artificial stream laboratory and conducted preliminary growth experiments which he plans to continue in 1995.

5.1.8 Fish swimming speeds

Prof Tom Hecht (Department of Ichthyology and Fisheries Science, Rhodes university) has an honours student investigating the maximum speed mullet can swim, in order to make recommendations for the deign of fish ladders. The middle channel in a system is blocked off, and the velocity in the two outer channels increased until the fish are no longer able to make headway. This project is currently underway.

5.2 TRAINING

Two students have registered with the IWR, in association with the artificial stream project, under the supervision of CGP. In both cases the students are full-time employees upgrading their qualifications with the support of their employers.

5.2.1 Chlorine tolerance testing

Ms Margot Williams (Natal Technikon) is investigating chlorine toxicity, and the effects of chlorinated sewage on macroinvertebrate communities. Her project is funded by the FRD, and has provided opportunities for collaboration with Umgeni Water, Pinetown Municipality, and also with the Pollution Research Group (University of Natal).

5.2.2 Whole effluent testing with textile effluent

Mrs Tandiwe Zokufa (Dept. Public Works, Water Affairs, Bisho) will investigate the tolerances of invertebrates from the Buffalo river to textile effluent and desalinated textile

effluent from the Da Gama textile mill. This will be the first use of artificial streams for whole effluent testing.

5.3 RESEARCH TEAM ACTIVITIES

5.3.1 Water Research Commission steering committees

CGP is or has been a member of the following steering committees:-

* Development of standard laboratory organisms for water quality studies (Co-Project Leader).
* Development of a laboratory river model to determine the environmental impact of xenobiotic compounds.
* Effects of pollution on the physiology of fishes in the Olifants River (eastern Transvaal).
* Development of Rapid Biological Assessment of water quality impacts in streams and rivers.
* The effect of water quality variables on riverine biotas.
* Development of a guide to assess non-point source pollution of surface water resources in south Africa..
* An assessment of the ecological impacts of interbasin transfer schemes in dryland environments.

5.3.2 Contracts

The following contract work has been undertaken during the artificial stream project:

* Ingwelala Share Block: Effects of a dam being constructed upstream of the Nhlaralumi River (Division of Forest Science and Technology, CSIR). August 1992. J'OK and CGP.
* A limited bioassessment of water quality in the Buffalo River upstream and downstream of a tributary, the Mlakalaka stream, and in the tributary itself. (BKS Incorporated, Consulting Engineers) April 1994. CGP (with Ms M Uys, IWR).
* Advice on water quality planning to Richard's Bay Minerals. July 1994. JO'K and CGP.

5.3.3 DWAF, WRC and FRD workshops

The following workshops have been organised#, or attended:

* Artificial Stream design and planning workshop. April 1992. JO'K and CGP#.
* Instream Flow Requirements work session for the Umgeni River (DWAF). October 1992. J'OK and CGP.
* Heavy metals workshop (WRC). February 1993. CGP.
* Biomonitoring workshop (DWAF). August 1993. J'OK and CGP.
* Instream Flow Requirements work-session for the Olifants River (DWAF). October 1993. JO'K and CGP.
* Bioassay workshop (WRC). November 1993. CGP and P-AG.
* Instream flow requirements workshop for the Luvuvhu River (DWAF), July 1994. J'OK and CGP.
* River classification workshop. South Africa/Australia.(DWAF, FRD, WRC) February 1994. JO'K# and CGP.
* Practical Application of Water Quality Management Workshop, PETCRU - Port Elizabeth Technikon, April 1994. JO'K, CGP and P-AG.

Workshop presentations are recorded in section 5.4 below.

5.3.4 Courses

The following courses have been organised#, or attended:

* Hydrology for Ecologists. Rhodes, IWR. February 1993. J.O'K# and CGP.
* MINTEQA2. Rhodes, IWR. May 1994. J'OK, CGP#, P-AG#, BM, and QAR.
* HSPF. Pretoria University, KNPRRP. July 1994. JO'K and CGP.
* PRIMER - multivariate statistics. UCT October 1994. J'OK and P-AG.
* Wordperfect, Rhodes University Computing Centre, April 1994. KS.

5.3.5 Travel and international contacts

The following international travel has mainly been funded by the WRC (additional funding sources are acknowledged):

Visits to artificial stream facilities in England, Canada and USA. Additional funds (R10 000) from Anglo American Chairman's Fund Travel Grant. January 1992. CGP:

* Institute of Freshwater Ecology, Wareham, England.
  Contact: Dr. Michael Ladle.
  Large (52m circuits) outdoor, recirculating systems.
* University of Toronto, Toronto, Canada.
  Contact: Dr. Rosemary Mackay.
  Small (< 1 litre volume) recirculating systems.
* Canadian Centre for Inland Waters, Hamilton, Canada.
  Contacts: Drs. Kristin Day and Janice Metcalfe-Smith.
  Ecotoxicology methods, and small stainless steel recirculating units.
* University of Windsor, Windsor, Canada.
  Contacts: Drs. Jan Ciborowski and Lynda Corkum.
  Small perspex recirculating units, and bio-indicator techniques. **Used in raceway design.**
* Stroud Water Research Centre, Philadelphia, Pennsylvania, U.S.A.
  Contact: Dr. Bernard Sweeney.
  Large and small recirculating laboratory systems.
* Purdue University, Indiana, U.S.A.
  Contact: Professor Patrick McCafferty.
  Aquatic insect rearing techniques.

Visit to Australia by JO'K with South African delegation:

* Water Studies Centre, Monash University, Victoria, Australia.
  Contact: Professor Barry Hart.
  In channel through-flow systems for salinity research.
* Centre for Catchment and In-stream Research, Griffiths University, Brisbane, Australia.
  Contacts: Dr. Brad Pusey, and Professor Angela Arthington.
  Large, outdoor, through-flow channels.
* Rural Water Commission, Armadale, Victoria, Australia.
  Contact: Dr. G.L. Bennison.
  Large laboratory recirculating system. **Used as a basis for large system design.**

Visit to South Africa by Prof Barry Hart (Water Studies Centre, Monash University, Victoria, Australia). Prof. Hart evaluated the project aims and the results of the artificial stream research and model design workshop held in April 1992. His recommendations were: 1) to omit any research on community responses, and rather to concentrate on single species; 2) to evaluate behavioural responses as well as mortality; 3) to attempt chronic testing only in later phases of the project; 4) to instigate salinity as the first variable of interest; 5) to consider copper, zinc, lead and cadmium as possible metals for future investigation, and to take speciation chemistry in account when dealing with metal ions; 5) to consider investigation of nutrients and algal responses in the future.

Visit to Zimbabwe to present papers at a meeting of the Hydrobiological Society of East Africa. December 1992. JO'K and CGP.

Visit to England to present a paper at Leicester University during May 1993. J'OK.

Visit to Canada and USA to present a paper at a meeting of the North American Benthological Society. Additional travel funding from Anglo American Chairman's Fund (R3 000) and Sappi Ltd.(R2 000). May-June 1993. CGP:

Visits to: Dr Dan Kurtak, Chewelah, Washington (blackfly control); Kananaskis Field Station (Canadian national research facility);Prof Doug Craig, University of Edmonton (artificial stream design and application); Abbot Laboratories, Chicago (aquatic insect rearing).

Visit to Denmark to present stream design and KNP tolerance tests at a SETAC meeting. Invited to participate by Dr James Everts (FAO, Senegal) in a session on aquatic ecotoxicology in arid zones. Additional travel funding from: Richard's Bay Minerals (R3 039); Anglo American Chairman's Fund (R3 000); Sappi Ltd (R5 000). June 1995, CGP and P-AG. P-AG will continue to visit the EAWAG ecotoxicity laboratories in Switzerland.

Invitation to SETAC in Vancouver November 1995 by Prof John Chapman (Centre for Ecotoxicology, New South Wales, Australia) to present our research in a session on Aquatic Toxicology: application and research in warm climates. CGP will find external funding to attend.

5.4 PUBLICATIONS AND PRESENTATIONS

* signifies publications relevant to this project.

5.4.1 Publications 1992-1995

Refereed journals

PALMER, C G AND O'KEEFFE, J H (1992) Feeding patterns of four macroinvertebrate taxa in the headwaters of the Buffalo River, eastern Cape. Hydrobiologia 228: 157-173.

PALMER, C G, O'KEEFFE, J H, PALMER, A, DUNNE, T, AND RADLOFF, S (1993) Macroinvertebrate functional feeding groups in the middle and lower reaches of the

Buffalo River, eastern Cape. Part 1: Dietary variability. Freshwater Biology 29: 441-453.

PALMER, C G, O'KEEFFE, J H, AND PALMER, A (1993) Macroinvertebrate functional feeding groups in the middle and lower reaches of the Buffalo River, eastern Cape. Part 2: Functional morphology and behaviour. Freshwater Biology 29: 455-462.

PALMER, C G, PALMER, A, O'KEEFFE, J, AND PALMER, R (1994) Macroinvertebrate community structure and altitudinal changes in the upper reaches of a warm temperate southern African river. Freshwater Biology 32:337-347.

* PALMER, C G, ROWLSTON, W S, JEZEWSKI, W A, AND SKOTNICKY, P I (1994) The design and research potential of an artificial stream system for the investigation of macroinvertebrate water quality tolerances. Water SA 20 (3): 247-258.

Non-refereed reports

* PALMER, C G, AND ROUX, D J (1993) Toxicity testing as a form of biomonitoring. In: Proceedings of a workshop on aquatic biomonitoring Ed. RGM Heath. HRI Report N0000/00/REQ/2893. Hydrological Research Institute, DWAF.

* PALMER, C G, ROUHANI, Q A, GOETSCH, P-A, AND O'KEEFFE, J H (1994) Development of a recirculating artificial stream system to investigate the use of macroinvertebrates as water quality indicators. Progress report (January 1993 - June 1994) submitted to the Water Research Commission.

O'KEEFFE, J H, PALMER, C G, AND HUGHES, D A (1994) Water quality modelling. Letaba River IFR workshop document.

O'KEEFFE, J H, PALMER, C G, AND HUGHES, D A (1994) Water quality in the Luvuvhu River, and methods of predicting the effects of flow modification. Luvuvhu River IFR workshop document.

PALMER, C G, AND O'KEEFFE, J H (1994) Aquatic invertebrates of the Luvuvhu River, and their habitat requirements. Luvuvhu River IFR workshop document.

Popular articles

* PALMER, C G (1993) Artificial stream laboratory constructed at Rhodes. SA Water Bulletin 19: 20-21.

* PALMER, C G (1994) The first artificial stream laboratory. Water February: 8-9.

* PALMER, C G (1994) The development of an artificial stream laboratory at Rhodes University, and its use in water quality management. The Naturalist 38 (1): 26-30.

Publications in preparation and in press

GOETSCH, P-A, STRYDOM, F G, AND DEACON, A (1994) Water quality of six main rivers of the Kruger National Park as monitored during the drought of 1991-1992. In preparation.

PALMER, C G (1994) Setae and microtrichia - structures for fine particle feeding in aquatic larvae. Accepted for publication in Microscopic Anatomy of Invertebrates, Vol 11A Insecta. Eds. F Harrison and M Locke. John Wiley and Sons, New York.

PALMER, C G, MAART, B, PALMER, A R AND O'KEEFFE, J H (In Press) An assessment of macroinvertebrate functional feeding groups as water quality indicators in the Buffalo River, eastern Cape Province, South Africa. Hydrobiologia.

Posters

WELDRICK, S, AND PALMER, C G (1992) A structural classification of setae used by brushers and filterers for the ingestion of fine organic particles. Annual Congress of the Southern African Society for Aquatic Sciences, Cape Town.

* ROWLSTON, W S, PALMER, C G, JEZEWSKI, W, AND SKOTNICKY, P (1993) The design and development of a recirculating artificial stream for the investigation of water quality tolerances of South African riffle dwelling macroinvertebrates. Poster presented at NABS, Calgary; SASAQS, Johannesburg and Water, the Lifeblood of Africa, Zimbabwe.

* GOETSCH, P-A, PALMER, C G, AND ROUHANI, Q A (1994) The survival of Sabie River macroinvertebrates in Selati River water. Symposium on integrated river basin management in the Olifants River basin. October 1994.

5.4.2 Presentations 1992-1995

* PALMER, C G (1992) Development of a recirculating experimental stream system to investigate the use of macroinvertebrates as water quality indicators Kruger National Park Rivers Research Programme. Second Annual Conference Pretoria.

PALMER, C G (1992) The functional morphology of selected southern African mayfly brushers and filterers. Annual Congress of the Southern African Society for Aquatic Sciences, Cape Town.

PALMER, C G, MAART, B, PALMER, A R, AND O'KEEFFE, J H (1992) An assessment of macroinvertebrate functional feeding groups as water quality indicators in the Buffalo River, eastern Cape Province, South Africa. Biodiversity, Production and Conservation of Aquatic Ecosystems in Africa. Harare.

PALMER, C G, O'KEEFFE, J H, AND PALMER, A R (1993) Macroinvertebrate functional feeding groups in the Buffalo River: dietary variability and feeding behaviour and morphology. Congress of the North American Benthological Society, Calgary, Canada.

PALMER, C G, O'KEEFFE, J H, PALMER, A R, AND PALMER, R W (1993) Macroinvertebrate community structure and elevation changes in the upper reaches of a warm, temperate southern African river. Presented at the congress of the North American Benthological Society, Calgary, Canada.

* PALMER, C G, ROUHANI, Q A, AND O'KEEFFE, J H (1993) The physico-chemical calibration of an artificial stream system. Southern African Society for Aquatic Scientists, Johannesburg.

* PALMER, C G ,ROUX, D J, VAN VUREN, J, DU PREEZ, H, SLABBERT, L, AND KÜHN, A (1993) The role of ecotoxicology in a national biomonitoring programme. Biomonitoring workshop, Roodeplaat Dam.

* PALMER, C G (1993) The use of an artificial stream in ecotoxicology. WRC Ecotoxicology workshop, Pretoria.

* PALMER, C G (1993) The use of an artificial stream system to investigate the toxicity of heavy metals to river invertebrates. WRC Heavy metals workshop, Johannesburg.

PALMER, C G, AND O'KEEFFE, J H (1993) Water resources of Region D Water 2010 conference, Grahamstown.

O'KEEFFE, J H, GOETSCH, P-A, WEEKS, D C, AND, FOURIE A (1993) A preliminary comparison of the Letaba and Sabie-Sand Rivers. Presented at the Third Annual Workshop of the Kruger National Park Research Programme, Magaliesberg, South Africa, September 1993.

* GOETSCH, P-A, AND PALMER C G (1993) Tolerances of selected macroinvertebrates of the Olifants River. Presented at Bioassay Workshop in Pretoria, South Africa, 11 November 1993.

* PALMER, C G (1994) The use of an experimental stream system to test riverine invertebrate responses to changing water quality. Afriwater 94 conference, Johannesburg.

* PALMER, C G, AND ROUHANI, Q A (1994) Calibration of an artificial stream; and the effects of handling procedures on test organisms. SASAQS conference, Port Elizabeth.

PALMER, C G, AND PECKHAM, B (1994) SA Water Act: 1) Environmental concepts and their implications; and 2) a review if existing legislation and suggested changes. SASAQS conference, Port Elizabeth; Law Teachers Society conference, Port Elizabeth.

PALMER, C G, ROWLSTON, W S J (1994) Water for the environment. Border Kei Development Forum Environmental Awareness Workshop, East London, October 1994.

* GOETSCH, P-A, PALMER, C G, AND ROUHANI, Q A (1995) The survival of Sabie River macroinvertebrates in Selati River water. To be presented at the Joint Symposium of the ZSSA and SASAQS at Grahamstown, South Africa, 27-30 June 1995.

* GOETSCH, P-A, AND PALMER, C G (1995) Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. To be presented at the Fifth SETAC Europe Congress, Copenhagen, Denmark, 24-28 June 1995.

* PALMER, C G, AND GOETSCH, P-A (1995) The use of an artificial stream system to investigate the tolerances of indigenous South African riverine invertebrates to selected water quality variables. To be presented at the Fifth SETAC Europe Congress, Copenhagen, Denmark, 24-28 June 1995.

Appendix 5.1

A through-flow, stream-side experimental stream in the Kruger National Park (KNP)

One recommendation from the April 1992 artificial stream workshop was that an experimental facility should be built in the KNP. It was not feasible to fund such an enterprise from the artificial stream project. It was suggested that it should be initiated and run by the National Parks Board. An assessment of the feasibility of this development was made in August 1992 (CGP). At that time Mr. Bill Rowlston (DWAF) was prepared to assist with the design of such a system.

Preliminary Design Ideas

- Three channels - either epoxy coated timber or galvanised steel, sunk into the ground. Trapezoidal in section, with a 25cm base, and ± 5cm in length.
- Maximum velocity 0.5 m sec^{-1} (At a maximum depth of 10cm, this will require a pump which can deliver 40 l sec^{-1}).
- Raw water from the Sabie River pumped by an existing pump, through the channels, into a gravel filter and back, by seepage, into the river. It is important to note that all the pumps currently in use have gland packing that leaks material into the water. If the raw water delivered into the channels is to be uncontaminated, a pump with a mechanical seal such as the ones to be installed in Grahamstown may have to be considered and budgeted for.

Table 5.1 shows information provided by Mr. Ferdie Gerber (August 1992) on the pumps which currently pump raw water from the Sabie River in the Park. There is no current information of how much spare capacity these pumps have, i.e. whether they could deliver sufficient water to 3 artificial channels. The Parks Board cannot guarantee a water supply at this stage, but are keen to be involved in any future planning.

Table 5.1 Pump stations - Sabie River.

1. Phabene	15 kl/hour 50 kl/hour (dam)	
2. Kruger gate	2,5 kl/hour	
3. Mkuhlu	1,5 kl/hour	
4. Skukuza	a) Raw water pump (Picnic pump station, staff village) b) Raw water pump (purification works) c) Raw water pump (on right bank of Sabie River, directly downstream of Nwaswitsaka River mouth)	a) 115 kl/hour (dams) b) 15 kl/hour (Golf course) c) 47 kl/hour (near Nwaswitsaka River mouth) d) 97 kl/hour (Sabie bridge)
5. Lower Sabie	a) Purification works b) Recirculation pipeline	a) 35 kl/hour 50 kl/hour (irrigation) b) 756 kl/hour

Of these options three should be considered further:

1. Picnic Pump Station
   Advantage: sufficient space within a fenced area.
2. Station on right bank of Sabie River, directly downstream of Nwaswitsaka River mouth
   Advantage: sufficient space within a fenced area; convenient for maintenance.
3. Lower Sabie-Recirculation Pipeline
   Advantage: large spare pump capacity.

Information would be required on the suction head on the selected pump (height of pump above river surface).

The following factors were discussed with Dr. Andrew Deacon:

1. Personnel: None of the KNP staff have time for intensive research. At best they could maintain the system. The system would be used experimentally by visiting researchers.
2. Funding: As previously suggested a separate motivation should be prepared. The possibility of the KNP budgeting for maintenance of the system should be considered.
3. System Size: The system should be designed primarily for macroinvertebrate work, with the possibility of working with small fish species such as rock catlets - but not of a size that would accommodate large fish species.
4. Locality: In addition to the three sites which are suitable, the following other sites were visited and considered:
   - The Skukuza Golf Course pump station

- The old sewage treatment works
- The ornamental stream near the research offices.

5. Durability: Once the system is built, it will not require a lot of maintenance and could be a permanent facility.

Recommendation

The recommendation was made that this proposal be kept in abeyance until after the first results of field verifications with the portable artificial streams are available. All considerations should then be re-evaluated. Since then it has proved more cost effective to transport raceways to the KNP, and to use them in the KNPRRP laboratory, which has been modified specially to accommodate them.

CHAPTER 6

EVALUATION - PITFALLS AND PROGRESS

Summary This chapter is a record of what to avoid, and what was learned. Pitfalls and progress from the two major parts of the project, i.e. design and construction, and tolerance testing, are listed and evaluated.

6.1 INTRODUCTION

In any project which involves pioneering development, the list of "what not to do" is often longer than the final protocols. Despite every effort to ask questions, and read the literature, there are things that in retrospect would have been better done differently. This chapter is a record of what to avoid, and what was learned. Each phase of the project is listed and evaluated.

6.2 DESIGN AND CONSTRUCTION

At the 1991 conference of the North American Benthological Society there was a session on artificial stream research. CGP corresponded with the organisers of the session, but their synthesis of the session was only published in December 1993, after the artificial stream laboratory had been built. However, this review paper (Lamberti and Steinman, 1993) offers interesting perspectives. One prime concern was that artificial stream research was becoming a slave to statistics, and that in order to meet the demands of independent replication, little attention was being paid to the link between laboratory results and field conditions. There is also a warning of the pitfalls of "over-engineering". These are aspects which received considerable attention in the design and construction phase of the stream project.

6.2.1 Pre-construction

Questions:
What sort of system should be built, where and how?

Progress:

* Overseas travel: This allowed a wide evaluation of different types of artificial stream systems, and clearly demonstrated Lamberti and Steinman's (1993) assertion that there is no "right" design but that each must suit the question being asked. Key observations were: (1) the large outdoor systems in Dorset were too labour intensive to run, and had very little operational control. However, large-scale systems had advantages of adequate organism:volume ratios. Flow-through systems had many advantages but would not be practicable in water-scarce Grahamstown. The final compromise was therefore: a high degree of operational control in an indoor laboratory, with little "reality" but at as large a scale as was practical taking into account the need for careful description of hydraulic conditions, and combined with small portable units that could be used in the field.
* Workshop: As there was considerable criticism of the project in the early phases, all interested parties, both peers and potential users, were invited to a workshop. The reasoning behind the project and alternative designs were presented and consensus

sought on both stream design criteria, and experimental designs. Critics then expressed their views, accepted the project concept, and agreed that the inevitable compromises were justified. A key decision was that it was not feasible to have nine independent systems with a random design due to the cost of pumps. The compromise of three 3-channel systems was therefore agreed upon.

* Co-operation: DWAF's agreement to be actively involved in design and construction was essential.
* Site: The discovery of an appropriate site near a back-up power supply, adequate water supply, and other aquatic researchers was a significant step forward. The Department of Ichthyology and Fisheries Science (DIFS, Rhodes University) allowed the use of part of one of their storage sheds.

Pitfalls:

* Over-engineering: Once it was decided that a larger-scale system capable of velocities from <0.5 to over 1 $m.sec^{-1}$ was necessary due to scale considerations, and that hydraulic conditions should be accurately described, we were committed to a highly engineered system. Since the aim of the project was to investigate water quality responses, it might have been possible to disregard the importance of hydraulics, and to begin with small-scale systems that were cheaper and easier to replicate. Disadvantages with the decision to build a larger-scale system included the loss of time due to problems associated with pumps, and difficulties encountered working at this larger scale.

6.2.2 Design and construction

Design and construction aspects are evaluated in Chapter 2. Additional considerations are presented below.

Question:
What materials should be used?

Progress:

* Chemically inert: All materials used had to be chemically inert so they would not affect water quality.
* Mechanically sound: The streams had to be robust, and user-friendly.

Pitfalls:

* Chemically inert: Pump impellers were initially cast-iron which rusted and left iron oxide deposits in the pipes. The pump interiors were then electro-less nickel plated, which was time-consuming and expensive. The plastic pipework will adsorb metal ions - precluding research using metals in the large systems (this despite professional advice to the contrary in the pre-construction phase). Because of the use of silicon glue and polystyrene it is not possible to acid-wash the large streams to remove adsorbed ions.
* Leaks: Both the large and small systems leak regularly, which increases maintenance costs and wastes time.
* Height: Channel velocity and pump delivery dictated that channels were too high to work in comfortably.
* Raceways: Although the raceways were deigned to be portable they are unwieldy and

heavy, and transport causes them to leak. Gutter designs that are modular might have been more appropriate. Raceways were also designed to run in the field off a battery; this proved not to be feasible as batteries do not run for sufficiently long periods. The choice of using windscreen wiper motors so as to run easily off a DC power source was therefore unnecessary. These motors overheat and do not always run well for protracted experimental periods.

* Laboratory: The laboratory is too small, and is difficult to work in comfortably.
* Temperature: Due to cost constraints it was decided to split temperature control between air conditioners and cooling units in the large systems. At the time it was envisaged that three raceways would be attached to each unit and recirculate the same water. This however exacerbates the problem of pseudoreplication. However, with raceways running as independent units the temperature range is limited to that provided by the air conditioners.
* Budget: Total end cost was difficult to estimate. Some items were rebought as sufficient funds were available at the end of the project for upgraded equipment (e.g. stainless steel bolts replaced cast-iron bolts throughout; and stainless steel cooling coils replaced plastic-coated coils).
* Over-engineering: The large systems may be somewhat "over engineered" for water quality research, but the systems offer unparalleled potential for a wide range of stream research activities. The large systems appear best suited to hydraulic habitat investigations, but for other water quality studies a less ambitious approach will suffice. Two students are currently working on aspects of flowing water toxicology and are using less sophisticated gutter channels with smaller pumps - so far with some success.

6.3 EXPERIMENTAL PHASE

Question:
How should tolerance experiments be run in the raceways and in the large streams?

Progress:

* Replication: Advantages include a design that has the potential for well-replicated experimental systems. With 15 independent raceways, small-scale replication is possible. Chapter 3 reports the water quality calibration in the large systems, with identical conditions monitored in all three units. However, when the large streams were to be used in tolerance tests one system would act as a control, and the other two as experimental units, with the channels as replicates. Unfortunately, this results in the much-mentioned pseudo-replication as the three replicate channels do not have independent water sources. However, there are three different populations of responding organisms, which represents a similar compromise to that advocated by Lamberti and Steinman (1993).
* Scale: The design of streams at two scales was excellent. Raceways have been successfully transported to the KNP and used in the KNPRRP laboratory.
* Co-operation: When planning the stream laboratory, the aim was to use the large systems to simulate the most "realistic" organism:volume ratios possible with a recirculating design, and then to use the raceways for field verification. Constraints with using raceways at river-side sites make this option unlikely. However, one of the strengths of South African research is the close contact between researchers using

experimental approaches, and those working in the field using a survey and correlative approach. Careful comparison of results should yield useful insights.

Pitfalls:

* Large streams: The large streams have proved more difficult to work with than expected, with a major problem being the retention of test organisms in the channels. Currently netted "boxes" are being constructed which will contain experimental organisms and fit into each channel. Originally we planned to use drift as an index of response. This would require large numbers of test animals, and is logistically difficult to design as return upstream is impossible. Use of animals in the large systems is still in the developmental phase, as it was in the raceways a year ago.
* Raceways: Most progress has been made in the use of the raceways, but this was not achieved without any problems. During tolerance tests experiments were always carried out in triplicate. We originally planned to conduct experiments in duplicate, but triplicate systems provide for duplication should one of the three raceways develop water quality problems (this has proved the case). This back-up is necessary as it is not possible to simply abandon an experiment and start again if problems develop in one of the duplicates. Replicate systems should be randomly arranged to prevent positional effects on mortality due to placement in the laboratory.

Question:
Which animals should be used for ecotoxicological experiments, and how should they be handled ?

Progress:

* The best taxa have been *Tricorythus* sp. and *Adenophlebia auriculata*. These are both medium-sized nymphs that are easy to see, count, handle and retain in experimental systems. The ancylid limpets (*Burnupia stenochorias*) are also useful, and will be more so now we know they are sensitive to handling.

Pitfalls:

* Baetid mayfly nymphs and ancylid limpets (*B. stenochorias*) were collected from the Bloukrans River at the railway bridge 10 km outside Grahamstown. A problem encountered during collection is that these mayfly nymphs could not be identified to species level when alive. Animals were therefore collected after death in the raceways during and at the end of the experiment, and then speciated. Tests therefore had to be carried out using multi-species mayfly populations.
* Collection methods proved to be a vital factor governing rates of mortalities in laboratory experimental systems. Several methods of collecting invertebrates were tested. Finally it was concluded that mayflies are best collected by carefully lifting rocks from the river bed and rinsing them over a collecting tray. They should then be placed on foam in a cooler box and aerated during transport back to the laboratory. If possible, enough mayflies should be collected to allow for acclimation mortality and initiate the experiment with 25-30 organisms. When collecting limpets they should be gently pried off rocks using a paintbrush. It has subsequently been shown that limpets should be collected and placed on plastic sheets in buckets for transport. They should also be acclimated to laboratory conditions while still in the buckets, before being transferred to raceways. Limpet numbers per raceway depend on availability,

but 20 organisms were normally used. Limpet mortalities during acclimation and during experiments were variable. Acclimation mortalities were generally above the recommended 10%, and control mortalities of 15-20% have to be accepted at this

stage. Unfortunately a range of experiments were carried out before collecting and handling methods had been finalised.

Mistakes:

* Transport and holding problems should have been finalised before the onset of experimental testing. Good quality river water should preferentially be used, though experiments using tap, river, rain and municipal water have been successfully carried out. Initial experiments also showed that replicated tolerance experiments should only be conducted after unreplicated range-finding tests, so as to present some indication of mortality.

Consequences:

* It has only now become clear that baetids from the Bloukrans River are an unsatisfactory source of test organisms, both because of the size of baetids, the mixture of species, and because of the poor water quality in the river.
* Limpet handling experiments should have been carried out sooner. Limpets are now grown in the laboratory on movable strips of plastic so they can be transferred to experimental systems without handling. Holding and salinity tolerance experiments indicate that in these systems it is unrealistic to expect either the zero control mortalities achieved in laboratory *Daphnia* populations or the 10% suggested by Coler and Rockwood (1989). Control mortalities should therefore be provisionally set at 15-20%.
* Successful tolerance experiments have not yet been conducted in the large systems. There is still work to be done on the behaviour of larger mayfly nymphs in the system. Retention of test taxa in the channels should be solved by the use of in-channel netted baskets.

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CHAPTER 7

CONCLUSIONS

Summary The aims of the project have been achieved, while the rearing of experimental invertebrates was transferred to another project. Aims and progress are briefly reviewed in this chapter. The artificial stream project is poised to contribute significantly to the development of environmental water quality guidelines, but the following factors must be taken into account: a supply of test organisms needs to be guaranteed, the inherent complexity of the natural environment acknowledged, the cost effectiveness of this method evaluated, and chronic testing as well as acute testing undertaken.

7.1 AIMS ACHIEVED

The primary aim of this project was the development of a facility where experimental research could be carried out under controlled laboratory conditions, on the water quality tolerances of organisms which live naturally in flowing water. This has been achieved and is the major accomplishment of the project.

The next aim was the development of methods for tolerance testing. In this regard a significant step forward came with the initiation of an independent project on the rearing of test populations. This has resulted in a relatively ready supply of limpets for experiments, and advice on successful transport of animals from the field. The further development of these production methods till two taxa can reliably be supplied, is essential for further work in the artificial stream laboratory.

Despite technical problems with the pumps in the large systems, and incompetent service from the suppliers which resulted in the loss of 3 months experimentation time, methods have been developed for the use of both the portable raceways and the large streams. Both systems have been calibrated, though additional behavioural calibration is required in the large streams. An important discovery has been the susceptibility to handling distress of limpets used in experiments. Protocols for minimal disturbance during collection and experimentation have been developed.

The results of salinity tolerance testing have contributed both data and method protocols. Information has been generated concerning the type of riverine organisms best to use for toxicity studies, as well as the water quality of choice. Comparative studies using a mixed baetid population from the polluted Bloukrans River (Eastern Cape), and the mayfly *Tricorythus* sp. from the unpolluted Sabie River (Mpumalanga), have clearly shown the advantages of using a clean water system and a reliable source of similar-sized invertebrates. Results have clearly shown that salinity tolerance is salt-specific, with a higher mortality response to sodium sulphate than sodium chloride.

Although research on the toxicity of metal ions was not a brief for this project, the considerable ground-work covered has ensured that metal ion toxicity testing is a feasible aim for Phase 2.

7.2 ENVIRONMENTAL WATER QUALITY GUIDELINES

In the immediate future the most useful application of this research is in the development and refining of environmental water quality guidelines. To achieve this goal test organisms must be available for toxicity testing, the complexity of the natural environment must be taken into account in interpreting data, the cost effectiveness of toxicity testing must be evaluated, and chronic testing as well as acute testing must be undertaken.

7.2.1 Supply of test organisms

The long-term success of tolerance testing in the artificial stream laboratory is **entirely** dependent on an adequate supply of test organisms. SLO is the acronym for the Standard Laboratory Organism project which is currently investigating life histories and optimal rearing conditions of selected organisms, establishing transport and holding techniques and facilities, and finally optimising growth and harvesting techniques.

Progress in the first three years has been encouraging. Two potential test organisms have been identified: the limpet *Burnupia stenochorias* and the mayfly *Adenophlebia auriculata.* Procedures for the transport and laboratory maintenance of these species has been established. A reproducing population of limpets has been maintained in the laboratory, which have been supplied to the artificial stream project. Optimal production conditions are under investigation. Field data on the population structure and life history of *A. auriculata* have been collected, and some progress has been made with laboratory fertilisation. Rearing of laboratory fertilised eggs to nymphs of a size suitable for experimentation, is underway.

This avenue of research must be pursued until a ready supply of these two test organisms can be guaranteed.

7.2.2 Natural complexity

The challenge of developing environmental water quality guideline should not be under-estimated. The aim of developing such guidelines is to maintain aquatic ecosystems in an ecologically functional state. The difficulty lies in the infinite complexity and variability of the environment. Firstly there is spatial variability: a river, for example, is a longitudinal system, with different water quality characteristics in the upper, middle and lower reaches. Pollutants can enter, be stored in, or be released from the air, the water or the sediments. Water and sediments will have different characteristics in different habitats, such as riffles or pools. Rivers in different geographical regions will again have a different suite of characteristics.

Chemical characteristics also vary. Toxicity of a pollutant to organisms may depend on the concentration of a specific chemical species, particularly in the case of metal ions. The presence of a particular chemical species depends on multiple factors such as pH, redox potential, and other ions present in the system. The rate of change of concentration, and the duration of contamination by a pollutant have vastly different effects on biota. Water quality variables can also interact either antagonistically or synergistically.

Organisms present in a river also vary spatially and temporally, with particular species characteristic of longitudinal reaches, habitats or seasons.

The designation of water quality guidelines that will ensure continued ecological function is therefore difficult. It is envisaged that the data requirements for the development of the first set of environmental guidelines will be as follows: acute tolerances of a representative of each of eight families of organisms; and chronic tests or acute-chronic ratios from at least one fish and one invertebrate species. It is hoped that the tolerances of this wide range of biota will allow the derivation of concentrations that will ensure the optimal survival, and therefore the functional integrity of the aquatic ecosystem.

In this way, the tolerance data generated through artificial stream research will contribute directly to the development of water quality guidelines. However, if we are to be confident of the applicability of the results in different geographical regions, and longitudinally down rivers, site-specific testing with local biota will be essential.

7.2.3 Cost-effectiveness

Tolerance testing in the artificial stream is slow and man-power intensive, and therefore expensive. By comparison the use of standard test organisms such as *Daphnia* is well established in ecotoxicology. There is no doubt that at the current stage of experimental development the techniques for toxicity testing using *Daphnia* are quicker and cheaper than using riverine invertebrates. However, *Daphnia* is not a flowing water organism, and its tolerances must be linked to those of riverine taxa before they can be applied with confidence. What is needed are comparative data and an evaluation of their respective contributions.

It is important to realise that in instances where there is not a complete data set of tolerances, safety factors will be imposed. When data are supplied, and confidence in the published value increases, the safety factors will be reduced or removed. Therefore, in the context of the imposition of guidelines nationally, many of which will initially incorporate safety factors, the contribution of tolerance data can only prove cost effective over time.

7.2.4 Acute and chronic testing

So far, the artificial streams have only been used for acute testing, and for the derivation of LC_{50} values. Clearly the concentration at which 50% of the population dies is not going to prove effective as a concentration aiming at protecting ecological function. LC_{50} values are useful measures of acute toxicity, because although they do not represent concentrations that are safe or harmless in aquatic habitats, they can be predictive of chronic toxic effects (Suter, 1990). However pollutants that are not toxic in 96 hours may well be toxic at longer exposure periods (APHA, 1992), and it is necessary to also assess chronic toxicity levels and determine MATCs (Slooff, 1983). Acute-to-chronic ratios determined from life-cycle tests should ideally be used for determining pollutant levels allowed in rivers, although NOEL values determined from shorter-duration chronic tests can also be used. Although knowledge of acute toxicities can be very important in predicting and preventing acute damage to aquatic life in the event of effluent spills (APHA, 1992), chronic tests are important in determining long-term tolerances. Another important aspect of chronic testing is the inclusion of multi-generation tests.

7.3 GENERAL

It is important to recognise that there was previously no knowledge whatsoever of the tolerances of indigenous riverine macroinvertebrates to any water quality variables, and that without such information we are at a loss to set realistic environmental guidelines. We are therefore at a stage in the development of environmental guidelines in which any results represent an advance in our knowledge, and it is in this respect that this project has been most successful.

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CHAPTER 8

RECOMMENDATIONS FOR ARTIFICIAL STREAM RESEARCH

Summary The artificial stream laboratory is designed to meet the needs of a wide range of research applications; both applied and in the field of general stream ecology. It is therefore important that clients and markets be identified. However, the most useful immediate application of tolerance testing is the development and refinement of environmental water quality guidelines. The project also has the potential for integrating water quality information into the IFR method. Finally, a range of general research possibilities are listed, and the contribution of artificial stream research to training.

8.1 INTRODUCTION

The artificial stream laboratory was designed with applications to water quality research in mind, but also to build a system with a wide range of potential applications. The role of artificial stream research is well established internationally. In a recent review, Lamberti and Steinman (1993) recognised seven areas where the frequency of this approach is growing, and has been useful:

* hydrodynamics (hydraulics)
* algal-nutrient dynamics
* macroinvertebrate growth
* grazer-algal interactions
* fish ecology
* disturbance
* **ecotoxicology**

Although the aim of the first phase of the project was to develop the infrastructure and methods for ecotoxicological research, the potential to use the system for research in all the above-mentioned areas, still exists.

There are two areas where the stream project is poised to make a contribution: national and site-specific environmental water quality guidelines; and water quality catchment modelling, where the aim would be to integrate water quality aspects into the Building Block Methodology of the Instream Flow Requirements (IFR) process (King *et al.*, 1994). In this chapter we recommend a research direction, some of which will be undertaken in Phase 2 of the stream project.

8.2 ENVIRONMENTAL WATER QUALITY GUIDELINES

8.2.1 National guidelines

The development of the first set of national environmental water quality guidelines is well underway. The method which will be used is based on results from tolerance testing using a wide range of biota, potentially including riverine invertebrates (Roux, 1994; Roux and

Jooste, in prep.). Safety factors will be imposed when data is insufficient. The results from the artificial stream tolerance testing can be used to reduce or remove these safety factors.

Recommendations:

* continue with salinity tolerance testing, and proceed with tolerance testing using a selected metal ion (planned for Phase 2);
* calculate guideline values for salinity with and without the data from the artificial stream project, compare the results and evaluate the contribution of the stream data (CGP and P-AG will carry this evaluation out as part of their sub-contract to the CSIR on the guideline project).
* repeat this procedure after metal ion results are available from Phase 2 of the stream project.

8.2.2 Site-specific guidelines

Once the national guidelines have been published, the next stage would be to legislate compliance in terms of tolerance testing. This would generally require the use of a suite of standard test organisms (e.g. *Selanastrum* and *Daphnia*). However it might prove useful to use riverine invertebrates to refine site-specific guideline in cases where specific goals for management have been defined. This could apply for the conservation of a water body, or to provide an industry with site-specific data on their receiving waters.

The general acceptance of the role of tolerance testing will only be reached with legislative requirement for these data to meet permit requirements.

Recommendation:

* collaborate with DWAF, so that when compliance is required of an industry in terms of tolerance testing, comparing the tolerances of standard test organisms and riverine fauna may be suggested.

8.2.3 Catchment modelling and IFR methods

One of the most successful applications of river ecology to water management has been the implementation of the Building Block methodology (King *et al.*, 1994), in the process of instream flow requirement (IFR) assessments. This process has concentrated on the water quantity requirements of rivers, particularly in the context of the development of impoundments for water supply. However it is of little use to ensure an adequate supply of water, if water quality impairment renders it unfit for use. It is therefore important to include the assessment of water quality requirements in the IFR methodology in rivers with water quality problems.

Application of the programme HSPF (Barnwell and Johanson, 1981) could be used to model water quality conditions using a daily time-step hydrological model together with available water quality data. Durations of concentrations could then be linked to tolerance data, and dilution requirements could be quantified. Critical seasons could also be identified.

Recommendation:

* pursue the investigation initiated informally between Dr Mark Dent (CCWR) and CGP to apply this approach to the Sabie River.

8.2.4 Standard Laboratory Organism (SLO) project

The SLO project is not yet at a stage where two test species can be supplied in sufficient numbers on demand. This is an essential step for progress with artificial stream tolerance testing research.

Recommendation:

* support SLO research for another 3 year period.

8.2.5 Comparison with *Daphnia*

Recommendation:

* compare the results of tolerance testing (firstly, salinity tolerances, and secondly, tolerances to a selected metal ion) using *Daphnia* and stream invertebrates on a cost-effective basis; and then define the role of tolerance data generated from indigenous fauna.

8.2.6 Tolerance testing

Recommendations:

* undertake chronic and well as acute tolerance testing;
* plan multi-species testing; and
* evaluate the effects of scale, and define the roles of the raceways and the large streams.

8.3 CLIENTS AND MARKETS

The WRC has funded artificial stream research in two phases:
1) 1992 - mid-1995: development of the stream laboratory and methods for its use, and
2) mid-1995 - mid-1998: data collection on tolerances to salinity and a selected metal ion, and comparison of results with *Daphnia*.

The SLO project (1993-1995) has also been an essential part of artificial stream research. Continued efforts in the SLO project are essential, and although it is unlikely that all problems will be solved by 1998, it will certainly be possible to define data that can be generated, together with costs and applicability.

Once the value of the data has been demonstrated, procedures will need to be repeated as data on a wide range of variables and organisms are collected. In addition, whole effluent testing should also be undertaken. The question arises of funding for this continued research. It is important to identify future clients and markets: the objects of "technology transfer". If the ongoing development and refining of environmental water quality guidelines is recognised by DWAF as a priority, the Department could be one such client. If tolerance testing becomes part of effluent permit requirements, and the value of site-specific testing with riverine organisms can be demonstrated, then industry becomes a potential client.

So far we have experienced difficulties in involving industry, although Sappi Ltd has funded travel for both CGP and P-AG. After the first donation CGP arranged to make a presentation of the research to the Research and Development director at Sappi, as well as to their

environmental officer. Since paper mill effluent is discharged directly into rivers, and Sappi have experienced problem spills, the company seemed a likely client. They expressed an interest, and CGP submitted a proposal for an M.Sc student to work specifically on paper mill effluent in one of their receiving waters. The company rejected the proposal as being too costly. In the future they might be interested in short term contracts use the raceways for site-specific testing.

CPG also made a presentation of the research potential of the raceways at Richards Bay Minerals (who have also made donations toward overseas travel). RBM expressed in interest and later contracted CGP and JO'K to advise on the planning on their in-house water quality research, but decided that they had no immediate need for running water tolerance testing.

There are many potential clients, but this kind of initiative is time consuming, and seems to have a low rate of immediate, direct success. The recognition of markets and promotion of research products is never articulated as an aim within the research programme - there is a real need to define who should do this, how, and when.

Recommendations:

* approach DWAF and investigate the possibility of their being a client for the generation of tolerance data for the development and refining of environmental water quality guidelines; and
* organise a workshop/course in Grahamstown on the application of artificial stream research specifically for potential clients in industry and the government sector.

8.4 OTHER APPLICATIONS

8.4.1 Hydraulic habitat assessment

The great strength of the large stream systems is their engineering rigour and the potential for accurate definitions of hydraulic conditions. They are ideal for research on the hydraulic habitat requirements of riverine biota, both invertebrates and small fish.

Instream Flow Requirement assessments would ideally use data on the flow requirements of key taxa in all life history stages. As flow rates drop, the hydraulic characteristics of habitats change, and the area of some habitats declines or completely disappear. There are field data on hydraulic habitat requirements of some fish and invertebrates (King and Tharme, 1994; Weeks *et al.*, in prep.), but no experimental research has been undertaken. This would be an ideal application for the stream laboratory, and would complement field research on flow assessment at the Freshwater Research Unit, UCT (King and Tharme, 1994).

Recommendation:

* encourage collaborative research where the artificial streams are used as an experimental complement to field research.

8.4.2 Fish ladders

The other immediate research use of the artificial stream is their use in quantifying the condition under which fish can swim, for application in fish ladder design. This is being initiated in an exploratory phase by Prof Tom Hecht (DIFS, Rhodes University).

8.4.3 General

The other potential directions and applications include:

* algal-nutrient dynamics - information on algal responses to nutrient enrichment could be applied to eutrophication problems in rivers;
* macroinvertebrate growth - an applied example is the need for growth studies of blackfly larvae in the blackfly control programme on the Orange River; and
* contribution to general stream ecology in the fields of grazer-algal interactions, fish ecology, and disturbance theory.

8.5 TRAINING AND NETWORKING

The field of ecotoxicology is embryonic in South Africa. IWQS (DWAF), Watertek (CSIR), and Umgeni Water are the main organisations which routinely undertake water-related toxicity testing. If toxicity testing is integrated in effluent discharge permits, the demand for testing will escalate and there will be a tremendous need for trained personnel. Training should become one of the major interests associated with the artificial stream laboratory. It would certainly be possible in the future to run specialist training courses. At the moment CGP offers a third-year course in water quality management and applied ecotoxicology.

There are also relatively few professionals working in this area, and collaboration and networking is valuable and ongoing. This is currently facilitated by WRC steering committees, and the newly established e-mail network.

There remains the need for international exposure and contact. Ecotoxicology is well established internationally; SETAC being the international organisation for ecotoxicological research. Countries, rather than individuals have to join, and South Africa does not yet have sufficient professionals in the field to make this possible. CGP and P-AG have been invited to present the artificial stream research at both the SETAC European conference in Copenhagen, and the second world conference in Vancouver. CGP has joined the Australasian Society for Ecotoxicology, and is in correspondence with Dr John Chapman (Centre for Environmental Toxicology (New South Wales, Australia). Given the fruitful collaboration between South Africa and Australia in the areas of water quantity requirements and river classification, there appears to be great potential for future contact.

Recommendation:

* plan an ecotoxicity course.

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