EXECUTIVE SUMMARY

Introduction

Apart from natural causes, agricultural practices and industrial activities have been identified as major contributors to increasing salinisation and deterioration of resource water quality both in South Africa and worldwide (Walmsley et al., 1999; Kefford et al., 2004). Pressure to develop infrastructure and provide food security has resulted in a rapid expansion of the industrial and agricultural sectors (Goetsch and Palmer, 1997). This expansion has increased pressure on the country's water resources and has resulted in elevated levels of inorganic salt pollution in rivers by increasing salinisation (Goetsch and Palmer, 1997; Kefford et al., 2004).

The South African National Water Act (No. 36 of 1998) provides for an ecological Reserve which is intended to protect fresh water ecosystems and resources from degradation as a result of misuse, and to maintain vital ecological functions within these systems (Palmer et al., 2004). Water quality guidelines are an important tool in the management of these water resources, aiming to adequately balance protection of aquatic ecological systems with sustainable human use needs. Jooste and Rossouw (2002) proposed guidelines or boundary values for inorganic salts to be included in the ecological Reserve. These boundary values for inorganic salts were derived as follows, acute lethality data (LC_{50}s) from the ECOTOX database maintained by the USEPA were projected to 336 h and the 5_{th} percentile determined as a lethality benchmark, analogous to the Fair/Poor boundary. Similarly, the 5_{th} percentile of available sublethal data was determined as the sublethality benchmark and analogous with the Natural/Good boundary value. The Good/Fair boundary was the mean value between Natural/Good and Fair/Poor values. It has been suggested however, that these guidelines might not be entirely appropriate as they were derived without including tolerances of South African biota. Furthermore, the accuracy for some salt boundary values have been questioned (Scherman, 2009; Scherman, 2010).

In order to address these issues, there is a need to increase understanding of the physiological responses of organisms to salinity and for the generation of toxicity response data from indigenous species which might improve the accuracy of the guidelines. In general, it is understood that biota react adversely to increases in salinisation, although the effects on individual species are poorly understood (Hart et al., 1991). In particular it may affect the osmoregulation of both invertebrates and vertebrate species while negatively affecting oxygen uptake (Schmidt-Nielsen, 1998). Consequently, it was decided to investigate the oxygen consumption of two fish species and the haemolymph osmolality of a fresh water crustacean. Furthermore, an alternative approach to deriving magnesium sulphate ($MgSO_4$) guideline boundary values using indigenous mayfly lethality data was investigated.

Indigenous mayfly responses to $MgSO_4$ exposure

The objective of this experiment was to compare sensitivities of three different mayfly species to $MgSO_4$ and generate 96 h lethality data. These data, together with other lethality data from organisms exposed to $MgSO_4$ in international studies, were used to calculate guideline values for $MgSO_4$ using a species sensitivity distribution (SSD) approach.

Nymphs of three different mayfly (Ephemeroptera) genera: *Afronurus barnardi* (Heptageniidae); *Tricorythus discolor* (Tricorythidae); and *Euthraulus elegans* (Leptophlebiidae) were collected from the Kat River, Eastern Cape, South Africa, and exposed to increasing concentrations of $MgSO_4$ in recirculating channel systems on three different occasions. Toxicity tests were conducted over a 10 day (240 h) period with LC_{50} values determined after 96 h considered acute endpoints, and LC_{50} values determined after 240 h considered chronic endpoints.

The geometric means of LC_{50}s over the three experiments were 3.16 g/L for *E. elegans*, 5.96 g/L for *T. discolor* and for 7.55 g/L for *A. barnardi*. An evaluation of the current Reserve boundary values was undertaken by combining these indigenous mayfly 96 h LC_{50} data (see Chapter 2) with international acute lethality data from the ECOTOX database (USEPA, 2004) and deriving protective concentration values (PCVs) according to methods outlined in Warne et al. (2005). A comparison of the current Reserve boundary values and the PCVs determined in this study show the PCVs to be more conservative at the Natural/Good boundary, but less conservative at the Good/Fair boundary and considerably so at the Fair/Poor boundary (Table 5.4).

In recent assessments of the water quality component of the ecological Reserve (Scherman, 2009; Scherman, 2010), the $MgSO_4$ boundary value guidelines have been shown to be inconsistent with EC and biotic response data assessed concurrently. This suggests that the salt is either being overestimated by the analytical tool TEACHA (Tool for Ecological Aquatic Chemical Habitat Assessment) which is used to determine the inorganic salt concentrations from the available salt ions found in solution, or that the guideline boundary values may be over-protective. This situation has particularly problematic implications when only desktop analyses of water quality data for water use licenses are undertaken, as biotic response data are generally not available for comparative assessment purposes. Consequently, the PCV derivation approach should be investigated further in order to determine if it may provide more realistic boundary values for $MgSO_4$. Although it is possible to use only acute lethality data in deriving guidelines and then apply an acute to chronic ratio (ARC), further research should investigate the use of chronic/sublethal data

Fish responses to NaCl and Na_2SO_4 exposure

The objective of this experiment was to determine whether a change in dissolved oxygen (DO) could be used as a measure of the physiological response in guppies, *Poecilia reticulata* and zebra fish, *Danio rerio* when exposed to increasing concentrations of sodium chloride (NaCl) and sodium sulphate (Na_2SO_4). By using fish species in toxicity tests a more comprehensive approach to toxicity testing is provided through incorporating another trophic level in addition to that of invertebrates. The two freshwater fish species used for this experiment were the guppy, *P. reticulata* and the zebra danio, *D. rerio*. Both species are exotic to South Africa, however are used globally in toxicity tests (Boisen et al., 2003). These two species were exposed to increasing concentrations of the inorganic salts Na_2SO_4 and NaCl in separate experiments.

A NOEC (no observed effect concentration) for NaCl of 0.5 g/L was determined for both *D. rerio* and *P. reticulata*. For Na_2SO_4, only a LOEC of 0.375 g/L for both species could be determined and a MATC (maximum allowable toxicant concentration) of 0.188 g/L was calculated by dividing the LOEC by two. These data indicate little difference in the sensitivity of the two species to either salt. As sublethal data were used in the derivation of the Natural/Good Reserve boundary values, physiological response data such as the oxygen consumption data measured in *D. rerio* and *P. reticulata* could be used to evaluate this boundary value. For NaCl, a NOEC of 0.5 g/L was determined for both species. When compared with the sublethal toxicity data used by Jooste and Rossouw (2002) to derive the Reserve boundary values for NaCl (Table 5.5) it is evident that the physiological response of oxygen consumption has the potential to contribute as a sensitive endpoint in the determination of a realistic but protective guideline. The types of sublethal endpoints used in the derivation of the Reserve boundary values (e.g. growth, reproduction etc) are not detailed in Jooste and Rossouw (2002) and thus it is difficult to interpret the significance of the difference in NOEC value obtained for *D. rerio* in the current study as compared to the NOEC listed in Table 5.5. A NOEC could not be obtained for oxygen consumption as a physiological response in Na_2SO_4 exposed *D. rerio* and *P. reticulata*, although a LOEC could, allowing the calculation of a MATC of 0.188 g/L. The MATC (calculated by dividing the LOEC by half) is sometimes, in the absence of a NOEC, used as a sublethal endpoint in guideline derivation. When comparing this endpoint to the NOECs used by Jooste and Rossouw (2002) to derive the Reserve boundary values for Na_2SO_4 (Table 5.5), it is again evident that oxygen consumption can contribute as a sensitive endpoint in the determination of suitable guidelines.

Indigenous crustacean response to NaCl and Na_2SO_4 exposure

Osmoregulatory capacity (OC) is the difference between the osmolality of haemolymph and that of the external medium (Charmantier et al., 1989) and has been suggested by Lignot et al. (2000) as a tool for monitoring physiological stress in crustaceans. The freshwater shrimp *Caridina nilotica* has been used as a model indigenous crustacean species in acute and chronic toxicity testing in South Africa (Slaughter et al., 2008). Therefore the physiological endpoint of osmoregulatory capacity (OC) was determined in *C. nilotica* exposed to increasing concentrations of sodium chloride (NaCl) and sodium sulphate (Na_2SO_4). *Caridina nilotica* used within this study were collected from the Bushmans River in Alicedale, South Africa.

Results generated (Chapter 4) indicate no evidence of osmotic stress in *C. nilotica* with haemolymph osmolality levels remaining steady with increasing exposure to the selected inorganic salts. At 96 h, shrimp exposed to the highest concentration of Na_2SO_4 died, but there was no evidence at 72 h that osmoregulatory capacity in these organisms was failing. Hence osmoregulatory capacity (OC) could

not be applied as an indicator for osmotic stress in *C. nilotica* exposed to the inorganic salts NaCl and Na_2SO_4.
Consequently, due to the hyper-hypo-regulatory mechanism employed by freshwater shrimp exposed in this project (Chapter 4), a negative impact on the osmoregulatory mechanism of these animals could not be determined for either salt and consequently NOECs could not be calculated. To successfully evaluate current Reserve boundary values using osmoregulation as endpoint, test organisms whose mechanisms of osmoregulation are measurably impacted by increasing concentrations of inorganic salts should be utilised. As internal haemolymph osmolality levels may vary between taxa, the use of multiple species is also recommended in order to increase confidence in derived guidelines.

Conclusions and future research
The lack of confidence in the $MgSO_4$ Reserve boundary value guidelines has recently led to a review of the guideline and a revision of derivation methods for salts being included as sub-tasks in a Water Research Commission (WRC) / Department of Water Affairs (DWA) proposal for further development of the water quality methods of the ecological Reserve, submitted in August 2010. Results from the current study, particularly the demonstration of the PCV derivation approach, could make a contribution to this project and should be further investigated.

Usually there are very few sublethality data available to derive the Natural/Good Reserve boundary value using the method described by Jooste and Rossouw (2002), leading to lower confidence in the resultant guideline. Although the most reliable PCVs are also derived using sublethality data, it is still possible to utilise acute lethality data in deriving PCVs and apply a default or, preferably, experimentally determined acute-to-chronic ratio. Ultimately, however, sublethal endpoints generated using indigenous aquatic organisms are necessary in order to derive realistic protective guidelines and the generation of these data should be prioritised.

Problematic issues encountered in producing and utilising sublethality endpoints at sub-organism levels in water quality management, such as osmoregulatory capacity, are well documented (Clark et al. 1999; Tannenbaum 2005; Forbes et al. 2006). Issues raised are: the inherent variability of the endpoints measured (mainly related to the assay protocol and the differences in tolerances at low levels of organisation among exposed individuals); complicated time- or dose-dependent responses are frequently measured, but are difficult to explain and to derive endpoints such as NOECs or EC_{50}s from; confounding nonchemical influences such as temperature, nutritional state, reproductive state and lifecycle stage often impact results and; there are unclear or undetermined links between suborganism endpoints and the fitness of the individual, and especially, fitness of the population and community. These issues need to be considered when undertaking sublethal toxicity tests, and applying these data to guideline derivation.

Lastly, the EWQ management approach to salinity should reconsider the use of electrical conductivity as an additional tool, particularly in combination with biological response data. The process to determine individual salt concentrations (TEACHA) is complex, not well understood and requires salt ion data that is often not available. In addition, the accuracy of the Reserve boundary values for some salts have been questioned (Scherman, 2009; Scherman, 2010). Electrical conductivity, however, is easy to measure and the data are readily available in most cases. Further research should be conducted to determine advantages and limitations of using electrical conductivity data, either alone or in combination with biological data, in EWQ management practices.

Capacity Building
This project was utilised as an opportunity to develop scientific thinking, experimentation and writing skills in a number of students and early career water scientists based within the Institute for Water Research at Rhodes University. Much of the experimental work was undertaken by undergraduate students, supported by the incumbent IWR research intern, and overseen by the project manager Dr Muller. A 3rd year undergraduate project was completed by Mr Guy Williams, who generated data for Chapter 2 of this report. Mr Greg Tutt completed his Honours project whilst generating data which contributed substantially to Chapter 3 of this report. In addition, three research interns worked in turn on this project whilst undertaking their MSc/PhDs. This project offered them training in research and scientific writing and broadened their aquatic scientific expertise.