
Total Dissolved Solids (TDS)

An aesthetic objective of ≤ 500 mg/L has been established for total dissolved solids (TDS) in drinking water. At higher levels, excessive hardness, unpalatability, mineral deposition and corrosion may occur. At low levels, however, TDS contributes to the palatability of water.

Definition

Total dissolved solids (TDS) comprise inorganic salts and small amounts of organic matter that are dissolved in water. The principal constituents are usually the cations calcium, magnesium, sodium and potassium and the anions carbonate, bicarbonate, chloride, sulphate and, particularly in groundwater, nitrate (from agricultural use).

Occurrence

Total dissolved solids in water supplies originate from natural sources, sewage, urban and agricultural runoff and industrial wastewater. In Canada, salts used for road deicing can contribute significantly to the TDS loading of water supplies. Concentrations of TDS in water vary owing to different mineral solubilities in different geological regions. The concentration of TDS in water in contact with granite, siliceous sand, well-leached soil or other relatively insoluble materials is usually below 30 mg/L.⁽¹⁾ In areas of Precambrian rock, TDS concentrations in water are generally less than 65 mg/L.⁽²⁾ Levels are higher in regions of Palaeozoic and Mesozoic sedimentary rock, ranging from 195 to 1100 mg/L⁽²⁾ because of the presence of carbonates, chlorides, calcium, magnesium and sulphates.^(1,3) Concentrations of TDS in some streams and small lakes in the arid western regions of Canada and the United States are often as high as 15 000 mg/L.^(3,4)

Concentrations of TDS, expressed as the sum of its constituents, were below 500 mg/L in 36 of 41 rivers monitored in Canada.⁽⁵⁾ In a survey of the Great Lakes, TDS levels ranged from 61 to 227 mg/L.⁽⁶⁾ The levels of TDS in all of the Great Lakes except Lake Superior increased between 1900 and 1970. A threefold increase in chlorides and a twofold increase in sulphates, sodium and potassium in Lakes Erie and Ontario⁽⁷⁾ increased the TDS concentration in those lakes by 50 to 60 mg/L.^(6,8-10)

Concentrations of TDS in drinking water in Canada are generally below 500 mg/L but are considerably higher in some locations, particularly the arid western regions. Levels of TDS in Newfoundland and Labrador were below 500 mg/L in 96% of 103 communities sampled from 1969 to 1989 (range 10 to 2263 mg/L; average 146 mg/L).⁽¹¹⁾ In Quebec, samples of distributed water taken at 19 plants between 1987 and 1989 contained TDS at mean concentrations ranging from 58 to 213 mg/L.⁽¹²⁾ Concentrations of TDS in distributed water from 31 plants in Ontario during 1987 and 1988 ranged from 91 to 470 mg/L.⁽¹³⁾ In Manitoba, TDS concentrations measured during 1988 in the treated water of 168 communities ranged from 56 to 2510 mg/L; concentrations were less than 500 mg/L in 19% of these communities.⁽¹⁴⁾ Levels of TDS in 1978 samples of community drinking water taken between 1970 and 1989 in Saskatchewan ranged from 6.5 to 5376 mg/L.⁽¹⁵⁾ Concentrations of TDS in 54% of 1042 communities surveyed in Alberta in October 1989 were below 500 mg/L (range <100 to 1000 mg/L).⁽¹⁶⁾ In British Columbia, concentrations of TDS in individual well water supplies ranged from 120 to 4662 mg/L; those in community (generally surface water) supplies were commonly less than 500 mg/L.⁽¹⁷⁾

Analytical Methods and Treatment Technology

The method most commonly used for the analysis of TDS in water supplies is the measurement of specific conductivity with a conductivity probe that detects the presence of ions in water. Conductivity measurements are converted to TDS values by a factor that varies with the type of water.^(18,19) The practical quantitation limit for TDS in water by this method is 10 mg/L.⁽²⁰⁾ High TDS concentrations can also be measured gravimetrically, although this method excludes volatile organics.⁽²¹⁾ The constituents of TDS can also be measured individually.

Total dissolved solids are not appreciably removed using conventional water treatment processes. In fact, the addition of chemicals during conventional water treatment generally increases the TDS concentration.⁽²²⁾ Certain treatment processes, such as lime-soda ash softening and sodium exchange zeolite softening, may

slightly decrease or increase the TDS concentration, respectively.⁽²³⁾ Demineralization processes are required for significant TDS removal. Although the technology is available to reduce TDS levels significantly, the economic cost may be a major constraint.⁽²³⁾ Reverse osmosis and electrodialysis would probably be the most economical processes for removing TDS from public water supplies.⁽²⁴⁾

Health Considerations

Recent data on health effects associated with the ingestion of TDS in drinking water have not been identified; however, associations between various health effects and hardness, rather than TDS content, have been investigated in many studies. These data are discussed in the section on hardness. As well, some of the individual components of TDS can have effects on human health. Effects that can be attributed to specific constituents are discussed in separate reviews for those constituents.

In early studies, inverse relationships were reported between TDS concentrations in drinking water and the incidence of cancer,⁽²⁵⁾ coronary heart disease,⁽²⁶⁾ arteriosclerotic heart disease⁽²⁷⁾ and cardiovascular disease.^(28,29) Total mortality rates were reported to be inversely correlated with TDS levels in drinking water.^(29,30)

Conversely, a summary of an Australian study reported that mortality due to all categories of ischaemic heart disease and acute myocardial infarction was increased in a community with higher levels of soluble solids, calcium, magnesium, sulphate, chloride and fluoride, alkalinity, total hardness and pH, when compared with a community in which levels were lower.⁽³¹⁾ No attempts were made to relate mortality due to cardiovascular disease to other potential confounding factors. The results of a limited epidemiological study in the former Soviet Union indicated that the average number of "cases" of inflammation of the gall bladder and gallstones over a five-year period increased with the mean level of dry residue in the groundwater.⁽³²⁾ It should be noted, however, that the number of "cases" varied greatly from year to year in one district, as did the concentration of dry residue in each district, and no attempt was made to take into account possible confounding factors.

Other Considerations

The presence of dissolved solids in water may affect its taste.⁽³³⁻⁴²⁾ The palatability of drinking water has been rated, by panels of tasters, according to TDS level as follows: excellent, less than 300 mg/L; good, between 300 and 600 mg/L; fair, between 600 and 900 mg/L; poor, between 900 and 1200 mg/L; and unacceptable, greater than 1200 mg/L.⁽³⁷⁾ Water with extremely low TDS concentrations may also be unacceptable because of its flat, insipid taste.

In addition to palatability, certain components of TDS such as chlorides, sulphates, magnesium, calcium and carbonates also affect corrosion or encrustation in water distribution systems.⁽²¹⁾ High TDS levels (above 500 mg/L) result in excessive scaling in water pipes, water heaters, boilers and household appliances such as tea kettles and steam irons.⁽⁴³⁾ Such scaling can shorten the service life of these appliances.⁽⁴⁴⁾

Rationale

1. The most important aspect of TDS with respect to drinking water quality is its effect on taste. The palatability of drinking water with a TDS level less than 600 mg/L is generally considered to be good. Drinking water supplies with TDS levels greater than 1200 mg/L are unpalatable to most consumers.

2. Concentrations of TDS above 500 mg/L result in excessive scaling in water pipes, water heaters, boilers and household appliances.

3. An aesthetic objective of ≤ 500 mg/L should ensure palatability and prevent excessive scaling. However, it should be noted that at low levels TDS contributes to the palatability of drinking water.

References

1. Rainwater, F.H. and Thatcher, L.L. Methods for collection and analysis of water samples. Geological Survey Water-Supply Paper, U.S. Department of the Interior. U.S. Government Printing Office, Washington, DC (1960).
2. Garrison Investigative Board. Water quality report (Appendix A). Garrison Diversion Study, report to the International Joint Commission (1977).
3. Durfor, C.J. and Becker, E. Constituents and properties of water. In: Water quality in a stressed environment: readings in environmental hydrology. W.A. Pettyjohn (ed.). Burgess Publishing Company, Minneapolis, MN (1972).
4. Rawson, D.S. and Moore, J.E. The saline lakes of Saskatchewan. Can. J. Res., 22: 141 (1944).
5. Fisheries and Environment Canada. Surface water quality in Canada. An overview. Water Quality Branch, Inland Waters Directorate (1977).
6. Upper Lakes Reference Group. The waters of Lake Huron and Lake Superior. Vols. I to III, Parts A and B. Report to the International Joint Commission (1977).
7. Great Lakes Water Quality Board. Great Lakes water quality 1974. Appendix A. Water Quality Objectives Subcommittee report to the International Joint Commission (1975).
8. Beeton, A.M. Indices of Great Lakes eutrophication. Publ. No. 15, Great Lakes Research Division, University of Michigan, Ann Arbor, MI (1966).
9. Kormondy, E.J. Concepts of ecology. Prentice-Hall, Englewood Cliffs, NJ. p. 182 (1969).
10. Vaughn, J.C. and Reed, P.A. Quality status of southern Lake Michigan. J. Am. Water Works Assoc., 62: 103 (1972).
11. Dominie, K. Personal communication. Newfoundland and Labrador Department of Environment and Lands (1989).

12. Durocher, H. Personal communication. Ministère de l'Environnement, Gouvernement du Québec (1990).
13. Vajdic, A. Personal communication. Ontario Ministry of the Environment (1989).
14. Rocan, D. Personal communication. Manitoba Department of Environment (1989).
15. Nargang, D. Personal communication. Saskatchewan Department of the Environment and Resource Management (1989).
16. Spink, D. Personal communication. Alberta Ministry of Environmental Protection (1989).
17. Willoughby, B.A. Personal communication. B.C. Ministry of Health (1989).
18. Ontario Ministry of the Environment. Outlines of analytical methods. A guide to the occurrence, significance, sampling and analysis of chemical and microbial parameters in water. February (1975).
19. Singh, T. and Kalra, Y.P. Specific conductance method for in situ estimation of total dissolved solids. *J. Am. Water Works Assoc.*, 67(2): 99 (1975).
20. Forbes, M. Personal communication. Canada Centre for Inland Waters, Burlington, Ontario (1988).
21. Sawyer, C.N. and McCarty, P.L. Chemistry for sanitary engineers. 2nd edition. McGraw-Hill Series in Sanitary Science and Water Resources Engineering, McGraw-Hill, Toronto (1967).
22. Department of National Health and Welfare. Water treatment principles and applications: a manual for the production of drinking water. Canadian Water and Wastewater Association (1993).
23. Canadian Council of Resource and Environment Ministers. Total dissolved solids. In: Canadian water quality guidelines. Prepared by the Task Force on Water Quality Guidelines. Environment Canada, Ottawa, March (1987).
24. Clark, J.W., Viessman, W., Jr. and Hammer, M.J. Water supply and pollution control. 3rd edition. Harper & Row Publishers, New York, NY (1977).
25. Burton, A.C. and Cornhill, J.F. Correlation of cancer death rates with altitude and with the quality of water supply of the 100 largest cities in the United States. *J. Toxicol. Environ. Health*, 3(3): 465 (1977).
26. Schroeder, H.A. Relation between mortality from cardiovascular disease and treated water supplies. Variation in states and 163 largest municipalities. *J. Am. Med. Assoc.*, 172: 1902 (1960).
27. Schroeder, H.A. Municipal drinking water and cardiovascular death rates. *J. Am. Med. Assoc.*, 195: 125 (1966).
28. Sauer, H.I. Relationship between trace element content of drinking water and chronic disease. *Univ. Ill. Bull.*, 71(108): 39 (1974).
29. Craun, G.F. and McCabe, L.J. Problems associated with metals in drinking water. *J. Am. Water Works Assoc.*, 67: 593 (1975).
30. Crawford, M., Gardner, M.J. and Morris, J.N. Mortality and hardness of local water supplies. *Lancet*, i: 827 (1968).
31. Meyers, D. Mortality and water hardness. *Lancet*, i: 398 (1975).
32. Popov, V.V. Cholelithiasis and calculi, hardness of drinking water. *Gig. Sanit.*, 33(6): 416 (1970).
33. Bruvold, W.H. and Pangborn, R.M. Rated acceptability of mineral taste in water. *J. Appl. Psychol.*, 50(1): 22 (1966).
34. Bruvold, W.H., Ongerth, H.J. and Dillehay, R.C. Consumer attitudes toward mineral taste in domestic water. *J. Am. Water Works Assoc.*, 59: 547 (1967).
35. Bruvold, W.H. Scales for rating the taste of water. *J. Appl. Psychol.*, 52: 245 (1968).
36. Bruvold, W.H. Mineral taste and the potability of domestic water. *Water Res.*, 4: 331 (1970).
37. Bruvold, W.H. and Ongerth, H.J. Taste quality of mineralized water. *J. Am. Water Works Assoc.*, 61: 170 (1969).
38. Cox, G.J., Nathans, J.W. and Vonau, N. Subthreshold-to-taste thresholds of sodium, potassium, magnesium and calcium ions in water. *J. Appl. Physiol.*, 8: 283 (1955).
39. Bryan, P.E., Kuzmurski, L.N., Sawyer, F.M. and Feng, T.H. Taste thresholds of halogens in water. *J. Am. Water Works Assoc.*, 65: 363 (1973).
40. Pangborn, R.M. and Bertolero, L.L. Influence of temperature on taste intensity and degree of liking of drinking water. *J. Am. Water Works Assoc.*, 64: 511 (1972).
41. Bruvold, W.H. and Pangborn, R.M. Rated acceptability of mineral taste in water. *J. Appl. Psychol.*, 50: 22 (1966).
42. Pangborn, R.M., Trabue, I.M. and Little, A.C. Analysis of coffee, tea, artificially flavoured drinks prepared from mineralized waters. *J. Food Sci.*, 36: 355 (1971).
43. Tihansky, D.P. Economic damages from residential use of mineralized water supply. *Water Resour. Res.*, 10(2): 145 (1974).
44. McQuillan, R.G. and Spent, P.G. The addition of chemicals to apartment water supplies. *J. Am. Water Works Assoc.*, 68: 415 (1976).

Chloride

An aesthetic objective of ≤ 250 mg/L has been established for chloride in drinking water. At concentrations above the aesthetic objective, chloride imparts undesirable tastes to water and to beverages prepared from water and may cause corrosion in the distribution system.

General

Chloride is widely distributed in nature, generally as the sodium (NaCl) and potassium (KCl) salts; it constitutes approximately 0.05% of the lithosphere.⁽¹⁾ By far the greatest amount of chloride found in the environment is in the oceans.

Underground salt deposits have been found in all Canadian provinces except British Columbia. Bedded deposits occur in southwestern Ontario, Saskatchewan and Alberta; dome deposits are found in Nova Scotia, New Brunswick, Ontario, Manitoba, Saskatchewan and Alberta.⁽²⁾

Sodium chloride is widely used in the production of industrial chemicals such as caustic soda (sodium hydroxide), chlorine, soda ash (sodium carbonate), sodium chlorite, sodium bicarbonate and sodium hypochlorite. In 1984, it was estimated that 4 078 000 t of sodium chloride were used by the chemicals industry.⁽²⁾ Sodium chloride and, to a lesser extent, calcium chloride (CaCl_2) are used for snow and ice control in Canada; 45% of all salt consumed in Canada is used for this purpose, compared with 25% in the United States and 14% in western Europe.⁽²⁾ In 1984, it was estimated that 3 560 800 t of sodium chloride were applied to Canadian roads.⁽²⁾ Potassium chloride is used in the production of fertilizers.^(1,2)

Occurrence

The presence of chloride in drinking water sources can be attributed to the dissolution of salt deposits,⁽³⁾ salting of highways to control ice and snow,⁽⁴⁻⁸⁾ effluents from chemical industries,⁽⁹⁾ oil well operations,⁽¹⁰⁾ sewage,⁽¹¹⁾ irrigation drainage,⁽¹²⁾ refuse leachates,⁽¹³⁾ volcanic emanations, sea spray and seawater intrusion in coastal areas.⁽¹⁾ Each of these sources may result in local contamination of surface water and groundwater. The chloride ion is highly

mobile and is eventually transported into closed basins or to the oceans.⁽¹⁾

Chloride is generally present at low concentrations in natural surface waters in Canada; concentrations are normally less than 10 mg/L and often less than 1 mg/L.^(12,14) The mean chloride concentration in 109 lakes in the Experimental Lakes Area (ELA) of northwestern Ontario was 0.8 mg/L in 1973; a chloride concentration of 0.9 mg/L was measured in a small acidic lake near Sudbury, Ontario, in the same year.⁽¹⁵⁾ The Great Lakes and waters in the St. Lawrence lowlands have somewhat higher concentrations of chloride (20 mg/L), largely because of industrial activities in the area.⁽¹⁾ The concentration of dissolved chloride in Canadian waters over the period 1980 to 1984 usually fell in the range <0.1 to 861 mg/L,⁽¹⁴⁾ but concentrations as high as 24 500 mg/L have been recorded in Bench Mark Creek in Alberta.⁽¹⁶⁾

Drinking water data for several Canadian provinces indicate that chloride concentrations are generally low, often less than 10 mg/L.^(14,17) Of 127 stations in Saskatchewan that analysed for chloride in 1975, only one recorded a chloride concentration greater than 50 mg/L; no station recorded a concentration greater than 250 mg/L.⁽¹⁸⁾ The same results were found for 56 stations in Nova Scotia that recorded chloride concentrations in drinking water during 1975.⁽¹⁹⁾ In Alberta, 51 out of 492 stations recorded chloride concentrations greater than 50 mg/L in 1976; 15 stations recorded concentrations greater than 250 mg/L.⁽²⁰⁾ In a 1987 analysis of 60 samples of treated water from the Lemieux Island water treatment plant in Ottawa, Ontario, the average chloride concentration was 5.5 mg/L (range 4.0 to 9.5 mg/L).⁽²¹⁾ The average concentration of chloride in U.S. public water supplies is about 11.5 mg/L⁽¹²⁾; in European water supplies, it is 52 mg/L.⁽²²⁾ Higher concentrations of chloride are most often present in drinking water derived from ground-water sources; this could be due to naturally high concentrations or to contamination. An estimated 25 to 50% of applied road salt enters groundwater.⁽²³⁾

Only limited data are available on chloride concentrations in air in Canada. A survey carried out in Edmonton over three one-month periods found the geometric means and ranges (in parentheses) of the

chloride concentrations in air to be as follows: November 1978, 1.97 $\mu\text{g}/\text{m}^3$ (0.3 to 9.0 $\mu\text{g}/\text{m}^3$); March/April 1979, 0.82 $\mu\text{g}/\text{m}^3$ (0.1 to 3.4 $\mu\text{g}/\text{m}^3$); and July/August 1979, 0.64 $\mu\text{g}/\text{m}^3$ (0.1 to 2.8 $\mu\text{g}/\text{m}^3$). For the total period of observation, the mean chloride concentration was 1.2 $\mu\text{g}/\text{m}^3$.⁽²⁴⁾ The chloride concentration in air above Lake Michigan was estimated to be 0.5 $\mu\text{g}/\text{m}^3$.⁽²⁵⁾

The chloride content of foods varies over a wide range; edible plants generally have concentrations below 0.5 mg/g, whereas meat and fish have concentrations up to 1.0 and 1.5 mg/g, respectively.⁽²⁶⁾

Canadian Exposure

Estimation of the daily intake of chloride in food is complicated by the widespread use of salt as a condiment. Approximately 600 mg of chloride per day are ingested in a salt-free diet.^(27,28) However, because of the addition of salt to food, the daily intake of chloride averages 6 g and may range as high as 12 g.⁽²⁹⁾

If one assumes that daily water consumption is 1.5 L and that the average concentration of chloride in Canadian drinking water is 10 mg/L, the average daily intake of chloride from drinking water can be calculated to be approximately 15 mg per person. The intake from water therefore constitutes only about 0.25% of the average intake from food.

If the average concentration of chloride in air in Canada is assumed to be 1.2 $\mu\text{g}/\text{m}^3$ and the daily respiratory volume is 20 m^3 , then the daily intake of chloride from air would be 0.024 mg.

Based on the above considerations, the total daily intake of chloride is about 6 g and comes almost entirely from food. Large deviations from this value are expected because of individual variations in the quantities of salt added to food during cooking and at the table.

Analytical Methods and Treatment Technology

Several analytical techniques may be used for chloride in water, including titration (e.g., potentiometric titration with silver nitrate), colorimetry (e.g., thiocyanate colorimetry), chloride ion selective electrode and ion chromatography.⁽³⁰⁾ Limits of detection range from 50 $\mu\text{g}/\text{L}$ for colorimetry to 5 mg/L for titration.

Because chloride is very soluble in water, it is not easily removed, and conventional water treatment processes are generally ineffective.⁽³¹⁾ A removal of 87% has been reported using a point-of-use treatment device employing granular activated carbon adsorption and reverse osmosis.⁽³²⁾ Chloride concentrations in water may increase during the treatment process if chlorine is used for disinfection purposes or if aluminum or iron chlorides are used for flocculation purposes.⁽¹⁷⁾

Health Considerations

Essentiality

Chloride is an essential element and is the main extracellular anion in the body. It is a highly mobile ion that easily crosses cell membranes and is involved in maintaining proper osmotic pressure, water balance and acid-base balance.

Until recently, it had been assumed that the physiological role of the chloride ion was simply that of a passive counterion. Over the past few years, however, several studies have suggested that the chloride ion may play a more active and independent role in renal function,^(33,34) neurophysiology⁽³⁵⁾ and nutrition.⁽³⁶⁾

Absorption, Distribution and Excretion

Absorption of chloride from the diet is considered to be essentially complete. Balance studies in young men have shown that 92% of the ingested chloride is excreted in the urine.⁽³⁷⁾

The amount of chloride in the intestinal contents declines as the contents move along the gastrointestinal tract. Typically, 540 mg of chloride enter the duodenum each day.⁽³⁸⁾ Chloride is absorbed in the jejunum by "solvent drag" and in the ileum and colon by active chloride transport coupled to bicarbonate secretion.^(38,39) Both of these processes are linked to sodium-based co-transport mechanisms that create the necessary osmotic and electrochemical gradients.

It has been estimated that the human body contains 0.15% chloride,⁽⁴⁰⁾ or 105 g/70 kg bw. Most of this chloride is extracellular, as shown by serum levels of 98 to 106 meq/L, compared with the approximate 1 meq/L for tissue cells.⁽⁴¹⁾ Stomach secretions are high in chloride, with concentrations between 45 and 155 meq/L in gastric residues. All body chloride is considered to belong to an exchangeable pool.⁽⁴²⁾

Body chloride concentrations are regulated by excretions, primarily via the kidneys. Both chloride and sodium are regulated by aldosterone.⁽⁴³⁾ The urinary excretion of chloride for adults is about 4.4 g/d, with a range of 2.2 to 13 g/d; the amount excreted is closely related to the amount of salt in the diet. Chloride loss in the faeces is low, with 14 to 110 mg excreted daily by this route. Significant additional daily losses of chloride occur in perspiration.⁽³⁷⁾

Toxic Effects

A role for chloride in sodium-sensitive hypertension has been proposed.^(44,45) Evidence seems to indicate that both sodium and chloride are required for a hypertensive effect.⁽⁴²⁾ Chloride by itself does not appear to cause hypertension in rats,⁽⁴⁶⁾ although red blood cells from human hypertensives show altered chloride handling.⁽⁴⁷⁾ The role of sodium in hypertension is under investigation (see sodium review); however, there

is no evidence that high chloride concentrations would be any more toxic than high sodium concentrations.

Other Considerations

The taste threshold for chloride is dependent on the associated cation and is generally in the range of 200 to 300 mg/L.⁽³¹⁾ Chloride concentrations detected by taste in drinking water by panels of 18 or more people were 210, 310 and 222 mg/L from the respective sodium, potassium and calcium salts.⁽⁴⁸⁾ The taste of coffee was affected when brewed with water containing chloride concentrations of 400, 450 and 530 mg/L from sodium, potassium and calcium chloride, respectively.⁽⁴⁸⁾

Chloride concentrations above 250 mg/L in drinking water may cause corrosion in the distribution system.⁽²³⁾ The chloride ion's ability to form soluble salts with many metal ions prevents the formation of films that could prevent the further corrosion of metal surfaces.⁽¹⁷⁾

Rationale

1. Chloride concentrations in the body are well regulated through a complex interrelated system involving both nervous and hormonal systems. Even after intake of large quantities of chloride through food and water, the chloride balance is maintained, mainly by the excretion of excess chloride via the urine. Therefore, a maximum acceptable concentration has not been set for chloride in drinking water.

2. Taste thresholds for chloride from sodium chloride, potassium chloride and calcium chloride in drinking water are 210, 310 and 222 mg/L, respectively; the taste of coffee is affected when brewed with water containing chloride concentrations of 400, 450 and 530 mg/L from the same salts. Chloride concentrations above 250 mg/L in drinking water may cause corrosion in the distribution system.

3. The aesthetic objective for chloride in drinking water is therefore ≤ 250 mg/L. Chloride concentrations in Canadian drinking water supplies are generally much lower than 250 mg/L.

References

1. National Research Council of Canada. The effects of alkali halides in the Canadian environment. NRCC No. 15019, Associate Committee on Scientific Criteria for Environmental Quality, Ottawa (1977).
2. Prud'Homme, M. Salt. In: Canadian minerals yearbook 1985 — review and outlook. Mineral Report 34, Mineral Resources Branch, Department of Energy, Mines and Resources, Ottawa (1986).
3. National Academy of Sciences. Nutrient and toxic substances in water for livestock and poultry. National Academy Press, Washington, DC (1974).
4. Murray, D.M. and Ennst, V.F.W. An economic analysis of the environmental impact of highway de-icing salts. National Technical Information Service Publication 253, U.S. Department of Commerce, Springfield, VA. p. 268 (1976).
5. Pollock, J.J. and Toler, L.G. Effects of highway de-icing salts on groundwater and water supplies in Massachusetts. U.S. Geological Survey, Boston, MA (1972).
6. Terry, R.C. Road salt, drinking water and safety. Ballinger, Cambridge, MA (1974).
7. Hutchinson, F.E. Effects of highway salting on the concentration of sodium chloride in private water supplies. Res. Life Sci., Fall: 15 (1969).
8. Ralston, J.G. De-icing salts as a source of water pollution. Ontario Ministry of the Environment, Toronto (1971).
9. Little, A.D. Inorganic chemical pollution of freshwater. U.S. Environmental Protection Agency, Washington, DC (1971).
10. Pettyjohn, W.A. Water pollution by oil-field brines and related industrial wastes in Ohio. Ohio J. Sci., 71: 257 (1971).
11. Pettyjohn, W.A. Water quality in a stressed environment. Burgess Publishing Co., MN (1972).
12. Bond, R.G. and Straub, C.P. Handbook of environmental control. Vol. 3. Chemical Rubber Co., Cleveland, OH (1973).
13. Schneider, W.J. Hydrologic implications of solid-waste disposal. U.S. Geol. Surv. Circ., 601-F: F1 (1970).
14. National Water Quality Data Bank (NAQUADAT). Water Quality Branch, Inland Waters Directorate, Environment Canada, Ottawa (1985).
15. Beamish, R.J. and Van Loon, J.C. Precipitation loading of acid and heavy metals to a small acid lake near Sudbury, Ontario. J. Fish. Res. Board Can., 34: 649 (1977).
16. Environment Canada. Detailed surface water quality data, Northwest Territories 1980–1981, Alberta 1980–1981, Saskatchewan 1980–1981, Manitoba 1980–1981. Inland Waters Directorate (1984).
17. World Health Organization. Sodium, chlorides and conductivity in drinking-water. Report on a WHO Working Group. EURO Reports and Studies 2, Regional Office for Europe, Copenhagen (1979).
18. Saskatchewan Department of the Environment. Personal communication.
19. Nova Scotia Department of Public Health. Personal communication.
20. Alberta Environment. Summary of water chemical analysis. Environmental Protection Services (1976).
21. Ontario Ministry of Environment. Ottawa (Lemieux Island) water treatment plant. Drinking Water Surveillance Program. Annual report 1987. Queen's Printer for Ontario (1988).
22. Zoeteman, B.C.J. and Brinckman, F.J.J. Human intake of minerals from drinking water in the European Communities. In: Hardness of drinking water and public health. Proceedings of the European Scientific Colloquium, Luxembourg, 1975. Pergamon Press, Oxford. p. 175 (1976).
23. McConnell, H.H. and Lewis, J. ... Add salt to taste. Environment, 14: 38 (1972).
24. Klemm, R.F. and Gray, J.M.L. A study of the chemical composition of particulate matter and aerosols over Edmonton. Report No. RMD 82/9, prepared for the Research Management Division by the Alberta Research Council (1982).
25. Klein, D.H. Fluxes, residence times and sources of some elements to Lake Michigan. Water Air Soil Pollut., 4: 3 (1975).
26. Diem, K. (ed.). Documenta Geigy. Scientific tables. 5th edition. J.R. Geigy, Basle, Switzerland. p. 501 (1962).

27. Dahl, L.K. Salt and hypertension. *Am. J. Clin. Nutr.*, 25: 231 (1972).
28. Meneely, G.R. A review of sources of and the toxic effects of excess sodium chloride and the protective effect of extra potassium in the diet. *Plant Foods Hum. Nutr.*, 23: 3 (1973).
29. Department of National Health and Welfare. Dietary standard for Canada. Health Protection Branch, Ottawa (1975).
30. Department of the Environment of the United Kingdom. Methods for the examination of waters and associated materials: chloride in waters, sewage and effluents 1981. Her Majesty's Stationery Office, London (1981).
31. World Health Organization. Guidelines for drinking-water quality. Vol. 2. Health criteria and other supporting information. Geneva (1984).
32. Regumathan, P., Beauman, W.H. and Kreusch, E.G. Efficiency of point of use treatment devices. *J. Am. Water Works Assoc.*, 75(1): 42 (1983).
33. Jaina, A., Kapuler, S., Govendo, S., Serban, I. and Eliahou, H.E. Blood pressure and renin activity in Na⁺, Cl⁻, or NaCl loading in rats. *Miner. Electrolyte Metab.*, 3: 276 (1980).
34. Toto, R.D., Hulter, H.N., Mackie, S. and Sebastian, A. Renal tubular acidosis induced by dietary chloride. *Kidney Int.*, 25: 26 (1984).
35. Sackmann, B. and Neher, E. Patch clamp techniques for studying ionic channels in excitable membranes. *Annu. Rev. Physiol.*, 46: 455 (1984).
36. Honeyfield, D.C. and Froseth, J.A. Effects of dietary sodium and chloride on growth, efficiency of feed utilization, plasma electrolytes and plasma basic amino acids in young pigs. *J. Nutr.*, 115: 1366 (1985).
37. International Commission on Radiological Protection. Report of the Task Group on Reference Man. ICRP Publication No. 23, Pergamon Press, Oxford. p. 379 (1975).
38. Weinberg, J.M. Fluid and electrolyte disorders and gastrointestinal diseases. In: *Fluids and electrolytes*. J.P. Kokko and R.L. Tanner (eds.). W.B. Saunders Co., Toronto (1986).
39. Schultz, S.G. A cellular model for active sodium absorption by mammalian colon. *Annu. Rev. Physiol.*, 46: 435 (1984).
40. National Academy of Sciences. Recommended dietary allowances. 9th edition. Washington, DC (1980).
41. Siggaard-Anderson, O. Blood gases and electrolytes. In: *Fundamentals of clinical chemistry*. N.W. Tietz (ed.). W.B. Saunders Co., Philadelphia, PA (1976).
42. Department of National Health and Welfare. Recommended nutrient intake for Canadians. Ottawa (1983).
43. Tortora, G.J. and Anagnostakos, N.P. Principles of anatomy and physiology. 4th edition. Harper & Row, New York, NY (1984).
44. Kurtz, T.W. and Morris, R.C., Jr. Dietary chloride as a determinant of "sodium-dependent" hypertension. *Science*, 222: 1139 (1983).
45. Whitescarves, S.A., Ott, C.E. and Jackson, B.A. Salt-sensitive hypertension: contribution of chloride. *Science*, 223: 1430 (1984).
46. Whiting, S.J. and Cole, D.E.C. Effects of dietary anion composition on acid-induced hypercalciuria in the adult rat. *J. Nutr.*, 116: 388 (1986).
47. Zidek, W., Losse, H., Lange-Asschenfeldt, H. and Vetter, H. Intracellular chloride in essential hypertension. *Clin. Sci.*, 68: 45 (1985).
48. Lockhart, E.E., Tucker, C.L. and Merritt, M.C. The effect of water impurities on the flavor of brewed coffee. *Food Res.*, 20: 598 (1955).

OSMOREGULATION IN FRESHWATER INVERTEBRATES IN RESPONSE TO EXPOSURE TO SALT POLLUTION

Report to the
Water Research Commission

by

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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 5th percentile determined as a lethality benchmark, analogous to the Fair/Poor boundary. Similarly, the 5th 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 LC₅₀s 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 LC₅₀ 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 MgSO₄ 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 MgSO₄. 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 Na₂SO₄ 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 (Na₂SO₄). 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 Na₂SO₄ 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 Na₂SO₄, 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 Na₂SO₄ 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 Na₂SO₄ (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 Na₂SO₄ 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 (Na₂SO₄). *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 Na₂SO₄ 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 Na₂SO₄.

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 MgSO₄ 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 EC₅₀s 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 sub-organism 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.

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ABBREVIATIONS

ACR	acute-chronic ratio
ANOVA	analysis of variance
BOD	biological oxygen demand
C₅₀	lethal concentration at which 50% of the tested population dies
COD	chemical oxygen demand
Conc	Concentration
CV	coefficient of variation
DEEEP	Direct Estimation of Ecological Effects Potential
DO	dissolved oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
EC	electrical conductivity
EC₅₀	effective concentration at which 50% of the tested population shown an effect
ECOTOX	USEPA database of ecotoxicology data
EWQ	environmental water quality
IWR	Institute for Water Research
LOEC	lowest observed effect concentration
MATC	maximum allowable toxicant concentration
MgSO₄	magnesium sulphide
NaCl	sodium chloride
Na₂SO₄	sodium sulphide
NOEC	no observed effect concentration
NWA	National Water Act
OC	osmoregulatory capacity
PC	protective concentration
PCV	protective concentration value
RDM	Resource Directed Measures
RUESC	Rhodes University Ethical Standards Committee
SDC	Source Directed Controls
SSD	species sensitivity distribution
Std. Dev.	Standard deviation
T	temperature
TEACHA	Tool for Ecological Aquatic Chemical Habitat Assessment
TSK	Trimmed Spearman-Kärber
UCEWQ	Unilever Centre for Environmental Water Quality
USEPA	United States Environmental Protection Agency
WRC	Water Research Commission
WQG	Water Quality Guideline

1 GENERAL INTRODUCTION

Conservation physiology was defined by Wikelski and Cooke (2006) as “the study of physiological responses of organisms to human alteration of the environment that might cause or contribute to population declines”. Wide arrays of disciplines, including environmental toxicology, are able to make contributions towards sustainable environmental management and conservation (Wikelski and Cooke, 2006). This research field may, therefore, be useful in contributing towards achieving a balance between water resource protection (and conservation of biodiversity) and resource use, a requirement in achieving long-term sustainability as required by the South African National Water Act (National Water Act, 1998). Wikelski and Cooke (2006) consider that physiological characteristics (of key species) may prove useful in predicting and anticipating environmental problems, thus ensuring that corrective management interventions can be instituted to achieve desired conservation measures.

This study was initiated as a developmental process towards establishing a possible role for physiological responses such as osmoregulation in water resource management tools. In this project a strong emphasis was placed on building capacity through the participation of students and early career scientists.

1.1 Managing environmental water quality

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). In order to achieve a balance of resource protection while ensuring long-term and optimal resource use (National Water Act, 1998) the Environmental Water Quality (EWQ) approach has been proposed for application in managing environmental water quality in both Resource Directed Measures (RDM) and Source Directed Controls (SDC) (Scherman et al., 2003). EWQ is an approach which recognises the value of using aquatic organisms for resource protection and monitoring, combining biomonitoring and ecotoxicology with the traditionally used physico-chemical measurements when defining ecologically acceptable water quality parameters (Palmer et al., 2004). In general it is understood that biota react adversely to increases in salinisation, although the effects on individual species is poorly understood (Hart et al., 1991). Urgent attention therefore needs to be paid to assessing the effects of salts on biota in water resources in order to optimise resource protection and resource utilization.

1.2 Endpoints in toxicology assessments in water resource management:

1.2.1 Developing water quality guidelines

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). In order to create ecological Reserves that adequately balance protection of aquatic ecological systems and sustainable human use needs, accurate and if possible site-specific water quality guidelines (WQGs) need to be created through an integrated understanding of physico-chemical, biomonitoring, and ecotoxicological data (Palmer et al., 2005).

Ecotoxicology provides valuable and highly reliable data for the creation of boundary values used to establish WQGs (Warne et al., 2005) as it relates biological responses of test organisms to physico-chemical values in a concentration-response relationship (Scherman et al., 2003). Although acute toxicity test data can be found in abundance internationally, it is widely recognized that site-specific data using indigenous species and long term chronic tests are ultimately required to provide precise

local ecospecs (Scherman et al., 2003). Indeed, the accuracy or reliability of using acute toxicity data has been much debated (Roux et al., 1996). Warne (1998) argued that acute data could not be used to show long term exposure effects and that it was important to incorporate sub-lethal chronic toxicity data in the process of deriving these guidelines.

Site specific WQGs for inorganic salts, proposed by Jooste and Rossouw (2002), were derived without including tolerances of South African biota (Slaughter et al., 2004). The need for widespread site- and indigenous species-specific water chemistry and toxicology testing is a reoccurring theme (Scherman and Palmer, 2000; Palmer et al., 2004; Warne et al., 2005; Browne, 2005; Palmer et al., 2005) and a major reason for the initiation of this study.

1.2.2 Toxicity assessment approaches

The current methodology for water quality assessments includes the determination of boundary values for specific salts, based on biological effects data. Aquatic ecotoxicology is central to the EWQ approach, although currently much of the data are based on acute (lethal) toxicity tests due to the paucity of chronic (sub-lethal) toxicity data. To add to uncertainty, even fewer data based on indigenous species are available. Extrapolation methods from acute to chronic endpoints have been shown statistically possible in deriving accurate chronic endpoints by exposing the freshwater shrimp *Caridina nilotica* to inorganic salts (Slaughter, 2005).

Riverine macroinvertebrates are excellent indicators of water-borne pollutants (they are in constant contact with pollutants in the water column), and are suitable laboratory-test organisms. They hold a key position in aquatic food chains but little information is available on their tolerances to increased salinities. Methods have been developed by UCEWQ for generating chronic toxicity test data for selected indigenous species, at both organism and sub-organism levels (Gordon et al., 2009). However, accurate interpretation of toxicity test results remains elusive as very little is known of the biology, and physiology, of these indigenous toxicity test species. Physiological functions, including endocrine control mechanisms, mediate the relationship of the organism to its environment (Ricklefs and Wikelski, 2002). Thus it has been argued that comparative physiology does have an important role to play in informing a variety of assumptions made in macro-ecology, including tolerances to pollutants (Chown et al., 2002).

1.3 Selected physiological responses to environmental water quality stress

In general freshwater animals are termed hyperosmotic, meaning they have a higher concentration of solute (or salts) than the water surrounding them. As a result freshwater animals constantly have to excrete water in order to maintain equilibrium, and in so doing lose some solutes (Schmidt-Nielsen, 1998). Freshwater animals therefore continually need to take up ions to replace those lost through diffusion to the environment (Boisen et al., 2003). Although some animals are able to tolerate and adapt to a wide range of salinities (euryhaline), most are stenohaline (have a narrow range of tolerance) (Schmidt-Nielsen, 1998). Therefore changes in salinity, for example through addition of inorganic salts from industrial effluents and agricultural runoff, are likely to affect the ability of organisms to effectively osmoregulate. This in turn may affect such factors as endocrine balance, and oxygen consumption following chronic exposures, with subsequent changes in physiological processes. Elevated energy expenditures may occur until a threshold of intolerance is reached. Thresholds may in turn differ between species even of the same genus (Rowe, 2002).

1.3.1 Oxygen consumption as a measure of physiological stress

An indirect indicator of metabolic rate in fish is the rate of oxygen consumption usually expressed in mg oxygen per gram dry weight of the test species per hour (Chech, 1990). Oxygen consumption has been used to assess the energetic cost of osmoregulation in several fish species when exposed to increasing salinities (Altinok and Grizzle, 2003; Zheng et al., 2000). Differences in oxygen consumption found in a range of species tested seem to be partly based on the developmental stage of the species (Moser and Hettler, 1989; Aristizabal-Abud, 1992) and their degree of euryhalinity or stenohalinity. As fish metabolic expenditures rise, ventilation-related osmotic and ionic activities will increase (Rao, 1968). In this study oxygen consumption could not accurately be measured and therefore changes in DO were used as a surrogate measure.

1.3.2 Osmoregulation as a measure of physiological stress

Main sites for osmoregulation in both fish and invertebrates are the gills, which are also responsible for active uptake of lost solutes. The sodium pump ($\text{Na}^+ + \text{K}^+ - \text{ATPase}$) is the main mechanism for moving ions across the gills in aquatic animals (Lucu and Towle, 2003). Freshwater fish primarily use their kidneys for maintaining water balance and excreting harmful substances. The mechanism of osmoregulation used is dependent on the developmental status of the animal, for example pre-larval fish osmoregulate largely through the skin, whereas larval stages regulate through the gills (Varsamos and Charmantier, 2005). Insects, in addition, possess a network of Malpighian tubules lined with secretory cells extending throughout much of the body cavity and attached to the alimentary canal between the midgut and hindgut, where ions get reabsorbed before waste is excreted (Dettner and Peters, 1999).

Osmoregulation can be monitored by measuring osmolarity or osmolality, depending on the mechanism used to determine endpoints. Osmolarity is the concentration of osmotically active particles in solution, which may be quantitatively expressed in osmoles of solute per litre of solution, whereas osmolality is expressed in osmoles of solute per kilogram of solvent (Schmidt-Nielsen, 1998). Osmolality of the haemolymph (in the case of macroinvertebrates) will give an indication of the osmotic concentration of the transport fluid when the animal is exposed to higher concentrations of inorganic salts.

1.4 Selection of environmental water quality stressors: Inorganic salts

South Africa is largely a semi-arid country with an average rainfall of 450 mm per annum, almost half the global average (DWA, 2004), making it a water-scarce country. Much emphasis is placed on the conservation and management of the water resource. In addition to meeting ecological needs (The Ecological Reserve) this resource also needs to meet human needs (Basic Human Needs Reserve) (NWA, 1998). Pressure to develop infrastructure and provide food security has resulted in the industrial and agricultural sectors expanding rapidly over the last few years (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). Three main causes for increased salinisation have been cited by Goetsch and Palmer (1997): the geology of the area, agricultural practices and industrial activities. This increase in salinisation can have severe impacts on the biota in these river systems. In particular it may affect the osmoregulation of both invertebrates and vertebrate species while negatively affecting the uptake of oxygen of these biota (Schmidt-Nielsen, 1998). Sodium sulphate (Na_2SO_4) and sodium chloride (NaCl) have been identified as suitable indicators of salinisation as most agricultural salts are dominated by NaCl while industrial salts are dominated by SO_4^{2-} (Dallas and Day, 1993). According to

Palmer et al. (2005) MgSO_4 is more toxic than Na_2SO_4 and NaCl making it the most toxic of the six common inorganic salts listed in the Reserve process (Jooste and Rossouw, 2002). It was specifically suggested by Browne (2005) that MgSO_4 be tested on indigenous South African organisms because no such tests have yet been undertaken. Thus, Na_2SO_4 , NaCl , and MgSO_4 were selected as the three salts to be tested for this study (Table 1.1).

Table 1.1 Choice of three inorganic salts used for this study

Salt (abbreviation)	Chemical structure	Reasons for choice
Magnesium sulphate (MgSO_4)	$\begin{array}{c} \text{O} \\ \\ \text{O}^- - \text{S} = \text{O} \\ \\ \text{O}^- \\ \text{Mg}^{2+} \end{array}$	<ul style="list-style-type: none"> - considered the most toxicologically important salt of those used in Present Ecological State assessments - therefore a core water quality variable for ecological water quality Reserve assessments. - consistently responsible for Poor to Fair water quality class classification (Jooste and Rossouw, 2002).
Sodium chloride (NaCl)	$\text{Na}^+ - \text{Cl}^-$	<ul style="list-style-type: none"> - dominant naturally-occurring salt of inland and south western parts of South African waters (Day, 1993). - dominates agricultural salts - necessary core water quality variable for ecological water quality Reserve assessments.
Sodium sulphate (Na_2SO_4)	$\begin{array}{c} \text{Na}^+ \quad \text{O}^- \quad \text{Na}^+ \\ \\ \text{O} = \text{S} = \text{O} \\ \\ \text{O}^- \end{array}$	<ul style="list-style-type: none"> - dominates industrial effluent - core water quality variable for ecological water quality Reserve assessments.

1.5 Selection of test organisms

Test organisms selected to investigate osmoregulatory responses to inorganic salt exposure are listed in Table 1.2.

Mayflies (Ephemeroptera) were selected as indigenous insect representatives as they are abundant in South African rivers, widespread, easy to collect and are established as suitable toxicity test organisms (Palmer et al., 2004). Mayflies have also been exposed to salts in previous toxicity tests (Goetsch and Palmer, 1997). Organisms were collected in the field and identified in our laboratories prior to conducting toxicity tests. Representatives from three different genera were collected and identified: Heptageniidae (*Afronurus barnardi*), Tricorythidae (*Tricorythus discolor*), and Leptophlebiidae (*Euthraululus elegans*) as used previously in Palmer et al. (2004).

The shrimp, *Caridina nilotica*, was chosen as indigenous crustacean representative. This species is frequently used as a toxicity test organism within UCEWQ for testing salts and other pollutants like pesticides and herbicides. The freshwater shrimp is widespread in South Africa and easy to collect. Organisms were field collected from a known relatively unimpacted reference site in the Eastern Cape, South Africa.

Two species of fish were chosen as representation of aquatic vertebrates: the guppy (*Poecilia reticulata*) and the Zebra fish (*Danio rerio*). Both species are commonly used in toxicity testing internationally and are not indigenous to South Africa. At the time of this study however, tests with

both species were warranted as there were no test protocols available for indigenous species at the time. Since then, Rall et al. (2010) have described breeding and toxicity test methods for indigenous fish such as *Barbus trimaculatus*, which could be considered for use in future testing. Zebrafish (*Danio rerio*) are easily bred and kept in captivity, and are commonly used as a test standard in toxicology studies. However little is known about their physiology when coping with osmoregulation (Boisen et al., 2003). The guppy (*Poecilia reticulata*) is a standard species for toxicology tests due to their ease of breeding in captivity and relatively short life cycle. Guppies and Zebra fish were obtained from a local breeder.

Table 1.2 Choice of test species to investigate osmoregulatory responses to inorganic salt exposure

Test animal	Common name	Reasons for choice
<i>Afronurus barnardi</i> , <i>Tricorythus discolor</i> , <i>Euthraulus elegans</i>	Mayflies	<ul style="list-style-type: none"> - abundant - indigenous - widespread in South Africa - easy to collect - toxicity test protocol exists (Goetsch and Palmer, 1997)
<i>Caridina nilotica</i>	Freshwater Shrimp	<ul style="list-style-type: none"> - indigenous - widespread in South Africa - easy to collect - used for lethal and sublethal toxicity testing, at UCEWQ-IWR laboratories (WRC project number K5/1313)
<i>Poecilia reticulata</i>	Guppy	<ul style="list-style-type: none"> - available in sufficient numbers from a local breeder - recommended for short term fish toxicity testing in the National Direct Estimation of Ecological Effect Potential (DEEEP) (DWAF, 2004).
<i>Danio rerio</i>	Zebra fish	<ul style="list-style-type: none"> - available in sufficient numbers from a local breeder - recommended for long term chronic fish development toxicity testing in the National Toxicity Monitoring Programme (DWAF, 2005).

2 FRESHWATER MACROINVERTEBRATE RESPONSES TO SELECTED INORGANIC SALTS

2.1 Introduction

The objective of this experiment was to compare sensitivities of three different mayfly species to magnesium sulphate (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 Reserve boundary values for MgSO_4 using a species sensitivity distribution (SSD) approach.

2.2 Materials and Methods

2.2.1 Experimental organisms

Nymphs of three different mayfly (Ephemeroptera) genera were used for this experiment: *Afronurus barnardi* (Heptageniidae), *Tricorythus discolor* (Tricorythidae), and *Euthraulus elegans* (Leptophlebiidae). These indigenous mayflies were collected from the Kat River, Eastern Cape, South Africa, and are considered an established species for toxicity testing in South Africa (Scherman et al., 2003). These three test species were exposed to increasing concentrations of magnesium sulphate (MgSO_4) in three separate experiments.

2.2.2 Experimental systems

The experimental design was based on the recirculating channel system described by Scherman and Palmer (2000), with the following minor adjustments to facilitate a ten day chronic test:

Test solutions were changed on day 4 and 8 to minimise build up of algae and nutrients within channels. Experiments were conducted at a constant temperature in a controlled environment room. Water quality parameters were recorded after each water change. Animals were fed by placing three disks of filter paper used to filter 250 mL Palmiet River water beneath stones in each channel for 6 h prior to each water change.

2.2.3 Experimental design and procedure

The number of organisms per channel per experiment is detailed in Table 2.1. Organism numbers varied between experiments. The organisms were given 36 h to acclimatise to laboratory conditions before test solutions were applied. Nymphs dying before the application of exposure solutions were removed at the start of the test and were not included in the statistical analysis.

Toxicity tests were conducted over a 10 day (240 h) period. LC_{50} values determined after 96 h were considered acute endpoints, while LC_{50} values determined after 240 h were considered chronic endpoints.

Table 2.1 Number of animals per species per channel for 3 experiments

Experiment	Organism number per channel		
	<i>A. barnardi</i>	<i>T. discolor</i>	<i>E. elegans</i>
Exp1	25	30	35
Exp2	35	35	22
Exp3	20	25	25

Water quality parameters (pH, temperature, EC) were recorded for each channel daily to ensure consistency within the channels. The test endpoint was defined as mortality or immobilisation assessed by prodding the organism and checking for movement. Acceptable control mortality was restricted to 10% for the 96 h period. Percentages reported for mortalities were based on the total number of dead organisms removed from the channels during the experiment and survivors present at the end. Emerged or escaped organisms did not contribute towards the data. Dechlorinated tap water was used as the solvent. Experiment one (Exp1) was undertaken in a different laboratory to experiment two (Exp2) and experiment three (Exp3).

The test animals for Exp1 and Exp2 were collected from a riffle at a minimally impacted reference site in close proximity to Hertzog village, Eastern Cape, South Africa on 19 March (Exp1) and 28 April (Exp2), 2006. Due to high flow conditions in the Kat River on 19 August 2006, animals were collected from the riffle at a reference Site on the Balfour River for Exp3. The Balfour is a relatively unimpacted tributary of the Kat River. Collection was carried out by sweeping selected nymphs off rocks and into buckets of river water with a paintbrush. They were transported to the laboratory in ice-cooled and aerated water by car within three h of collection. Dissolved oxygen (DO), water temperature, pH and electrical conductivity (EC) were measured in the field at the time of collection.

2.2.4 Data analysis

Data were analysed using either Probit or Trimmed Spearman-Kärber (TSK) regression analysis to provide LC_{50} values. Only data unsuitable for Probit analysis (i.e. greater than 10% mortality in the control and deviations from increased mortality with increased concentration assumption) were subjected to TSK analysis. The acute (96 h) LC_{50} values were used in conjunction with acute (<96 h) LC_{50} data on eleven other taxa from the ECOTOX toxicity database (USEPA, 2004) and subjected to a Species Sensitivity Distribution (SSD). The SSD was produced using the Burr Type III regression analysis run on BurrliOz software. The concentration divisor was calculated as the geometric mean of the acute/chronic ratios (ACR) of the LC_{50} values generated in this study. The ACRs were calculated using 96 h and 240 h LC_{50} s. The SSD was used to calculate species protection parameters for 'Natural' (95%), 'Good' (90%) and 'Fair' (80%) conditions as defined by the South African water quality management boundary classification (Warne et al., 2004). The boundary values produced from the SSD were compared to the benchmark boundary values currently in use for Reserve assessments in South Africa (Table 5.1).

2.3 Results

Mapped distributions of the three test species indicate that they inhabit a wide range of South African river systems. *E. elegans* (Figure 2.1) is found country-wide, inhabiting most river systems including the Orange-Vaal, Great Fish, Great Berg, Incomati and Olifants-Klip River systems. *T. discolor* (Figure 2.2) is similarly widely distributed. Both species are found in river systems bordering or flowing through neighbouring countries, suggesting further distribution into Zimbabwe, Mozambique, Lesotho and Swaziland. Both *E. elegans* and *T. discolor* are found in all six of the water quality management regions proposed by Day et al. (1998). *A. barnardi* (Figure 2.3) appears to have a more restricted range, being found mostly in eastern regions including the Incomati, Limpopo, Olifants-Kliprivier, Tugela, Umvoti and Vaal River systems. This species is therefore largely absent from the pure waters of the southern and western coasts, the highly mineralized chloride/sulphate waters of the arid interior and alkaline soda carbonate/temporary hard carbonate waters of the upper Orange/Vaal region (Day et al., 1998).

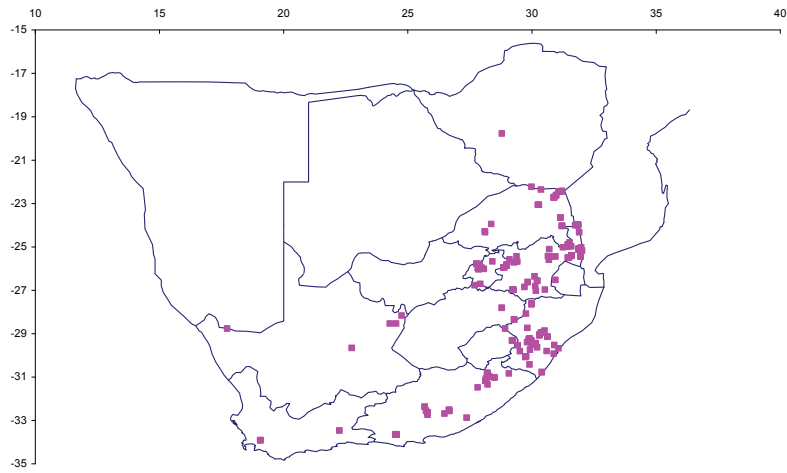


Figure 2.1 Distribution of *E. elegans* in South Africa based on information from the Albany Museum.

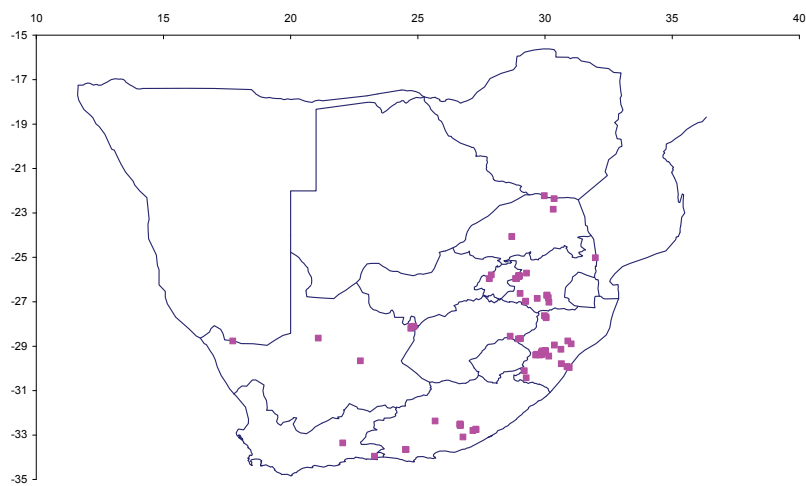


Figure 2.2 Distribution of *T. discolor* in South Africa based on information from the Albany Museum.

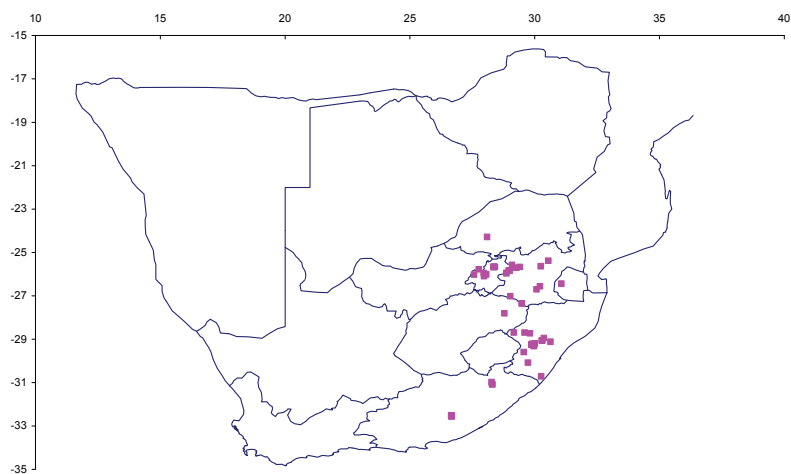


Figure 2.3 Distribution of *A. barnardi* in South Africa based on information from the Albany Museum.

2.3.1 Test conditions

Water quality parameters measured during experiments in the laboratories (Tables 2.2, 2.3 and 2.4) indicate that consistent water temperatures were maintained in all experiments. Coefficients of variation (CV) did not exceed 2.2%.

Table 2.2 Summary of water parameters per channel over 10 days for Exp1

Concentration (g/L)	Mean EC (mS/m)	CV (%)	Mean pH	CV (%)	Mean T (°C)	CV (%)
0.0	58.73	14.36	7.4	4.45	16.3	1.22
1.2	138.21	6.89	7.6	4.80	16.3	1.22
1.6	160.33	7.72	7.6	4.50	16.3	1.22
2.1	190.08	3.99	7.6	4.70	16.3	1.22
2.9	227.87	5.89	7.7	4.46	16.3	1.22
3.8	272.60	3.73	7.6	4.86	16.3	1.22
5.0	326.76	3.87	7.7	4.23	16.3	1.22
6.75	403.12	2.87	7.7	4.17	16.3	1.22
9.0	501.30	4.39	7.8	4.03	16.3	1.22
12.0	609.08	3.99	7.8	4.69	16.3	1.22

Table 2.3 Summary of water parameters per channel over 10 days for Exp2

Concentration (g/L)	Mean EC (mS/m)	CV (%)	Mean pH	CV (%)	Mean T (°C)	CV (%)
0.0	43.28	2.35	7.8	3.66	16.49	2.14
1.5	128.31	2.42	7.9	3.94	16.49	2.14
2.0	148.80	2.78	8.1	1.48	16.49	2.14
2.7	181.93	3.48	8.1	0.96	16.49	2.14
3.5	154.75	32.42	8.2	1.61	16.49	2.14
4.7	621.91	11.04	8.2	1.94	16.49	2.14
6.3	267.65	47.89	8.2	1.76	16.49	2.14
8.4	397.10	3.07	8.2	1.72	16.49	2.14
11.25	493.80	2.65	8.2	1.47	16.49	2.14
15.0	609.24	2.42	8.2	1.51	16.49	2.14

The trends in test channel EC values were different for each experiment. In Exp1 (Table 2.2) the CV for the control solution was high (14.36%), while those of all the other concentrations remained lower than 7.5%. Although slight rises in conductivity occurred over time, no channel in Exp1 experienced a change of more than 50.0 mS/m over 10 days. The control solution in Exp2 (Table 2.3) did not exhibit such high EC variability. Very high CVs occurred in the channels containing 3.5 g/L, 4.7 g/L and 6.3 g/L however, indicating high variation of concentration. Deviations of over 300 mS/m from expected conductivity values were recorded for these channels, with coefficients of variation reaching 47.89% in the 6.3 g/L solution. No uniform pattern of deviation was apparent as values decreased and increased independently and unpredictably. Only these three channels were affected. EC values in Exp2 were consistently lower per concentration compared to those in Exp1. The highest concentration in Exp2, for example, had almost exactly the same EC value as the highest concentration in Exp1 despite being 2 g/L stronger. EC values of control solutions in Exp1 and Exp2 were over four times those at the collection site at the time of collection. Exp3 (Table 2.4) had very consistent EC values in all channels throughout the entire duration of the experiment. CV values stayed below 1% over the 10 day period.

Table 2.4 Summary of water parameters per channel over 10 days for Exp3

Concentration (g/L)	Mean E.C. (mS/m)	CV (%)	Mean pH	CV (%)	Mean T (°C)	CV (%)
0.0	27.51	0.08	7.36	0.05	17.8	0.03
1.5	117.09	0.04	7.41	0.03	17.8	0.03
2.0	143.24	0.05	7.50	0.02	17.8	0.03
2.7	179.00	0.02	7.48	0.02	17.8	0.03
3.5	212.25	0.05	7.50	0.02	17.8	0.03
4.7	261.31	0.05	7.49	0.03	17.8	0.03
6.3	328.84	0.03	7.43	0.02	17.8	0.03
8.4	406.79	0.04	7.45	0.02	17.8	0.03
11.25	508.69	0.03	7.46	0.02	17.8	0.03
15.0	632.48	0.04	7.41	0.02	17.8	0.03

Average pH (Tables 2.2, 2.3 and 2.4) increased slightly with solution concentration, although the difference between the highest concentration and control never exceeded 1 unit of pH in any of the experiments. The pH values in Exp2 were consistently higher than those in Exp1 and Exp3, even at similar EC values.

2.3.2 Organism response – Probit and TSK analysis

Probit results for 96 h tests on *E. elegans* (Table 2.5) provided LC₅₀ values which differed by 7.54 g/L between the upper and lower 95% confidence limits. The maximum difference between LC₅₀s from the three experiments was 5.12 g/L. The 95% confidence limit ranges for all LC₅₀s for *E. elegans* were below 2.2 g/L. The LC₅₀ value for Exp2 was possibly skewed due to low sample size (Table 2.1) and a trim of nearly 15% of the data contributing to this number. The geometric mean of all LC₅₀s for *E. elegans* was 3.16 g/L.

Table 2.5 LC₅₀ values and type of regression analysis for 96h tests for each species by experiment

Experiment	Species	Regression model	Spearman-Kärber trim (%)	LC ₅₀ (g/L)	95% confidence limits
Exp1	<i>E. elegans</i>	PROBIT	0	1.37	(0.12 - 2.29)
Exp2	<i>E. elegans</i>	TSK	7.33	6.49	(5.51 - 7.66)
Exp3	<i>E. elegans</i>	PROBIT	0	3.56	(2.85 - 4.31)
Exp1	<i>T. discolor</i>	PROBIT	0	2.79	(1.99 - 3.57)
Exp2	<i>T. discolor</i>	PROBIT	0	10.92	(7.59 - 14.36)
Exp3	<i>T. discolor</i>	TSK	18.01	6.95	(5.99 - 8.06)
Exp1	<i>A. barnardi</i>	TSK	8.73	7.63	(6.59 - 8.83)
Exp2	<i>A. barnardi</i>	TSK	7.89	7.48	(6.65 - 8.41)
Exp3	<i>A. barnardi</i>	PROBIT	0	7.56	(6.19 - 9.64)

The LC₅₀ values calculated for *T. discolor* also exhibited wide variation. The highest 95% confidence limit was 12.37 g/L higher than the lowest. The LC₅₀ calculated for Exp2 had a 95% confidence limit range of 6.77 g/L while those for Exp1 and Exp3 ranged over 1.58 g/L and 2.07 g/L respectively. The geometric mean for all three LC₅₀s for *T. discolor* is 5.96 g/L.

Although both Exp1 and Exp2 for *A. barnardi* required approximately 16% data trims the LC₅₀s for all three experiments were remarkably similar. The LC₅₀s differed by a maximum of 0.15 g/L, and the

95% confidence limits by 3.45 g/L. The geometric mean of the LC₅₀s from all three experiments is 7.55 g/L.

A comparison of LC₅₀s and their 95% confidence limits for all species from all three experiments reveals that they do not differ significantly from one another (Figure 2.4). The large confidence ranges for all species overlapped, and the difference between LC₅₀s did not exceed 10 g/L.

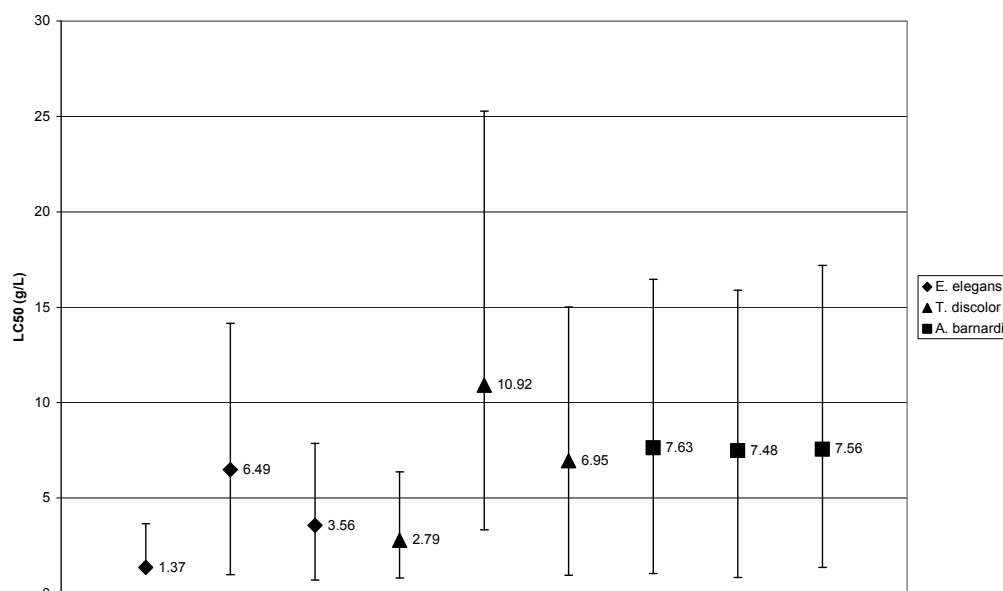


Figure 2.4 Plot of comparative 96 h LC₅₀ values for each species for three experiments with 95% confidence values

2.3.3 Species sensitivity distribution

LC₅₀ values for eight other taxa were used in conjunction with those calculated by this study. These comprised *Lymnaea* sp. (Pond snail), *Villorita cyprinoides* (Black clam), *Pimephales promelas* (Fathead minnow), *Oryzias latipes* (Medaka/high-eyes), *Lepomis macrochirus* (Bluegill), *Gambusia affinis* (Western mosquitofish), *Daphnia magna* (Water flea) and *Ceriodaphnia dubia* (Water flea) (see Appendix Table A.1). The geometric mean of LC₅₀s was determined wherever multiple results were available. All LC₅₀s were calculated for an acute time period (<96 h). The ACR was calculated as 1.441 and was applied as the concentration divisor (Figure 2.5). The calculated protection concentrations were higher than the current Reserve boundaries for MgSO₄ (Table 5.2) at the Good/Fair and Fair/Poor boundaries but almost half that of the Natural/Good boundary value.

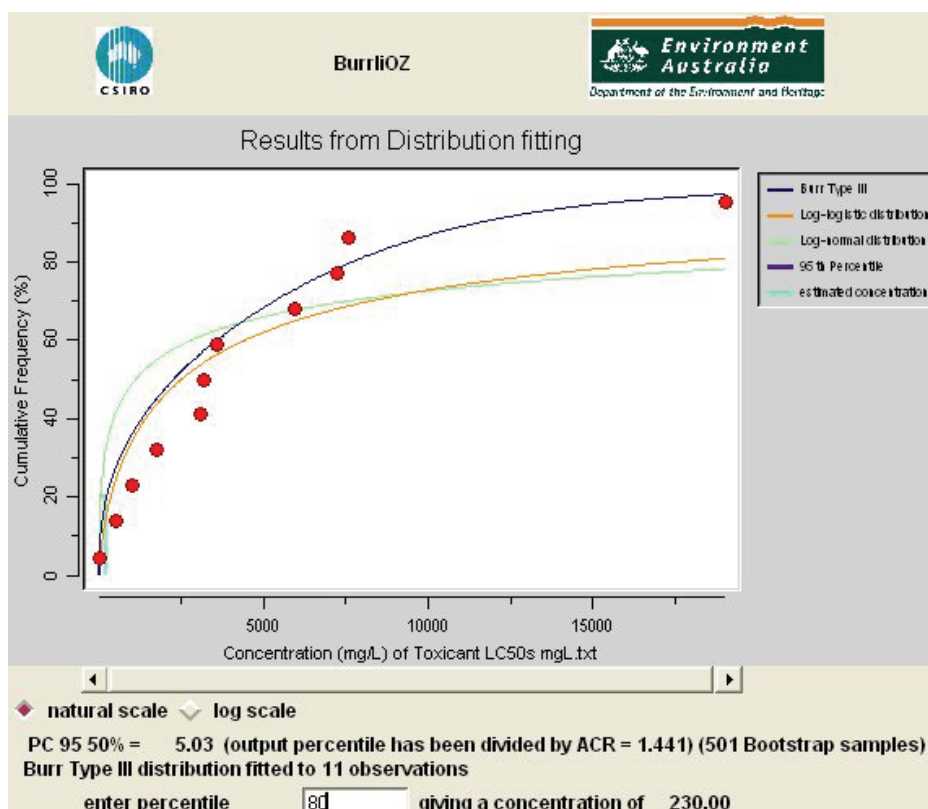


Figure 2.5 Species sensitivity distribution (SSD) for MgSO_4

2.4 Discussion

Exp2 exhibited a number of variations from the results provided by Exp1 and Exp3, especially in terms of water quality and mortality rates. The EC readings for the concentrations were consistently lower at the same MgSO_4 concentration than those in Exp1. Additionally, the deviations of three mid-range channels from the expected conductivity range in an unpredictable manner reduced the reliability of Probit analyses based upon reduced data sets, necessitating the use of the TSK method.

There was a disparity between the EC values in Exp2 and Exp3 for the same MgSO_4 concentrations. The anomalous EC data for the three mid-range channels in Exp2 could explain why such different LC_{50} values resulted from this experiment. These anomalies can possibly be attributed to some form of contamination in the three channels as it was apparent from the beginning of the experiments. Although water for both laboratories originates from the same source there are certainly differences in piping materials and distance the water travels. Due to the divalent property of MgSO_4 this inorganic salt reacts readily with other ions when in aqueous solution (Péqueux, 1995). It is possible that differences in piping material between the two laboratories may have resulted in differing ion contents of the tap water. These slight differences could have affected the bioavailability and speciation of the MgSO_4 between experiments.

Despite the problems raised by the anomalous results in Exp2, results from Exp3 appear to support LC_{50} results obtained from this experiment. The fact that the LC_{50} s for the three species in all experiments are not significantly different from one another suggests that the responses of these taxa to MgSO_4 are fairly uniform. In fact it appears from LC_{50} data recorded by Browne (2005) for mayflies exposed to NaCl and Na_2SO_4 and LC_{50} values obtained in this study for MgSO_4 that mayflies respond in a similar manner to these three different salts (Figure 2.6).

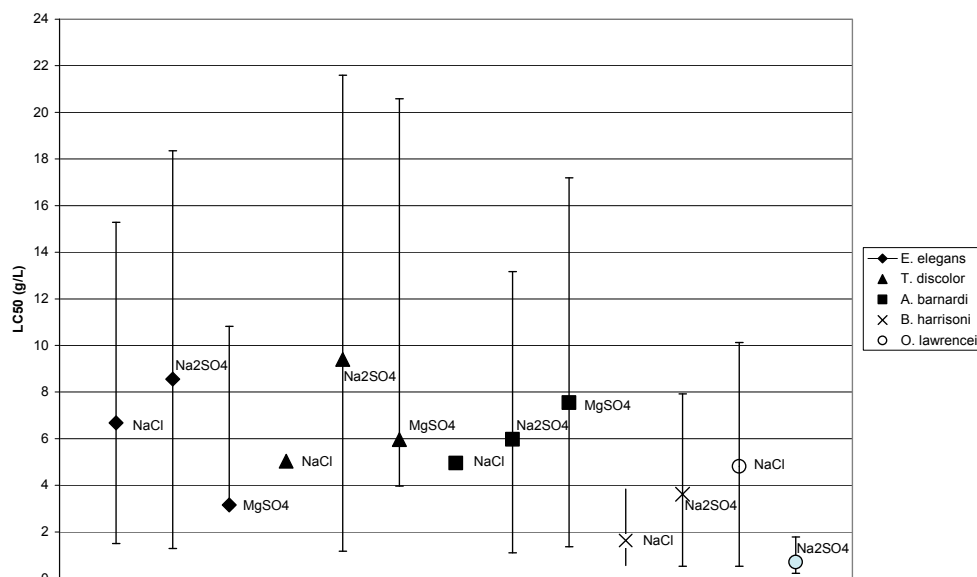


Figure 2.6 Comparison of LC₅₀ values and 95% confidence limits between NaCl, Na₂SO₄ and MgSO₄ for five species of indigenous mayflies.

It is apparent that the differences in tolerance to MgSO₄ between mayfly species are no larger or more significant than the differences in tolerance to multiple salts within each species. There are no trends in tolerance which apply to all species, and although *A. barnardi* appears to indicate that MgSO₄ is more toxic than Na₂SO₄ which is in turn more toxic than NaCl, the confidence limits overlap significantly and the trend is not reflected by the other species. This lack of a general trend in tolerance to inorganic salts within or between taxa reiterates the need for increased species and salt specific ecotoxicity tests on indigenous organisms. Without these specific data the accuracy of boundary values based upon LC₅₀s extrapolated from exotic organisms or other chemicals may be drastically reduced (Warne et al., 2005). It is also important to note that natural intraspecies tolerance variation can lead to broad confidence limits being applied. This might reduce the accuracy and relevance of LC₅₀s, especially in cases where only one toxicity test has been undertaken. It must be recognized however, that although an increase in sample size and replication could reduce this margin of uncertainty, the logistical implications of testing more than 400 individuals per species are a limiting factor. In addition it is clear that natural variation in salt tolerance is an important factor in the widespread regional distribution of the taxa involved and is thus probably unavoidable. It is conceivable that significant regional differences in tolerance could exist. This would further the case for the need for extensive site-salt-species specific ecotoxicology in South Africa.

The SSD results provide an interesting contrast to the MgSO₄ Reserve boundary values proposed by Jooste and Rossouw (2002). The much lower 95% protection concentration conflicts with the expectation that the current Reserve boundary value for Natural/Good is conservative. The 90% and 80% boundaries are conversely much less conservative than the Reserve boundary values for Good/Fair and Fair/Poor. The assessment of the Kat River, Eastern Cape (Muller, 2005a), indicates that concentrations between 23.6 mg/L (Good) and 48.1 mg/L (Poor) were present within a system that is considered to be in a 'Good' condition for most other water quality parameters. An example from the Leeuspruit (Muller, 2005b) also shows that concentrations of MgSO₄ vastly exceeding the Fair/Poor boundary (124 mg/L - 142 mg/L) are found within a system which could be categorized as largely 'Fair' based upon other water chemistry parameters. Such disparities suggest that the boundaries produced in this study have the potential to provide a more realistic representation of background prevalence and ecosystem tolerance of MgSO₄ within South African systems.

It should be noted that the SSD, and hence the protective concentration values, in this study are based on preliminary and to some extent limited data. No data for algae, and very little for macroinvertebrates were available. For greater accuracy, multiple ACRs from each group should be combined to provide a more representative divisor. The presence of the highly sensitive estuarine clam *Villorita cyprinoids* in the SSD made a significant difference to the estimated protective concentration values. This exemplifies how such a small taxon sample size can skew the resulting protective concentration values. Hence more toxicology testing of MgSO_4 on indigenous organisms from all trophic levels is critical for definition of accurate and relevant boundary values and WQGs for MgSO_4 in South Africa.

3 OXYGEN CONSUMPTION IN TWO SPECIES OF FISH IN RESPONSE TO INCREASED CONCENTRATIONS OF SELECTED INORGANIC SALTS

3.1 Introduction

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 of 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.

3.2 Materials and Methods

3.2.1 Experimental organisms

Two freshwater fish species were used for this experiment following an approval by the Rhodes University Ethical Standards Committee (RUESC). Test species were the guppy, *Poecilia reticulata* and the zebra danio, *Danio rerio*. Both species are exotic to South Africa, however are used globally in toxicity tests (Boisen et al., 2003). Guppies are cultured on large scale for ornamental purposes and were obtained from a local breeder. The danios were obtained from a wholesaler dealing in ornamental fish. These two species were exposed to increasing concentrations of the inorganic salts Na_2SO_4 and NaCl in separate experiments.

3.2.2 Experimental systems

Numerous experimental systems have been used to determine the tolerance of biota to salinity, however static systems are used as a simple standard for rapidly testing many species (Kefford et al., 2003; Kefford et al., 2004). These systems are utilised across the world (Kefford et al., 2004) and were used in this study to determine the effects of salinity on the test species. Tests conducted in this study made use of static systems without replacement (non-renewal). This meant that test organisms were exposed to the same test solution for the duration of the test (USEPA, 1994). Static test systems were favoured as they are simple, cost effective (compared with flow through systems) and require few resources (USEPA, 1994). Some of the major disadvantages are the possible DO depletion due to biological oxygen demand (BOD) and chemical oxygen demand (COD) (USEPA, 1994) as well as the accumulation of waste products. The effects of this were hoped to be controlled by the short experimental time and by purging the fish for 24 h prior to testing. Any effects that occurred as a result of this would be expected to appear in the controls, and treatments could be measured relative to this.

Respirometers were used as static test systems to determine the oxygen consumption of aquatic organisms over time. A pilot study revealed that a plastic respirometer of 2.4 L volume was suitable for the test species as dissolved oxygen (DO) did not drop below 5 mg/L over the experimental period. As with static systems for toxicity tests, static respirometers have the disadvantage of decreased DO levels over time, yet unlike flow through respirometers they are not subject to frequent calibrations, baseline errors and effects of dilution rate (Steffenson, 1989).

3.2.3 Experimental design and procedure

Prior to the start of the experiment, physio-chemical water quality parameters were recorded. These included water hardness (mg/L CaCO_3), pH, conductivity (mS/m), temperature ($^{\circ}\text{C}$) and dissolved oxygen (mg/L). In addition light intensity was recorded. Dechlorinated tap water was used for all experiments. Respirometers were filled completely so as to eliminate air gaps. Thereafter one test specimen was added and the lid was screwed on tightly underwater.

To minimise confounding factors, respirometers were randomly placed on the test bench, with a colour assigned to each experimental period. The colours facilitated faster removal of respirometers at the end of each experimental period (24, 48, 72 and 96 h) when 60 respirometers were removed and water quality parameters measured. The experiment was conducted in a constant environment room where the temperature was maintained at $22 \pm 2^{\circ}\text{C}$ with a photoperiod of 14:10 h light:dark (Slabbert, 2004) to simulate South African summer conditions.

Electrical conductivity was measured using an AMEL 160 conductivity (mS/m) meter, pH was measured using a Cyberscan pH 5000 and DO (mg/L) was measured using a Cyberscan DO 1500.

In the first experiment DO was measured by chemically fixing the oxygen and titrating the sample using the modified Winkler method (Mackereth et al., 1978). Statistical analyses of this data when compared with that of the Cyberscan DO meter revealed no significant difference in trends between the two methods. The Winkler method was therefore discarded in favour of the DO probe as a time saving tool due to the number of samples that required processing every 24 h.

To avoid oxygen depletion within the static systems, acclimation of fish in the respirometers was not undertaken. Static respirometers do not facilitate sampling without the introduction of atmospheric oxygen. For this reason a destructive sampling method was used, where respirometers sampled at the end of each exposure period were not re-introduced into the experiment.

Six sub-lethal concentrations were tested. The control concentration was 0 mg/L with increasing concentrations of 0.5, 1, 2, 4 and 8 g/L for NaCl and 0.375, 0.75, 1.5, 3, 6 g/L for Na_2SO_4 . These concentrations were derived using concentrations less than LC_{50} values obtained from the ECOTOX database (USEPA, 2004), as the endpoint of this experiment was oxygen consumption, not mortality. All six concentrations contained fish. The 0 mg/L concentration acted as one control, in addition there were controls for each salt concentration at 0 and 96h, these contained no fish. Each concentration as well as the controls comprised 10 replicates across the four exposure periods.

3.2.4 Data analysis

Dissolved oxygen (DO) data were analysed using Statistica software package and a multifactorial ANOVA (analysis of variance). A multi-stage Neuman-keuls test was used to show significant

differences between treatments. Temperature and pH were also recorded and means, standard deviations and coefficient of variations (CV) were determined.

The lowest value that was not significantly different from the control would indicate the no observed effect concentration (NOEC). This value could be incorporated into water quality guidelines and in doing so help in the refinement of these values.

3.3 Results

3.3.1 *Poecilia reticulata*

NaCl

For the NaCl experiment a mean pH of 7.73 was determined and a minimum and maximum pH of 7.19 and 8.36 were recorded respectively. A CV value of 3.2% was calculated. A mean temperature of 23.25°C was determined with minimum and maximum temperatures of 22.1°C and 23.6°C being recorded respectively. A CV value of 0.8% for temperature was calculated.

In the test without fish, available DO within the control showed a general decreasing trend from the start of the experiment (0 h) to the completion of the experiment (96 h) (Figure 3.1). A significant ($F=4.03$ and $p=0.0037$) change in DO was observed for concentrations of 0.5, 1 and 2 g/L.

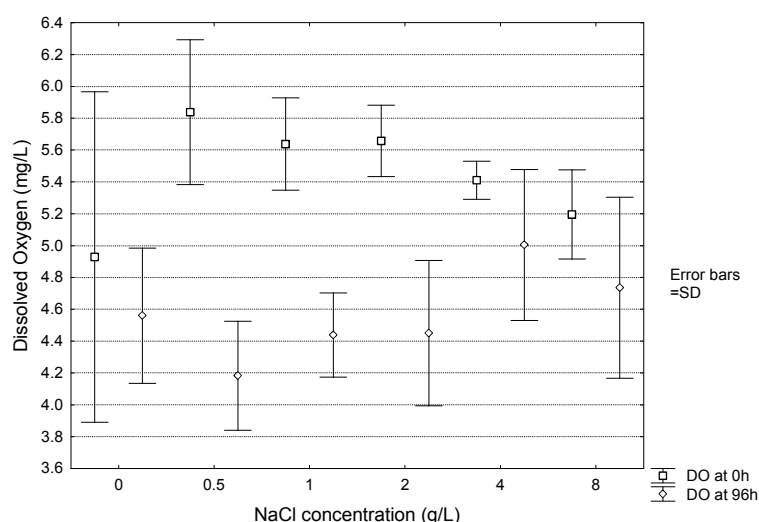


Figure 3.1 Changes in dissolved oxygen (mg/L) at different NaCl concentrations at 0 h and 96 h. These concentrations did not contain fish and measured natural changes on DO over 96 h of exposure.

During the 96 h toxicity test, which contained fish, a significant difference was found across treatments between the 0 g/L concentration and the other treatments and between the 8 g/L concentration and the other treatments at the 0 h time interval (Figure 3.2) ($F=3.02$, $p=0.017$). This difference was also reflected with change in DO over time 0 h to 24 h period (Figure 3.3). No significant difference was found between treatments at the 24 h time interval ($F=1.21$, $p=0.314$) (Figure 3.2). Significant differences ($F=5.45$, $p=0.0004$) were observed for the 48 h time period at the 1 g/L and 8 g/L treatments. At the 72 h time period a significant difference was noted for the 1 g/L concentration ($F=7.32$, $p=0.00003$) while at the culmination of the experimental period (96 h) a significant difference was found at the 2 g/L concentration (Figure 3.2). In addition to the general

decrease in available oxygen from 0 h to 24 h (Figure 3.3), a significant change is also reflected at the 48 h time period for 1 g/L concentration (Figure 3.3).

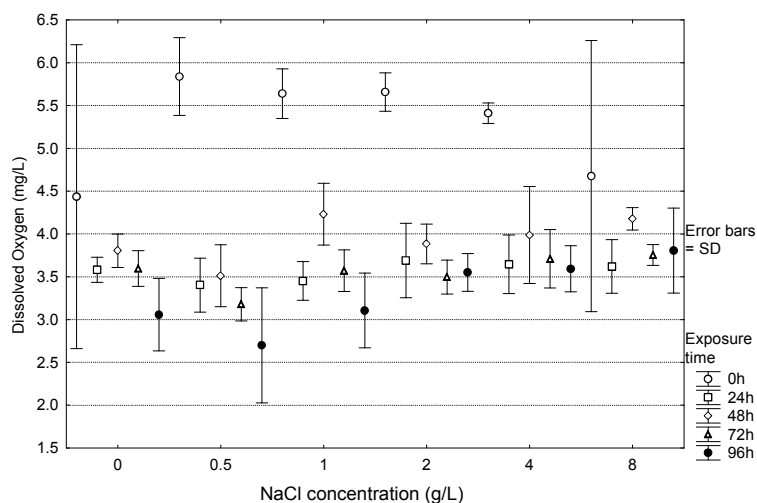


Figure 3.2 Change in dissolved oxygen (mg/L) in response to *Poecilia reticulata* exposed to increasing concentrations of NaCl over 5 exposure times

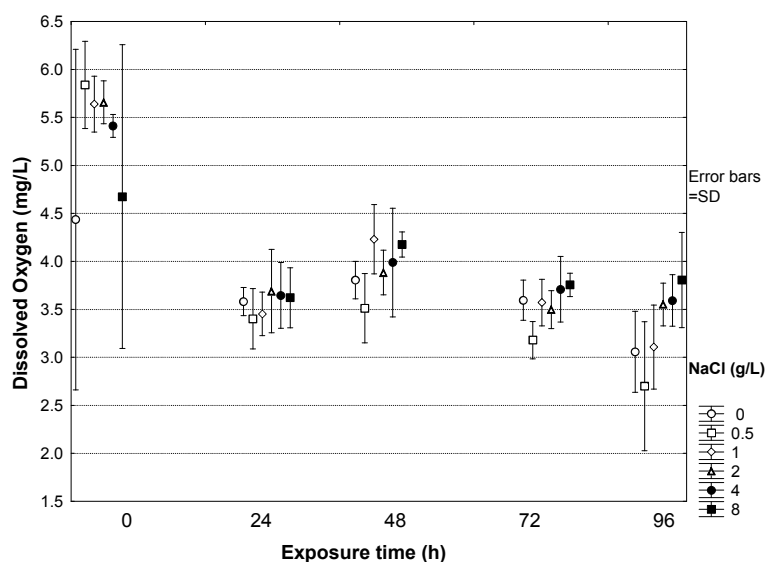


Figure 3.3 Change in dissolved oxygen (mg/L) in response to *Poecilia reticulata* exposed to increasing concentrations of NaCl per concentration over time

Na_2SO_4

For the Na_2SO_4 experiment, a mean pH of 7.35 was determined and a minimum and maximum pH of 6.6 and 7.81 were recorded respectively. A CV value of 2.2% was calculated. A mean temperature of 22.32°C was determined with minimum and maximum temperatures of 21°C and 23.2°C being recorded respectively. A CV value of 2.0% for temperature was calculated.

DO changed significantly ($F=20.43$, $p<0.00005$) from the 0 h to 96 h time period showing a decrease across all concentrations for the controls that contained no fish (Figure 3.4).

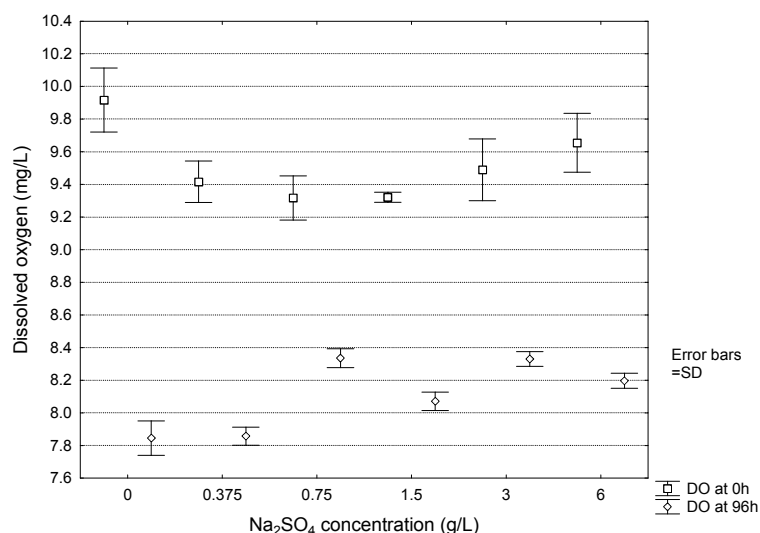


Figure 3.4 Changes in dissolved oxygen (mg/L) at different Na₂SO₄ concentrations at 0 h and 96 h. These concentrations did not contain fish and measured natural changes on DO over 96 h of exposure.

The 96 h toxicity test containing fish showed significant decreases in DO across all concentrations over duration of the experiment. Most notable were the changes from 0 h to 24 h and 48 h respectively ($F=157.78$, $p<0.0005$) (Figure 3.5). These decreases in available DO are also reflected in the change of DO over the exposure time with each concentration (Figure 3.6).

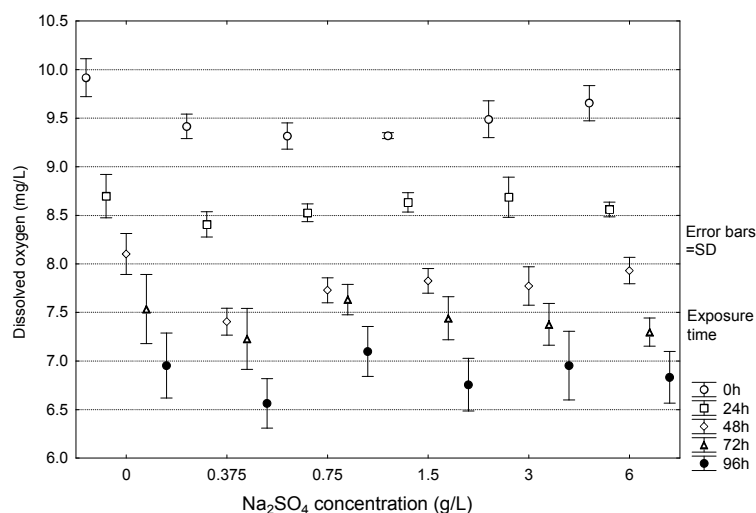


Figure 3.5 Change in dissolved oxygen (mg/L) in response to *Poecilia reticulata* exposed to increasing concentrations of Na₂SO₄ over 5 exposure times

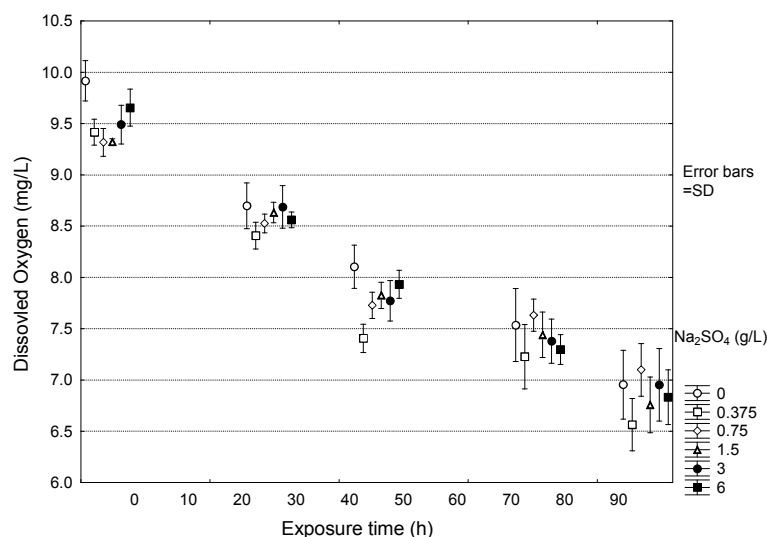


Figure 3.6 Change in dissolved oxygen (mg/L) in response to *Poecilia reticulata* exposed to increasing concentrations of Na_2SO_4 per concentration over time

3.3.2 *Danio rerio*

NaCl

All DO decreased significantly ($F= 21.12$, $p<0.005$) across the controls containing no fish. This was observed for all treatments with NaCl (Figure 3.7).

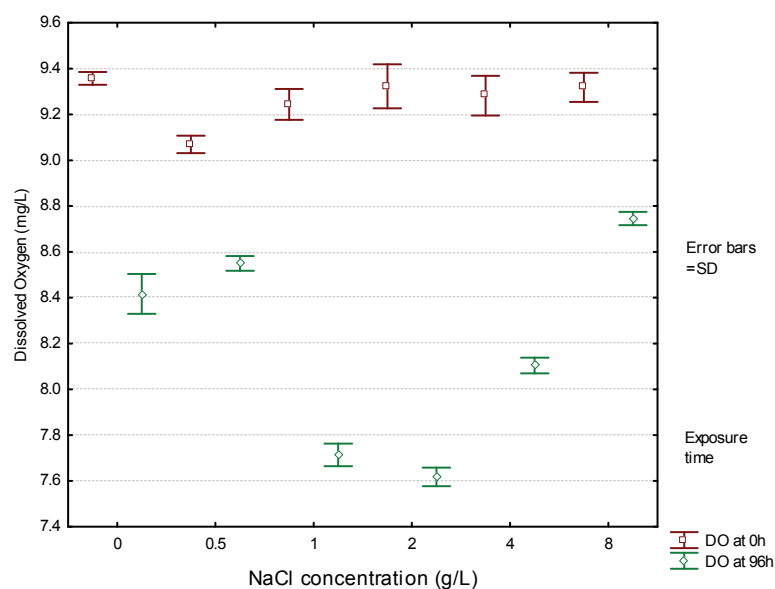


Figure 3.7 Changes in dissolved oxygen (mg/L) at different NaCl concentrations at 0 h and 96 h. These concentrations did not contain fish and measured natural changes on DO over 96 h of exposure.

Similar trends were seen with this experiment (Figures 3.8 and 3.9) as with the Na_2SO_4 experiment with *P. reticulata* (Figures 3.5 and 3.6). A significant decrease in DO was seen over the 96 h exposure period, notably around the 24 h and 48 h periods and 0.5 g/L and 1 g/L concentrations ($F= 5.1$, $p=0.0007$; $F=6.36$, $p=0.0001$) (Figures 3.8 and 3.9).

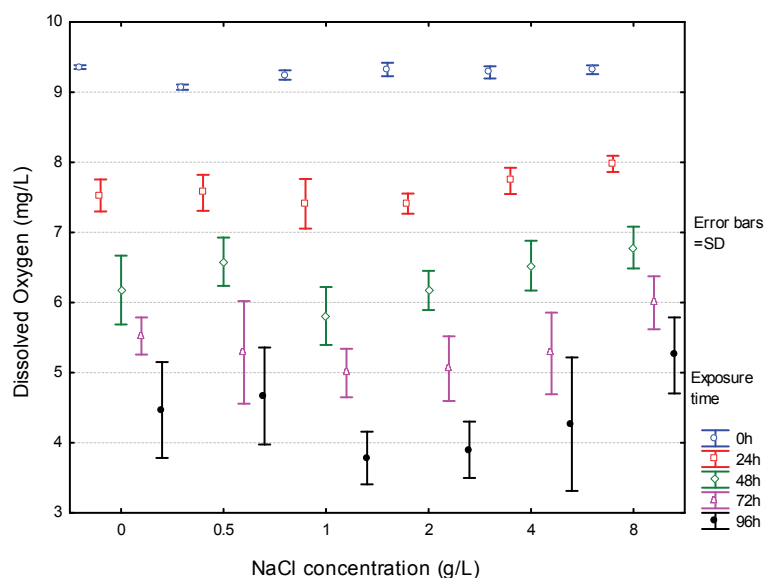


Figure 3.8 Change in dissolved oxygen (mg/L) in response to *Danio rerio* exposed to increasing concentrations of Na_2SO_4 over 5 exposure times

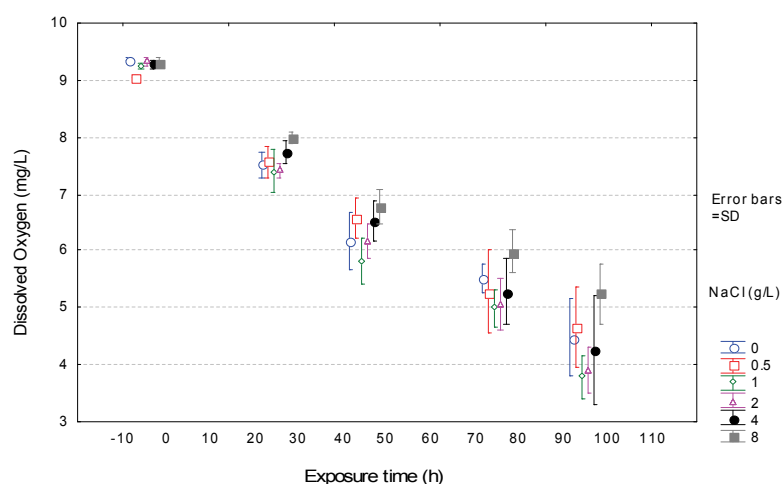


Figure 3.9 Change in dissolved oxygen (mg/L) in response to *Danio rerio* exposed to increasing concentrations of NaCl per concentration over time

Na_2SO_4

As seen with the other experiments, DO significantly decreased from the start of the Na_2SO_4 experiment (0 h) to the end of the experimental period (96 h) ($F=40$, $p<0.0005$) (Figure 3.10). Similar to the other experiments these controls also contained no fish.

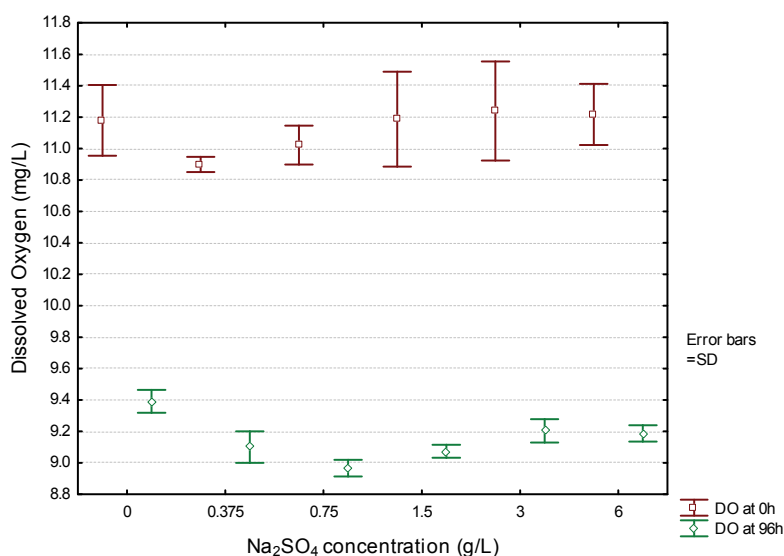


Figure 3.10 Changes in dissolved oxygen (mg/L) at different Na₂SO₄ concentrations at 0 h and 96 h. These concentrations did not contain fish and measured natural changes on DO over 96 h of exposure.

The *D. rerio* experiment also showed a decrease in available DO over the 96 h period. Significant differences were found between 0 h and 24 h and between 0 h and 48 h ($F=180.12$, $p<0.0005$; $F=108.88$, $p=0.005$) (Figures 3.11 and 3.12). These trends were also reflected in Figure 3.12, where a decrease in available DO was seen over concentrations over the 24 h and 48 h time periods, particularly for the 0.375 g/L and 0.75 g/L concentrations.

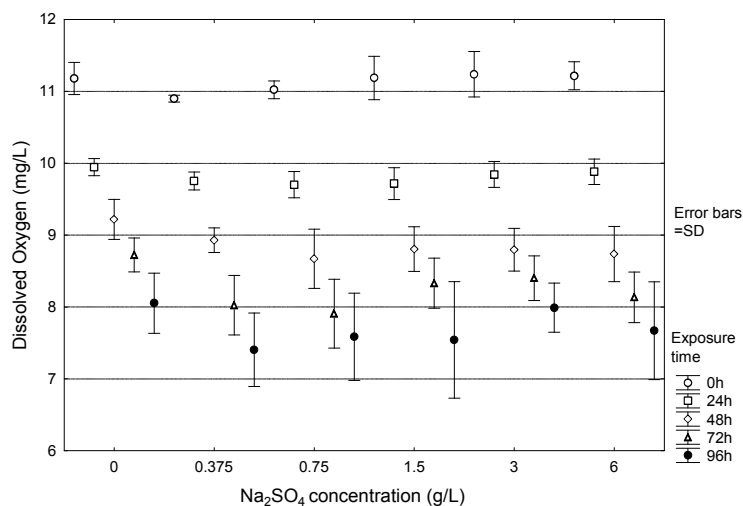


Figure 3.11 Change in dissolved oxygen (mg/L) in response to *Danio rerio* exposed to increasing concentrations of Na₂SO₄ over 5 exposure times

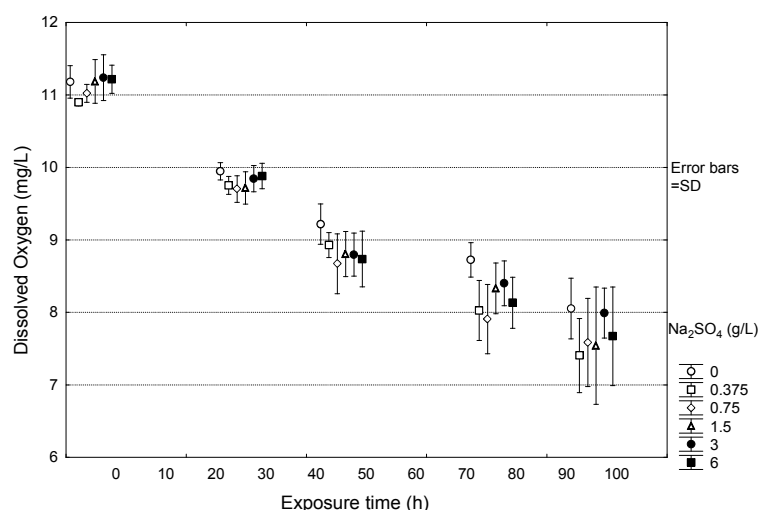


Figure 3.12 Change in dissolved oxygen (mg/L) in response to *Danio rerio* exposed to increasing concentrations of Na_2SO_4 per concentration over time

3.4 Discussion and Conclusion

Temperature and pH were recorded for all concentrations over the 96 h period. From the results the CV values were very low (<3.2%) for both parameters. Based on this it is unlikely that these parameters had a confounding effect on the experiment as changes in DO were measured as relative changes. It has been therefore decided to exclude these parameters in data analysis.

The NaCl experiment using *P. reticulata* as test species revealed that a concentration of 1 g/L was the lowest concentration to show a response, particularly at the 24 and 48 h exposure periods. When testing the same salt on *D. rerio* it was found that the same concentration was the lowest to yield a response, however this occurred at the 48 and 96 h exposure periods. These differences in response times may be explained by differences in the abilities of these species to conform or regulate when exposed to inorganic salt toxicants. Most fishes are osmoregulators, maintaining osmotic balance by regulating their internal osmotic environment to maintain cellular function even when the external environment fluctuates (Helfman et al., 2002). Some species are able to tolerate large osmotic changes in their environment (euryhaline), while others are only able to tolerate small changes (stenohaline). Freshwater fish are hyperosmotic to their environment and therefore gain water while losing salts to the environment, as a result salts need to be actively transported back across the gills to maintain homeostasis (Helfman et al., 2002).

The ability of freshwater fishes to adapt to increasing salt concentrations is species specific and is dependent on several factors, including (but not limited to) gill to body surface ratio, hormonal and endocrine control, oxygen levels and temperature fluctuations (Lagler et al., 1962).

Guppies (*P. reticulata*) are neither catadromous nor anadromous and are only able to adapt to changes in salinity in a gradual manner (Daikoku, 1980). Sudden changes, such as those experienced during a rapid influx of salt toxins may affect the ability of these fishes to osmoregulate and be reflected in the oxygen consumption of these organisms (Daikoku, 1980). Data gathered on euryhaline species showed a wider tolerance to salinity than guppies, reflecting a better developed ability of these species to osmoregulate and therefore to adapt to rapid changes in salinities (Daikoku, 1980).

Oxygen consumption has been used in relation to physiological activity when assessing stress caused by pollutants and, in addition to indicating metabolic rate, it has been used to provide an index for stress through toxin exposure (Grobler et al., 1989; Palanivelu et al., 2005).

These results showed that oxygen consumption could be used as a physiological response variable to stressors such as the inorganic salts Na_2SO_4 and NaCl . These data indicate that the NOEC (no observed effect concentration) for NaCl may be found at the 0.5 g/L concentration for both *D. rerio* and *P. reticulata*. The LOEC for Na_2SO_4 appeared to be at a concentration of 0.375 g/L for both species and seeing that this was the lowest concentration tested a MATC (maximum allowable toxicant concentration) of 0.188 g/L was calculated by dividing the LOEC by two. This indicated that there appeared to be no difference between the sensitivity of the two species, as both responded to the same concentrations, albeit that *D. rerio* appeared to lag behind *P. reticulata* by 24 h.

These sublethal data could prove useful in refining water quality guidelines. In addition, a protocol for testing fish species in this manner has now been established and may be incorporated into future toxicity testing involving the use of oxygen consumption as a physiological response in aquatic organisms. Further analysis could still be done on these data to show how metabolic rate is affected over time with respect to the given salts and the given concentrations. This may explain some variability in the data and the response of the two species. While this study may provide baseline data, it would prove very useful to conduct such tests on indigenous species and provide data that is more environmentally accurate for local conditions.

4 OSMOREGULATORY RESPONSES OF FRESHWATER SHRIMP TO INCREASED CONCENTRATIONS OF SELECTED INORGANIC SALTS

4.1 Introduction

Crustaceans inhabit a wide range of aquatic biotopes (marine, semi-marine, brackish, estuarine, and freshwater) and use a wide variety of different osmoregulatory mechanisms in different salinities. The two main mechanisms split crustaceans into two categories: osmoregulators and osmoconformers (Anger, 2001). Most marine crustaceans are osmoconformers where the internal osmotic pressure equals the external one of the medium (marine environment) which is more or less stable (Péqueux, 1995). Osmoregulators actively keep the internal concentration of body fluids (haemolymph, blood) different from external media which involves a fair amount of energy expenditure in changing environmental conditions. Among osmoregulators there are different mechanisms involved in maintaining the internal osmotic concentration. Hyper-regulators actively replace passively lost ions in dilute media through ion pumps whereas hypo-regulators in hypersaline media actively excrete ions (Anger, 2001). Hyper-hypo regulators are able to maintain their haemolymph osmolality at a relative constant level (hyper-regulation in low salinities and hypo-regulation in high salinities) (Péqueux, 1995). This form of osmoregulation is very common in Decapoda, Branchiopoda, Isopoda, Copepoda, and Mysidacea in particular amongst semi terrestrial and terrestrial forms (Péqueux, 1995).

Effects of increased salinities on crustaceans include decreases in longevity and fecundity in *Daphnia magna* (Martínez-Jerónimo and Martínez-Jerónimo, 2007) and *Branchipus schaefferi* (Sarma et al., 2005). An increase in salinity may also result in limitations of growth rates as shown for *Daphnia carinata* (Hall and Burns, 2002) and *Daphnia magna* (Teschner, 1995, Arner and Koivisto, 1993).

The 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 for this experiment was osmoregulatory capacity (OC) of the freshwater shrimp, *C. nilotica* (Decapoda: Atyidae) in response to increasing concentrations of sodium chloride (NaCl) and sodium sulphate (Na₂SO₄).

4.2 Materials and Methods

4.2.1 Experimental organisms

Freshwater shrimp, *C. nilotica* (Crustacea: Decapoda), collected from the Bushmans River in Alicedale, South Africa, were used in this study. These animals are indigenous to southern Africa (Hart 1983) and have been used in toxicity testing (Slaughter et al., 2008). Shrimp were subjected to increasing concentrations of inorganic salts (NaCl and Na₂SO₄) in two separate experiments.

4.2.2 Experimental systems

The respirometer system (see Chapter 3) was adjusted for the freshwater shrimp. A 350 mL plastic respirometer used with one individual shrimp each was determined to suit the experimental requirements best.

4.2.3 Experimental design and procedure

Caridina nilotica were collected the Bushmans River in Alicedale, South Africa, using a SASS net and returned to the laboratory in aerated cooler boxes. Test animals acclimatised in an aerated 40 L glass aquarium for 48 h at 26°C water temperature and a 8:16 h light/dark cycle.

The following concentrations of sodium chloride (NaCl) and sodium sulphate (Na₂SO₄) were made up in 25 L buckets: NaCl: 0.5, 1, 2, 4, 8 g/L; Na₂SO₄: 0.375, 0.75, 1.5, 3, 6 g/L. These concentrations were derived by using less than LC₅₀ values from the IWR-UCEWQ toxicity database and are the same concentrations used in Chapter 3 of this report for fish. For each concentration, fifty respirometers (350 mL) were half filled with experimental solution and one animal was added into each of these plastic jars. The respirometers were then fully submerged in experimental solution and closed with a lid under the surface to prevent oxygen from entering. The experiments were conducted at 20°C room temperature and with a 8:16 h light/dark cycle. Electrical conductivity was measured using an AMEL 160 conductivity (mS/m) meter, pH was measured using a Cyberscan pH 5000 and dissolved oxygen (mg/L) was measured using a Cyberscan DO 1500. These water quality parameters were measured prior to the experiment (0 h) and thereafter at 12, 24, 48, 72, and 96 h from respirometers after being removed from the experiment to measure haemolymph osmolality in the shrimp.

Ten shrimp from each concentration were removed from the experiment after 12, 24, 48, 72, and 96 h. As a control, twenty shrimp were taken directly from the 40 L tank at the start of the experiment as a control (0 h). Shrimp length was measured from eye socket to tail tip using a caliper. The tail was then cut off behind the last pleopod with a scalpel. Guts were removed and discarded. A syringe needle was inserted into the heart of the shrimp and 20 units sodium citrate was injected. Any excess fluid coming out of the opening of the tail-cut or through any other openings was collected with another syringe.

Osmolality of the sodium citrate/haemolymph mixture was measured using an Osmometer (Advanced Micro-Osmometer 3320) located at the Department of Zoology, Rhodes University. Sodium citrate without haemolymph was measured as a blank and subtracted from the osmolality reading to get adjusted osmolality.

4.2.4 Data analysis

Data were tested for normality using the Shapiro-Wilk-Test and for homogeneity of variance using the Levene-Test. Significant differences were established using the one-way ANOVA and t-test for normally distributed data sets and the Kruskal-Wallis-Test for non-parametric data sets. The Statistica software package was used for all analysis.

4.3 Results

4.3.1 NaCl

Water quality parameters for 0.5 g/L NaCl at 0 h were not measured due to problematic meters. DO and pH data are normally distributed ($p < 0.05$) and homogenous ($p < 0.05$). EC data are normally distributed but not homogenous ($F = 14.94$, $p = 0.00$) due to different concentrations of salt used in the experiments. Coefficient of variance (CV) values for pH and EC were below 7% and CV values for DO were up to 20% (Table 4.1).

Table 4.1 Summary of water quality parameters per concentration (Conc) over 96 h exposure for NaCl measured from random respirometers

Conc (g/L)	Mean pH	Std. Dev.	CV (%)	Mean EC (mS/m)	Std. Dev.	CV (%)	Mean DO (mg/L)	Std. Dev.	CV (%)
0.0	7.08	0.31	4.36	66.24	0.04	6.74	4.10	0.27	6.67
0.5	7.90	0.24	3.10	178.65	0.04	2.50	3.22	0.54	16.66
1.0	8.06	0.15	1.89	316.14	0.11	4.05	4.12	0.63	15.41
2.0	8.30	0.37	4.47	450.85	0.15	3.21	4.18	0.24	5.68
4.0	8.00	0.19	2.41	795.92	0.25	3.09	3.50	0.70	20.07
8.0	8.10	0.15	1.79	1465.97	0.72	4.86	3.96	0.47	12.07

EC values were translated into medium osmolality values by measuring them with the Osmometer (see Table 4.2) and were used as such for further analysis.

Table 4.2 Overview of NaCl concentrations with their respective EC and osmolality values

Concentration (g/L)	EC (mS/m)	Medium Osmolality (mOsm/kg)
0.0	66.24	3
0.5	178.65	21
1.0	316.14	27
2.0	450.85	58
4.0	795.92	108
8.0	1465.97	212

Only DO values of the lowest concentration tested (0.5 g/L) differed significantly from the control (0.0 g/L) ($p=0.01$) all other concentrations were not significantly different from the control (Figure 4.1). After an exposure time of 24 h all DO values were significantly different from the start of the experiment (0 h) (24 h $p=0.01$, 48 h $p=0.03$, 72 h $p=0.00$, 96 h $p=0.00$) (Figure 4.2).

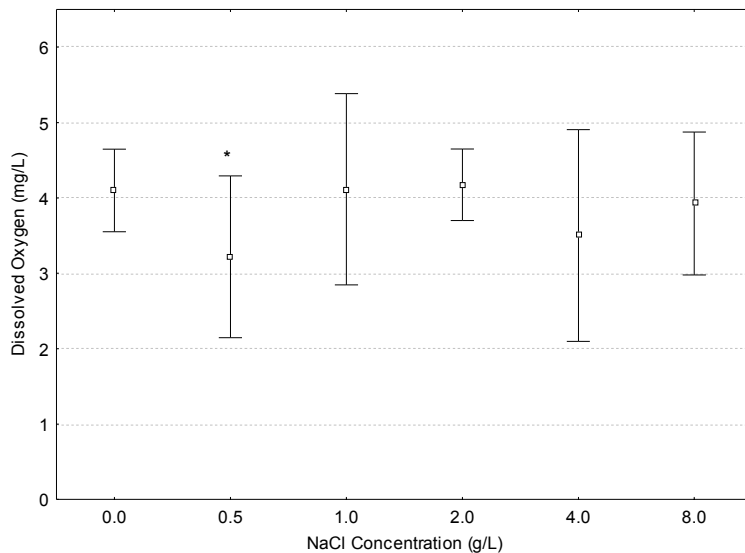


Figure 4.1 Dissolved Oxygen of all exposure times combined over all NaCl concentrations (error bars are standard deviation, significant differences are marked with an *)

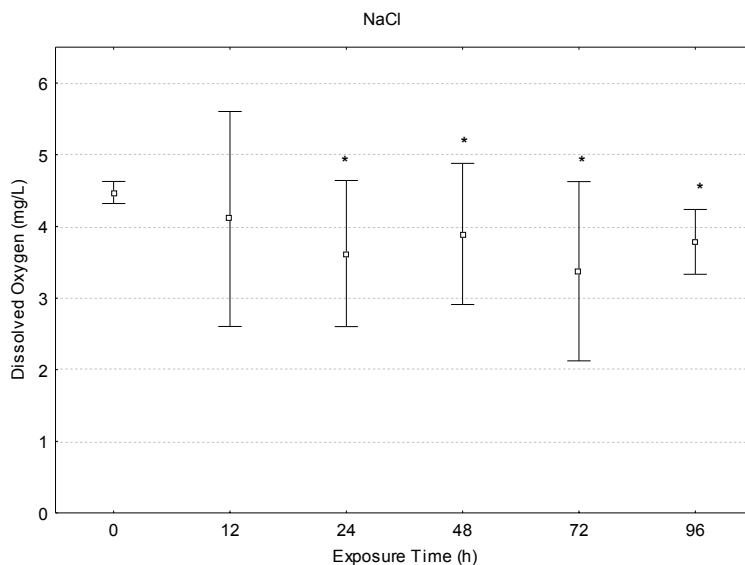


Figure 4.2 Dissolved Oxygen of all NaCl concentrations combined over time (error bars are standard deviation, significant differences are marked with an *)

Haemolymph osmolality data were not normally distributed ($W=0.97920$, $p=0.00$) and not homogenous grouped as time ($F=6.499995$, $p=0.00$) but homogenous when grouped as concentration ($F=1.37213$, $p=0.25$). Only osmolality values of the lowest concentration (0.5 g/L) were significantly different from the control ($p=0.01$) (Figure 4.3). Haemolymph osmolality values of 12 ($p=0.00$), 24 ($p=0.00$), 48 ($p=0.00$) and 72 h ($p=0.00$) of exposure differed significantly from 0 h. After 96 h of exposure the haemolymph osmolality was not different from the start of the experiment ($p=1.00$) (Figure 4.4).

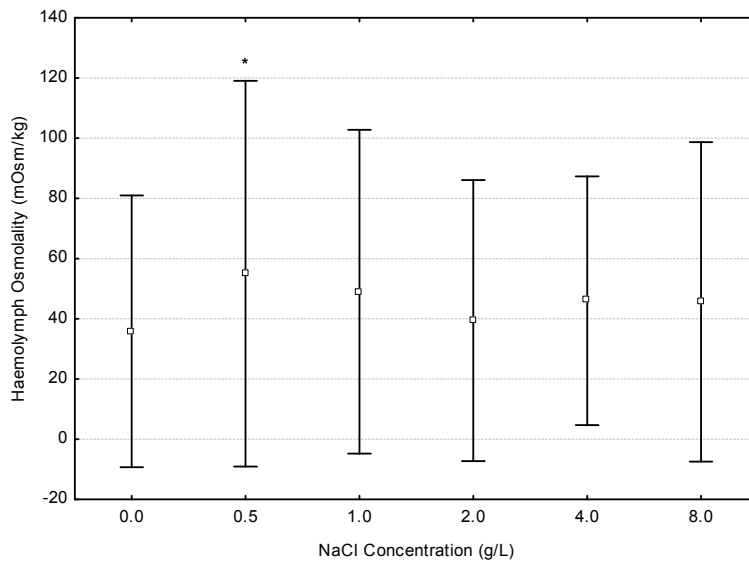


Figure 4.3 Haemolymph osmolality of all exposure times combined over all NaCl concentrations (error bars are standard deviation, significant differences are marked with an *)

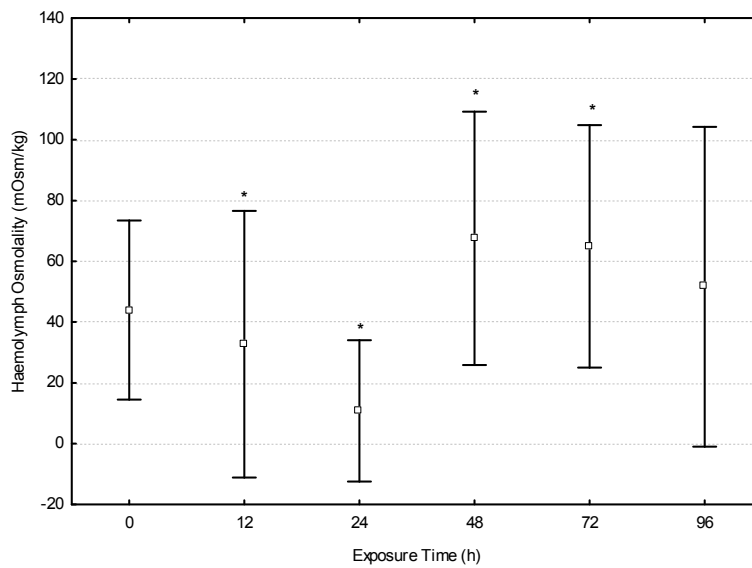


Figure 4.4 Haemolymph osmolality of all NaCl concentrations combined over time (error bars are standard deviation, significant differences are marked with an *)

There were significant differences of haemolymph levels in shrimp between the start of the experiment (0 h) and 12 h in the control (0.0 g/L $p=0.02$) and the highest concentration (8.0 g/L $p=0.04$) (Figure 4.5). Differences were found between 0 h and 24 h in the control ($p=0.00$), 1.0 g/L ($p=0.02$), 2.0 g/L ($p=0.00$) and 4.0 g/L NaCl ($p=0.01$). Differences are significant in 0.5 g/L between 0 h and 48 h ($p=0.03$) and 96 h ($p=0.00$) and in 1.0 g/L between 0 h and 72 h of NaCl exposure ($p=0.02$).

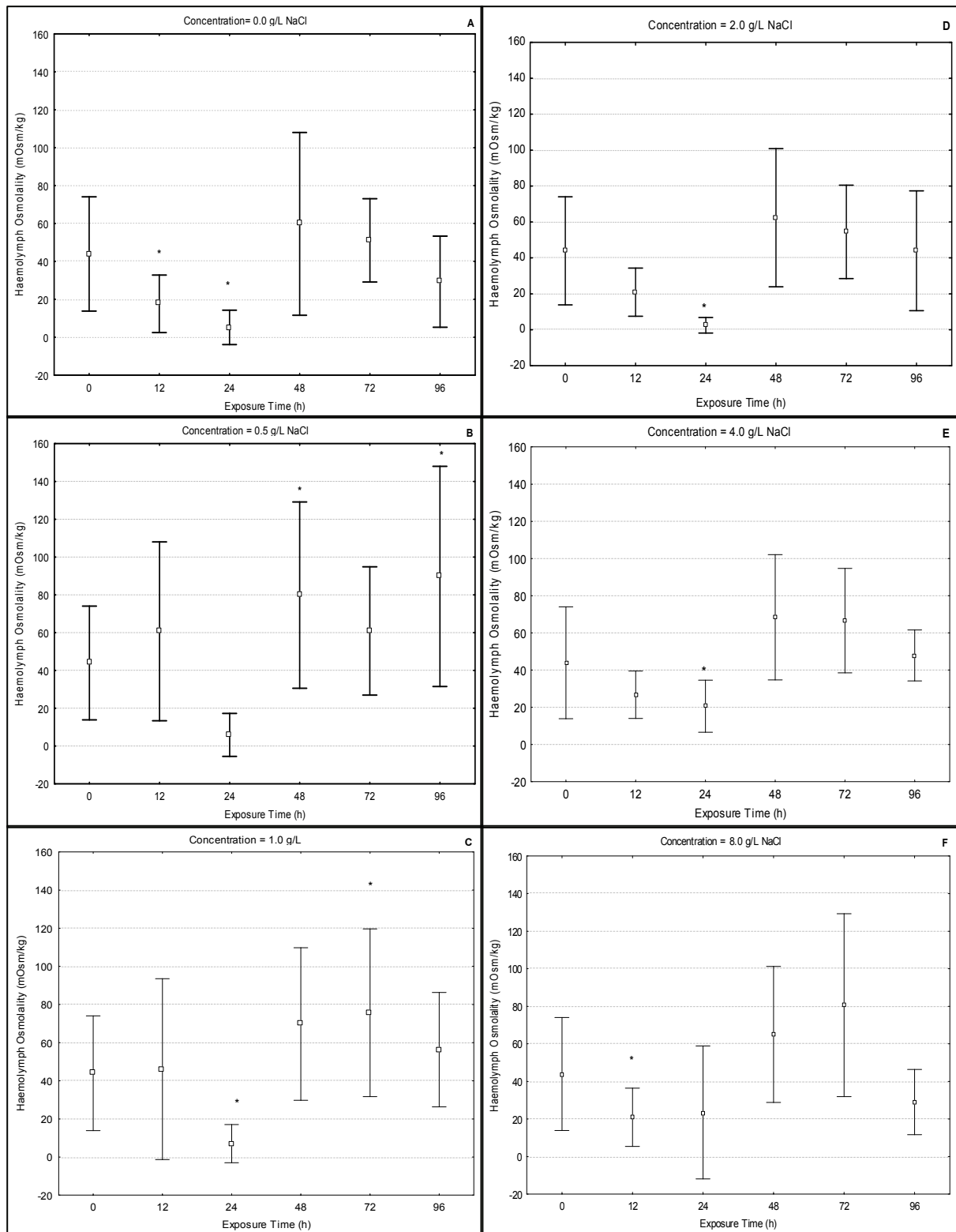


Figure 4.5 Haemolymph osmolality for each NaCl concentration over all exposure times (error bars are standard deviation, significant differences are marked with an *)

Differences between haemolymph levels in the control (0.0 g/L) and the two lowest concentrations of NaCl (0.5 g/L and 1.0 g/L) were significant after 12 h of exposure ($p=0.00$) and after 96 h ($p=0.00$ and 0.01 respectively) (Figure 4.6). After 24 h the control differed significantly from 4.0 g/L ($p=0.00$) and 8.0 g/L ($p=0.01$) NaCl concentration. After 72 h of exposure 8.0 g/L differed significantly from the control ($p=0.02$).

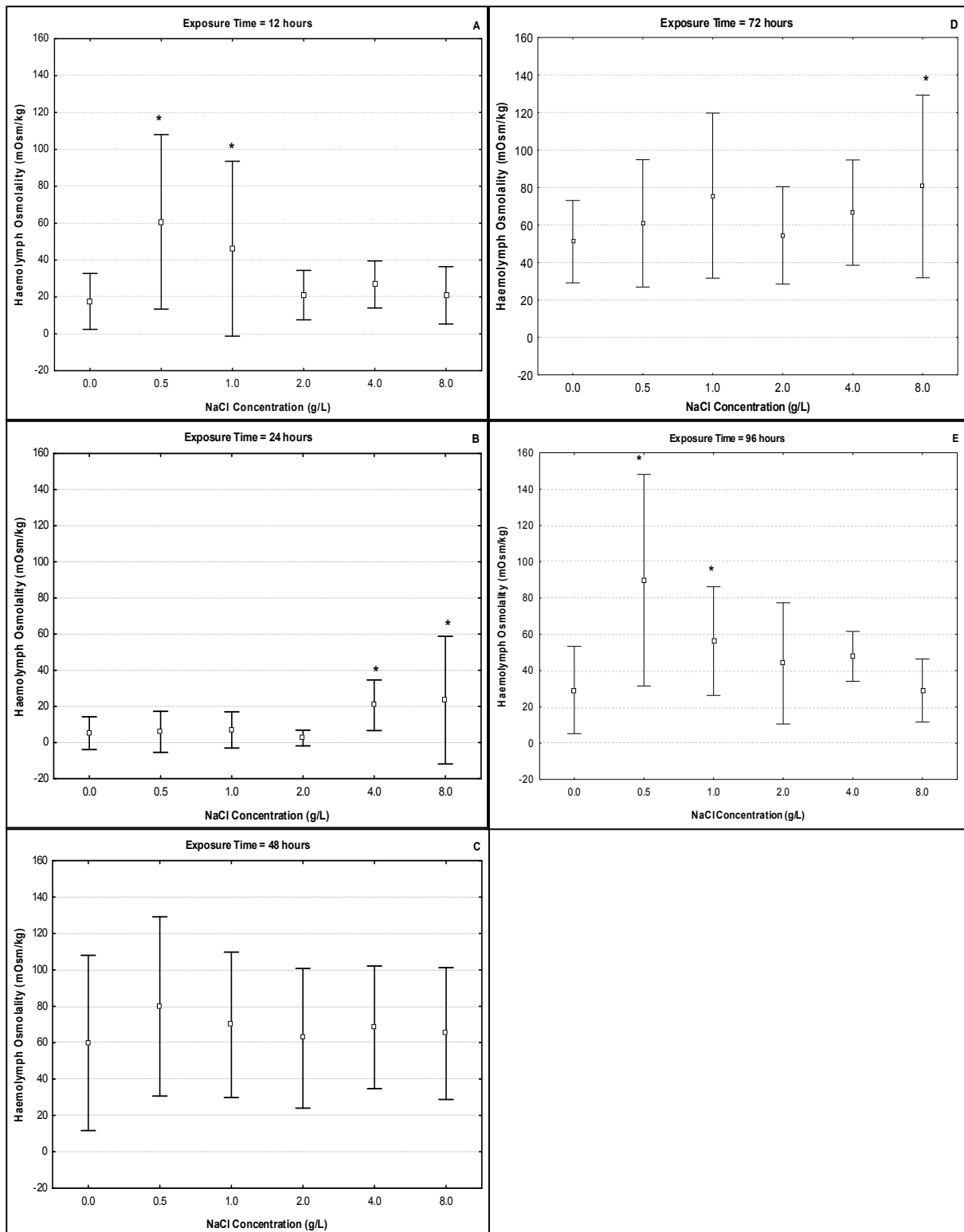


Figure 4.6 Haemolymph osmolality for each exposure time over all NaCl concentrations (error bars are standard deviation, significant differences are marked with an *)

Haemolymph osmolality stayed very constant over different exposure concentrations even beyond the isosmoticity line (Figure 4.7). Only the lowest concentration was significantly different from the control (second line of circles from the left) (see Figure 4.3). The linear fit line (horizontal line) stayed at a constant level with no significant rise or fall ($p=0.79$).

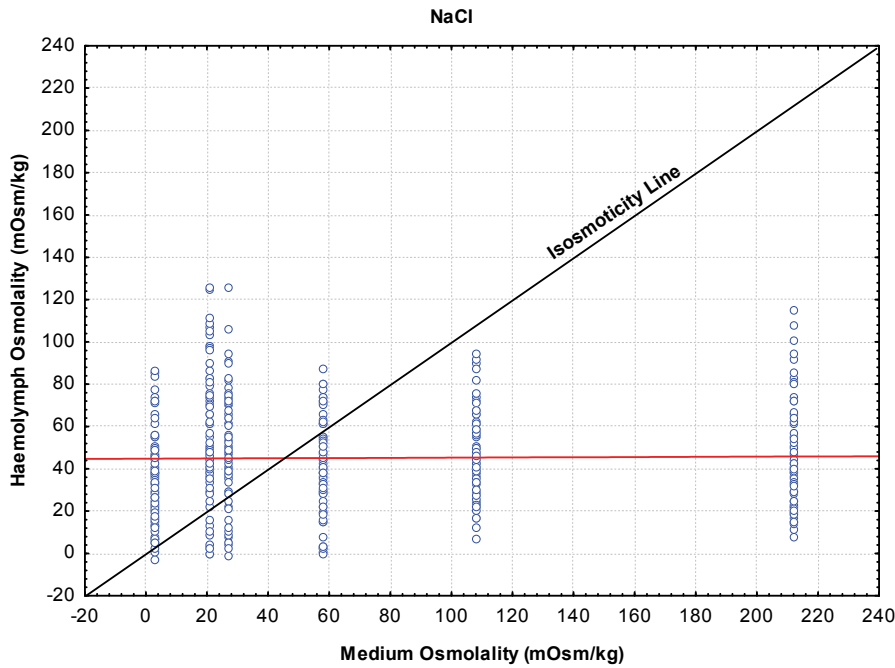


Figure 4.7 Haemolymph Osmolality vs. Medium Osmolality of *C. nilotica* exposed to NaCl (Isosmoticity Line at Medium Osmolality = Haemolymph Osmolality)

When calculating the osmoregulatory capacity (OC) of freshwater shrimp with regards to different NaCl concentrations the following formula was used (Charmantier et al., 1989):

$$\text{Haemolymph osmolality} - \text{Medium osmolality} = \text{Osmoregulatory Capacity (OC)}.$$

A positive OC indicates that the test organism is hyper-regulating, a negative value indicates that the test organism is hypo-regulating and a null value indicates that the test organism is osmo-conforming. The freshwater shrimp, *C. nilotica*, was hyper-regulating up to a NaCl concentration of 1g/L (27 mOsm/kg) and hyporegulating from a NaCl concentration of 2 g/L (58 mOsm/kg) and higher (Figure 4.8). Consequently, it appears that *C. nilotica* used in this study were hyper-hypo-regulating.

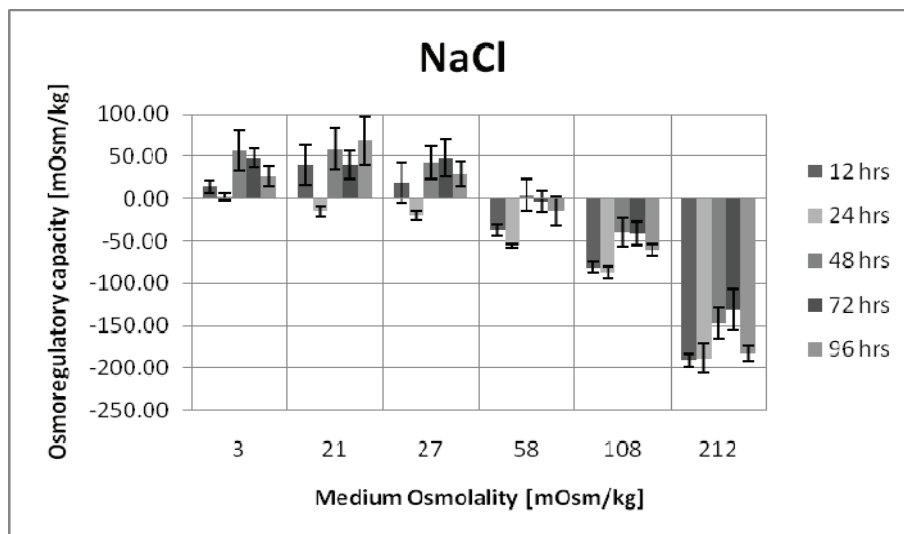


Figure 4.8 Osmoregulatory capacity (OC) at different NaCl concentrations (medium osmolality) for different exposure times

4.3.2 Na₂SO₄

Water quality parameters for 6.0 g/L Na₂SO₄ at 96 h were not measured due to all shrimp having died. The DO and pH data were normally distributed ($p < 0.05$) and homogenous ($p < 0.05$), while all EC data were normally distributed but not homogenous ($F = 4.76$, $p = 0.02$) due to different concentrations of salt used in the experiments. Coefficient of variance (CV) values for pH and EC were below 5% and for DO up to 15% (Table 4.3). The EC values were translated into medium osmolality values by measuring them with the Osmometer (see Table 4.4) and were used as such for further analysis.

Table 4.3 Summary of water quality parameters per concentration (Conc) over 96 h exposure for Na₂SO₄ measured from random respirometers

Conc (g/L)	Mean pH	Std. Dev.	CV (%)	Mean EC (mS/m)	Std. Dev.	CV (%)	Mean DO (mg/L)	Std. Dev.	CV (%)
0	7.73	0.18	2.28	53.36	0.01	1.29	4.51	0.67	14.82
0.375	8.19	0.13	1.59	88.75	0.02	1.24	4.56	0.60	13.26
0.75	8.28	0.13	1.54	166.37	0.06	3.34	4.63	0.49	10.52
1.5	8.40	0.14	1.62	287.68	0.05	1.80	4.33	0.46	10.59
3	8.58	0.11	1.30	465.65	0.14	3.04	4.60	0.60	13.03
6	8.74	0.11	1.25	799.64	0.39	4.82	3.95	0.43	10.84

Table 4.4 Overview of Na₂SO₄ concentrations with their respective EC and osmolality values

Concentration (g/L)	EC (mS/m)	Medium osmolality (mOsm/kg)
0.000	53.36	3
0.375	88.75	11
0.750	166.37	14
1.500	287.68	23
3.000	465.65	39
6.000	799.64	77

Values of DO of all concentrations were not significantly different from the control (Figure 4.9), whereas all DO values for different exposure times differed significantly from the start of the experiment at 0 h (all p -values < 0.00) (Figure 4.10).

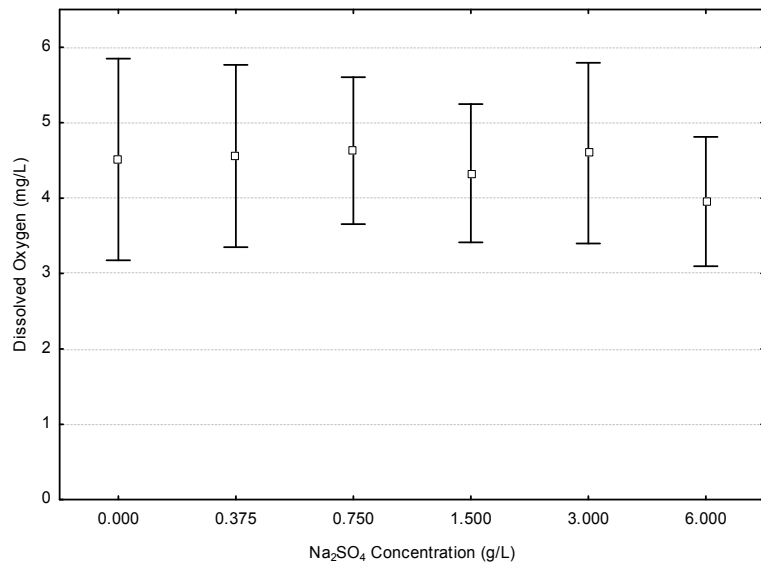


Figure 4.9 Dissolved Oxygen of all exposure times combined over all Na₂SO₄ concentrations (error bars are standard deviation, significant differences are marked with an *)

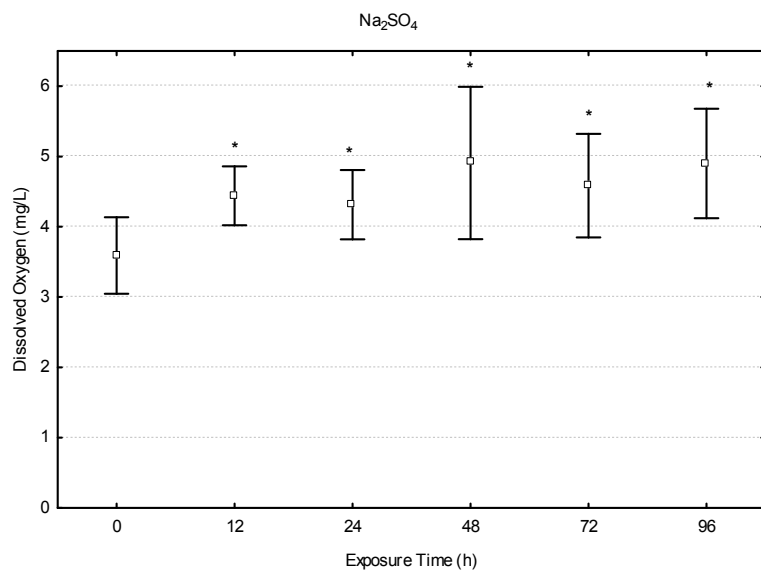


Figure 4.10 Dissolved Oxygen of all Na₂SO₄ concentrations combined over time (error bars are standard deviation, significant differences are marked with an *)

Haemolymph osmolality data were not normally distributed ($W=0.91983$, $p=0.00$) but homogenous ($F=0.005060$, $p=0.99$). Osmolality values of all concentrations were not significantly different from the control (Figure 4.11). The only values differing from the start of the experiment (0 h) were the 12 h haemolymph osmolality values ($p=0.00$) (Figure 4.12).

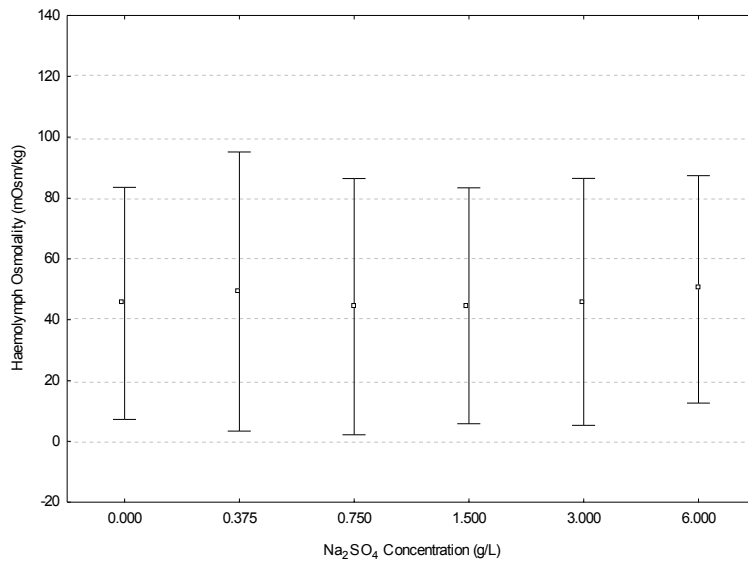


Figure 4.11 Haemolymph osmolality of all exposure times combined over all Na₂SO₄ concentrations (error bars are standard deviation, significant differences are marked with an *)

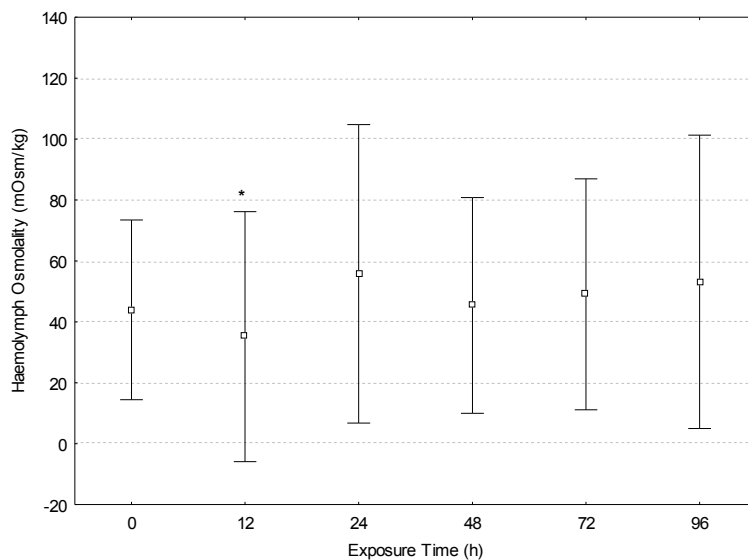


Figure 4.12 Haemolymph osmolality of all Na₂SO₄ concentrations combined over time (error bars are standard deviation, significant differences are marked with an *)

There was a significant difference between haemolymph levels at 0 h and 12 h in the control (0.0 g/L $p=0.03$) and the two lowest concentrations (0.375 g/L $p=0.03$ and 0.75 g/L $p=0.00$) (Figure 4.13). For the highest concentration (6 g/L) there were no 96 h data since all animals died before the end of the experiment.

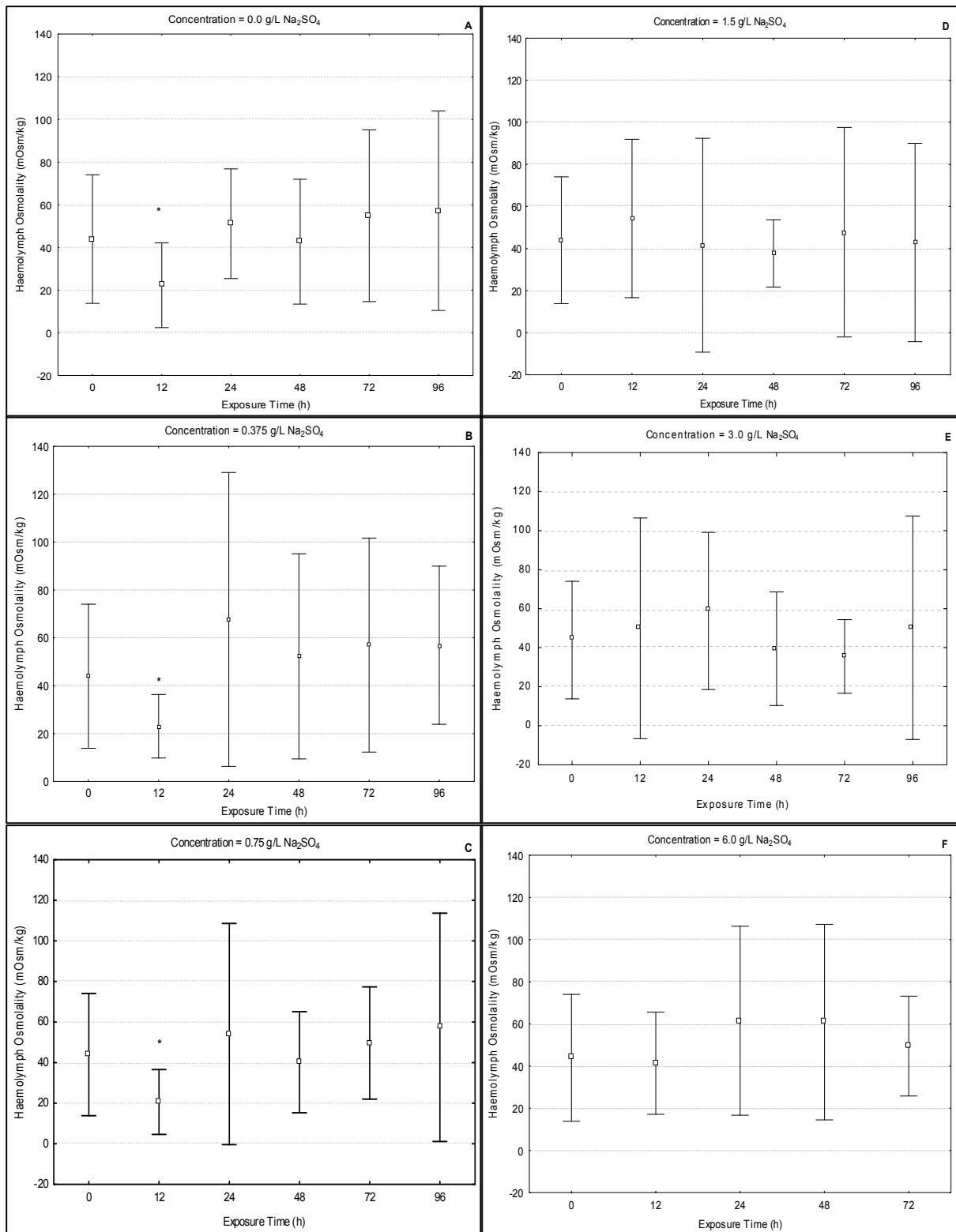


Figure 4.13 Haemolymph osmolality for each Na_2SO_4 concentration over all exposure times (error bars are standard deviation, significant differences are marked with an *)

While there was a significant difference in haemolymph levels after 12 h of exposure between the control (0.0 g/L) and 1.5 g/L ($p=0.00$) and 3.0 g/L ($p=0.03$), there are no other significant differences at later exposure times (Figure 4.14).

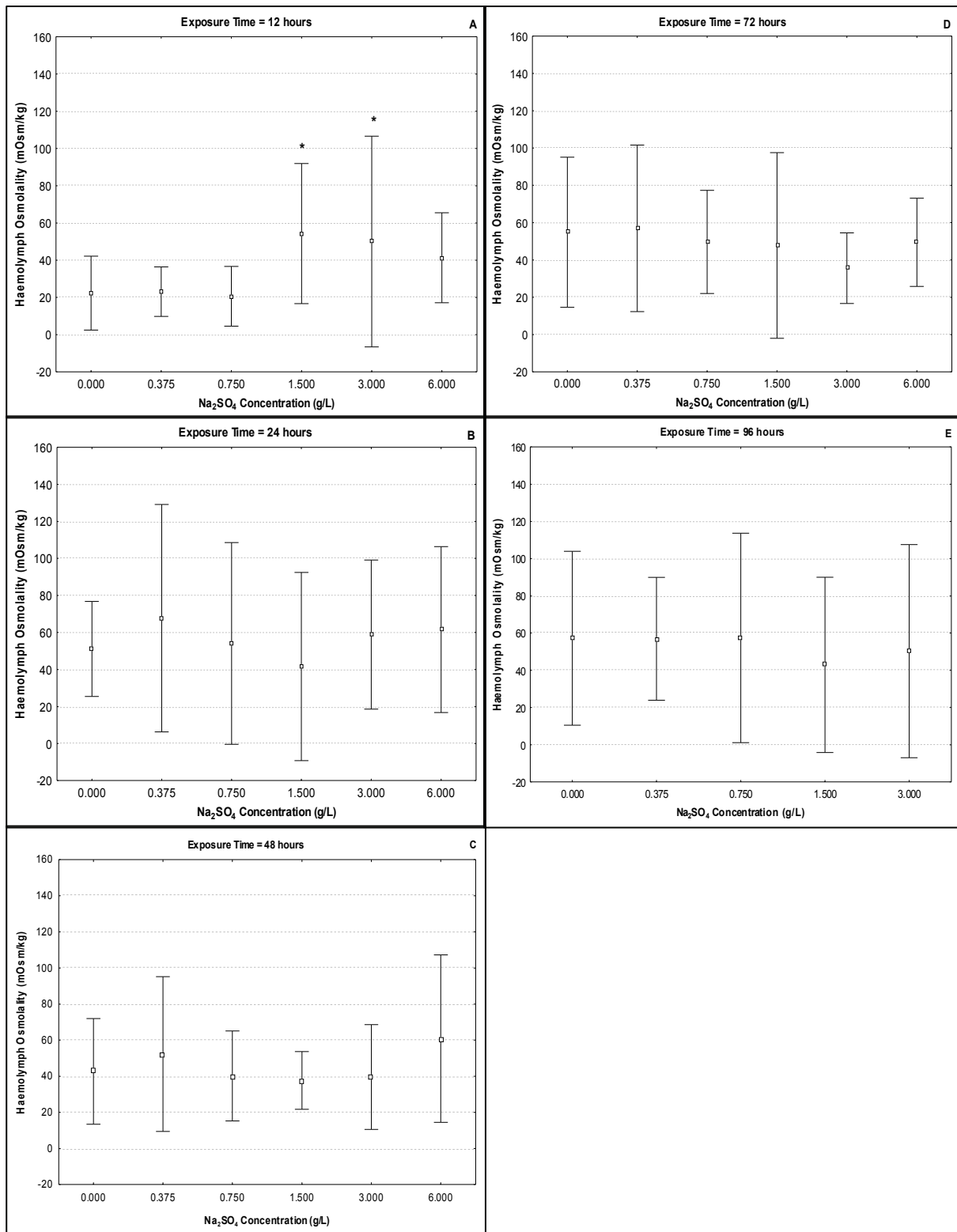


Figure 4.14 Haemolymph osmolality for each exposure time over all Na_2SO_4 concentrations (error bars are standard deviation, significant differences are marked with an *)

The haemolymph osmolality stayed very constant over different exposure concentrations (Figure 4.15). Mortality was 100% in the highest concentration (6.0 g/L) after 96 h of exposure. The linear fit line (horizontal line) rose slightly as the Na_2SO_4 concentration rose but this was not significant ($p=0.28$).

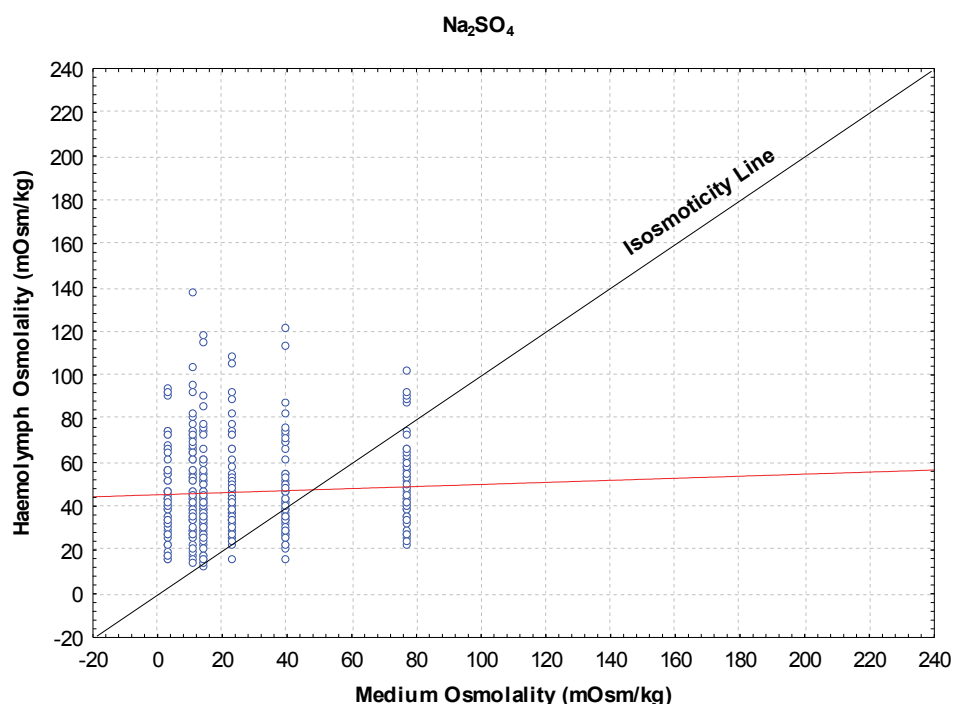


Figure 4.15 Haemolymph Osmolality vs. Medium Osmolality of *C. nilotica* exposed to Na₂SO₄ (Isosmoticity Line at Medium Osmolality =Haemolymph Osmolality)

When calculating the osmoregulatory capacity (OC) of the freshwater shrimp with regards to different Na₂SO₄ concentrations, the results show that *C. nilotica* was hyper-regulating up to a Na₂SO₄ concentration of 3 g/L (39 mOsm/kg) and hyporegulating from a Na₂SO₄ concentration of 6 g/L (77 mOsm/kg) and higher (Figure 4.16). As with exposure to NaCl, *C. nilotica* in this study hyper-hypo-regulated when exposed to increasing concentrations of Na₂SO₄.

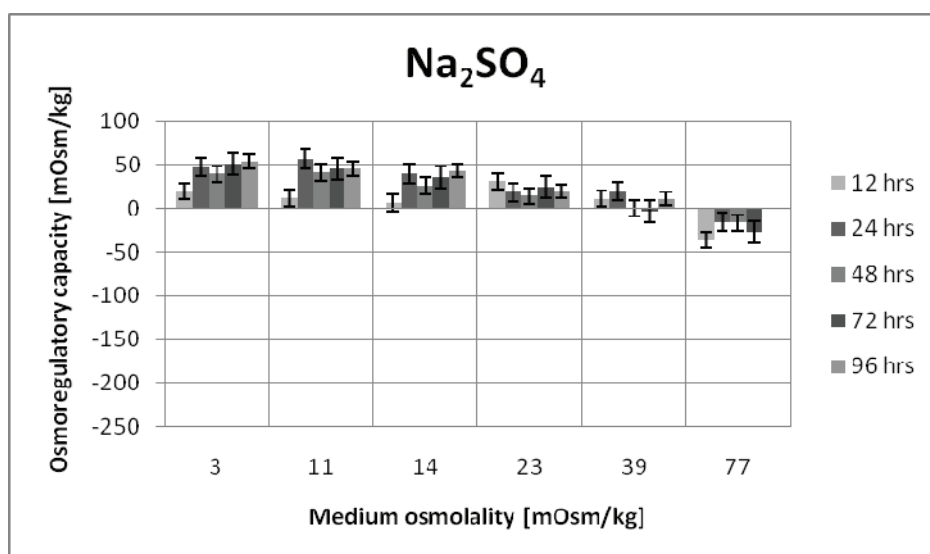


Figure 4.16 Osmoregulatory capacity (OC) at different Na₂SO₄ concentrations (medium osmolality) for different exposure times

4.4 Discussion and Conclusion

Looking at the relationship between oxygen levels in the test solutions and haemolymph osmolality in shrimp exposed to NaCl we found that at the lowest concentration (0.5 g/L NaCl) the DO was lower and the haemolymph osmolality was higher than in the control (Figure 4.1 and Figure 4.3). At this low concentration of NaCl, an increased uptake of Na^+ - and Cl^- - ions seems to take place which results in a higher respiration rate and therefore a lower DO value.

DO levels in the NaCl exposure experiments decreased significantly over time (Figure 4.2) whereas DO levels in experiments with Na_2SO_4 were significantly higher over the course of the experiment compared to the control (Figure 4.10). Shrimp exposed to NaCl were very active and higher heart rates were observed compared to control organisms, whereas organisms exposed to Na_2SO_4 were less active with lower heart rates compared to control animals (personal observation). The lower DO values measured during NaCl exposure could be the result of a higher respiration rate due to increased overall activity in the test organisms. Higher DO values in the Na_2SO_4 experiment could be the result of a measurement error of the control value, which seems very low at 3.62 mg/L dissolved oxygen compared to the control value of 4.5 mg/L for the NaCl control.

When treated with Na_2SO_4 , shrimp haemolymph osmolality decreased within the first 12 h of exposure in the control and the two lowest concentrations, whereas when exposed to NaCl all treatments showed decreased haemolymph osmolality between 12 and 24 h, except for 0.5 g/L where osmolality increased at 48 and 96 h. Haemolymph osmolality levels dropped when shrimp were exposed to NaCl and Na_2SO_4 during the first 24 h of the experiments, which might be due to the medium osmolality being lower than the haemolymph osmolality from field conditions. Therefore the exposure time of 12 to 24 h could be considered as acclimatisation time and thus only values determined after 96 h are discussed further.

Exposure to the two lowest NaCl concentrations (0.5 g/L and 1 g/L) resulted in a significantly higher haemolymph osmolality value at 96 h, whereas all other exposure concentrations were not different from the control. There were no significant differences in haemolymph osmolality at any exposure periods in the Na_2SO_4 exposure experiments. This might be due to the fact that Na^+ - and Cl^- -ions can be taken up more readily because of a smaller diameter of the ions, whereas SO_4^{2-} -ions are much bigger, divalent (can form two bonds with other ions or molecules), and require more energy for diffusion (Péqueux, 1995).

Plotting shrimp haemolymph osmolality against medium osmolality shows, that the freshwater shrimp, *C. nilotica*, is a hypo-hyper-osmoregulator, since haemolymph osmolality levels remain at around the same mean when hypo- and hyper-regulating. This means that the internal ion concentration of the shrimp is higher at lower external ion concentrations (hyper) and the internal ion concentration is lower in higher external concentrations. The point at which internal and external ion concentrations are equal is called the isosmotic point. For some animals (most of the marine crustaceans for example) the ion concentration budget stays close to the isosmotic point, these are called osmoconformers as opposed to osmoregulators because they conform their internal ion concentration to that of the external medium. This means that osmoconforming organisms are confined to a more or less stable environment and would die in places of high salinity fluctuations like estuaries and ephemeral rivers (Péqueux, 1995).

According to results generated in this study (Chapter 4), there was no evidence of osmotic stress in *C. nilotica*, haemolymph osmolality levels stayed the same when exposed to different concentrations of 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 the osmoregulatory capacity of 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 Na₂SO₄.

5 ASSESSING THE USE OF PHYSIOLOGICAL RESPONSES IN MANAGING ENVIRONMENTAL WATER QUALITY

In this chapter, acute lethality data for three indigenous mayfly species (Chapter 2), sublethal physiological response data for two fish species (Chapter 3), and an indigenous shrimp (Chapter 4) are discussed in terms of their usefulness in assessing the Reserve benchmark boundary values for selected inorganic salts. These boundary values for inorganic salts were derived by Jooste and Rossouw (2002) (Table 5.1), whereby acute lethality data (LC₅₀s) from the ECOTOX database maintained by the USEPA were projected to 336 h and the 5th percentile determined as a lethality benchmark, analogous to the Fair/Poor boundary. Similarly, the 5th 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.

In this report, Reserve boundary value results for inorganic salts are reported according to the Natural/Good/Fair/Poor classification system as detailed in Jooste and Rossouw (2002) (Table 5.1). However, current EWQ management encourages the use of the classification system A-F (DWAf, 2008). A conversion table between the two systems is available in DWAf (2008). Electrical conductivity values are also included in this report for comparative purposes (Table 5.2).

Table 5.1 Current Reserve boundary values for inorganic salts in the South African ecological Reserve (Jooste and Rossouw, 2002)

Variable	Categories and associated salt concentration		
	Natural	Good	Fair
MgSO ₄	<16 mg/L	16-27 mg/L	27-37 mg/L
Na ₂ SO ₄	<20 mg/L	20-36 mg/L	36-51 mg/L
NaCl	<45 mg/L	45-217 mg/L	217-389 mg/L

Table 5.2 Current electrical conductivity boundary values in the South African ecological Reserve (DWAf, 2008)

Variable	Categories and associated electrical conductivity		
	Natural	Good	Fair
Electrical conductivity	<30 mS/m	30.1 - 55.0 mS/m	55.1 – 85.0 mS/m

5.1 Evaluation of the current Reserve benchmark boundary value for MgSO₄ using lethality data

An evaluation of the current Reserve boundary values was undertaken by combining indigenous mayfly 96 h LC₅₀ data (generated in 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). The derivation process involved subjecting the acute data to a SSD and obtaining the 5th, 10th and 20th percentiles of the data. These percentiles are considered to be protective of 95%, 90% and 80% of the organisms used in the derivation process, i.e. the protective concentration (PC). These PCs are analogous of the Natural/Good, Good/Fair and Fair/Poor categories respectively (Table 5.3).

A comparison of the current Reserve boundary value and the PCVs determined in this study show the PCV 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 only in the derivation of the PCVs (this may include the need to generate these data), as these data are considered to provide more reliable boundary values than the use of acute values and some ARCs.

Table 5.3 Relationship between ecological categories, protective concentrations and linear distribution percentiles as determined using methods outline by Warne et al. (2005).

Category	Level of protection (PC)	Percentile
Natural	>95	<5 th
Good	>90	> 5 th < 10 th
Fair	>80	> 10 th < 20 th
Poor	<80	> 20 th

Table 5.4 Protection concentration values (PCVs) for MgSO_4 calculated using three indigenous mayfly species and eight other taxa available from ECOTOX database (USEPA, 2004)

MgSO_4	Categories and associated salt concentration		
	Natural	Good	Fair
Current Reserve boundary value	16 mg/L	27 mg/L	37 mg/L
PCVs	7.25 mg/L	41 mg/L	230 mg/L

5.2 Evaluation of the current Reserve benchmark boundary values for NaCl and Na_2SO_4 using physiological response data

Oxygen consumption was determined as a sublethal physiological response endpoint in two species of fish exposed to the salts NaCl and Na_2SO_4 (Chapter 3). 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 *Danio rerio* and *Poecilia reticulata* could be used to evaluate this boundary value. For NaCl , a no observed effect concentration (NOEC) of 500 mg/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 Na₂SO₄ exposed *D. rerio* and *P. reticulata*, although a lowest observed effect concentration (LOEC) could, allowing the calculation of a MATC (maximum allowable toxicant concentration) of 188 mg/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 Na₂SO₄ (Table 5.5), it is again evident that oxygen consumption can contribute as a sensitive endpoint in the determination of suitable guidelines.

Table 5.5 Sublethal toxicity data used in the derivation of the Natural/Good ecological Reserve boundary values for NaCl and Na₂SO₄ (Jooste and Rossouw, 2002).

NaCl		Na ₂ SO ₄	
Organism	NOEC (mg/L)	Organism	NOEC (mg/L)
<i>Anguilla anguilla</i>	14 142	<i>Anabaena sp.</i>	384
<i>Anguilla anguilla</i>	30 000	<i>Cyprinidae sp.</i>	4 500
<i>Astacus astacus</i>	86	<i>Daphnia magna</i>	1 920
<i>Baetis tricaudatus</i>	8 000	<i>Gambusia affinis</i>	849
<i>Ceriodaphnia dubia</i>	704	<i>Myriophyllum spicatum</i>	2 161
<i>Lemna minor</i>	5 186	<i>Navicula seminulum</i>	1 900
<i>Chlorella vulgaris</i>	590	<i>Oncorhynchus mykiss</i>	704
<i>Danio rerio</i>	5 031	<i>Pectinatella gelatinosa</i>	44 904
<i>Pectinatella gelatinosa</i>	41 366	<i>Spartina alterniflora</i>	25
<i>Pimephales promelas</i>	4 000	<i>Spartina cynosuroides</i>	1 094
<i>Stenonema modestum</i>	5	<i>Tricorythus sp.</i>	7 340

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.

5.3 Conclusions and Future Research

The lack of confidence in the MgSO₄ 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 EC₅₀s 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 sub-organism 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.

6 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.

6.1 Undergraduate

This project funded a 3rd year project for **Mr Guy Williams** in Zoology who generated the data for Chapter 2 of this report.

6.2 Postgraduate

This project funded the Honours project of **Mr Greg Tutt** who generated the data and contributed substantially to Chapter 3 of this report.

6.3 Staff Development

Three research interns worked in turn on this project whilst undertaking their MSc's/PhDs. This project offered them training in research and scientific writing and broadened their aquatic scientific expertise:

Ms Nosiphiwo Ketse – previously disadvantaged (MSc student and research Intern until 2006)

Mr Andrew Slaughter (PhD student and research Intern until 2008)

Ms Alexandra Holland (PhD student and research Intern since 2008)

7 REFERENCES

- Altinok I and Grizzle JM (2003) Effects of brackish water on growth, feed conversion and energy absorption efficiency by juvenile euryhaline and freshwater stenohaline fishes. *Journal of Fish Biology* 59, 1142-1152.
- Anger K (2001) *The Biology of Decapod Crustacean Larvae*. Crustacean Issues 14, A.A. Balkema Publishers.
- Aristizabal-Abud EO (1992) Effects of salinity and weight on routine metabolism in the juvenile croaker, *Micropogonias furnieri* (Desmarest 1823). *Journal of Fish Biology* 40, 471-472.
- Arnér M and Koivisto S (1993) Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. *Hydrobiologia* 225, 291-299.
- Boise AMZ, Amstrup J, Novak I and Grosell M (2003) Sodium and chloride transport in soft and hard water acclimated zebrafish (*Danio rerio*). *Biochimica et Biophysica Acta*. 1618, 207-218.
- Browne S (2005) The role of acute toxicity data for South African freshwater macroinvertebrates in the derivation of water quality guidelines for salinity. MSc thesis, Rhodes University, Grahamstown, South Africa.
- Charmantier G, Bouaricha N, Charmantier-Daures M, Thouet P and Trilles J-P (1989) Salinity tolerance and osmoregulatory capacity as indicators of the physiological state of penaeid shrimps. European Aquaculture Society, Special Publication 10, 65-66.
- Chech JJ Jr. (1990) Respirometry. in Schreck CB and Moyle PB (Eds). *Methods for fish biology*. American Fisheries Society, Bethesda, Maryland, USA, 335-362.
- Chown SL, Addo-Bediako A and Gaston KJ (2002) Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 131 (4), 587-602.
- Clark JR, Reinert KH and Dorn PB (1999) Development and application of benchmarks in ecological risk assessment. *Environmental Toxicology and Chemistry* 18, 1869-1870.
- Daikoku T (1980) Changes in osmotic and ionic concentrations of various tissues of the guppy, *Poecilia reticulata*, with adaptation to sea water, and the effect of dietary trimethylamine on these changes. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 66, 189-195.
- Dallas HF and Day JA (1993) The effect of water quality variables on riverine ecosystems: A review. Water Research Commission (WRC) Report No. TT 61/93. Water Research Commission, Pretoria, South Africa.
- Day JA (1993) The major ion chemistry of some southern African saline systems. *Hydrobiologia* 267, 37-59.
- Day JA, Dallas HF and Wackernagel A (1998) Delineation of management regions for South African rivers based on water chemistry. *Aquatic Ecosystem Health & Management* 1, 183-197.

Dettner K and Peters W (1999) Lehrbuch der Entomologie. Gustav Fischer Verlag, Stuttgart, Germany.

DWAF (2004) National water resource strategy. Department of Water Affairs and Forestry, Private Bag x313, Pretoria 0001, South Africa.

DWAF (2005) National toxicity monitoring programme for surface waters. Draft conceptual design framework and record of decision report. Version 1.23. Pretoria, South Africa.

DWAF (2008) Methods for determining the Water Quality component of the Ecological Reserve. Prepared by P-A Scherman of Scherman Consulting for Department of Water Affairs and Forestry, South Africa.

Forbes VE, Palmqvist A and Bach L (2006) The use and misuse of biomarkers in ecotoxicology. *Environmental Toxicology and Chemistry* 25, 272-280.

Goetsch PA and Palmer CG (1997) Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Archives of Environmental Contamination and Toxicology* 32, 32-41.

Gordon AK, Slaughter AR and Muller WJ (2009) The application of chronic (sublethal toxicity endpoints to the development of resource quality objectives. WRC Report No 1484/1/09, Water Research Commission, Pretoria, South Africa.

Grobler E, Van Vuren JHJ and Du Preez HH (1989) Routine oxygen consumption of *Tilapia sparrmanii* (Cichlidae) following acute exposure to atrazine. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 93, 37-42.

Hall CJ and Burns CW (2002) Mortality and growth response of *Daphnia carinata* to increases in temperature and salinity. *Freshwater Biology* 47, 451-458.

Hart BT (1983) Implication of trace metal studies for water quality management. Proceedings, OSS Workshop, Environmental Protection in the Alligator Rivers Region, Jabiru.

Hart BT, Maher B and Lawrence I (1999) New generation water quality guidelines for ecosystem protection. *Freshwater Biology* 42 (2), 347-359.

Helfman GS, Collette BB and Facey DE (2002) The Diversity of Fishes. Blackwell Science Inc. Malden, USA.

Jooste S and Rossouw JN (2002) Hazard-based Water Quality EcoSpecs for the Ecological Reserve in Fresh Surface Water Resources. Report No N/0000/REQ0000. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.

Kefford BJ, Papas PJ and Nuggeoda D (2003) Relative salinity tolerance of macroinvertebrates from the Barwon River, Victoria, Australia. *Marine Freshwater Research* 54, 755-765.

Kefford BJ, Palmer CG, Pakhomova L and Nuggeoda D (2004) Comparing test systems to measure the salinity tolerance of freshwater invertebrates. *Water SA* 30, 499-506.

Lagler KF, Bardach JE and Miller RR (1962) Ichthyology. John Wiley and Sons, Inc., New York, USA.

Lignot J-H, Spanings-Pierrot C and Charmantier G (2000) Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in Crustaceans. *Aquaculture* 191, 209-245.

Lucu C and Towle DW (2003) $\text{Na}^+ + \text{K}^+$ -ATPase in gills of aquatic crustacean. *Comparative biochemistry and physiology. Part A, Molecular and integrative physiology* 135 (2), 195-214.

Mackereth FJH, Harona J and Taling JF (1978) Water analysis: Some revised methods. *Freshwater Biological Association (Scientific Publication no.36)*.

Martínez-Jerónimo F and Martínez-Jerónimo L (2007) Chronic effect of NaCl salinity on a freshwater strain of *Daphnia magna* Straus (Crustacea: Cladocera): A demographic study. *Ecotoxicology and Environmental Safety* 67, 411-416.

Morel FMM and Hering JG (1993) Principles and applications of aquatic waterchemistry. John Wiley and Sons, Inc., New York, USA.

Moser ML and Hettler WF (1989) Routine metabolism of juvenile spot, *Leiostomus xanthurus* (Lacépède) as a function of temperature, salinity and weight. *Journal of Fish Biology* 35, 703-707.

Muller WJ (2005a) Kat River Reserve Determination Study - Technical component: Present ecological state assessment of environmental water quality in the Kat River. Unpublished Water Research Commission Report. Project No. K5/1496. Institute for Water Research, Rhodes University, Grahamstown, South Africa.

Muller WJ (2005b) Integrated environmental water quality assessment of the Leeuspruit for Sasol planning purposes. Confidential report to Sasol R&D, Sasolburg, South Africa.

National Water Act (1998) Government Gazette, 398: no. 19182. Republic of South Africa (RSA) Cape Town: Office of the President.

Newman MC and Aplin MS (1992) Enhancing toxicity data interpretation and prediction of ecological risk with survival time modeling: an illustration using sodium chloride toxicity to mosquitofish (*Gambusia holbrooki*). *Aquatic Toxicology* 23, 85-96.

Palanivelu V, Vijayavel K, Ezhilarasibalasubramanian S and Balasubramanian MP (2005) Impact of fertilizer (urea) on oxygen consumption and feeding energetics in the fresh water fish *Oreochromis mossambicus*. *Environmental Toxicology and Pharmacology* 19, 351-355.

Palmer CG, Berold RS and Muller WJ (2004) Environmental water quality resources management. WRC Report No. TT 217/04, Water Research Commission, Pretoria, South Africa.

Palmer CG, Muller WJ and Davies-Coleman HD (2004) Applied aquatic ecotoxicology: sub-lethal methods, whole effluent testing and communication. WRC Report No 1245/1/04, Water Research Commission, Pretoria, South Africa.

Palmer CG, Rossouw N, Muller WJ and Scherman P-A (2005) The development of water quality methods within ecological Reserve assessments, and links to environmental flows. *Water SA* 31, 161-170.

Péqueux A (1995) Osmotic regulation in Crustaceans. *Journal of Crustacean Biology* 15(1), 1-60.

Rall VE, Engelbrecht JS, Musgrave H, Rall LJ, Williams DBG and Simelane R (2010) Development of protocols for acute fish toxicity bioassays, using suitable indigenous freshwater fish species. WRC Report No. 1313/2/10, Water Research Commission, Pretoria, South Africa.

Rao GMM (1968) Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. Canadian Journal of Zoology 46, 781-786.

Ricklefs RE and Wikelski M (2002) The physiology/life-history nexus. Trends in Ecology and Evolution 17, 462-468.

Roux DJ, Jooste SHJ and MacKay HM (1996) Substance-specific water quality criteria for the protection of South African freshwater ecosystems: methods for derivation and initial results for some inorganic toxic substances. South African Journal of Science 92(4), 198-206.

Rowe CL (2002) Differences in maintenance energy expenditure by two estuarine shrimp (*Palaemonetes pugio* and *P. vulgaris*) that may permit partitioning of habitats by salinity. Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology 132 (2), 341-351.

Sarma SSS, Beladjal L, Nandini S, Cerón-Martínez G and Tavera-Briseño K (2005) Effect of salinity stress on the life history variables of *Branchipus schaefferi* Fisher, 1834 (Crustacea: Anostraca). Saline Systems 1 (4), 1-7.

Scherman P-A (2009) Ecological Reserve Study for the Inkomati catchment: Water quality component. Prepared for Water for Africa.

Scherman P-A (2010) Ecological Reserve Study for the Orange River catchment: Water quality component. Prepared for Rivers for Africa.

Scherman P-A and Palmer CG (2000) A protocol for acute toxicity testing using riverine invertebrates in artificial stream systems. Version 1. Department of Water Affairs and Forestry, South Africa.

Scherman P-A, Palmer CG and Muller WJ (2003) Use of indigenous riverine invertebrates in applied toxicology and water resource-quality management. WRC Report No. 955/1/03, Water Research Commission, Pretoria, South Africa.

Schmidt-Nielsen K (1998) Animal physiology - adaptation and environment. Cambridge University Press, Cambridge, UK.

Slabbert L (2004) Methods for direct estimation of ecological effect potential (DEEEP). Water Research Commission (WRC) Report No. 1313/01/04. Water Research Commission, Pretoria, South Africa.

Slaughter AR (2005) The contribution of chronic toxicity data for indigenous aquatic macroinvertebrates to the development of protective salinity guidelines for South African freshwater resources. MSc thesis, Rhodes University, Grahamstown South Africa.

Slaughter AR, Palmer CG and Muller WJ (2008) A chronic toxicity protocol using *Caridina nilotica* (Decapoda: Atyidae) and the generation of salinity toxicity data. African Journal of Aquatic Science 33 (1), 37-44.

Steffenson JF (1989) Some errors in respirometry of aquatic breathers: how to avoid and correct them. Fish Physiology and Biochemistry. 6(1), 49-59.

Tannenbaum LV (2005) A critical assessment of the ecological risk assessment process: A review of misapplied concepts. *Integrated Environmental Assessment and Management* 1, 66-72.

Teschner M (1995) Effects of salinity on the life history and fitness of *Daphnia magna* variability within and between populations. *Hydrobiologia* 307, 33-41.

USEPA (1994) Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. EPA/600/4-91/002, Cincinnati, United States Environmental Protection Agency, USA.

USEPA (2004) data acquired from the ECOTOX database <http://cfpub.epa.gov/ecotox/> in 2004, United States Environmental Protection Agency, USA.

Varsamos A, Nebel C and Charmantier G (2005) Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology* 141 (4), 401-429.

Walmsley RD, Walmsley JJ and Breytenbach R (1999) An overview of water quality management of South Africa's major port-catchment systems. WRC Report No. 794/1/99, Water Research Commission, Pretoria, South Africa.

Warne MJ (1998) Critical review of methods to derive water quality guidelines for toxicants and a proposal for a new framework. Supervising scientist report 135, Supervising Scientist, Canberra.

Warne MS, Palmer C and Muller WJ (2004) Water quality guideline development programme (WQGD). Development of pilot guidelines for selected organic toxicants/toxicity effects. I. Protocol for aquatic ecosystem guideline development. Report to the South African Department of Water Affairs and Forestry [commercial-in-confidence]. CSIRO Land and Water Client report. Adelaide: CSIRO.

Warne MStJ, Palmer CG and Muller WJ (2005) Development of pilot guidelines for selected organic toxicants: 1. Protocol for aquatic ecosystem guideline development. Department of Water Affairs and Forestry, Water Quality Guideline Development Programme, South Africa.

Wikelski M and Cooke SJ (2006) Conservation physiology. *Trends in Ecology and Evolution* 21, 38-46.

Zheng Y, Zhu H and Luo Y (2000) Assessment on the situation of water quality at Xiangshan Bay. *Chin. J. Mar. Environ. Sci.* 19, 56-59. (in Chinese with English abstract).

MVLWB

Water and Effluent Quality Management Policy

March 31, 2011



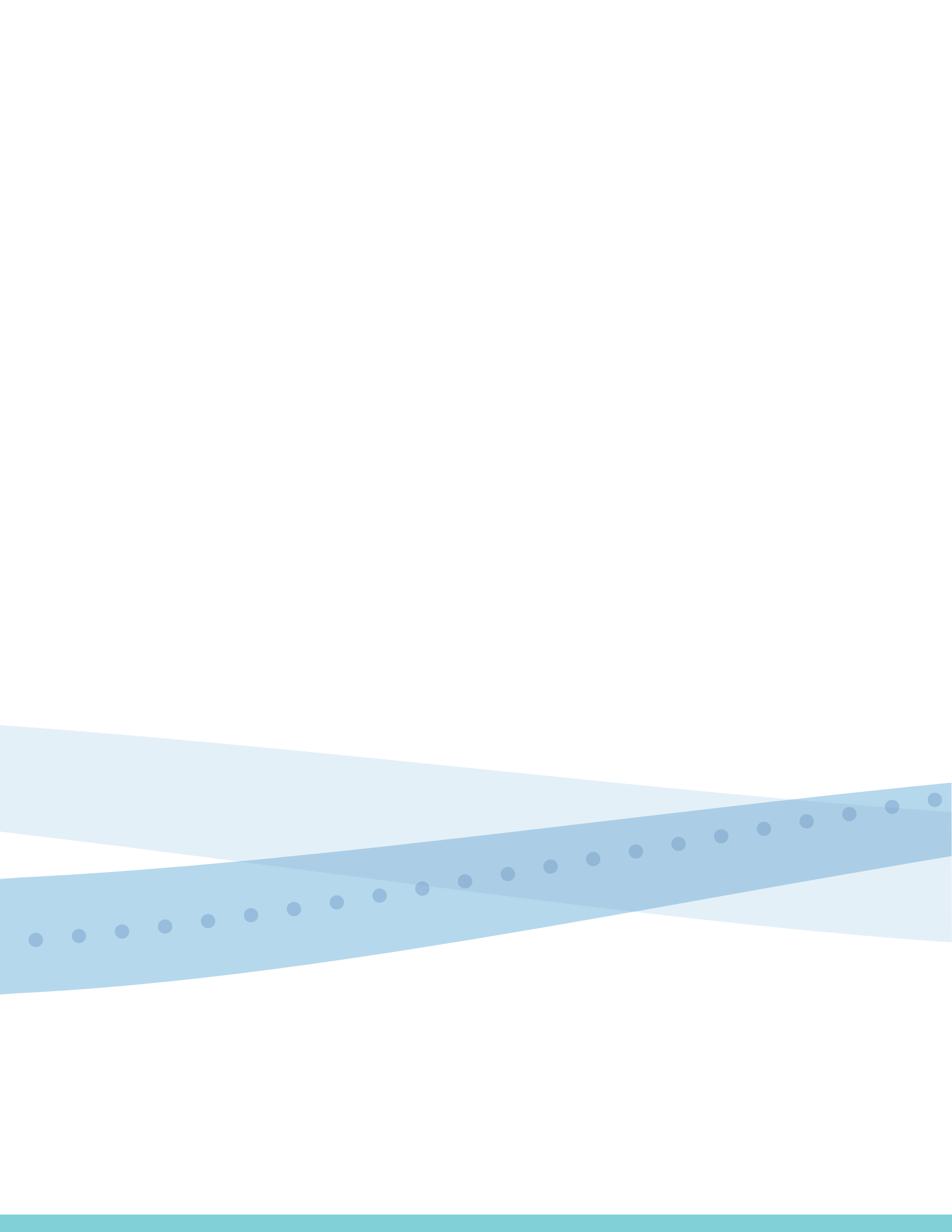
Mackenzie Valley Land and Water Board



MVLWB



“Sharing responsibility—working together to make the best decisions for the land, water, and people.”



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Definitions and Acronyms

TERM	DEFINITION
AEMP	aquatic effects monitoring program
Boards	Land and Water Boards of the Mackenzie Valley, as mandated by the MVRMA
CCME	Canadian Council of Ministers of the Environment
effluent quality criteria (EQC)	Numerical or narrative limits on the quality or quantity of the waste deposited to the receiving environment
GLWB	Gwich'in Land and Water Board
mixing zone	An area adjacent to the effluent outfall within which waste is deposited and first mixes with water in the receiving environment.
Mackenzie Valley	That part of the Northwest Territories bounded on the south by the 60th parallel of latitude, on the west by the Yukon Territory, on the north by the Inuvialuit Settlement Region as defined in the Agreement given effect by the <i>Western Arctic (Inuvialuit) Claims Settlement Act</i> , and on the east by the Nunavut Settlement Area as defined in the <i>Nunavut Land Claims Agreement Act</i> , but does not include Wood Buffalo National Park.
MVLWB	Mackenzie Valley Land and Water Board
MVRMA	<i>Mackenzie Valley Resource Management Act</i>
NWT	Northwest Territories
project	Any activity that requires a water licence

proponent	Applicants for water licences
receiving environment	The natural environment that, directly or indirectly, receives any deposit of waste (as defined in the <i>NWT Waters Act</i>) from a project
SLWB	Sahtu Land and Water Board
SNP	Surveillance network program
stakeholders	Term includes industry, federal agencies, the territorial government, Aboriginal governments, and organizations, communities, and other interested parties.
type A water licence	A water licence required as per Column IV of Schedules IV to VIII of the Northwest Territories Waters Regulations SOR/92/203
type B water licence	A water licence required as per Column III of Schedules IV to VIII of the Northwest Territories Waters Regulations SOR/92/203
waste	As defined in section 2 of the <i>NWT Waters Act</i> ¹
WLWB	Wek'èezhii Land and Water Board

¹"waste" is defined, in section 2 of the *Northwest Territories Waters Act*, as:

- (a) any substance that, if added to water, would degrade or alter or form part of a process of degradation or alteration of the quality of the water to an extent that is detrimental to its use by people or by any animal, fish or plant, or
- (b) water that contains a substance in such a quantity or concentration, or that has been so treated, processed or changed, by heat or other means, that it would, if added to any other water, degrade or alter or form part of a process of degradation or alteration of the quality of that water to the extent described in paragraph (a), and, without limiting the generality of the foregoing, includes
- (c) any substance or water that, for the purposes of the *Canada Water Act*, is deemed to be waste,
- (d) any substance or class of substances prescribed by regulations made under subparagraph 33(1)(b)(i),
- (e) water that contains any substance or class of substances in a quantity or concentration that is equal to or greater than a quantity or concentration prescribed in respect of that substance or class of substances by regulations made under subparagraph 33(1)(b)(ii), and
- (f) water that has been subjected to a treatment, process or change prescribed by regulations made under subparagraph 33(1)(b)(iii)."

1.0 Purpose of This Policy

The Land and Water Boards of the Mackenzie Valley regulate the use of water and the deposit of waste into water through the issuance of water licences.

The purpose of the Water and Effluent Quality Management Policy (the Policy) is to describe the Boards' approach to managing the deposit of waste to the receiving environment through enforceable terms and conditions set in water licences. Such terms and conditions include ², but are not limited to, effluent quality criteria (EQC), activities related to waste management, monitoring programs, adaptive management planning, and/or other management plans.

The Boards have set out this Policy in order to ensure that Board licensing decisions are clear, timely, consistent, and transparent. By referencing this Policy, proponents, stakeholders, and other interested parties will be able to make more informed submissions to the Boards which will, in turn, lead to more efficient and effective Board processes and decisions.

The Boards recognize that this Policy will need to be supported by more detailed guidelines and specific procedures including, but not limited to, setting site-specific water quality standards, collecting baseline information, establishing mixing zones, and developing plans for monitoring and waste management. A complete list of items currently identified as requiring more guidance is noted in the text of the Policy and itemized in Appendix A. These guidelines and procedures will further address the level to which the Policy will apply to different projects and authorizations (e.g., type A or type B water licence applications, etc.).

2.0 Authority

The Boards' authority to develop and implement this Policy is granted under sections 65, 102, and 106 of the MVRMA.

The authority to set limits on the amount of waste discharged from a project is given to the Boards under paragraph 14(4)(c) of the **Northwest Territories Waters Act**, which states that any waste produced by an undertaking "will be treated and disposed of in a manner that is appropriate for the maintenance of:

- (i) water quality standards prescribed by regulations made under paragraph 33(1)(h) or, in the absence of such regulations, such water quality standards as the Board considers acceptable, and
- (ii) effluent standards prescribed by regulations made under paragraph 33(1)(i) or, in the absence of such regulations, such effluent standards as the Board considers acceptable."

No regulations for water quality or effluent standards have been prescribed by the Governor in Council under paragraphs 33(1)(h) or 33(1)(i) of the **Northwest Territories Waters Act**. This Policy outlines the process for setting water quality and effluent standards during water licencing.

3.0 How These Policy Was Developed

This Policy was developed by the Water/Effluent Quality Guidelines Working Group, one of the Standard Procedures and Consistency Working Groups established by the Boards in 2008.

This Policy is based on input from Board staff, consultants, and numerous publically available documents and is consistent with past and present practices of the Boards. During the development of the Policy and prior to public distribution, members of the Boards reviewed the draft Policy and provided input on the document and, in particular, on the "Guiding Principles" (section 5, below). On April 29, 2010, a draft of this Policy was distributed to all organizations that regularly participate in the proceedings of the Gwich'in, Sahtu, Wek'èezhii and/or Mackenzie Valley Land and Water Boards as a licensee, a reviewer,

² see section 15 of the **Northwest Territories Waters Act** for terms and conditions that may be set in a water licence

or other interested party. The comment deadline for this review was July 5, 2010. The Policy was revised by the Water/Effluent Quality Guidelines Working Group with consideration of all the comments received. The revised Policy was put before the Boards on December 8, 2010 and approved. The Policy is effective starting March 31, 2011.

4.0 Application of This Policy

This Policy will be applied by all the Land and Water Boards (Boards) operating under the **Mackenzie Valley Resource Management Act** (MVRMA) including the:

- Mackenzie Valley Land and Water Board
- Gwich'in Land and Water Board
- Sahtu Land and Water Board
- Wek'èezhìi Land and Water Board.

This Policy applies to all projects that require a water licence. Specifically, this Policy applies to the terms and conditions of a water licence as set by the Boards to manage the deposit of waste to the receiving environment.

This Policy outlines the types of information that a proponent must submit to a Board as part of the process of setting water licence terms and conditions to manage the deposit of waste. In all cases, the Boards will set the terms and conditions of a water licence based on the evidence presented during the water licensing process. Although the same types of information will be required from each proponent, the amount of detail required will vary depending on the size, type, stage, and duration of the project under consideration. The appropriate level of information required from the proponent will be described in relevant guideline documents (see Appendix A).

This Policy will be applied to all new or renewal water licence applications received after the effective date of the Policy. In the case of existing water licences, this Policy may be applied if there is a proposal to

amend any terms and conditions of the water licence, including EQC. Amendments to water licences are considered ³ upon request of the proponent or by a Board's own motion (if the amendment appears to be in the public interest).

5.0 Guiding Principles

The following principles have been adopted by the Boards and will guide the Boards' decisions on any matter related to the deposit of waste from a project to the receiving environment. The principles are not listed in any order of priority.

1. Sustainable Development: Meeting the needs of the present without compromising the ability of future generations to meet their own needs.
2. Pollution Prevention: The use of processes, practices, materials, products, or energy that avoid or minimize the creation of pollutants and waste and reduce overall risk to human health and the environment.
3. Precaution: Where there are threats of serious or irreversible damage, the lack of full scientific certainty should not be used as a reason for postponing cost effective measures to prevent environmental degradation.
4. Polluter Pays: The polluting party should pay for the restoration of damage done to the natural and built environments.
5. Integrated Watershed Management: The cooperative and coordinated stewardship of shared water resources where decisions are made in a watershed context and for the greatest collective benefit for all Canadians and in particular for residents of the Mackenzie Valley.
6. Multiple Uses and Values: Decisions should address multiple, diverse, and sequential uses of water – many of which depend at the same time on the same water body.

³ section 18 of the *Northwest Territories Waters Act*

7. Shared Responsibility: In our co-management system, all stakeholders have a responsibility to meaningfully participate in decisions that will affect water.
8. Jurisdiction Best-Placed: Although policy development should take place at all jurisdictional levels, policy implementation should be the responsibility of the level most appropriate to resolving the issue at hand.

6.0 Objectives for Regulating the Deposit of Waste

The Boards regulate the “quantity, concentration, and types of waste”⁴ that may be deposited from a project to the receiving environment. In accordance with the guiding principles listed in section 5, the Boards regulate, through water licence requirements, the deposit of waste such that the following two objectives are met:

1. Water quality in the receiving environment is maintained at a level that allows for current and future water uses.

Protection of water quality in the receiving environment is the primary objective. The level of protection will be defined by the water quality standards⁵ that have been set site-specifically for the receiving environment in question. Effluent Quality Criteria (EQC) will be set for a project to ensure that water quality standards will be met. A Board may set other terms and conditions in the water licence that, in its opinion, will aid in achieving this objective.

2. The amount of waste to be deposited to the receiving environment is minimized.

The Boards expect proponents to identify and implement waste prevention and/or minimization measures, whenever feasible. Implementation of such measures may be stipulated in the terms and conditions of a water licence. The Boards can assess how these measures are expected to impact effluent from a project in order to set EQC that proponents can reasonably and consistently achieve.

There are several different types of water licence requirements (e.g., EQC, management plans, monitoring, etc.) that can be used by the Boards to ensure that, collectively, the water licence meets the objectives above. The key requirements, also called terms and conditions, used in water licences are described in section 7, below. The types of information that the Boards require to set the terms and conditions necessary to achieve the objectives above are summarized in section 8.

7.0 Typical Water Licence Requirements Used to Regulate the Deposit of Waste

Boards will set terms and conditions in a water licence to ensure that the objectives set out in section 6.0 for regulating the deposit of waste are met. Such terms and conditions will be set on a project-specific basis, but the types of requirements typically used by the Boards are described below.

7.1 Waste Management Practices

The Boards may require proponents to use practices that are known to be effective in managing waste and protecting the environment. Such practices may be stipulated directly as conditions in water licences or through Board-approved management plans that describe the proponent’s practices (see also subsection 7.4).

⁴ paragraph 15(1)(b) of the *Northwest Territories Waters Act*

⁵ *The Northwest Territories Waters Act* states that “any waste that would be produced by the appurtenant undertaking will be treated and disposed of in a manner that is appropriate for the maintenance of water quality standards” (subsection 4(c) of the *Northwest Territories Waters Act*). There is no definition of the term “water quality standard” in the *Northwest Territories Waters Act*, but the Boards believe it to be equivalent to the more widely accepted term “water quality objective” which has been defined by the Canadian Council of Ministers of the Environment (CCME) as: “a numerical concentration or narrative statement that has been established to support and protect the designated uses of water at a specified site.” (CCME (1999), Canadian Environmental Quality Guidelines. Guidelines and Standards Division, Winnipeg, MB.)

In general, waste management practices should be guided by the waste prevention/minimization hierarchy⁶ of preferred options, as follows:

1. Source reduction – waste should be prevented or reduced at the source whenever feasible;
2. Reuse/recycle – waste that cannot be prevented should be reused or recycled in an environmentally safe manner whenever feasible;
3. Treatment – waste that cannot be prevented or recycled/reused should be treated in an environmentally safe manner whenever feasible; and
4. Discharge – discharge or deposit of waste into the environment should be employed only as a last resort and must meet EQC.

An example of prescribing a management practice would be a condition in a water licence stipulating the use of a specific erosion control method known to reduce the amount of sediment that enters water (i.e., source reduction). In all cases, the intent of prescribing specific management practices is to achieve the objectives listed in section 6.

7.2 Effluent Quality Criteria

Once all reasonable measures have been taken to limit the amount of waste, concerns may still exist about the quantity, concentration, and type of waste to be deposited, and in these cases the Boards will set EQC in the water licence. EQC define the maximum allowable concentrations (e.g., mg/L), quantities (e.g., kg/year), or limits (e.g., pH range) of any contaminant or parameter of the waste which, in the Boards' opinion, has the potential to adversely affect water quality in the receiving environment. Sampling and analysis of effluent will be specified in the Surveillance

Network Program (SNP) of the water licence and the proponent must ensure that the waste discharged meets the EQC in order to remain in compliance with the water licence.

Figure 1 illustrates, with an example, the relationship between EQC and the receiving body's water quality standards. At a minimum, EQC for a project must be set at levels that will ensure water quality standards for the receiving environment will be met. As no pre-defined water quality standards have been established for water bodies in the NWT, the level of water quality to be maintained in the receiving environment has been, and will continue to be, decided on a site-specific basis⁷ (note: information that the Boards will consider when setting site-specific water quality standards is outlined in Section 8). On a case-by-case basis, the Boards may decide to define a mixing zone between the point of effluent discharge and the point at which water quality standards need to be met. Guidelines on when mixing zones may be prescribed as well as how such zones will be defined will be developed by the Boards as noted in Appendix A.

Note that in accordance with the Boards' objective to minimize waste discharge, proponents are expected to minimize and, where feasible, to prevent waste from entering water in the NWT. Therefore, and consistent with the CCME nondegradation policy⁸, the Boards may set EQC that are more stringent than what is necessary to meet water quality standards in the receiving environment. When making this determination, the Boards will ensure that EQC are set at levels that the proponent can reasonably and consistently achieve.

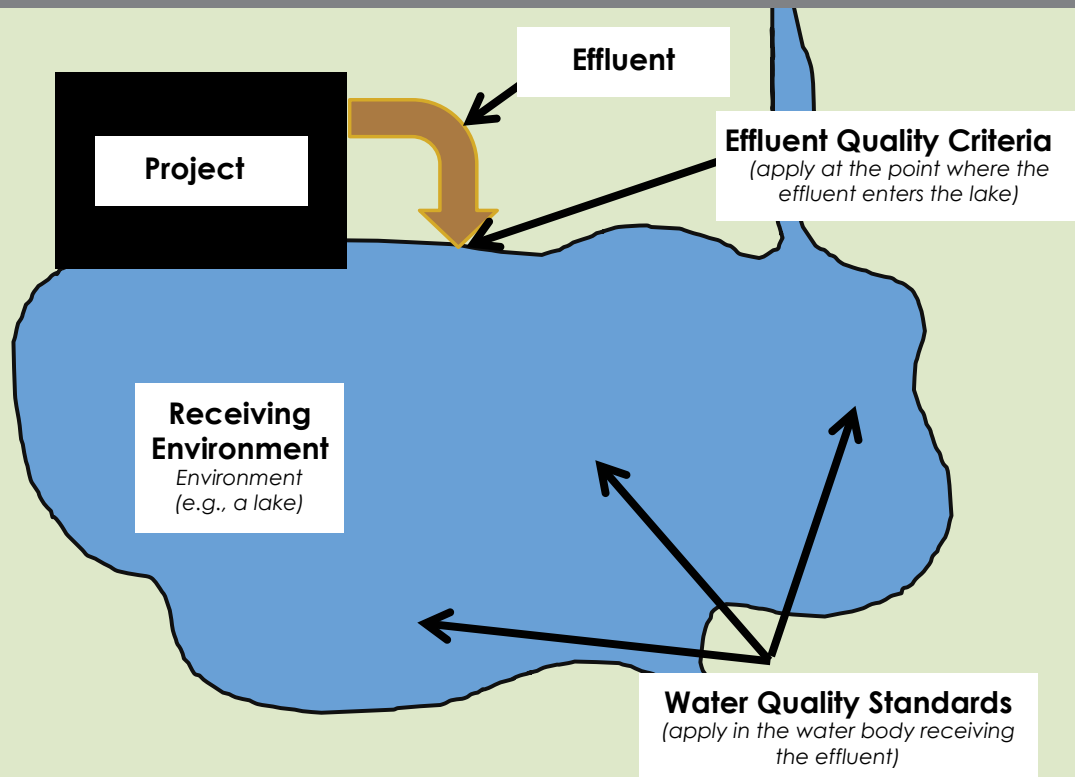
Further details on the procedure for setting EQC will be addressed in the guidelines that will be developed by the Water/Effluent Quality Guidelines Working Group to support this Policy.

⁶ the waste prevention/minimization hierarchy as written above has been adapted from the following reference: F. Henry Habicht II. Memorandum: EPA Definition of Pollution Prevention. U.S. Environmental Protection Agency, May 28, 1992.

⁷ MacDonald Environmental Sciences Ltd. (2006), Toward the Development of Northern Water Standards, prepared for Indian and Northern Affairs. Chapter 3.

⁸ "For waters of superior quality or that support valuable biological resources, the CCME nondegradation policy states that the degradation of the existing water quality should always be avoided." CCME (1999), Canadian Environmental Quality Guidelines. Guidelines and Standards Division, Winnipeg, MB.

Figure 1: An Example of the Relationship Between Effluent Quality Criteria and Water Quality Standards



Water quality standards define the quality of water that must be maintained in the receiving environment. In this example, water quality standards would be defined for the lake into which effluent is being discharged from a project. EQC would be set as described in section 7.2 and would, in this example, apply at the point at which the effluent discharge enters the lake (i.e., the end-of-pipe)

7.3 Monitoring Requirements

Environmental monitoring programs are essential for providing the information needed to determine if the waste prevention/minimization and water quality protection measures (including EQC) are successfully meeting their stated objectives. Monitoring will be required for various activities during the construction, operation and closure of a project; the most common monitoring programs are described below:

1. Surveillance Network Programs (SNPs), consist of specific sites within a development at which water quality and quantity are measured; sampling requirements are decided on a site-specific basis. SNPs are designed to aid the proponent and the regulators in ensuring that waste management activities are being effective. Typically, one of the SNP stations is assigned to the end-of-pipe and is the point at which the proponent must comply with the

EQC. Other SNP stations are often located at points of waste transfer or treatment prior to the end-of-pipe to ensure that the waste-handling system is working as expected and to identify any source control issues as they arise.

2. Aquatic Effects Monitoring Programs (AEMPs) monitor the short- and long-term effects of a project on the wider receiving environment; such programs are currently only required of projects that require a type A water licence. AEMPs in particular can tell us if the water quality standards set for a receiving environment are being met. In addition to water quality monitoring, AEMPs often include requirements for monitoring biota at different levels of the food chain (e.g., plankton, small-bodied fish, large-bodied fish, etc.) to ensure the water quality standards as set are sufficiently protective and to identify any effects that were not originally predicted. Monitoring results can be used to guide adaptive management actions as described below. Guidelines for the development of AEMPs are available (Appendix A).

7.4 Adaptive Management

While selecting the best possible approach to water and effluent quality management is very important, the use of adaptive management acknowledges that it can be difficult to predict all the effects of projects and developments on water resources. As a result, adaptive management involves monitoring the effects of actions and, where necessary, adjusting actions based on the monitoring results. For example, if monitoring results show the effects of a project on the environment are different or worse than predicted, further mitigation measures may be prescribed or EQC may be changed appropriately. While the concept of adaptive management has been integrated, to a certain extent, into the water licensing process, the Boards are developing further guidelines specifying how the principles of adaptive management will be applied to projects.

7.5 Management Plans

As discussed in section 7.1 above, the Boards may require the submission of management plans that will detail how certain aspects of the waste prevention/minimization hierarchy (e.g., source control, reuse/recycle, and/or treatment of waste) or other environmental protection methods will be implemented. Such management plans as the Boards deem necessary will be required by the terms and conditions of a water licence. In general, the water licence will stipulate the management objectives but will allow the proponent to describe how, for their project, those objectives can be best achieved. In general, management plans will require Board approval (to ensure the plan is able to meet the stated objectives) prior to implementation by the proponent. Management plans may include, but not be limited to: waste management plans, spill contingency plans, site-water management plans, erosion and sediment control plans, and closure and reclamation plans. Please refer to Appendix A for a list of management plan guidance documents that are either approved or require development.

8.0 Information Required to Regulate the Deposit of Waste

In their water licence applications, proponents are required to submit the information necessary for the Boards to set appropriate water licence terms and conditions. Most of the information requirements are listed in the standard water licence application form⁹. This section of the Policy is only meant to highlight some specific types of information that the Boards consider when setting terms and conditions that, collectively, will result in a water licence that meets the objectives stated in section 6.

8.1 Information Required from the Proponent

The types of information required from proponents include, but are not limited to:

- Information on proposed waste prevention and minimization measures for a project;

⁹ as set out in Schedule III and Section 6 of the Northwest Territories Waters Regulations SOR/93-30.

- Technically accurate predictions of the concentration and quantities of waste that the proponent proposes to deposit after all feasible proposed waste prevention and management measures have been employed;
- Predictions of how the effluent, once discharged, will mix and disperse in the receiving environment;
- Recommended site-specific water quality standards for the project's receiving environment including the evidence upon which the recommendations are based. During the water licencing process, the proponent's proposed standards will be evaluated by all parties and a final decision on the applicable standards will be made by the Boards. Information that the Boards will consider with respect to applicable water quality standards includes, but is not limited to, the items listed below. Note that this information should be submitted by the proponent to support the proposed site-specific standards.
 - Pre-development (baseline) conditions of the receiving waters (e.g., water quality, water quantity as well as the resident species of plants, animals, and fish that live in or use the water);
 - Traditional Knowledge, including knowledge about the environment, knowledge about interacting with the environment, and environmental values;
 - Traditional and potential uses of the receiving water bodies (e.g., sustenance, recreational, cultural, etc.)
 - Cultural significance of the water bodies to local residents;
 - Inputs of waste from other projects or activities located in the same watershed

or region in order to evaluate potential cumulative effects;

- Published water quality guidelines (e.g., CCME Guidelines) and scientific studies that are relevant and appropriate for the receiving waters, based on the information listed above; and
- Measures and suggestions, including predictions and limits of acceptable change, listed in Reports of Environmental Assessment or Environmental Impact Review.

In gathering information for their applications, proponents can and should avail themselves of relevant information that has already been collected by other parties or through other initiatives (e.g., governmental agencies, regional land use or water management plans). As well, and although the CCME has published some guidance documents on the development of site-specific water quality standards, the Boards recognize the need to develop specific guidance for proponents that provides details on the above information requirements and describes how each of the above factors will be considered during a water licensing process (also see Appendix A). While the same types of information will be required by each proponent, the amount of detail required will often vary depending on the size, type, and duration of the project.

Finally, some of the information listed above (in particular the information needed to set water quality standards), requires stakeholder input prior to the submission of the application. As listed in Appendix A, public engagement policy and guidelines are currently under development by the Boards. Pre-submission engagement, which is described in more detail under section 8.2, is key to an efficient and effective regulatory process.

8.2 Stakeholder Involvement and Community Participation

Although the onus is on the proponent to provide the information (as outlined above) in their applications, the Boards believe that the best decisions will be made only if all parties share their relevant expertise and knowledge during the water licensing process. Having input from all stakeholders, with a variety of backgrounds, expertise, values, and interests, is invaluable to the Boards in making fair and balanced decisions that provide for the optimum benefit of the residents of the management areas, the Mackenzie Valley, and all Canadians. There are several opportunities during the water licensing process for stakeholder input.

Firstly, the Boards require proponents to engage impacted communities and Aboriginal governments/organizations prior to making submissions to the Boards. The purpose of this engagement is to provide an opportunity for all parties involved to learn from each other, to develop a relationship based on mutual respect and trust, and to explore solutions to stakeholder concerns that meet the needs of all parties. Proponent engagement with stakeholders needs to be ongoing and continue during the water licence proceeding and for the life of the project. Evidence of these efforts must be filed with the Boards.

After a water licence application is submitted, the Boards distribute all documents to stakeholders for review and comment. The Boards encourage stakeholders to provide comments and recommendations specific to the project to help develop water licence terms and conditions. As well, for all type A water licence applications the Boards are required to call a public hearing. The Boards also have the option of holding public hearings for type B water licence applications. Public hearings provide an opportunity for stakeholders to present directly to the Boards with their input on specific applications. The Boards consider all contributions and statements

important, whether they are based on Traditional Knowledge, scientific knowledge, local values or other relevant information.

The Boards also involve stakeholders when developing policy and guidance documents. Stakeholder input helps shape policies and guidelines that are clear, transparent, and reflect the interests and values of stakeholders.

As noted in Appendix A, the Boards are developing a policy and guidelines to describe how stakeholders are involved with Board processes.

8.3 Consideration of Other Applicable Legislation

In addition to the information sources discussed above, the Boards recognize that there is other legislation that must be adhered to. For example, the Boards may not include any conditions in water licences relating to the deposit of waste that are less stringent than the provisions of regulations made under subsection 36(5) of the *Fisheries Act*.

(Note that proponents must adhere to all legal requirements (e.g., *Fisheries Act*, Metal Mining Effluent Regulations, etc.) relevant to their respective operation. It is the proponent's responsibility to be aware of and comply with these requirements.)

9.0 Policy Implementation

Section 106 of the *MVRMA* gives the MVLWB the responsibility to "issue directions on general policy matters or on matters concerning the use of land or waters or the deposit of waste that, in the Board's opinion, require consistent application throughout the Mackenzie Valley". This Policy is issued under section 106 and, as such, the MVLWB will establish the procedures necessary to ensure that this Policy is appropriately implemented and periodically reviewed.

Under the authority outlined in section 2, the MVLWB may establish working groups from time to time to address specific policy, technical, or scientific matters related to effluent and water quality management and the water licensing process, including the development of guidelines.

Individual Land and Water Boards (MVLWB, GLWB, SLWB, and WLWB) are responsible for processing, administering, and monitoring water licences in accordance with this Policy.

10.0 Measuring Performance and Reviewing the Policy

Mechanisms will be required to monitor and measure performance and to evaluate the effectiveness in achieving the Policy objectives articulated above. In accordance with the principles of a management systems approach (i.e., plan-do-check-act), the MVLWB will develop a performance measurement framework that specifies reporting requirements against the Policy objectives including indicators, sources of information, and frequency of reporting. This Policy will be reviewed and amended as necessary within that framework. The framework will also describe how stakeholders will be involved in the Policy review process.

APPENDIX A: Guidelines/Strategies That Will Support Implementation of This Policy

SUBJECT AREA	GUIDANCE REQUIRED	AVAILABILITY OF NWT-SPECIFIC GUIDANCE
Cumulative effects	Cumulative effects assessment strategy.	INAC's Environmental Stewardship Framework (www.ainc-inac.gc.ca/ai/scr/nt/ntr/pubs/CEG-eng.asp)
	Cumulative impact monitoring tools.	Not yet available.
EQC setting	Municipal wastewater discharge.	<ul style="list-style-type: none"> Guidelines for the Discharge of Treated Municipal Wastewater in the Northwest Territories (1992), prepared by Indian and Northern Affairs Canada (INAC) for the NWT Water Board. Environment Canada is developing recommendations for municipal wastewater discharge limits in northern Canada (under the CCME Canada-wide Strategy for the Management of Municipal Wastewater Effluent). Also, see information on the Northern Research Working Group at http://www.mvlwb.ca/nrwg.
	Setting site-specific water quality standards.	Not yet available but under development by INAC.
	Collection of baseline information for water bodies.	Not yet available.
	Establishment and characterization of mixing zones.	Not yet available.
	General objectives for effluent discharges.	Not yet available.
	Guidance document from INAC on technologies for mining effluents in the NWT.	INAC, Water Resources Division. 2002: Applicable Technologies for the Management of Mining Effluents in the Northwest Territories. Prepared by Lakefield Research Limited in association with SENES Consultants Limited.

Management Plans	Closure and reclamation planning.	Mine Site Reclamation Guidelines (2007 INAC). Also, joint guidelines are being developed by INAC and the Land and Water Boards.
	Spill contingency planning.	Guidelines for Spill Contingency Planning (2007), INAC.
	Adaptive management planning.	<ul style="list-style-type: none"> • Guidance has been developed in draft by the WLWB and was under public review until December 6, 2010. • Some guidance provided in INAC's Guidelines for Designing and Implementing Aquatic Effects Monitoring Programs for Development Projects in the NWT (2009).
	Waste management planning.	Guidelines have been developed and will be presented for approval by the MVLWB in December 2010.
Monitoring	Aquatic effects monitoring programs.	Guidelines for Designing and Implementing Aquatic Effects Monitoring Programs for Development Projects in the Northwest Territories, (2009), INAC.
	Establishment of surveillance network programs for water licences.	Not yet available.
	Sampling and Testing Requirements (elements).	<ul style="list-style-type: none"> • For drinking water: Summary Table – NWT Drinking Water Sampling and Testing Requirements (http://www.hlthss.gov.nt.ca/pdf/brochures_and_fact_sheets/environmental_health/2007/english/nwt_drinking_water_sampling_and_testing_requirements.pdf).
Stakeholder engagement	Public Engagement and Consultation Policy.	Under development by the Boards.
	Public Engagement Guidelines.	Under development by the Boards.



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NWT Water Stewardship: **A Plan for Action**

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Introduction

Photo Credit: J.Skelton

This document, *NWT Water Stewardship: A Plan for Action* (Action Plan), describes action items that put into motion the vision of the *Northern Voices, Northern Waters: NWT Water Stewardship Strategy* (the Strategy) developed by water partners in the NWT. The Action Plan, which lays out a partnership approach to improve and enhance water stewardship at all levels, designates lead water partners and deliverable dates for each action item.

The Action Plan is a living document and is subject to ongoing reviews and audits to ensure its implementation continues to advance the intent of the Strategy. Annual status updates will be published to track and report on progress. Subsequent Action Plans will outline activities beyond 2015.

Background

In June 2008, the Government of the Northwest Territories (GNWT), represented by the Department of Environment and Natural Resources (ENR), and the Government of Canada, represented by Indian and Northern Affairs Canada (INAC), released the discussion paper *Towards an NWT Water Resources Management Strategy for the Northwest Territories*. Under the guidance of an Aboriginal Steering Committee, ENR and INAC sought feedback on the discussion paper from water partners throughout the NWT. Input was reviewed and workshops held, resulting in a draft Water Stewardship Strategy in November 2009. Further workshops and dialogue with NWT water partners, as well as national and international water policy innovators, led to the final *Northern Voices, Northern Waters: NWT Water Stewardship Strategy* (the Strategy) released by the Ministers of ENR and INAC in May 2010.

The Strategy states a vision for water stewardship in the NWT: “*The waters of the Northwest Territories will remain clean, abundant and productive for all time.*” This vision reflects and advances the deep fundamental relationship NWT residents have with water by stressing an ecosystem-based approach, which honours traditional northern values and beliefs in protecting a vital natural resource. It encourages all water partners to work together to share ideas and knowledge (traditional, local and western scientific) in order to make sound decisions that promote responsible water use in economic and community development.

New water stewardship partners are welcome and the strengthening of existing partnership is encouraged. Ongoing and clear communication must occur to ensure all water partners participate fully in their respective roles, responsibilities and accountabilities. Engaging Aboriginal governments, community governments and other organizations is critical for the successful implementation of the Strategy.



Photo Credit: PehdzehKiNdeh-KZeigler

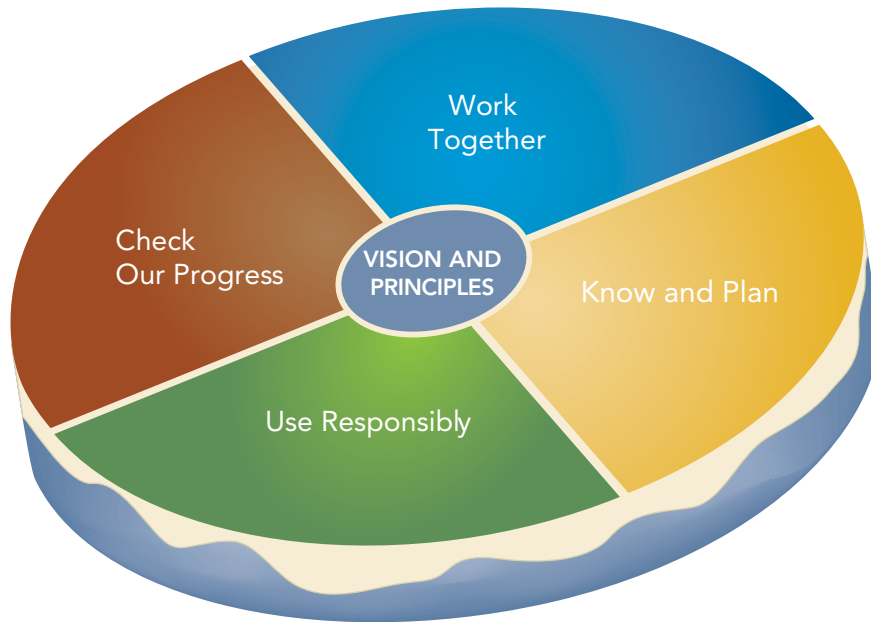
An aerial photograph of a wetland landscape. A winding waterway, possibly a river or stream, flows through a lush green forest. The water reflects the surrounding trees and sky. The forest is dense and appears to be a mix of deciduous and coniferous trees. The overall scene is serene and natural.

Action Plan Overview

Photo Credit: Donna Mulders

The Strategy describes broad actions necessary to achieve the vision, goals and objectives for water stewardship in the NWT. The Action Plan is based on these broad actions, further expanded from the “Keys to Success” in the Strategy. ENR, INAC and the water partners reviewed these broad actions in detail. In developing the Action Plan, the results of this review were considered by the Aboriginal Steering Committee (ASC), ENR and INAC working group.

Action items fall within the four components of water stewardship in the NWT: Work Together; Know and Plan; Use Responsibly; and Check Our Progress. Each component requires concentrated effort to ensure the actions taken are guided by the Strategy vision, goals and guiding principles. These four components are described below.



NWT Water Stewardship drum diagram illustrates the four components of water stewardship in the NWT.

Work Together

Actions ensure a cooperative environment to support water managers and water partners in sharing information, building capacity and working together.

Know and Plan

Actions ensure the implementation of multi-disciplinary aquatic monitoring and research programs. These programs consider traditional, local and western scientific knowledge and use of this information in the planning of water stewardship activities.

Use Responsibly

Actions ensure decision-makers have the tools necessary to make well-reasoned decisions. These tools should work well together and be easy to use in a consistent manner.

Check Our Progress

Actions ensure progress is made in achieving the vision. This includes measuring and reporting progress. Reporting results of, and responses to, audits and reviews must be transparent.

Keys to Success, Timeframes, Lead Agencies, Partners and Action Items

The Action Plan highlights actions for each component and its associated Keys to Success. Estimated deliverable dates, lead agency(s) and other partners are also included.

1. Work Together

Actions ensure all water partners have the information and resources needed to collaboratively achieve the vision and goals of the Strategy and to effectively integrate the vision and objectives of the Strategy with other resource planning and management processes in the NWT.

In order to Work Together, work falls into four main areas:

- Partnerships
- Information Management
- Communication and Engagement
- Transboundary Discussions, Agreements and Obligations

1.1

Work Together – Partnerships

Partnerships are essential for water stewardship in the NWT. No one agency is entirely responsible for water stewardship and no agency or individual is without responsibility for it. Partnerships can take many forms, including partnerships among decision-makers, funding partnerships, networking partnerships and data sharing partnerships.

Key to Success 1.1 A	Integrate the NWT Water Stewardship Strategy with current territorial watershed and natural resource planning and management frameworks, such as the Environmental Stewardship Framework and regional land use plans.	
	Lead Agency: ENR/INAC	Partners: All Water Partners
	Action Items	Deliverable Date
1	Water partners identify and share existing policies, strategies, frameworks, procedures, regional land use plans, interim measures agreements and other agreements that are related to the Strategy.	April 2012
2	Evaluate existing policies, strategies, frameworks, procedures, etc. for consistency with the Strategy and identify gaps or conflicts.	April 2013
3	Revise existing and future policies, strategies, frameworks, procedures, etc. to address gaps.	September 2013 and On-going
4	Engage public in the Strategy and Action Plan review processes.	On-going

Key to Success 1.1 B	Identify and facilitate the development of partnerships that support the NWT Water Stewardship Strategy, including establishing criteria for effective partnerships.	
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify current and possible key partners and their resource capacity for potential contributions.	September 2011 and On-going
2	Strengthen ongoing communication between all partners.	On-going
3	Publish routine updates of water partners and their activities.	April 2012
Key to Success 1.1 C	Establish an extended network of partners prepared to commit resources to research and monitoring priorities.	
	Lead Agency: ENR Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify an extended network of partners who could fund research and monitoring priorities and determine their resource capacity.	September 2011 and On-going
2	Document intent; share with partnership network.	April 2012
3	Strengthen communication between all partners.	On-going
4	Publish routine updates of partners and their activities.	September 2012 and Annually
Key to Success 1.1 D	Develop clear descriptions of the roles and responsibilities of the respective water partners.	
	Lead Agency: ENR Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify current roles and responsibilities of water partners.	September 2011
2	Create and routinely update responsibility matrix.	April 2012 and Annually
3	Communicate roles and responsibilities of water partners to all interested parties.	September 2012 and Annually

Key to Success 1.1 E	Develop collaborative processes among water partners to identify and resolve issues impeding coordinated watershed data collection, data sharing and management decisions.	
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify information management partners and/or programs.	April 2012
2	Define degree of involvement, commitment and capacity for each water partner.	April 2012
3	Undertake a gap analysis of issues impeding coordinated watershed data collection, sharing and management decisions undertaken by partners.	April 2013
4	Strengthen collaboration and coordination among water partners through routine communication.	On-going
5	Communicate results to all interested parties.	On-going
Key to Success 1.1 F	Routinely assess partners' capacity to partner actively in initiatives and address shortfalls.	
	Lead Agency: ENR/INAC Partners: Regulatory Boards, NWT Communities, Other Federal Departments, GNWT	
	Action Items	Deliverable Date
1	Identify challenges for water partner involvement in water stewardship activities.	April 2012 and Annually
2	Determine water partner capacity and shortfalls through routine dialogue and formal or informal reviews.	April 2012 and Annually
3	Develop and implement capacity building initiative through collaborative partnerships.	Annually
Key to Success 1.1 G	Develop community capacity to strengthen community involvement in water stewardship activities, including education, training, and research and monitoring programs.	
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Communities assess their desired roles and responsibilities in terms of water stewardship.	April 2012
2	Work with self-identified communities to undertake a needs assessment relative to their desired roles and responsibilities.	September 2012
3	Use workshops and other means to share experiences and adopt best practises through community engagement, involving youth and elders.	September 2012 and On-going
4	For each community, develop and strengthen relationships with water partners to enhance capacity.	April 2013 and On-going

Key to Success 1.1 H	Collaboratively develop and implement an approach that provides for effective use of traditional, local and western scientific knowledge in water stewardship decision-making processes.	
	Lead Agency: ENR/INAC Partners: Environment Canada, Fisheries and Oceans Canada, Regulatory Boards, Aboriginal Governments	
	Action Items	Deliverable Date
1	With collaborative input from traditional, local and western scientific knowledge holders, develop an effective approach to inform water stewardship decisions.	April 2012
2	Design effective tools to improve decision-making.	April 2013

1.2 Work Together – Information Management

Water stewardship activities, including decision-making at all levels, must be supported by accurate and current data. This can be achieved by enhancing gathering, storing, processing and delivering geographic information, or spatially referenced information and developing standard protocols for data collection, data sharing and data exchange. Traditional knowledge is an inherent part of the Strategy and Action Plan. Enhancing and developing traditional knowledge protocols ensures the collection and application of traditional knowledge is conducted in a respectful manner.

Key to Success 1.2 A	Undertake a review of existing geomatics capacity and capabilities in the NWT with respect to the collection and analysis of water related imagery, data and information and recommend means to improve these.	
	Lead Agency: ENR Partners: INAC, Regulatory Boards, Other Federal Departments, NWT Communities	
	Action Items	Deliverable Date
1	Undertake a review of existing water-related geomatics and/or remote sensing needs and potential uses in water stewardship action items.	April 2012
2	Share information about existing water-related geomatics and/or remote sensing uses to interested water partners.	April 2012
3	Identify opportunities to collaborate among water partners.	On-going
Key to Success 1.2 B	Assess the feasibility of filling identified gaps in water quality and quantity monitoring and research through use of alternative technological tools (e.g., remote sensing, aerial photography).	
	Lead Agency: ENR Partners: INAC, Regulatory Boards, Other Federal Departments, NWT Communities	
	Action Items	Deliverable Date
1	Identify alternative technological tools that could be used to fill identified gaps in water quality and quantity monitoring and research.	April 2012
2	Assess the potential to expand or improve current geomatics applications.	December 2012
3	Determine the feasibility of using any of these tools in the NWT within current monitoring and research programs.	April 2013

Key to Success 1.2 C	Improve data management for water-related monitoring programs, including Surveillance Network Programs.	
	Lead Agency: INAC/Regulatory Boards Partners: Environment Canada, Fisheries and Oceans Canada, GNWT, Industry	
	Action Items	Deliverable Date
1	Develop a formatting template for submitting monitoring and compliance data.	September 2012 and On-going
2	Identify and develop a data storage strategy.	December 2012
3	Standardize sampling protocols, including quality assurance and quality control.	April 2013
Key to Success 1.2 D	Share monitoring and research program findings with water partners and the public.	
	Lead Agency: INAC Partners: GNWT, Fisheries and Oceans Canada, Environment Canada, Industry, Regulatory Boards	
	Action Items	Deliverable Date
1	Identify existing monitoring and research reporting mechanisms of all water partners.	December 2011
2	Provide training on NWT Discovery Portal use and access protocols.	April 2012
3	Begin to share information on monitoring and research programs using the NWT Discovery Portal.	April 2013
4	Water partners populate the NWT Discovery Portal with all monitoring and research program findings.	April 2014 and On-going
Key to Success 1.2 E	Prepare and update, regularly, a comprehensive inventory of water use information, on a watershed basis.	
	Lead Agency: INAC/ENR Partners: All Water Partners	
	Action Items	Deliverable Date
1	Regularly update water use inventory and distribute to water partners.	April 2012 and Annually
2	Develop or adapt existing informatics infrastructure as necessary to systematically collect and store water use data by watershed.	April 2014 and Annually

Key to Success 1.2 F	Implement data collection, data sharing and data exchange protocols and tools to ensure effective and efficient data sharing among water partners.	
	Lead Agency: ENR/INAC Partners: Other Federal departments, GNWT, Regulatory Boards, NWT Communities	
	Action Items	Deliverable Date
1	Identify all NWT water-related data sets that water partners hold.	April 2012
2	Prioritize data sharing needs amongst water partners.	September 2012
3	Develop and implement data collection, sharing and exchange protocols for specific data sets.	April 2013 and On-going
4	Improve public access to data using the NWT Discovery Portal and other identified approaches.	On-going
Key to Success 1.2 G	Inventory all traditional knowledge protocols currently in place and developed by Aboriginal governments, communities and regions.	
	Lead Agency: Aboriginal Steering Committee Partners: Aboriginal Governments, NWT Communities, Regulatory Boards, INAC, ENR	
	Action Items	Deliverable Date
1	Collect all available traditional knowledge protocols and share among water partners.	September 2011
Key to Success 1.2 H	Develop and implement processes that promote use of traditional knowledge in ways that help ensure water stewardship activities that respect community values.	
	Lead Agency: Aboriginal Steering Committee Partners: Aboriginal Governments, NWT Communities, Regulatory Boards, INAC, ENR	
	Action Items	Deliverable Date
1	Support the implementation of traditional knowledge protocols.	On-going
2	Include traditional knowledge in partner planning activities and decision-making processes.	On-going
3	Engage with Aboriginal governments and communities to identify ways that traditional knowledge can be used in water stewardship activities.	April 2012

1.3

Work Together – Communication and Engagement

Good communication and engagement is necessary for building effective relationships among water stewardship partners and the public. Ongoing promotion is required to keep the public informed and aware of water stewardship activities.

Key to Success 1.3 A	Develop an approach to effectively maintain communications amongst water partners on the progress of implementing the NWT Water Stewardship Strategy.	
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify water partners and maintain membership in the partners' communication working group and Aboriginal Steering Committee.	On-going
2	Develop communications protocols for action item leads.	April 2012
3	Develop and implement a framework and mechanism for communicating with all water partners.	April 2012
4	Report on progress at regular intervals.	April 2012 and Bi-annually
Key to Success 1.3 B	Develop an approach to communicate effectively with interested organizations and the public on the progress of implementing the NWT Water Stewardship Strategy.	
	Lead Agency: INAC/ENR Partners: All Water Partners	
	Action Items	Deliverable Date
1	Coordinate and develop activities to celebrate Canada Water Week and World Water Day.	April 2011 and Annually
2	Identify existing communication tools and modify as needed.	September 2011 and On-going
3	Develop and maintain a dedicated NWT Water Stewardship web site.	September 2011 and On-going
4	Develop and implement public education and information plans, particularly targeting NWT youth.	April 2012
5	Support NWT water conferences to provide updates on water research and monitoring activities and Strategy implementation.	April 2012 and Annually
6	Publish regular reports on programs and implementation activities.	September 2012 and Annually

Key to Success 1.3 C	Develop a process for community organizations and individuals to collaborate routinely and effectively towards collectively achieving the vision of Water Stewardship in the NWT for the benefit of many.	
	Lead Agency: INAC/ENR Partners: Aboriginal Governments, Other Federal Departments	
	Action Items	Deliverable Date
1	Engage and collaborate with Aboriginal governments, NWT communities and the public on the implementation of the Strategy.	On-going
2	Broaden the scope of water partners and their contributions, through public participation.	On-going
3	Collect and evaluate feedback to improve water stewardship actions.	On-going
4	Increase or strengthen opportunities to work together for water partners.	On-going

1.4 Work Together – Transboundary Discussions, Agreements and Obligations

Successful transboundary discussions, agreements and obligations with upstream jurisdictions help ensure the waters of the NWT remain clean, abundant and productive for all time. Mackenzie River Basin jurisdictions agreed to a transboundary negotiations schedule that starts with the Slave River. Aboriginal governments will be involved in transboundary negotiations.

Key to Success 1.4 A	Negotiate transboundary water agreements with Alberta and other upstream Mackenzie River Basin jurisdictions.	
	Lead Agency: GNWT/INAC Partners: Aboriginal Governments, Other Federal Departments	
	Action Items	Deliverable Date
1	Identify commitments and obligations in legislation and agreements related to transboundary waters.	September 2011 and On-going
2	Collect and share all Slave River background information in support of negotiations and develop a common report for all relevant jurisdictions.	September 2011 and On-going
3	Determine transboundary negotiating team and support processes, including Aboriginal participation and engagement.	September 2011
4	Develop NWT interests, mandates and options to inform transboundary negotiations in partnership with Aboriginal governments.	December 2011
5	Sign transboundary agreement with Alberta for the Slave River.	December 2012
6	Advance bilateral agreements with all Mackenzie River Basin jurisdictions for the Hay, Liard and Peel River transboundary waters (repeat steps 1-5).	December 2011 and On-going
7	Provide updates on transboundary discussions, negotiations, obligations and implementations of agreements to NWT partners and the public.	On-going

2. Know and Plan

Actions support the development and implementation of collaborative research and monitoring programs. The incorporation of traditional, local and western scientific knowledge in these programs improves the collective understanding of health and diversity in the NWT.

In order to Know and Plan, work falls under two main areas:

- Aquatic Ecosystems, Water Quality and Quantity
- Community-based Monitoring

2.1

Know and Plan – Aquatic Ecosystems, Water Quality and Quantity

Considerable research and monitoring efforts is needed to more fully understand aquatic ecosystems, water quality and quantity in the NWT. Knowledge gaps must be identified to set priorities for filling those gaps. Development of consistent research and monitoring protocols and water valuation/ecosystems services methodologies can assist in monitoring and mitigating impacts and cumulative effects on NWT waters.

Key to Success 2.1 A	Undertake a review of existing aquatic monitoring programs, practices and research activities in the NWT, and identify and prioritize gaps.	
	Lead Agency: ENR/INAC Partners: Fisheries and Oceans Canada, Environment Canada, Regulatory Boards, Industry, Academia	
	Action Items	Deliverable Date
1	Identify existing monitoring programs and research activities in the NWT and compile into a report.	December 2011 and On-going
2	Review current monitoring and research activities for adequacy and identify and compile information gaps.	September 2012 and On-going
3	Collaborate with water partners to prioritize gaps with regard to goals of the Strategy.	April 2013 and On-going
4	Develop or improve existing monitoring and research activities to address identified gaps.	December 2014
Key to Success 2.1 B	Review factors that could impact aquatic ecosystem health to determine the priority in program delivery.	
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify factors that could impact aquatic ecosystem health in the NWT.	April 2012
2	Conduct risk assessment in relation to these factors and identify priorities.	April 2013
3	Assess adequacy of current monitoring and research to address potential impact of above factors.	September 2013
4	Expand and adjust program delivery according to identified priorities.	April 2014 and On-going

Key to Success 2.1 C	Determine consistent approaches to undertake research and monitoring to increase our understanding of the aquatic ecosystem, including transboundary watersheds.	
	Lead Agency: ENR/INAC Partners: Regulatory Boards, Aboriginal Governments, Other Federal Departments, Academia	
	Action Items	Deliverable Date
1	Review existing research and monitoring protocols and assess the need for adaptation.	April 2012
2	Ensure existing NWT research and monitoring protocols, including traditional knowledge protocols, are available and used by all water partners.	April 2013
3	Develop new or adapt existing research and monitoring protocols.	April 2014 and On-going
Key to Success 2.1 D	Develop and implement collaborative ecosystem-based research and monitoring programs.	
	Lead Agency: ENR/INAC Partners: Regulatory Boards, Fisheries and Oceans Canada, Environment Canada, Academia, Aboriginal Governments	
	Action Items	Deliverable Date
1	Develop potential collaborative ecosystem-based research and monitoring programs, ensuring that traditional knowledge can be fully considered.	April 2012 and Annually
2	Develop collaborative partnerships that can enhance ecosystem-based water stewardship in the NWT.	April 2012 and Annually
3	Select potential aquatic ecosystem health indicators, following the completion of a discussion paper and a community and experts workshop.	September 2012
4	Assess and set aquatic ecosystem health indicators and thresholds.	April 2015
5	Identify the sensitivity of northern aquatic species to toxins produced by industrial activities.	April 2015
Key to Success 2.1 E	Working with knowledgeable partners, assess current strategies and develop a NWT relevant approach in valuation of water and ecosystem services.	
	Lead Agency: ENR Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify partners with expertise in determining water and ecosystem service valuation approaches.	September 2013
2	Involve Aboriginal governments and other NWT water partners in developing water and ecosystem service valuation approaches.	April 2014
3	Develop an NWT tailored approach to water and ecosystem service valuation.	April 2015

Key to Success 2.1 F	Review existing water quality and quantity monitoring information (surface and groundwater), and identify capacity requirements to fill the gaps.	
	Lead Agency: INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Complete literature review of existing monitoring programs and activities.	December 2011
2	Complete gap analysis of water quality and quantity monitoring capacity.	September 2012
3	Prioritize water quality and quantity monitoring capacity needs.	April 2013
Key to Success 2.1 G	Develop and implement collaborative research and monitoring programs for water quality and quantity that integrate with existing programs.	
	Lead Agency: ENR/INAC/Regulatory Boards Partners: All Water Partners	
	Action Items	Deliverable Date
1	Identify potential collaborative water quality and quantity research and monitoring programs.	April 2012 and On-going
2	Develop collaborative partnerships that can enhance water quality and quantity research and monitoring programs.	April 2013 and On-going
3	Identify and implement methods to improve analytical capabilities for Surveillance Network Programs and other water-related monitoring programs.	April 2015
Key to Success 2.1 H	Enhance, where needed, the existing water quality and quantity monitoring network (surface and groundwater) in the NWT.	
	Lead Agency: INAC Partners: Environment Canada, Aboriginal Governments, ENR	
	Action Items	Deliverable Date
1	Complete detailed needs analysis regarding site locations.	September 2012
2	Establish monitoring agreements with interested agencies.	April 2013 and On-going
3	Implement monitoring agreements.	September 2013 and On-going
4	Evaluate effectiveness of the monitoring network.	December 2014 and On-going

Key to Success 2.1 I	Develop and implement collaborative research and monitoring programs for environmental stressors that can contribute to cumulative effects on NWT watersheds.	
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Assess existing programs that identify factors contributing to cumulative effects.	September 2012
2	Assess adequacy of existing monitoring and research programs, including protocols, reporting and data management.	April 2013
3	Implement cumulative effects research and monitoring programs.	September 2013 and On-going
4	Evaluate effectiveness of the research and monitoring programs.	April 2014 and On-going

2.2

Know and Plan – Community-based Monitoring

Community-based monitoring fosters a wide range of innovations, including increased awareness of water stewardship issues, improved traditional knowledge collection and application as well as increased, direct community involvement in research and monitoring program design. Opportunities for community-based research and monitoring programs are being explored and pilot projects funded, including transboundary watersheds.

Key to Success 2.2 A	Explore, develop and implement opportunities for community-based research and monitoring programs.	
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Determine which communities wish to participate in community-based monitoring.	September 2012
2	Support the communities in identifying priorities that will inform program design, including workshops.	April 2012
3	Identify and solicit funding resources for capacity building and program implementation.	September 2012
4	Implement community-based research and monitoring programs.	April 2013 and On-going

Key to Success 2.2 B	Work with partners on community source water protection.	
	Lead Agency: ENR	Partners: NWT Communities, Aboriginal Governments, INAC
	Action Items	Deliverable Date
1	Assess options and develop a model for source water protection planning in consideration of the needs of all NWT communities.	September 2011
2	Undertake community engagement to support source water protection planning.	September 2012
3	Update community watershed maps and distribute to all communities.	September 2012
4	Identify and develop capacity and support the implementation.	September 2013

3. Use Responsibly

Actions support sound water stewardship through the development and implementation of programs, practices and guidance for environmental assessment, regulatory and enforcement processes.

In order to Use Responsibility, work falls into three main areas

- Policy, Procedures and Protocols
- Evaluate and Amend Existing Legislation
- Compliance

3.1 Use Responsibly – Policy, Procedures and Protocols

An overarching protocol for developing, reviewing and implementing specific water-related policies, procedures and guidelines is needed to ensure consistency and strengthen water stewardship in the NWT.

Key to Success 3.1 A	Develop or update policy, procedures and protocols in a consistent, transparent manner that enhances NWT water stewardship actions and decisions.	
	Lead Agency: ENR/INAC Partners: Other Federal Governments, Aboriginal Governments, Regulatory Boards	
	Action Items	Deliverable Date
1	Research approaches to address water stewardship issues, seeking expertise from others nationally and globally.	April 2012
2	Modify or develop policy, procedures and protocols to ensure transparent decisions are made.	September 2013 and On-going
3	Implement policy, procedures and protocols.	On-going
4	Evaluate policy, procedures and protocols.	On-going
Key to Success 3.1 B	Develop an overarching protocol for developing, reviewing and implementing water-related regulatory procedures and guidelines.	
	Lead Agency: Regulatory Boards Partners: Aboriginal Governments, Other Federal Departments, GNWT, Industry, Academia	
	Action Items	Deliverable Date
1	Regularly update NWT mine site reclamation guidelines collaboratively with water partners.	September 2011 and On-going
2	Determine effective procedures for ensuring that the Water and Effluent Quality Management Policy and supporting guidelines are implemented as intended.	April 2012
3	Develop guidelines (e.g., for setting Effluent Quality Criteria) to support the Policy.	April 2013
4	Regularly update Aquatic Effects Monitoring Program guidelines collaboratively with water partners.	April 2013 and On-going

Key to Success 3.1 C			Implement the Canada-wide Strategy (CWS) for Municipal Waste Water Effluent (MWWE) in the NWT.
	Lead Agency: GNWT/Environment Canada/INAC Partners: Regulatory Boards, NWT Communities		
	Action Items		Deliverable Date
1	Develop and implement a work plan for the CWS for MWWE, guided by outcomes and advice of the CWS for MWWE Northern Working Group.		April 2012
2	Analyze existing information and address gaps through more research and monitoring for implementing the CWS for MWWE.		April 2013
3	Engage communities, governments, agencies and others to develop a viable approach to implementing the CWS for MWWE, increasing the awareness of MWWE management in the NWT.		September 2013
4	Develop appropriate northern performance standards and effluent discharge objectives based on risk to human health and the environment.		April 2014
5	Work with relevant agencies to apply standards and guidelines, including progress reports.		On-going

3.2

Use Responsibly – Evaluate and Amend Existing Legislation

Routinely evaluate current legislation and amend as required to ensure NWT water stewardship is improved.
Review existing legislation to identify gaps and ensure outcomes are consistent with water stewardship goals.

Key to Success 3.2 A			Evaluate and amend legislation in a consistent, transparent manner that enhances NWT water stewardship actions and decisions.
	Lead Agency: INAC Partners: Other Federal Departments, Aboriginal Governments, Regulatory Boards, ENR		
	Action Items		Deliverable Date
1	Evaluate federal legislation which can affect water stewardship actions and decisions.		April 2012 and On-going
2	Amend legislation, as necessary, to enhance water stewardship actions and decisions.		On-going
3	Continue to identify and engage in opportunities intended to improve NWT water stewardship.		On-going

3.3

Use Responsibly – Compliance

Work with NWT communities to build capacity to ensure a community water license is in place and that communities comply with the terms and conditions of their water license.

Key to Success 3.3 A	Engage communities and other water partners to identify issues relating to water license applications and compliance and work to address these issues.	
	Lead Agency: GNWT /INAC Partners: NWT Communities, Regulatory Boards	
	Action Items	Deliverable Date
1	Communicate benefits for communities to comply with municipal water licences.	December 2011 and On-going
2	Identify needs, such as training and support, that would enable communities to apply for, or comply with, their community water licence.	December 2012
3	Based on type(s) of facilities, develop and implement a community plan to address the issues identified by the community.	April 2013 and On-going
4	Develop communications or other promotional materials to increase industry recognition of community limitations and industry responsibilities on matters that could impact community facilities; for example, use of municipal infrastructure for waste disposal.	December 2012 and On-going

4. Check Our Progress

Check Our Progress is an active feed-back loop to ensure that water stewardship initiatives undertaken are working and that there is progress towards the vision of the Strategy. The evaluation criteria for Check Our Progress must be objective, accountable and directly linked to desired outcomes.

In order to Check Our Progress, work falls into two main areas:

- Routine Checks
- Formal Audits

4.1 Check Our Progress – Routine Checks

Develop and implement regular reviews of the NWT Water Stewardship Strategy and the Action Plan to ensure progress is being made and to adjust actions as necessary.

Key to Success 4.1 A		
Assess implementation progress of the Action Plan through annual reviews amongst partners.		
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Form a progress assessment team.	September 2011
2	Develop an evaluation framework to measure progress and program success.	April 2012
3	Report progress based on identified success measurement criteria.	September 2012 and Annually

4.2 Check Our Progress – Formal Audits

Undertake formal audits to determine progress, identify emerging challenges and actions required to deal with new challenges.

Key to Success 4.2 A		
Conduct a comprehensive audit of the NWT Water Stewardship Strategy every five years.		
	Lead Agency: ENR/INAC Partners: All Water Partners	
	Action Items	Deliverable Date
1	Form an audit team.	April 2012
2	Develop formal audit process and criteria.	April 2013
3	Develop an independent audit structure.	April 2014
4	Complete the audit with support from the audit team and water partners.	April 2015
5	Publish audit results and distribute findings.	September 2015 and every 5 years
6	Use audit results to develop subsequent water Action Plans.	Beyond 2015

More information on the NWT Water Stewardship Strategy and Action Plan
can be found at www.enr.gov.nt.ca



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