Arch. Environ. Contam. Toxicol. 32, 32–41 (1997)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Salinity Tolerances of Selected Macroinvertebrates of the Sabie River, Kruger National Park, South Africa

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Received: 1 April 1996/Revised: 21 July 1996

Abstract. Salinization has been identified as the most important problem facing the managers of South African freshwaters. Laboratory-based toxicity tests were conducted to assess the tolerance of selected macroinvertebrates to elevated salt concentrations. Since the Kruger National Park is the focus of river research in South Africa, and the Sabie River is the least mineralized river in the park, 96-h acute toxicity tests were conducted using Sabie River water and an ephemeropteran mayfly *Tricorythus* sp. found in the river. Experiments were conducted in flowing water systems known as *raceways*. The tolerance of the mayfly to two sodium salts, sodium chloride and sodium sulphate, was assessed at a range of selected conductivity levels/concentrations. The results indicated that mortality cannot be linked only to conductivity or total dissolved solid (TDS) concentrations, but also to the nature of the salt. Sodium sulphate was considerably more toxic to *Tricorythus* sp. than sodium chloride. Causes of mortality and implications for the development of water quality guidelines for the natural aquatic environment are discussed.

The water quality and quantity management strategies of South African rivers have had to be reviewed in the light of increasing demands on the resource. Rapid population growth and associated rural, urban, and industrial development have resulted in conflicting demands for water, necessitating a new approach to water quality management. A new Receiving Water Quality Objectives (RWQO) approach to water quality management has replaced the former uniform effluent standard approach (van der Merwe and Grobler 1990). The objective of the RWQO strategy is to maintain the quality of water in a water body fit for the use of its recognized users on a sustainable basis. Five user categories have been identified, namely: the domestic sector, industry, agriculture, recreation, and the natural aquatic environment (DWAF 1993). Since the natural aquatic environment is considered the resource base as well as a user (DWAF 1995b), its effective management and protection should ensure water of sufficient quality for all other users. This approach focuses on the protection of aquatic biota and, by deriving conservative water quality guidelines for biota, also some protection to aquatic habitats and processes (DWAF 1995a).

An essential component of evaluating impacts on aquatic ecosystems is the development of critical values for selected variables against which conditions can be tested. This information also can be used for issuing permits for point-source effluent discharges, and for assessing improvements in water quality from regulatory efforts (Roux *et al.* 1996). Although many international guidelines or criteria exist, *e.g.*, Canadian (CCREM 1987), American (USEPA 1986), and Australian (ANZECC 1992) guidelines, it has become necessary to develop guidelines specifically for South African conditions. South Africa is a semiarid country with low, irregular, and seasonal rainfall. This has resulted in a wide range of aquatic ecosystems adapted to different water quality regimes and flow patterns within the country. Also, many rivers are highly regulated, which leads to unnatural flow conditions and stressed aquatic ecosystems. These ecosystems are thus susceptible to changes in water quality (DWAF 1995a).

Before establishing water quality standards or guidelines, it is necessary to develop scientifically derived criteria to protect the aquatic environment. Toxicity tests therefore should provide some information about pollutant levels acceptable to key indicator species.

To derive effective water quality guidelines for the protection of freshwater ecosystems, there is a minimum requirement of laboratory toxicity data (DWAF 1995a). The more representative the data available, the more accurate the guidelines imposed. Since both acute and chronic toxicity data are required for aquatic insects (Roux *et al.* 1996) for the development of guidelines, this paper will describe the methods developed to conduct toxicity tests using riffle-dwelling macroinvertebrates and the application of these techniques at a field site in the Kruger National Park (KNP), South Africa.

This research was conducted as part of a project to develop a recirculating stream system in order to investigate the use of indigenous macroinvertebrates as water quality indicators (Palmer *et al.* in press). Although macroinvertebrates occupy a

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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. Benthic macroinvertebrates are also widely considered to be good indicators of environmental pollution (Chapman *et al.* 1982).

According to the Department of Water Affairs and Forestry (DWAF 1991), salinization and eutrophication are the main factors affecting water quality in South Africa. Of these, salinization, 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). The three main causes of increasing salinity in South African freshwaters are natural salinization due to factors such as the geomorphology or geological formations of an area, agricultural activities, and urban and industrial activities (Davies and Day 1986). Salinity, or the effect of increasing salt levels on selected riverine invertebrates, was therefore selected as the first water quality variable to be investigated by the artificial stream project.

Material dissolved in the water column is commonly measured as total dissolved solids (TDS), electrical conductivity (EC), or salinity (Dallas and Day 1993); there is a strong correlation between these variables in most waters. TDS represents the total quantity of dissolved material, organic and inorganic, ionized and unionized in a water sample, while salinity is specifically a measure of inorganic salts only (Day 1990). Conductivity is a measure of the ability of a sample of water to conduct an electrical current, and therefore a measure of the ionic material. In waters where most dissolved material is ionic, TDS and EC therefore correlate closely. The ions most commonly contributing to the conductivity or TDS of natural waters include 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^{-}), and sulphate (SO_4^{2-}). In South Africa the waters of the northeastern Highveld region 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).

Sodium chloride (NaCl) was selected because it is a common salt, and sodium sulphate (Na_2SO_4) because it is an important pollutant resulting from mining in the region (Mpumalanga province) adjacent to the KNP. Sodium, chloride, and sulphate ions have been shown to contribute significantly to high TDS levels recorded in the Olifants River system (Mpumalanga), particularly during droughts (van Vuren *et al.* 1994).

Study Area

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, that addresses the water quality and quantity requirements of the rivers flowing through the KNP. The aim of the program 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).

There are six major rivers flowing through the KNP: the Luvuvhu, Shingwedzi, Letaba, Olifants, Sabie, and Crocodile Rivers (Figure 1). Of these rivers, 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), and consequently may be the most vulnerable to any changes in the catchment that 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 of 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 diluting effect of volume.

Materials and Methods

This study employed 96-h acute toxicity tests. Test animals were not fed during this period. The test criterion was mortality, and LC_{50} levels were determined, *i.e.*, the concentration of test solution causing 50% mortality of test organisms. Mortality was defined and observed as immobility of the test animal.

Acute LC_{50} experiments were carried out in two phases. The first phase was unreplicated range-finding (RF) tests using two species of indigenous invertebrates from a riffle area of the Sabie River: the mayfly *Tricorythus* sp. (Order Ephemeroptera, Family Tricorythidae) and a caddisfly *Chimarra* sp. (Order Trichoptera, Family Philopotamidae). *Tricorythus* sp. and *Chimarra* sp. were selected for toxicity testing to evaluate tolerance differences among riffle-dwelling invertebrate species. The second phase was replicated definitive tests using only *Tricorythus* sp. *Tricorythus* sp. was selected for definitive testing as low mortalities were recorded under laboratory conditions, and high densities along much of the Sabie River provided a reliable source of experimental animals.

The conductivity and concentration ranges of industrial-grade sodium sulphate (Na_2SO_4) and sodium chloride (NaCl) selected for range-finding and definitive tests are listed in Table 1. Controls were Sabie River water and salt solutions prepared in river water. Conductivity values/salt concentrations were selected for definitive testing on the basis of unreplicated range-finding mortality results. The highest and lowest conductivities were repeated to determine the upper and lower limits of mortality, and two other conductivities selected around the potential LC_{50} value.

Experimental Stream Systems

Ecotoxicity studies were carried out in perspex experimental stream systems known as *raceways* (Figure 2). Each raceway is constructed from 5-mm perspex, and set into and glued to a perspex base that is reinforced by an additional 25-mm marine-plywood base. The channel width is 125 mm and the working length 470 mm (Palmer *et al.* 1994). Experiments are conducted using 12.5-L river water per raceway, which is within the APHA (1992) recommendation of a testing chamber of minimum 8 L capacity. Since macroinvertebrates are riffle-dwelling animals in constant contact with flowing water, raceways had to be flowing water systems. The current is generated by a 330-mm diameter perspex paddle wheel, which is driven by a Bosch CHP 12v small electric windscreen-wiper motor screwed to the

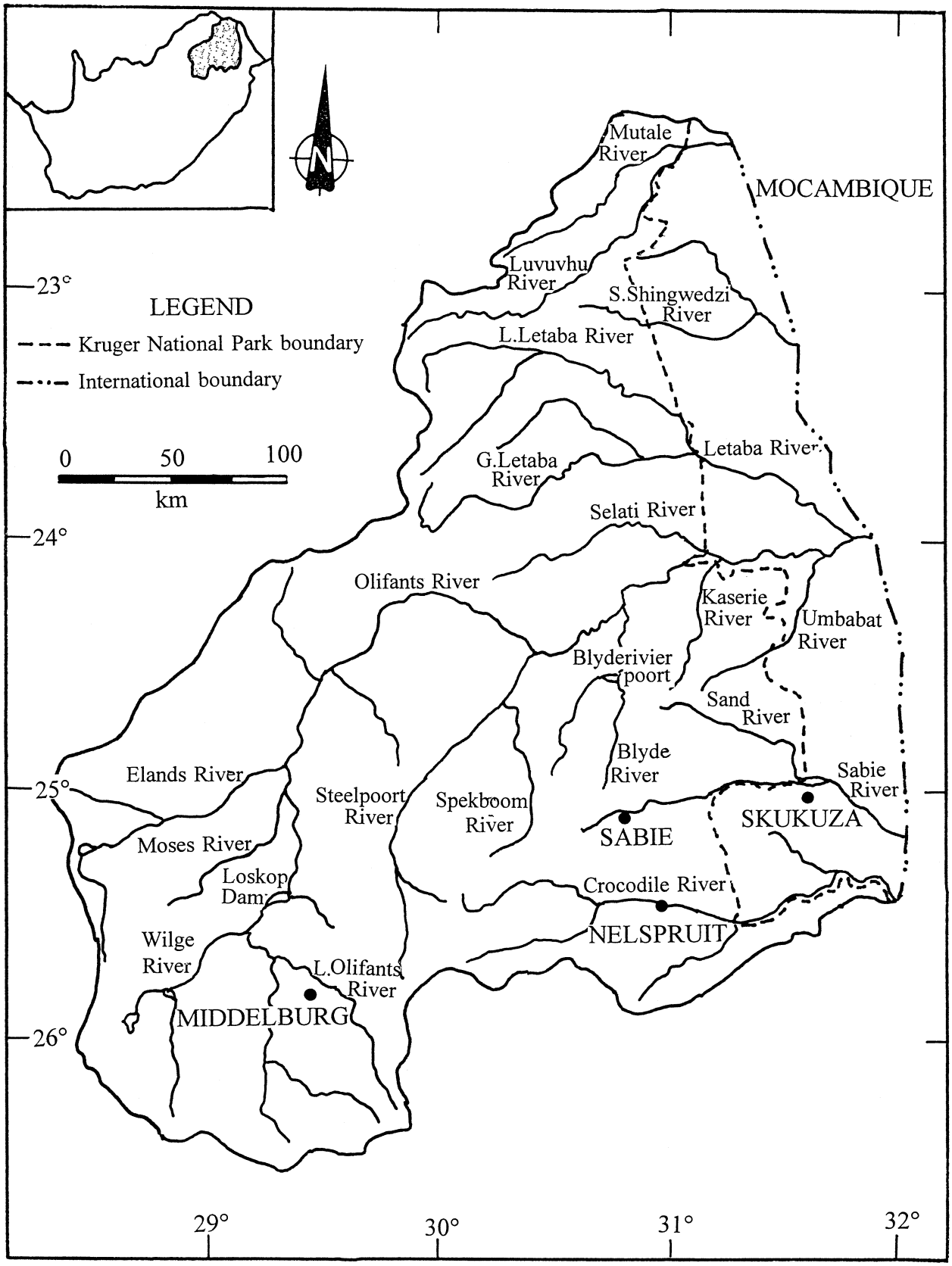

Fig. 1. The major river systems of the Kruger National Park (Breen *et al.* 1994)

Table 1. 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	100 mS/m	50 mS/m	100 mS/m
0.66 g/L	0.52 g/L	0.20 g/L	0.52 g/L
250 mS/m	250 mS/m	100 mS/m	250 mS/m
1.83 g/L	1.35 g/L	0.66 g/L	1.35 g/L
500 mS/m	500 mS/m	200 mS/m	400 mS/m
3.65 g/L	2.80 g/L	1.46 g/L	2.10 g/L
750 mS/m	750 mS/m	600 mS/m	600 mS/m
5.50 g/L	4.23 g/L	4.40 g/L	3.38 g/L
1000 mS/m	1000 mS/m		
7.34 g/L	5.65 g/L		

wooden base in the center of the raceway well. The motor speeds of 45 and 60 r/min enable the paddle wheel to operate at peripheral speeds of either 0.75 or 1 m/s, respectively. This constant recirculation of water also aids oxygenation. To facilitate easy observation of the invertebrates, they were confined to a specific area of the raceway by placing screens at each end of a selected channel. Four kaolinite stones were placed within this channel to serve as a substrate. Screens also served as another substrate, particularly for emerging animals. Emerging animals were prevented from leaving the raceways by screens placed over each raceway.

Laboratory Design

Experiments were conducted at the KNP Rivers Research Programme laboratory at Skukuza in the KNP. Due to the design of the laboratory, temperature and light conditions could not be strictly controlled. Laboratory temperatures were maintained between 14° and 23°C with the use of two air-conditioning units, and OSRAM biolux tubes provided wavelengths of light similar to sunlight. Lighting was generally maintained at a 12:12-h light:dark cycle. To compensate for positional effects, replicate raceways were arranged randomly around the laboratory.

Experimental Procedure

Each raceway was filled with 12.5 L river water prior to the animals being placed on kaolinite stones in the raceways. Invertebrates were collected in the field, sorted in the laboratory into 25 *Tricorythus* sp. and 20 *Chimarra* sp. individuals per raceway, and then placed into each raceway. Invertebrates then were acclimated unfed in the raceways for 36 h. After the acclimation period, all dead animals were removed and numbers between raceways equalized before the addition of the salt solution. Salt solutions were prepared with Sabie River water. Acclimation mortalities ranged between 4 and 8% for *Tricorythus* sp. and 4–12% for *Chimarra* sp. Twenty percent (2.5 L) of the total water volume in each raceway was replaced daily with fresh preprepared salt solution up to the required conductivity. This method is known as a partial renewal system and prevents the buildup 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 the test solution would be preferable, but is not practical in these large experimental systems.

Since the toxicity of any pollutant is influenced by environmental conditions such as pH and dissolved oxygen, the following physicochemical conditions were monitored daily in each raceway: temperature, pH, dissolved oxygen (DO), and EC. A Hanna HI 193 meter was used for oxygen measurements, a Checkmate CCA475627 kit for pH readings, and an Amel digital conductivity meter (model 160, graphite electrode model 193) for EC measurements. Mortalities were noted twice daily and nutrient concentrations, *i.e.*, ammonium, nitrate, nitrite, and phosphate, were monitored spectrophotometrically every second day using a Merck Spectroquant 118 photometer. Ammonium was analyzed by the indophenol blue method, nitrate was measured by reacting samples with nitrospectral in concentrated H_2SO_4 to produce a dark red-colored nitro compound, nitrite was measured 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).

At the onset and termination of each definitive experiment, the microbial population of each raceway was monitored using a pour plate method (after Biolab culture media catalogue and DWAF Analytical Methods Manual 1992). Two unfiltered water samples (1 × 600 ml, 1 × 200 ml) were also taken from each raceway for full chemical analysis. These analyses were carried out by the analytical laboratories of the Institute for Water Quality Studies (IWQS), a division of DWAF. One sample was preserved with preprepared 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 macroelements; and the other sample was analyzed for both dissolved and acid-extractable trace metals. Trace metals were analyzed by 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 (autoanalyzer). 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 (methods after APHA 1992).

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

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

To statistically test for significance of mortalities recorded between replicates of definitive experiments, and between mortalities at different salt concentrations, the percentage cumulative mortality data were arcsin-transformed and one-way analysis of variance tests (ANOVA) carried out at a 95% confidence level. The computer package Statgraphics, version 5.0, was employed for this analysis (Statgraphics Corporation 1990).

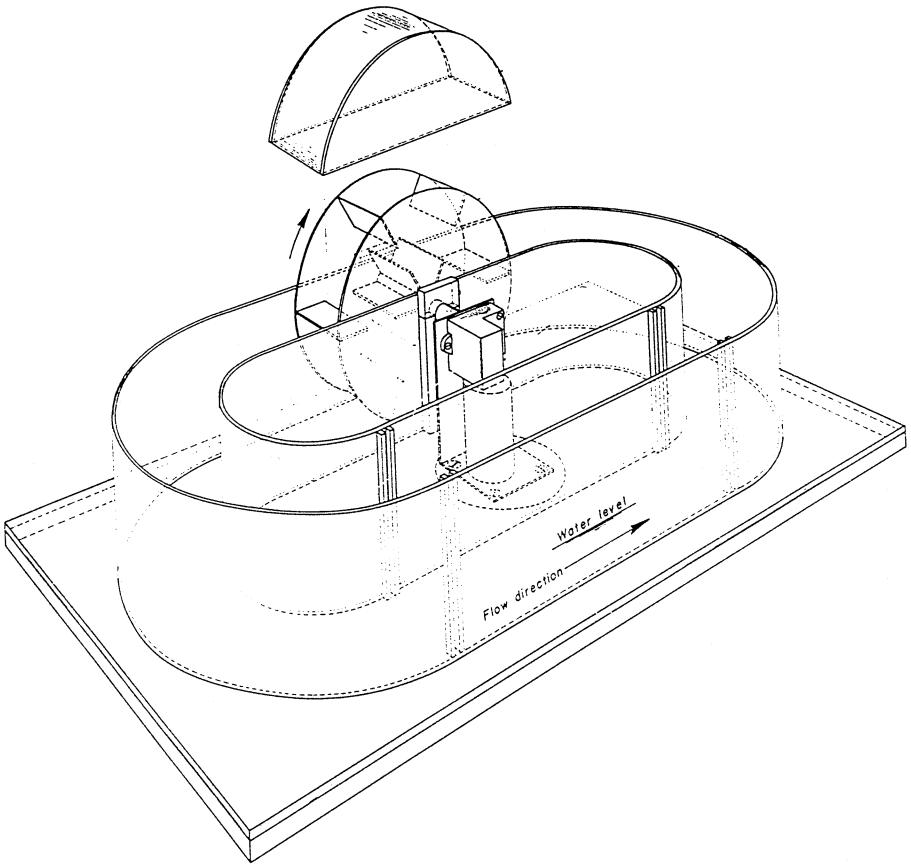

Fig. 2. Diagram of a portable raceway used for ecotoxicity studies

Results and Discussion

Since the toxicity of any pollutant is influenced by water quality and water chemistry conditions, it is essential to examine water quality throughout toxicity testing. Although the partial renewal system employed by this study helps prevent the buildup of toxins and metabolites in the water, it would be preferable to replace 100% of the test solution daily. Total replacement, however, is not practical in these large experimental systems. Since river water was used as the experimental medium instead of a defined synthetic medium, it was necessary to constantly monitor conditions in the experimental raceways so as to aid the interpretation of mortality data.

Physico-Chemical Constituents and Nutrient Concentrations

Tables 2 and 3 show the range of physico-chemical variables and nutrient concentrations recorded during both range-finding and definitive tests using sodium sulphate and sodium chloride, respectively. Little change was seen in physico-chemical conditions: pHs fluctuated within the 6.5 to 9.0 range prescribed by the Canadian guidelines for the protection of aquatic life (CCREM 1987), and DO levels were consistently above the 65.0% oxygen saturation guideline value of Hart (1974).

Total ammonia is the sum of unionized ammonia (NH_3) and the ammonium ion (NH_4^+), with the toxicity of ammonia being directly related to the concentration of the unionized form (NH_3). The ammonium ion therefore has little or no toxicity (Williams *et al.* 1986). At low to medium pH values, the ammonium ion dominates, but ammonia is formed as pH increases. However, the concentration of ammonia decreases with increasing ionic strength and increasing salinity (Williams *et al.* 1986). At 15°C and pH 7.0 (general conditions of this study), the recommended guideline for total ammonia is 2.2 mg/L (USEPA 1986). According to Gammeter and Frutiger (1990), NH_3 only contributes 0.27% to the total ammonia under these conditions, and NH_4^+ the remaining 99.73%. Since the highest ammonium concentration reached throughout the experiments was 2.10 mg/L, ammonia levels should be well below the 0.02 mg/L guideline for the protection of aquatic life (Hart 1974).

Current nitrite standards for the protection of aquatic life are the U.S. (Chiaudani and Premazzi 1988) and Canadian (CCREM 1987) guidelines of 0.2 mg/L, and the 0.1 mg/L NO_2 UK guideline (Gardiner and Zabel 1989). Nitrate guidelines include the 6.6 mg/L NO_3 Special Effluent Standard for South African conditions (Dallas and Day 1993), and the U.S. 396 mg/L NO_3 guideline for the protection of warm-water fish (Chiaudani and Premazzi 1988) (all references cited in Dallas and Day 1993). Nutrient concentrations fluctuated within these ranges during the experiments, with little difference noted between the control and experimental systems.

Phosphate results shown in Tables 2 and 3 are for total reactive orthophosphate (TRP), *i.e.*, the orthophosphate content

Table 2. Ranges of physico-chemical constituents and nutrient concentrations (expressed in mg/L) 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	<0.01–2.10	<0.2–2.3	<0.02–0.17	<0.06–2.10
50 mS/m	6.96–7.07	10–13	87.6–97.0	<0.01–0.10	<0.2–3.3	<0.02–0.24	<0.06–1.69
100 mS/m	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	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	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 3. Ranges of physico-chemical constituents and nutrient concentrations (expressed in mg/L) 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	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	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	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	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

of samples that have not been filtered, hydrolyzed, or digested (APHA 1992). No significant difference was noted between the TRP levels of the control and experimental systems.

Microbial Populations

The common water bacterium, *Klebsiella rhinosderomatis*, was identified in water samples from all raceways. Concentrations increased from approximately 1,500 at day 0 to about 3,200 colonies/ml by day 4, in both the control and experimental systems. A second pseudomonad bacterium also was identified at very low numbers in all raceways.

Water Chemistry

The analysis of samples showed little fluctuation of most parameters during the duration of the experiments. Total alkalinity fluctuated between 62 and 101 mg/L during both sodium sulphate and sodium chloride experiments. These values were consistently above the USEPA (1986) guideline of 20 mg/L $CaCO_3$, while hardness fluctuated around 69.4 mg/L. Increases in DOC concentrations were evident throughout the duration of each experiment, with the extent of change depending on the experimental salt solution and its conductivity. The amount of DOC initially present was dependent on the river water collected. Fluctuations during experiments may be ascribed to biological activity and DOC acting as a ligand and binding site for free ionic species (Ure and Davidson 1995). Concentrations therefore would 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 concentrations of relevant ionic species, *i.e.*, sodium, chloride, and sulphate ions. Table 4 represents the concentrations of these ions and TDS levels at various levels of conductivity or salt concentrations.

The analysis of kaolinite stones did not show any bound metals present above detection level. Since kaolin is a 1:1 layer structured aluminum-silicate, the clay has a low surface area and low cation and anion exchange capacity (P. Kempster, IWQS, personal communication). Its contribution to changing water quality is therefore minimal.

Mortality Results

Figures 3 to 6 show the percentage cumulative mortalities recorded during range-finding and definitive experiments for both sodium sulphate and sodium chloride experiments.

The statistical analysis of cumulative mortalities recorded during sodium sulphate definitive experiments showed that at 50 mS/m no significant difference could be detected between mortalities in the control and experimental systems ($f = 16.65$; $P < 0.00001$). At 100 mS/m, both the control and experimental mortalities were replicated, and a significant difference was evident between them ($f = 6.80$; $P = 0.0001$). The same result was seen at 200 mS/m ($f = 4.92$; $P = 0.0012$) and at 600 mS/m ($f = 6.62$; $P = 0.0003$). Mortalities recorded at 50 mS/m were significantly different from mortalities at all other conductivities, while there was no statistical difference between results of 100 mS/m and 200 mS/m. The difference between these and the results noted at 600 mS/m was significant ($f = 5.77$; $P < 0.00001$).

The LC_{50} value for sodium sulphate was experimentally observed to be at 100 mS/m (Figure 4B), *i.e.*, 0.66 g/L Na_2SO_4. The data could not be analyzed using the probit program since the probit model requires a normal distribution of concentration response data (APHA 1992).

Mortalities recorded for the sodium chloride control and experimental systems at 100 mS/m were not replicable or significantly different from each other ($f = 6.57$; $P = 0.0001$). The control and experimental mortalities were replicable at 250 mS/m and were significantly different from each other ($f =$

Table 4. Concentrations of ionic species and TDS levels (expressed in mg/L) as monitored during definitive experiments

Test	TDS	Na^+	SO_4^{2-}	Cl^-
Sodium sulphate				
50 mS/m—0.20 g/L	432	108	212	
100 mS/m—0.66 g/L	907	256	548	
200 mS/m—1.46 g/L	1816	563	1139	
600 mS/m—4.40 g/L	6334	1968	4193	
Sodium chloride				
100 mS/m—0.52 g/L	684	220		350
250 mS/m—1.30 g/L	1694	614		915
400 mS/m—2.10 g/L	2714	986		1568
600 mS/m—3.18 g/L	4417	1656		2473

13.75; $P < 0.00001$). Similar results were observed at 400 mS/m ($f = 9.02$; $P < 0.00001$) and 600 mS/m ($f = 6.08$; $P = 0.0003$). Results recorded at 100 mS/m were significantly different from those recorded at all other conductivities, while the results of 250 mS/m were not significantly different from those of 400 and 600 mS/m. The results noted at 400 and 600 mS/m also were not significantly different from each other ($f = 3.66$; $P = 0.0003$).

The mortality data were statistically analyzed, using the probit program to determine the LC_{50}. Chi-square heterogeneity for the two duplicate sets run was 0.73 and 1.79, respectively. Due to the lack of significance between mortalities at 250, 400, and 600 mS/m (Figures 6B–D), the LC_{50} could only be allocated to a range between 400 and 800 mS/m, *i.e.*, 2.2 and 4.5 g/L NaCl.

One of the aims of this research was to assign a guideline value to salinity or conductivity for the protection of aquatic ecosystems, and subsequently the natural environment, for South African conditions. Current guidelines include the Australian TDS guideline for the protection of the natural environment (Hart *et al.* 1992), *i.e.*, 1,000 mg/L, which is equivalent to 150 mS/m. An important finding of this research was the difficulty associated with assigning a single guideline value for conductivity. It is 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 (Figure 4B), while no significant mortality was noted at this conductivity 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, while the statistical analysis of definitive test results could only identify a LC_{50} range of 400 to 800 mS/m.

Since assigning a single guideline value for conductivity does not appear feasible, it is necessary to identify the exact cause of mortality and then 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, since most of the TDS levels are above the DWA (1986) recommended limit of 350–550 mg/L. Although these high TDS levels would affect the osmoregulatory functioning of the organism (Dallas and Day 1993), it has been reported that the rate of change of TDS rather than the absolute concentration causes mortality (van Vuren *et al.* 1994). Table 4, however, suggests that mortality may not only be linked to TDS concentrations. Since the TDS

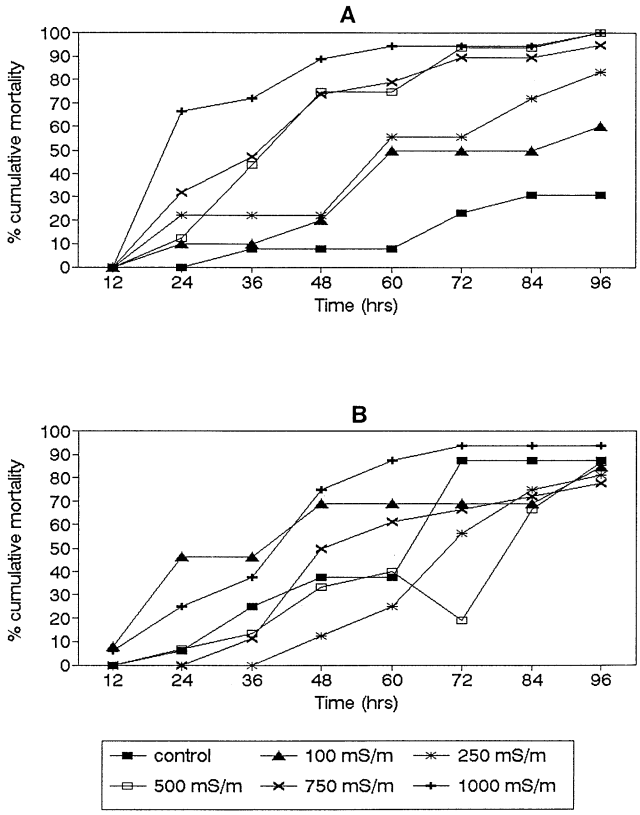

Fig. 3. Cumulative mortality of test organisms during the sodium sulphate RF experiment. (A) *Tricorythus* sp.; (B) *Chimarra* sp.

concentration at the LC_{50} for sodium sulphate is 907 mg/L (Table 4), one would expect the LC_{50} for sodium chloride to be at the same TDS concentration if TDS were solely responsible for mortality, *i.e.*, approximately 200 mS/m or 1.0 g/L. If the sodium cation (Na^+) were causing mortality, LC_{50}s should again be at the same Na^+ concentration for both salts. However, the sodium chloride LC_{50} is between 400 and 800 mS/m, suggesting that some other factor may be responsible for the elevated toxicity of sodium sulphate. Mortality is observed at a much lower concentration of sulphate ions when using sodium sulphate than chloride ions when using sodium chloride for tolerance tests, *i.e.*, 500 mg/L SO_4^{2-} versus 1,500 to 2,500 mg/L Cl^-. These data suggest the greater toxicity of sulphate ions to *Tricorythus*. This concentration of sulphate far exceeds the 250 mg/L guideline value suggested by Kühn (1991).

The toxicity tests provide only an indication of the salinity tolerance of a selected riverine macroinvertebrate species. It becomes necessary to investigate the physiology of these organisms so as to determine whether mortality is caused by a disruption of osmoregulatory functioning through elevated TDS concentrations, or whether any of the chemical species (Na^+, SO_4^{2-}, or Cl^-) may be disrupting essential enzymatic processes. Additional toxicity tests also should be carried out to determine whether the sulphate ion is responsible for mortality, or whether there is some molar influence related to the sodium cation.

To summarize, it is very difficult to draw any generalized conclusions regarding invertebrate tolerances from these acute

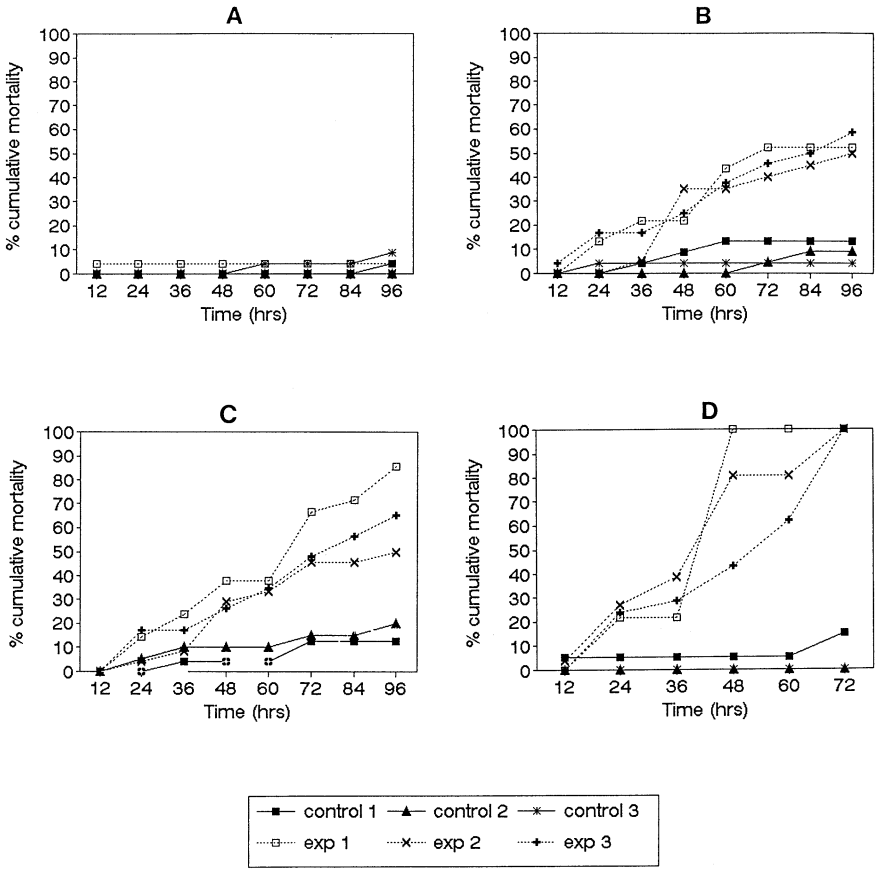

Fig. 4. Cumulative mortality of *Tricorythus* sp. during sodium sulphate definitive experiments. (A) 50 mS/m or 0.20 g/L; (B) 100 mS/m or 0.66 g/L; (C) 200 mS/m or 1.46 g/L; (D) 600 mS/m or 4.40 g/L

toxicity tests, particularly when using indigenous riverine fauna as test organisms. Guidelines already generated by DWAF (1993) for other designated water users interpret salinity guidelines in terms of geomorphological areas, indicating that each biogeographic area or catchment may have to be assessed separately. This may be essential, since the degree of mineralization of rivers will affect the acclimation of its resident fauna. This in turn will affect the salinity tolerance levels of the riverine fauna. Guidelines also will need to be linked to land-use patterns within the catchment as the water chemistry of each water body will affect the speciation and bioavailability of any pollutant present. This will again have a distinct influence on the tolerance of the fauna, and their subsequent behavior during toxicity studies.

The results presented here demonstrate many of the difficulties associated with ecotoxicity testing using riverine macroinvertebrates, but they also present an alternative to regulatory testing using laboratory-reared *Daphnia*. Information on *Daphnia* tolerances is of little use to the development of water quality guidelines for South African conditions if their tolerances cannot be related to the natural biota. This information then can be used as a basis for subsequent long-term studies, thus establishing ultimate concentration limits to preserve aquatic life.

Acknowledgments. We thank Qurban Rouhani and Kekere Soxujwa for assistance with data collection, Margi Rogers and Gay Youthed for administrative and technical assistance, the Institute for Water Quality Studies, Pretoria, for the analysis of chemical samples, and the Water Research Commission of South Africa for funding the research project.

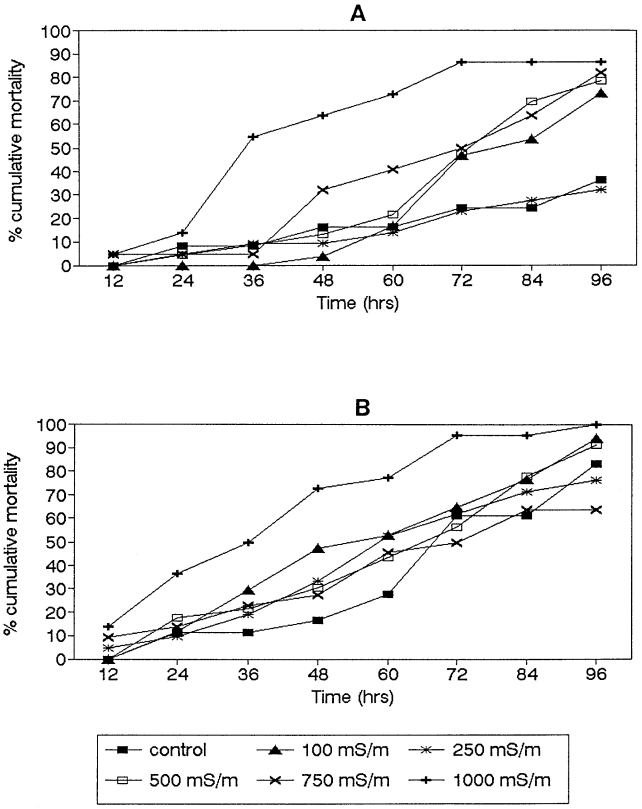

Fig. 5. Cumulative mortality of test organisms during the sodium chloride RF experiment. (A) *Tricorythus* sp.; (B) *Chimarra* sp.

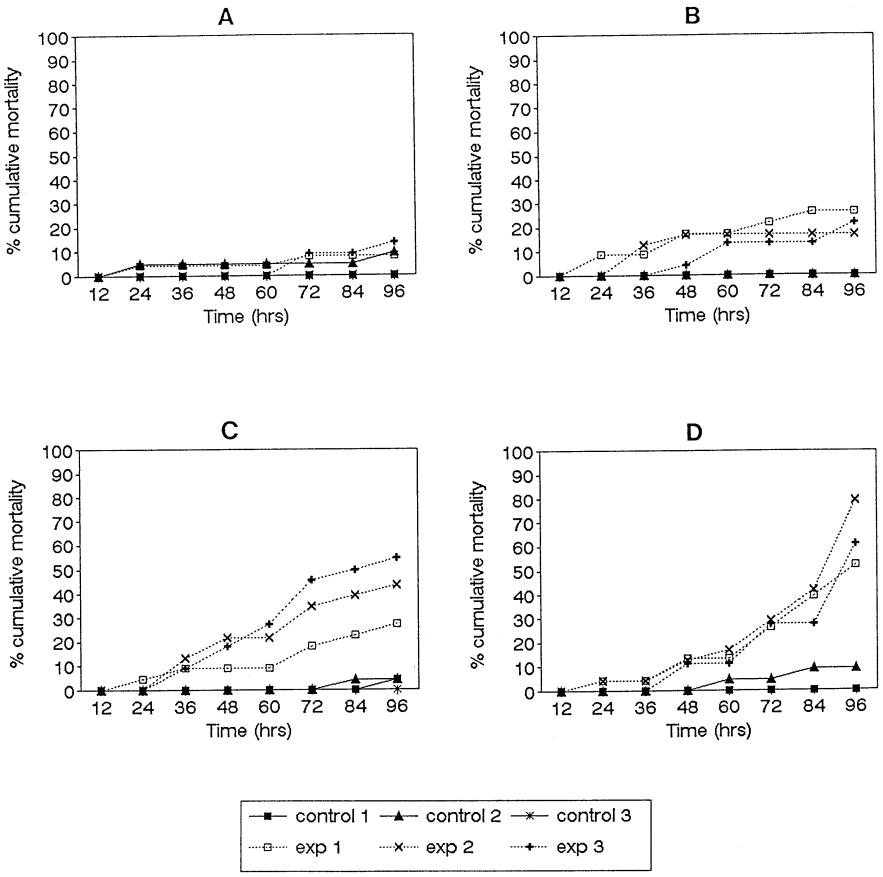

Fig. 6. Cumulative mortality of *Tricorythus* sp. during sodium chloride definitive experiments. (A) 100 mS/m or 0.52 g/L; (B) 250 mS/m or 1.30 g/L; (C) 400 mS/m or 2.10 g/L; (D) 600 mS/m or 3.38 g/L

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