EFFECT OF PULLANTS ON THE PHYSIOLOGY OF FISH IN THE OLIFANTS RIVER (EASTERN TRANSVAAL)

by

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Report to the Water Research Commission on the project entitled

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Project Leader : Professor J.H.J. van Vuuren
Professor H.H. du Preez

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EXECUTIVE SUMMARY

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Project K5/350

by

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3. Executive Summary

3.1 Background and motivation

Information on the potential pollution of the Olifants River and the effect thereof on water quality and river biota is limited. Little is known about the pollution by mining, industrial and agricultural activities along the river before it reaches the Kruger National Park. Pollution of the Olifants River probably occurred gradually over a number of years. It is therefore not possible to immediately determine the extent or impact of pollution on aquatic vertebrates.

The destructive effect of man's activities on the aquatic environment creates conditions of chronic stress in the form of sublethal pollution. The increase in susceptibility to diseases and a decrease in reproductive success of natural fish populations are probably caused by the increase of pollutants in the aquatic environment (Pickering, 1989). Fish are in direct contact with the external environment and any change in it will affect the physiological processes and thus the survival of the organism (Blaxhall, 1972).

Monitoring of the bioaccumulation of metals in a river system is essential because it serves the following purposes: (i) Gauging the extent of accumulation both temporal and spacial; (ii) Assessing organism health and (iii) human health. Spacial monitoring of bioaccumulation may produce data that would identify potential unknown areas with high concentrations while at known discharges it will provide some information regarding the trend of bioaccumulation, which will in turn be used to identify stability, improvement or deterioration. The accumulation of pollutants in aquatic biota can result in effects on survival and reproduction (EPA, 1991; Timmermans, 1993). For instance, in invertebrates it can affect life cycles or predator-prey interactions. The accumulated levels in aquatic organisms may also pose a risk to animals such as birds and mammals that feed on them, resulting in a decline in population numbers (Lloyd, 1992).

The monitoring of concentration levels in fish or other organisms which are used as food is essential because it aids in the protection against the consumption of contaminated food. Furthermore, the detected levels can be judged against standards set for food in general (Mance, 1987).

It is very important for aquatic toxicology biologists to determine the effect of different pollutants on aquatic ecosystems, even before these substances/chemicals are released into the environment. Until recently, most of the toxicological studies were conducted as acute exposure experiments where death of the test organism was taken as the only measurable variable (Larsson *et al.*, 1985). It is clear, however, that dysfunction of physiological systems occurs long before death. It is important in the conservation of river biota in an area such as the Kruger National Park (KNP) to determine the effect of pollution on the survival of aquatic organisms long before effects are noted. This will ensure that the necessary precautions are in place before aquatic organisms are negatively affected by effluents containing sublethal levels of pollutants.

Aquatic toxicology should therefore be aimed at the identification of nonvisible subtle pollutant-induced changes in the organism. Side-effects of pollution on the different biological levels, which include reactions on the subcellular level of an individual to changes which are reflected by the entire population, should be studied.

The sublethal effects of pollutants are normally of a biochemical nature since the majority of the pollutants react on the basic level of cellular organisation. Pollutants react with enzymes and metabolites of enzymatic reactions or interact

with membrane systems of other cellular components. Such primary interactions between pollutants and cellular components lead to functional and structural changes at a higher level of cellular and functional organisation, eg. osmoregulation, hormonal regulation, nervous and muscle function, immune reactions, respiration and circulation (Waldichuk, 1974; Larsson *et al.*, 1985). The determination of values for these different physiological variables employed to assess the effect of pollutants on the "health" of freshwater fish can be used in the same manner as established chemical indicators of human health.

In view of the abovementioned it is therefore of utmost importance to determine the effect of pollutants on the physiology of fish as an aquatic vertebrate, before the full impact of pollution on a specific water body can be determined. Determining critical levels of pollutants in the aquatic environment and establish guidelines according to those concentrations determined, will enhance measures to conserve river biota. The biota of the Olifants River will benefit to a large extent from the establishment of critical levels of pollution. Information of this nature can be utilised in the management of the KNP-Rivers since precautionary measures can be taken to conserve vulnerable aquatic organisms.

3.2 Objectives

Water and sediment quality is important in pollution assessment and samples were taken for analysis at selected sites along the Olifants River in the KNP. The same sampling was done at two sites outside the KNP in the Olifants and Selati Rivers before their confluence. This was done to determine the water quality entering the KNP, which pollutants (specifically metals) are present in the water, and whether dilution of pollutants was evident downstream.

At the same sites tissue and organ samples from different fish species were taken to determine the bioaccumulation levels of selected metals (Zn, Cu, Fe, Cr, Ni, Mu, Pb & St) in fish. The values obtained were compared with values from similar investigations. These surveys were conducted over three years to determine whether fluctuations in water metal levels were reflected in the bioaccumulation of these metals. Tissue samples were taken seasonally since drought and flood conditions could have had an effect on metal concentrations in the water. Fish were sampled at four sites in the study area, the Olifants River inside the KNP and one in the Selati River (Fig. 3.1). Representatives of selected genera according to habitat preference were used as sample organisms. Species of the family Cyprinidae are important for this kind of study since these species showed a clear sensitivity to pollutants during mortalities in the Eastern Transvaal rivers.

Experimental exposure of fish to pollutants under controlled environmental conditions was done after the identification of those metals which may pose a threat to the survival of fish in the river system. Furthermore, metals were selected according to concentration and toxicity eg. highly toxic, toxic and less toxic (Hellawel, 1986). A flow-through system was constructed for the exposure experiments through which the effect of the pollutant on fish physiology under controlled conditions was determined. Selected physiological variables were employed to measure these effects. Critical levels of pollutants after acute exposure as illustrated by the LC50 determination (8.2.1) as well as sublethal effects were established. If these values are exceeded, the survival of the fish population will be severely affected. *Oreochromis mossambicus* and *Clarias gariepinus* were used in the exposure experiments. Enzymatic changes and the effect thereof on the metabolism were also determined through the evaluation of the reaction of specific chemical variables to specific metals. Values determined for these chemical variables were used to determine the effects on the cellular and biochemical aspects of haematology, osmotic and ionic regulation, and metabolism.

3.3 Results and Conclusion

The Phalaborwa area has many point sources of pollution from both sewage treatment works as well as from mining, industrial and agriculture effluents. Sewage treatment plants are point sources of nutrients, such as nitrates and phosphates, which can lead to eutrophication problems. The mines and industries in the area are point sources of pollution containing constituents, such as calcium, fluorides, magnesium, phosphates, potassium, sodium, sulphates and metals (Theron, *et al.*, 1992). The extensive copper-mining activities in this region give rise to the concern that copper and other metals such as Fe, Zn, Mn, Cr, Ni and Pb, may increase to levels in the water which might have an affect on the physiology of fish.

3.3.1 Water and sediment

The water quality for the Selati River at locality 7 was found to be stressful to the aquatic life due to chemical constituents that exceeded the recommended guideline limits. Variables of special concern were: sodium, fluoride, chloride, sulphate, potassium, the total dissolved salts and the metal concentrations (except strontium). Effluents of the phosphorus extraction mining company and copper extraction mining company in the Phalaborwa area, as well as upstream inflow into the Selati River contributed to the high TDS concentrations in this river. The anionic component mainly responsible for the high TDS concentrations was sulphate. Furthermore, the Selati River had a negative influence on the water quality of the Olifants River after the confluence of the two rivers. This was clearly illustrated by the concentrations of some chemical constituents detected in the water at Mamba Weir. The negative influence of the Selati River was more pronounced during low flow periods (e.g. droughts or winter months) when limited water releases from the Phalaborwa Barrage reduced the dilution effect of the water on chemical constituent levels. However, most of the chemical constituent concentrations (not the metal concentrations) did decrease from the western side of the KNP to the eastern side, due to the dilution of the water through the tributaries of the Olifants River. At locality 3 (near Balule) some chemical constituents increased again in concentration, especially from April 1990 to February 1991 (8.1.1). The frequent occurrence of reed beds in that part of the river was the possible explanation for this. Most of the time, the water quality of the Olifants River in the KNP complied with the recommended guideline limits, except for the metal concentrations at most localities. However, the high metal concentrations in the water did not necessarily indicate conditions toxic to aquatic life. The water of the Olifants River is, amongst other features, hard (as $CaCO_3$), and this decreases the bioavailability of the metals to aquatic life and therefore decreasing the toxicity of the metals. Higher metal concentrations were detected in the sediment than in the water, due to the adsorption of metals on sediment particles. This indicated the chronic nature of metal pollution in the area. A large variation was detected in the metal concentrations of the water and sediment, making it difficult to establish the order of metal occurrence in the study area (Fig. 3.1). According to the sediment metal concentrations (which fluctuated less than the water metal concentrations), the general order from April 1990 to February 1991 for localities 1 to 6 was: Fe > Mn > Cr > Ni > Zn > Sr > Pb > Cu. For locality 7 in the Selati River it was Fe > Mn > Sr > Cu > Cr > Ni > Zn > Pb. The sediment at Pionier dam (control in KNP) had an occurrence pattern of metals similar to that of localities 1 to 6, except that more zinc than chromium was detected in the sediment. In the Selati River (at locality 7) much higher copper and strontium concentrations were detected in the

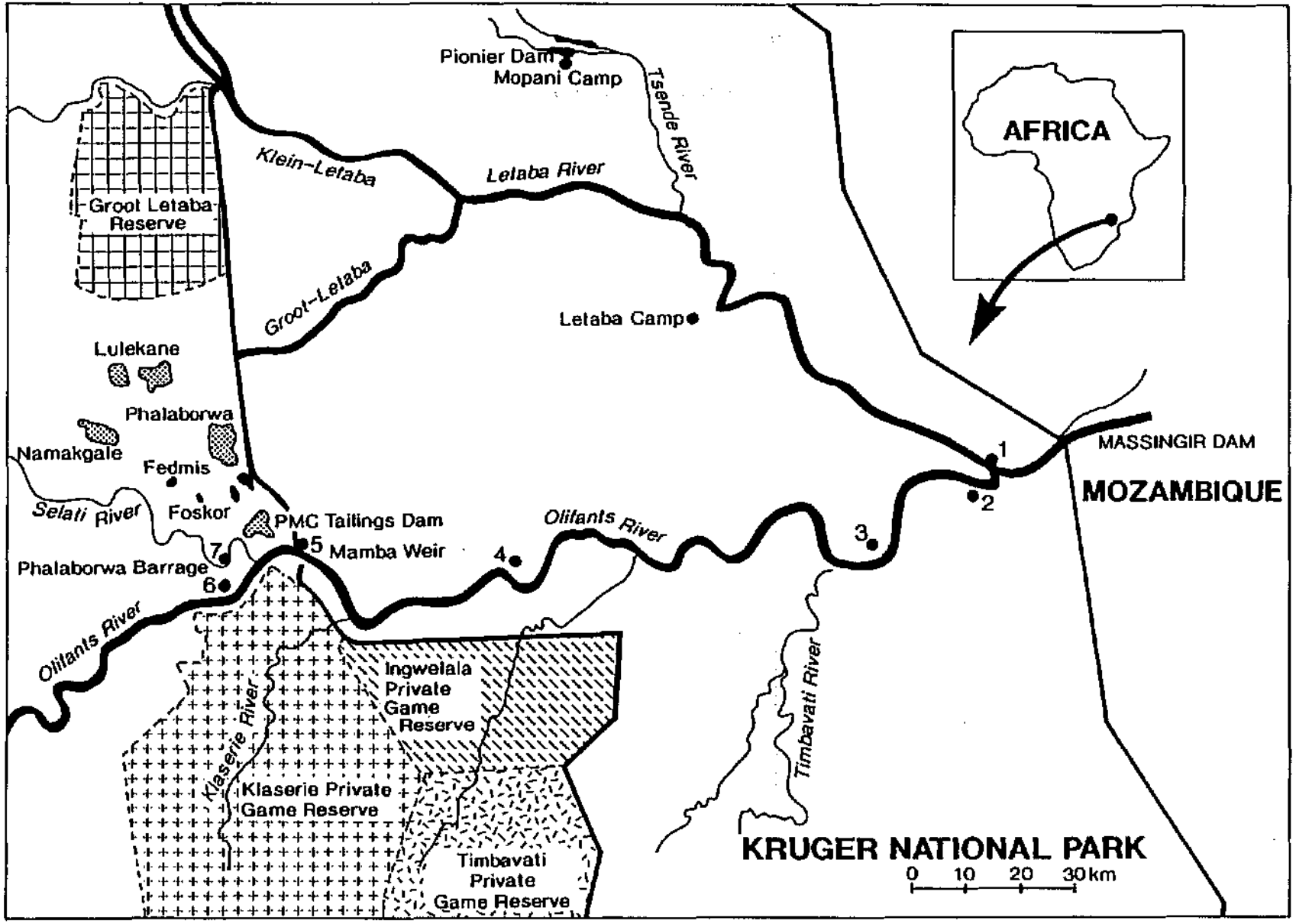

Figure 3.1

The study area in the Lower Olifants River Catchment, indicating the sampling localities 1 to 7

sediment than in the Olifants River (at localities 1 to 6). This indicated that these two metals originated from a local source which was not connected to the KNP. The Water 2 water quality index which was developed with water quality data obtained from this project is a useful tool in assessing the water quality of the Olifants River (8.1.2).

3.3.2 Bioaccumulation of metals

The accumulated metals (Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn) in the organs and tissues of *Barbus marequensis* gave a good indication of the metal levels to which the fish were exposed, especially when compared with the metal concentrations of a fish species from a polluted system (Germiston lake). *Barbus marequensis* seemed to have been chronically exposed to zinc, copper, lead and nickel, probably at sub-lethal levels. In addition, the fish at locality 7 seemed to have been chronically exposed to iron, chromium and manganese, also probably sub-lethally.

Metals were usually taken up *via* the gut and/or *via* the gills. The high metal concentrations in the gut contents of *B. marequensis* were not only due to the food ingested by the fish, but also to the metal-rich sediment associated with the food (*B. marequensis* is a benthic feeder). In the summer of 1990/91 the heavy rainfall increased the solubility of the metals and therefore metals could be taken up *via* the gills, and maybe even the skin more easily, leading to a higher accumulation of metals in the fish. The various metals were distributed differently in the organs and tissues of *B. marequensis*, and *Clarias gariepinus*, indicating that it is not necessarily the same organs that should be sampled for the analysis of different metals. It is therefore possible that, in using the wrong organs, an incorrect conclusion can be drawn in the assessment of the extent of metal pollution in an area. The suggested organs and tissues that should be sampled for the analysis of Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn in fish, as well as the organs and tissues of *B. marequensis* that accumulated the highest concentrations of these metals, are indicated in Table 3.1. Muscle tissue should always be sampled to test its fitness for human consumption. Apart from this, the gills, gut, liver and bony tissues seem to be good representative organs and tissues in general metal pollution surveys. If, however, surveys are being done on specific metals, organs and tissues as illustrated in Table 3.1, should be sampled. Seasonal differences in the bioaccumulation of the metals in the organs and tissues of fish did occur. Zinc is known to be essential for gonad development, especially for females, and therefore displayed a seasonal trend. The role of the other metals in gonad development (if any) is, however, not certain and cannot be related to this process as yet. Moreover, seasonal differences were related to the available metal concentrations that were taken up during a season.

3.3.3 Acute toxicity

The determined 96-hour LC50 values of copper for juvenile *O. mossambicus* were 2,61 mg/l and 2.78 mg/l, and the incipient LC50 values were 2.95 mg/l and 3.32 mg/l for copper at 29 ± 1°C, respectively. Results also indicated that seasonal variations in water temperature can alter the lethality of copper to fish. *O. mossambicus* was more susceptible to copper at the higher temperature. This may be because the fish had a relatively higher metabolic rate, at 29 ± 1°C, thus more water and copper passed through the gills. The higher water turnover in the gills could thus be an argument for a greater sensitivity to copper at the higher temperature, because more water containing copper was transported to the blood.

The 96-hour LC50 value of copper for adult *C. gariepinus* was 1,38 ± 0,08 mg/l at 21 ± 1 °C 1,29 ± 0,25 mg/l at 28 ± 1° C. For juvenile *C. gariepinus* the LC50 value of copper was 1,20 ± 0,04 mg/l at 21 ± 1° C and 1,30 ± 0,9 mg/l at 28 ± 1° C.

Results indicated that water temperature may alter the lethality of copper to *C. gariepinus*. Copper toxicity therefore appears to be enhanced by temperature as stressor.

The difference in LC50 values due to changes in seasonal water temperature may occur due to a transient change in the metabolic rate of the fish.

The 96-hour LC50 value of manganese for juvenile *O. mossambicus* was determined to be 1.723 g/l Mn, while the incipient LC50 value was 1.46 g/l Mn. These concentrations are much higher than the manganese concentrations occurring in the environment, which rarely exceeds one mg/l. Effluents of mines can, however, contain manganese concentrations that will have sublethal effects on fish. The highest manganese concentration detected in the water of the study area was 16.5 mg/l. Attention should therefore be given to the performance of chronic manganese toxicity tests in the future, in order to verify the existing water quality guideline of one mg/l Mn as a maximum concentration for the protection of aquatic life.

Despite increased sophistication in toxicity testing, increased awareness of the variety of processes operating in a natural system and the integration of information from a system of tests in various hazard evaluation protocols, the values obtained for a specific system and pollutant can always be refined. In the Olifants River, copper is one of several metals which pose a threat to the conservation status of the river. The LC50 values found during this study, can be used as an indication of the levels at which copper becomes lethal for this specific species. In a larger management-control program like the Kruger National Park, these results must be seen merely as the limits within which the concentration of copper can be regarded as lethal for *C. gariepinus* in the Olifants River. Future research should focus on the further development and validation of acceptable prediction strategies such as Water 2. In general, toxicants occur in mixtures in natural waters, and therefore the interaction of toxicity is an important factor which must be taken into account when assessing the hazard of an environmental pollutant to aquatic life. Additional studies on the effects of toxicants, singly and in mixtures, on biochemical and physiological processes, are needed to gain more knowledge of their interactions and toxic effects.

3.3.4 Haematology

After the exposure to sublethal concentrations of copper, several effects have been reported in fish. These include decreased survival, growth and reproduction, tissue damage in the gills as well as a decreased activity of gill NA^+-K^+-ATPase. Fish also accumulate copper in certain organs and tissues, particularly the gills and liver. Copper also affects the whole body, energy metabolism, swimming performance, various blood variables and, by binding to proteins, has a detrimental effect on enzymes in various metabolic pathways. Secondary stress responses have also been reported. This happens when fish are affected by various stressors and respond by a series of biochemical and physiological stress reactions.

Exposure of *C. gariepinus* to sublethal copper concentrations, results in certain physiological changes in the blood chemistry and metabolism.

Morphological changes in the gills of *C. gariepinus*, although not lethal, had a significant effect on the respiratory and osmoregulatory function of the gills. These morphological changes can be regarded as primary induced changes, which will inevitably lead to further secondary and physiological changes or responses affecting various organ systems. These physiological changes can either be seen as an initial response to the toxicant or as an adaptation reaction to retain a normal condition. Either of these responses will affect the performance of the organism in totality, however, leading to a decreased ability to survive.

The exposure of *O. mossambicus* to sublethal copper concentrations over short-term (96 hours) and long-term (4 weeks) periods, at 29± 1° C (summer) and 19 ± 1° C (winter), in experimental flow-through systems provides information on the tolerance of this species to sublethal copper concentrations. By determining the physiological and pathological changes in the blood of these fish, the risk and harmfulness of copper pollution were established. Apart from the sublethal exposures, acute toxicity tests were also conducted in flow-through systems in order to determine the 96-hour LC50 and incipient LC50 values of copper for juvenile *O. mossambicus*, at both temperatures.

During the exposure to sublethal copper concentrations as well as during the acute toxicity tests, at both temperatures, visible sublethal effects were observed, such as darkening of skin pigmentation, erratic swimming, increased coughing action, operculum movement and mucus secretion. Haemorrhaging was evident at the pectoral fins, operculum and the nose of exposed fish. These changes could be ascribed to the effect that copper had on the physiology of the fish. The exposed individuals also showed obvious signs of stress. These stress conditions resulted in an initial increase in the secretion of hormones derived from the adenohypophysis-internal axis. An increase in these so-called stress hormones resulted in changes in the values or blood coagulation, general haematology, osmoregulation and differential white blood cell counts.

There were delays in the blood coagulation times, as well as decreases in the shear modulus (elasticity) of the clots formed, after the exposure of *O. mossambicus* to copper at both temperatures. Copper was found to induce haemophilia at 29 ± 1° C and 19 ± 1° C, whilst at the latter it also induced thrombocytopenia. Haemophilia as well as thrombocytopenia results in haemorrhage in the body tissue. The haemorrhage occurred as a result of a disturbance in the cascade of reactions that leads to the conversion of fibrinogen to fibrin necessary for blood coagulation. This could be ascribed to the impaired production of thrombocytes. Although the fibrinogen concentrations were not determined in the present study, the decreases in the elasticity of the clots formed was an indication of a lower fibrinogen level. To determine which coagulation factor(s) is/are influenced by the presence of copper in water, and will thus lead to a defective coagulation process, it would be necessary to test every coagulation factor separately. Since the liver was concerned with the synthesis of some of the coagulation factors, it was presumed that there was damage to the liver. The damage that metals cause to the liver was confirmed by Gey van Pittius (1991).

An increase in the number of leucocytes (leucocytosis) is a normal reaction of the fish body to attacks by foreign substances, such as metals, which can alter the normal physiological function in the fish. Significant increases in the number of lymphocytes (lymphocytosis) and eosinophils (eosinophilia) combined with significant decreases in monocytes (monocytopenia) and neutrophils (neutropenia), are indicative of changes (infections) that set in

after short-term (96 hours) exposures to metals. This finding was confirmed by the number of leucocytes of *O. mossambicus* that increased at both temperatures to protect the body against infections that may have been caused by copper.

Exposure of *O. mossambicus* to sublethal copper concentrations fives rise to certain physiological changes in the general haematology. Morphological changes in the gills (Wepener, 1990; Van der Merwe, 1992), as reflected by the decreases in the plasma sodium, potassium, calcium and chloride concentrations, although not lethal, had a significant effect on the respiration and osmoregulatory function of the gills. These morphological changes can be regarded as primary changes, which will inevitably lead to secondary, physiological changes as well as responses that could affect various organ systems. Changes can be seen as an initial response to the toxicant (copper) or as an adaptation reaction to retain a normal condition.

Considering the fact that the copper concentrations (0.16 and 0.40 mg/l), administered to *O. mossambicus*, fall within the existing water quality guideline, as prescribed by Kempster *et al.* (1982) for South African freshwater (0.1-2.0 mg/l), it appears that existing values should be revised. Because although all the fish did not die of these concentrations, there were definite changes in the physiology of these fish.

If extrapolated, although, fish in the Olifants River may visually seem unaffected by metals such as copper, their physiological condition does not necessarily reflect a state of normality, but rather a state where it had to adapt physiologically to survive the current conditions. Although adaptation to changing environmental conditions in nature is not an uncommon phenomenon, it is essential that developers, economists and even scientist do not use this as an excuse for irresponsible and indiscriminate exploitation of riverine water.

3.4 Recommendations

It is recommended that a more intensive study on the water and sediment quality of the study area should be undertaken. The interaction between the water and the sediment with regard to metal distribution should be investigated, as well as the bioavailability of the metals to the fish. This can best be achieved by combining the field study with experimental work, in order to determine the effects of the physical and chemical environment on the metal toxicity. Water and sediment samples should be increased to at least ten per locality, thereby decreasing the variation in metal concentrations. Monitoring can be limited to localities 2, 3, 5, 6 and 7, giving special attention to locality 3 to determine the role of the reed beds. Sampling should also be performed higher up in the Olifants River catchment, in order to determine the influence of these mining, industrial and agricultural activities in the area. Biological monitoring should not only include a sensitive fish species, but also sensitive plant and invertebrate species. All of the biological species need be sampled only at localities 5, 6 and 7, as well as higher up in the catchment, and only the fish organs suggested in Table 9.1 should be used. The number of fish should be increased to 20 - 30 individuals, however, and the fish size should be large enough for one gram of dried tissue to be made available. Working on a dry weight basis, as well as the large n-value, will decrease the large variation in metal concentrations.

It seems important that methods should be established and adopted because although the results of the present study suggest that blood coagulation, general haematology, osmoregulation and differential white blood cell counts can be used as sensitive indicators in detecting and evaluating the sublethal effects of aquatic pollution

caused by copper, it is inevitable that individual variation will hamper the drawing of conclusions. The study of fish blood is of practical importance when conducting experimental pollution studies in a laboratory and does not have the same impact during field studies.

It is important to remember that, in general, pollutants rarely occur singly. Thus, for the purpose of environmental protection, it is necessary to know the lethal and sublethal toxicity to various aquatic species (such as algae and macroinvertebrates) of mixtures under various environmental conditions. Therefore, future studies on the lethal and sublethal effect of copper, on its own and in mixtures, on biochemical and physiological processes under various environmental conditions, are of the utmost importance in gaining more knowledge of its interactions with other metals and its toxic effects.

Laboratory experiments where fish (or other aquatic organisms) are exposed under controlled conditions to different metals, according to toxicity, will provide important information on lethal and sublethal levels of the metals. This information is essential for the improvement of water quality guidelines for metals and the "health" status of fish can also be assessed. These experiments are also important in developing improved water quality guidelines for metals which could aid water quality managers, engineers as well as consultants in assessing the impact of metals on the aquatic environment. The further development and expansion of a water quality index (Water 2) which already exists needs further refinement. The final product for the Olifants River could be tested for suitability of use in other river systems in South Africa.

For future management it is recommended that drastic measures should be taken in order to reduce the impact of mining activities on the water quality of the Selati River and also, indirectly, the Lower Olifants River (especially during low flow periods). It is important for enough water to be released in the Olifants River from Phalaborwa Barrage in order to dilute the Selati River water, especially during low flow periods (e.g. droughts and winter periods). If the water quality of the Selati River cannot be improved, it should at least be maintained at its present status, for a further degradation in water quality cannot be afforded.

TABLE 3.1
SUMMARY OF FISH ORGANS IMPORTANT IN METAL POLLUTION SURVEYS

	Zn	Cu	Fe	Cr	Ni	Mn	Pb	Sr
Bile		*		*	*			
Blood				*	*		*	
Gill				*	*	*	*	*
Gonads (F)	*							
Gonads (M)	*							
Gut		*	*	*	*			
Kidney		*		*	*			
Liver		*		*	*	*	*	*
Muscle	*	*	*	*	*	*	*	*
Opercular bone	*					*	*	*
Scales	*					*	*	*
Skin	*		*					
Vertebrae	*			*	*	*	*	*

* Fish organs to sample for metal analysis

Organs of *B. marequensis* with highest metal concentrations

Histopathological studies should be done in addition to metal analysis

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7. GENERAL INTRODUCTION

7.1 Overview and scope of study

It is well documented that pollutants such as trace metals and organic compounds can be accumulated by aquatic biota (Förstner & Wittmann 1979; Phillips 1980; Ellis 1989; EPA 1991). Bioaccumulation - that is, the uptake and retention of chemicals in the body of an organism (Roux 1994) - may take place via absorption through the gills (eg. fish, crabs) and/or through ingestion of contaminated food (EPA 1985). The extent of bioaccumulation of a specific chemical can, however, be influenced by factors related to the organism itself (eg. species, physiological condition, growth, age, sex, pollutant, interactions and physical/chemical of the environment (Mand 1987).

The monitoring of the bioaccumulation of pollutants in surface water (eg. metals) is essential because it serves the following purposes: (i) influence on human health (ii) assessing the extent of accumulation both temporal and spacial (Mance 1987) and (iii) determining the state of organism health. The monitoring of concentration levels in fish and any other organisms which are used as food is essential because it aids in the protection against the consumption of contaminated food. Furthermore, the detected levels should be judged against standards set for food in general (Mance 198). Spacial monitoring of bioaccumulation may produce data that would identify potential unknown areas with high concentrations, while at known discharges it will provide some information regarding the area being effected. Temporal changes in bioaccumulation will provide information regarding the trend of bioaccumulation, which will in turn be used to identify stability, improvement or deterioration (Mance 1987). The accumulation of pollutants in aquatic biota can result in effects on survival and reproduction (EPA, 1991; Timmermans, 1993). The accumulated levels in aquatic organisms may also pose a risk to animals such as birds and mammals that feed on them, resulting in a decline in population numbers (Lloyd, 1992).

Information concerning the lethal and sublethal effects of pollutants (eg. metals) form an integrated part of ecosystem health assessment programmes and of procedures followed to develop water quality guidelines for the environment. The lack of data information is presently affecting the establishment of water quality guidelines by the Department of Water Affairs and Forestry for the environment for Southern Africa. These guidelines are presently based on overseas data. It is therefore very important for freshwater biologists in South Africa to study both the lethal and sublethal effects of water quality variables.

The general objective of acute tests with pollutants (eg. metals) is to determine the concentration that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled laboratory conditions. The acute lethality tests have been useful in providing estimates of the concentration of pollutants (eg. metals) that cause direct irreversible harm to organisms (such as fish). Furthermore, these tests provide practical means for (a) deriving estimates for upper limits of concentrations that produce toxic effects, (b) evaluating the effects of other water quality variables (eg. pH, hardness, temperature, etc.) on the toxicity of pollutants (eg. metals) and (c) developing understanding of the concentration-response relationships (Macek, *et al.*, 1978). The acute toxicity test exposures are also used to develop chronic and other associated sublethal tests.

Sublethal pollution induces chronic stress in the aquatic environment. The increase in susceptibility to disease and impairment of the reproductive ability of fish can be directly related to the concentration of pollutants in the natural habitat of aquatic organisms (Pickering, 1989).

It is therefore important for the toxicologist to determine the biological effect of pollutants in aquatic ecosystems, before these substances are released into the environment. The majority of toxicological surveys in the past were conducted to establish the effect of pollutants under acute exposure where the death of the organism was used as the only measurable variable (Larsson *et al.*, 1985). It is, however, a fact that the normal physiological functioning of an organism is affected long before death comes. It is therefore important in the conservation of river biota in the KNP to obtain information on the effects of pollution on the survival of aquatic organisms whereby preventative measures can be taken. Sublethal pollution can have detrimental effects on aquatic organisms if it is not identified early.

Aquatic toxicology should therefore be focussed on the subtle pollution-induced changes. The side effects of pollution can be studied at different levels of biological organisation which range from induced reactions on sub-cellular level of an individual organism to changes reflected by the whole population.

Sublethal effects of pollutants are usually of biochemical nature since the basic level of cellular organisation is usually affected. Pollutants affect enzyme function and metabolites of enzymatic reactions. They also bind to and interact with membrane systems as well as other cellular components. These primary interactions between pollutants and the different cellular components result in functional and structural changes on a higher level or organisation which lead to the impairment of essential functions such as osmoregulation, hormone regulation, nerve and muscle function, respiration, immune reactions and circulation.

From the abovementioned it is clear that it is essential to establish the effect of pollutants on the physiology of fish as an aquatic vertebrate before the total extent of pollution on a specific waterbody can be determined. The provision of critical levels of pollutants in an aquatic environment ensures that preventative measures can be taken to protect the river biota. Prevention rather than cure is the primary objective.

7.2 Metal Pollution

The greater part of the Southern African sub-region is arid to semi-arid. More than half of South Africa receives less than 600 mm of rainfall per year and the variability of rainfall, coupled with hydrological extremes such as long droughts and unpredictable floods, results in an erratic and limited supply of water (Department of Water Affairs, 1986).

Metals were natural components of freshwater systems and originate from the erosion of rock. However, metal pollution in rivers is increasing world-wide due to the growth in mining, industrial and agricultural activities, as well as a proliferating human population. The most important metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Abel, 1989). Some of these metals are essential trace elements to living organisms (such as copper and zinc), while others (such as lead and cadmium) are non-essential, having no known biological function. All metals are, however, toxic to aquatic organisms when present at elevated levels, causing direct or indirect effects such as histological damage or a reduction in the survival, growth and reproduction of the species (Heath, 1987). The toxicity of metals can be influenced by various factors, of which environmental conditions (eg. temperature, pH, water hardness) are the most important ones. These conditions determine the chemical speciation of the metals (Abel, 1989) and consequently the bioavailability of the metals to aquatic organisms. Other factors influencing metal toxicity are interactions between

pollutants, the developmental stage of the organism and interspecific variations in susceptibility to metals (Hellawell, 1986).

In view of the consequences of metal pollution in aquatic ecosystems, it is undoubtedly essential to monitor river systems which may be affected directly or indirectly by mining and industrial activities on a regular basis. Metal concentrations in the water can then be compared to the metal concentrations proposed by existing water quality guidelines. The fitness of the aquatic environment to which the aquatic organisms are exposed can thereby be assessed. In order to obtain a reliable and general assessment of the metal pollution in question, the purely physical and chemical monitoring of the water and sediment should, however, be supported by biological monitoring (Abel, 1989). This supportive monitoring is based on the fact that living organisms can provide useful information on the chemical quality of the water as they have experienced it throughout their lives, while a chemical analysis can only indicate the conditions prevailing at the instant of sampling (Abel, 1989). Fish are good organisms to use in biological monitoring for a number of reasons. They are known to accumulate metals in their organs and tissues, they are readily identified, they can be sampled easily and quantitatively and they have a cosmopolitan distribution (Hellawell, 1986). Their economic importance as a resource is also an added feature of great importance. Fish can therefore provide valuable information in addition to the water and sediment data.

It is therefore important that an adequate water supply of an acceptable quality and quantity must be ensured for optimum utilisation by all the water users. In the past, water resource management has primarily been devoted to water storage and transportation for off-stream users. In recent years the aquatic ecosystem has been recognised as a legitimate water user requiring specific water allocation in order to preserve ecological integrity and functioning (Department of Water Affairs, 1986; Walmsley and Davies, 1991).

7.3 Aims

The Kruger National Park (KNP), one of the world's foremost conservation areas and an important contributor to South Africa's tourist industry, has been identified as an important water user sector to which adequate water must be allocated (Department of Water Affairs, 1986). In an attempt to address the management problems associated with water resource utilization, the Department of Water Affairs and Forestry (DWAF) initiated a multidisciplinary research programme aimed at developing a protocol for managing the allocation of water for ecological purposes.

The water quality of the rivers which drain through the KNP is deteriorating under pressures of an increasing rate of development to the west of the KNP (Moore *et al.*, 1991). The Olifants River was identified as a river with high silt loads, salinity and pollutant levels (Venter and Deacon, 1992). The KNP is regarded as an environmentally sensitive area due to its position at the lower end of the catchment basin and any further development and changes in resource management policies could have an influence on this sensitive area (Theron *et al.*, 1991a). In order to manage a system such as the Olifants River, the system must be divided and delineated into manageable entities e.g. catchments, sub-catchments, river reaches and tributaries (DWAF, 1992).

Information on the levels of metal pollution in the Olifants River in the KNP is limited and the impact on the aquatic environment unknown. Specific research had to be carried out to establish the present levels of pollution in this section of the river.

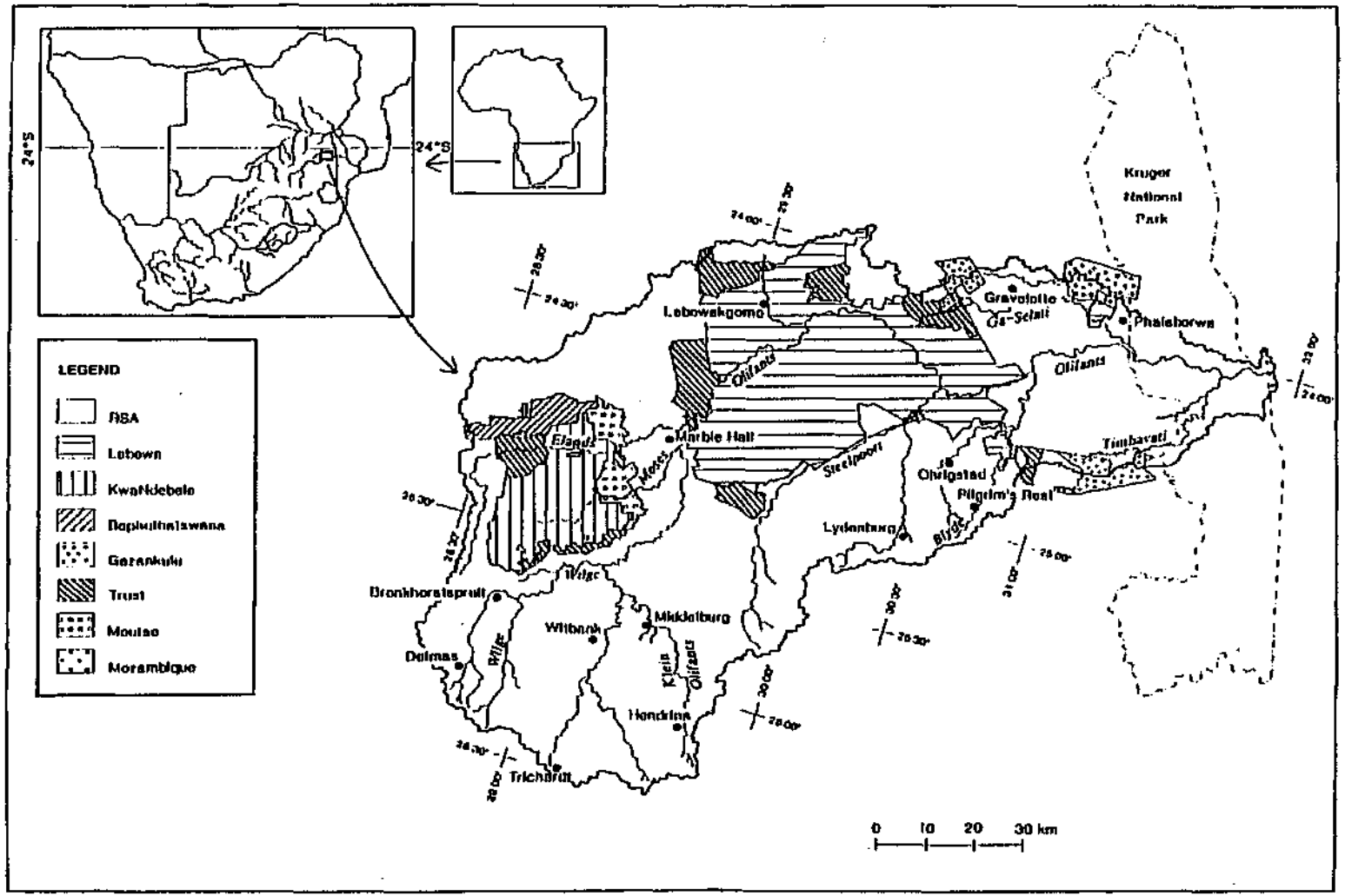

Figure 7.1
The Olifants River Catchment, indicating the involved co-basin states. (From: Theron et al., 1991a)

This research was done by collecting water and sediment samples at selected sites along the river from the western to the eastern border. Two sites outside the KNP at Phalaborwa Barrage and in the Selati River were also included to establish the quality of the water entering the KNP.

Together with water and sediment samples, tissue and organ samples of selected fish species were also collected to determine the levels of bio-accumulation of metals in these organisms. These samples were taken and analysed over a period of three years to make provision for any fluctuation in pollution levels. Furthermore, sampling was done seasonally since pollutant levels can fluctuate during high and low flow (summer and winter) as well as during floods and drought. Fish were collected at three pre-selected localities in the KNP and one in the Selati River. Representatives of selected genera were used as indicators.

Experimental exposure of fish to metals present at high concentrations and according to sensitivity (Hellawell, 1986) was carried out to determine the effect thereof on the physiology of the species concerned. Blood chemistry (haematology and metabolism) and ionic regulation of the systems were investigated. Fish were exposed to sublethal concentrations of the pollutants in a continuous flow system under controlled environmental conditions. By employing this exposure technique, critical levels of pollution affecting fish could be determined. Acute toxicity testing was done to determine lethal concentrations of the metal to fresh water. Different fish species were selected for LC_{50} experimentation.

7.4 General description of the Olifants River basin

The Olifants River catchment area consists of over 54 575 km^2 with an estimated mean runoff of more than 1 900 million m^3. The water resources of the Olifants River are shared by six different self-governing nations and provinces i.e. South Africa, Mozambique and former Eastern and Northern Transvaal.

The Olifants River is the second largest river in the Transvaal province of the Republic of South Africa. Together with its tributaries of which the major ones are the Wilge, Klein Olifants, Moses, Elands, Steelpoort, Blyde, Selati and Timbavati Rivers, it drains a catchment area of approximately 20% of the Transvaal (Theron *et al.*, 1991b). The Olifants River originates in the Bethal-Breyten area and flows in an easterly direction through the Drakensberg before crossing the Kruger National Park into Mozambique, where it flows into the Indian Ocean after its confluence with the Limpopo River (Fig. 7.1).

Topographically, on the basis mainly of altitude and relief, the catchment can be divided into four zones (Steffen *et al.*, 1991). These zones are: the Highveld in the south (1 200 m - 1 800 m above sea level), the Springbok flats in the west (900 m - 1 200 m above sea level), the Transvaal Drakensberg/Strydpoort escarpment zone in the centre of the basin (1 500 m - 2 400 m above sea level) and the Lowveld in the east (300 m - 900 m above sea level).

The climate of the basin is warm to hot sub-tropical, with seasonal rainfall occurring predominantly during the summer months (October to March) and peaking in January. Rainfall generally varies with altitude. Low rainfall of below 600 mm per annum occurs in the Lowveld and Springbok Flats. Moving towards the Highveld and escarpment zone, the rainfall gradually increases to 800 mm per annum. However, it increases rapidly with altitude along the escarpment to as much as 2 000 mm per annum (Kleynhans, 1992).

Three of the identified biomes in South Africa occur in the Olifants River catchment, namely the grassland, savanna and forest biomes. "A biome is a broad ecological unit which represents large, natural and reasonably homogeneous areas of

biotic and abiotic features. The biotic component is closely related to physical factors, particularly soil type and climate" (Steffen *et al.*, 1991). The grassland biome comprises mainly the Highveld , as well as the southern and western part of the escarpment. The vegetation is dominated by hemicryptophytes of the Poaceae, with *Themeda triandra* being the most widespread species. The canopy cover decreases with lower rainfall. Sweet grass occurs in drier regions, while sour grass occurs in areas where the rainfall exceeds 625 mm. Trees are uncommon, although they do occur in high altitude areas east of the escarpment. The savanna biome comprises the greater part of the Springbok Flats and the Lowveld, as well as the north-eastern parts of the escarpment. The vegetation consists of graminoid hemicryptophytes and perennial woody plants. It is well adapted to withstand both drought and fire. Most of the savanna biome is used for livestock grazing and game ranching. The forest biome covers a small portion of the catchment and is more or less centred around Mica. The vegetation consists mainly of evergreen woody plants. A multi-layered structure can be distinguished, with perennial woody plants and herbaceous species as the understorey, while epiphytes, ferns and lianas comprise the sub-canopy (Steffen *et al.*, 1991).

The study area of concern to this project comprises the lower part of the river basin from the point just before the Olifants River enters the KNP (Phalaborwa region) through to the area just before it enters Mozambique. The lower Olifants River catchment covers an area of 12 180 km (Fig. 7.2).

7.4.1 *Factors affecting water quality management; Olifants River*

7.4.1.1 Physical factors

Regional geology

The geology and geomorphology are not only one of the main determinants of topography, but also determine the extent to which water and minerals occur and thus the chemical characteristics of the water. The lower Olifants catchment consists of the following geological groups: Gravelotte, Rooiwater Complex, Mashimale Suite and Karoo Sequence. Most of the area is underlain by basalts of the Basement Complex.

In the low rainfall areas (mean annual rainfall for the study area is < 600 mm), the residual soils, where developed, are thin, and consist of loose aggregates of quartz and feldspar, derived mostly from mechanical disintegration of the parent rock. The study area has a Weinert N-value (which denotes the relationship between evaporation and rainfall) smaller than 5, which indicates that chemical, rather than mechanical, weathering of soils occur. The granites of the Basement Complex and the shales and mudstone of the Karoo Sequence could produce mineralized waters.

Geohydrology

The eastern half of the study area has a dolomitic outcrop, where the main aquifers are dolomitic krast-type aquifers with good potential for groundwater and boreholes. Towards the western border of the study area, Granitic Regions occur and it is expected that the groundwater originating from the granitic types of rocks will produce waters dominated by sodium, calcium, silica, iron and magnesium concentrations. The degree of mineralization is therefore expected to be high.

Hydrology

In terms of ecological water requirements, only low-flow hydrology will be discussed. Because the Olifants River is a major source of water for the KNP, a stable source of water is needed to secure the ecosystem and sustain species diversity. The way in which the water resources is managed in the Olifants River basin will determine the flow regime available to the KNP.

The Olifants River has only recorded cessation of flow once (1968) and remains a perennial river. At present the summer flow is approximately 60 million m^3 per month during the rainy season and 8 or less for the winter. It is predicted that future development would result in zero flow for 70 percent of the time during the month of October. This would represent a summer and winter monthly flow average of 15 and 5 million m^3 respectively.

Soil properties

The evaluation of the soil properties of a river basin serves as an indication of the potential for afforestation, irrigation or human settlement. The predominant soil group is of the Hutton form. These are well-drained red apedal soils varying from deep to shallow and loams to clays.

The Olifants River catchment in the study area has no potential for either afforestation or irrigation but shows moderate to high potential for human settlement. However, there is a limiting factor to settlement as found in the erosion hazard and the bedrock depth.

7.4.1.2 Water users as impacting factors

There are many different user sectors in the Olifants River basin (e.g. power stations, agriculture, afforestation, aquaculture, lifestock farming, etc.) making it one of the most intensively utilized water resources in South Africa. Only the water users in the direct vicinity of the KNP are discussed in this section, however.

Domestic and Industrial

Phalaborwa is the regional service centre with a population close to 10 000. The growth of the town can mainly be attributed to mining activities, and to a lesser extent, industrial development (which are by and large service industries to the mining activities). The growth of the developed sector is, however, projected to stabilize and even decrease.

On the other hand, a town like Namakgale, which is situated close to Phalaborwa and serves mainly as a dormitory town for the mines and industries in Phalaborwa, is expected to increase in population and grow to become one of the 20 largest towns in the whole basin. The current population of Namakgale is approximately 24 000.

Both of these towns extract water from the Olifants River system (either directly from the river or tributaries or from ground water sources) for domestic and industrial use. The estimated demand for water from these user sectors is projected to increase from 15,2 million m^3 per annum in 1987 to 28,6 million m^3 per annum in the year 2010.

The Phalaborwa Barrage is the main storage dam in the study area and most of the domestic and industrial water supply of Phalaborwa and surrounding

areas is extracted from it (approximately 141 million m^3 p.a.). The water level of the barrage is usually kept almost full with an approximate base inflow of 1,5 $m^3.s^{-1}$ (mean annual runoff under present conditions is estimated to be 1201 million m^3 p.a.). This base inflow is released as compensation for the KNP. When insufficient inflow into the barrage occurs, supplementary water is released from the Blyderivierspoort dam. It is important to note that the present capacity of the barrage has been reduced by 45% due to siltation.

Mining

The study area and the immediate vicinity have considerable mineral deposits. There are a total of 45 mines in this region, of which ten have closed down. Of these mines, six do not use water for mining purposes and six have yet to be commissioned. The remainder of the mines, all of which need water for production, mine copper, emerald, asbestos, magnetite, phosphate, clay, feldspar, fertilizers and slate. Furthermore, there are two gold mines, two mica mines, two crushed-stone mines, two platinum mines, three andalusite mines and three chromium mines.

The present water usage is estimated at 46 million m^3 p.a. with an expected increase of 7 million m^3 by the year 2010. Two of the mines in the Phalaborwa area receive water from the Phalaborwa Barrage and account for 84 percent of the total water consumption of mines in this region. According to projections these mines will use the maximum total allowance permitted in the foreseeable future.

Ecological systems

The water requirements of a riverine ecosystem are predominantly non-consumptive. Water allocated and released down the Olifants River may be used by the various ecological systems without there being much consumption or loss of water from the system. River ecosystems are longitudinal extensions through the catchment and any alteration in the status of the water quality or quantity will have an influence on the downstream reaches. The water requirements of the ecological system can therefore not be separated from those of the upstream river reaches.

The water requirements of the natural environment can be defined in broad terms as being the quality and quantity of water, and its temporal and spatial distribution necessary to maintain water-dependent ecosystems as a renewable resource. This would entail a recovery from stressed conditions to the original unstressed conditions without any loss of its components and diversity of species. The maintenance of the ecosystem as a renewable resource thus implies that species diversity would remain the same in stressed and unstressed conditions, but the number of species present would be reduced in the case of stressed conditions.

In practice these requirements are generally ignored and the flow needed for maintaining ecosystems is often diverted to supply other demands. Preliminary estimates from the KNP Rivers Research Programme indicate that average monthly flow rates during the critical dry periods should be at an absolute minimum of 1 m^3/s^{-1} in winter and 10 m^3/s^{-1} during the summer in the KNP. Furthermore, the water delivered to the system should be of such a quality that is maintains riverine ecological systems at a sustainable level in periods of drought.

7.4.1.3 Pollution

Major industrial and mining operations have developed in the Phalaborwa area and have resulted in changes in water pH and concentrations of inorganic determinants. Agriculture in the upstream catchment has also contributed to the pollution of the Olifants River by increasing phosphate and nitrate concentrations and releasing biocide residues.

Poor cultivation management in the agriculture sector and bad mining practices have caused soil erosion resulting in an increase in sediment loads. This had had a detrimental effect on not only benthic organisms, but also on the breeding, feeding and refuge areas of fish.

At point sources of pollution, the origin, volume and concentration of the effluent can be quantified. The major point sources in the lower catchment of the Olifants River are municipal (treated sewage), mining and industrial discharges. The point sources of pollution in the vicinity of the KNP discharge most of the effluent into the Selati River (see section on the Selati River).

The volumes and concentrations of effluent cannot normally be quantified for a non-point source and the origin is therefore usually diffuse. The discharge is difficult to relate to a specific location, as the discharge may reach the water body through overland runoff, atmospheric precipitation, groundwater infiltration or drainage and leaching from geological formations, mines and waste disposal sites. As in the case with point sources of pollution, discharges from diffuse sources mostly reach the Olifants River via the Selati River and will be discussed in the following section on the Selati River basin.

One major source of diffuse pollution of the Olifants River is high silt concentrations. Bad land practices in highly populated rural areas have led to soil erosion in the upper reaches of the Olifants River. The release of water carrying extremely high loads of suspended solids (24 000 and 77 000 mg/1) from the Phalaborwa Barrage has resulted in catastrophic fish kills (Venter and Deacon, 1991).

7.4.1.4 Political factors

The water resources of the Olifants River system are of joint interest to South Africa and Mozambique. There is an increasing demand for water for domestic, industrial and agricultural use and agreements on joint utilization have to be founded on a sound factual basis. Within the co-basin states there are different degrees of development and in order to achieve satisfactory joint water utilization, flow regulation, flood control and pollution control, it is imperative that there should be a high level of co-operation and joint control. It is therefore of the utmost importance that uniform criteria for water quality and quantity be adopted.

7.4.1.5 Socio-economic factors

The choice of land in the Lower Olifants catchment is restricted due to the mining potential in a large part of the area. The potential industrial development in mining related and the possibility exists that processing and manufacturing sectors may develop. In contrast, there is a moderate potential for agricultural development.

In the light of these factors it should be expected that there would be a population increase in the proclaimed towns, but the opposite has occurred. The population growth rate in both the developed rural and urban areas has stabilized and is beginning to decline. In terms of the total population, the developed portion has decreased from 8% in 1970 to 5% in 1985. Although the population in the developing society increased by 156 000 in the past 15 years, the greatest population growth occurred in the rural settlements and not in the proclaimed towns.

The reason for this can be found in the fact that the economically active portion of the population in the developed areas has increased by only three per cent in the past 15 years. The economically active population, as a percentage of the total developing population, declined between 17 and 40% over the same period. This is due to the high growth rate of this sector of the community and the lack of employment opportunities.

Projections are that by the year 2010, approximately 40% of the developed population and 34% of the developing population will fall into the economically active age group. This implies that the creation of job opportunities would have to be increased sixfold in order to meet the job demand. However, it is unlikely that the expansion in the job-creation sector would be able to meet this demand.

Presently, domestic water users, irrigation and mining consume significant quantities of water available from local resources. If the mining potential is fully realised and exploited to its full potential, the water requirements of these sectors would increase. The increased population in the rural areas would also increase water utilisation for domestic and irrigation purposes. If left unmanaged, this situation could lead to increased soil erosion and pollution of an already scarce water source.

7.4.1.6 Technological factors

As mentioned in the previous section, the employment opportunities in the study area are limited. The potential job-creation sectors, the mining and associated industrial sectors, are becoming increasingly more mechanized and technologically innovative. This is a limiting factor to the various sectors to create new jobs. Advanced technological processes would most probably require more water for its functioning, this placing a further burden on the water resources of the Olifants River.

7.4.1.7 Legislative factors

The purpose of the legal system is to maintain order and stability in a society to protect it by keeping pace with ever-changing needs. Traditional attitudes towards South African water resources have been that they should be divided fairly among human users and that their chemical, biological and aesthetic quality should be protected. The water law is regarded as a part of conservation law, aimed at allocating and preserving water for the benefit of interrelationships within the environment (Uys, 1992).

It has, however, been evident that the aquatic ecosystem has largely been ignored in the Water Act (Act No. 54 of 1956) and that this Act is outdated. According to Uys (1992), the main deficiencies of the act are the restricted user sectors entitled to water rights, the limited purposes of lawful water utilization, the uncertain meaning of the clause "public interest" and the unsound distinction between public and private water. It has been suggested that the incorporation of some Roman water law principles, such as natural

justice and all water being common, i.e. available for use by each and everyone but subject to government control, would negate most of the untenable provisions of the Water Act, and provide a sound foundation for the reconstruction of the South African water allocation system (Uys, 1992).

In an attempt to bring about a framework for environmental law, the President's Council of South Africa (1993) proposed a principle of "the user/polluter must pay". Rehabilitation costs would also have to be carried by the offending party.

7.4.2 *Factors affecting water quality management, Selati River*

The Selati River is a major influence on the water quality of the Olifants River in the KNP. The catchment area of the Selati River is on the eastern side of the Wolkberg Mountains (part of the Drakensberg Range) and flows eastward for approximately 140 km towards its confluence with the Olifants River, close to the western boundary of the KNP.

7.4.2.1 Physical factors

Regional geology

The following geological groups are found in the Selati River catchment: the Basement Complex, Gravelotte Group, Rooiwater Complex, Mashimale Suite and Transvaal Sequence. The weathered granite of the Basement Complex is eroded easily and may contain high concentrations of sodium and calcium. The felsite and gabbro from the Rooiwater Complex are also easily eroded and contain iron and titanium-rich magnite seams. Water from this complex may also contain sodium and calcium.

Hydrology

The low-flow hydrology of the Selati River is extremely eratic. The upper reaches can no longer sustain existing irrigation areas. There is no flow recorded for 30% of the time during the months from April to December at the Olifants River confluence. The present flow conditions range from 0,1 million m^3 in March to 0,03 million m^3 in January.

According to the Selati River Basin study in carrying out by Theron *et al.*, (1992) the Selati River has a low reliability to contribute to the flows in the Olifants River. The present study has, however, shown (See section 8.1.1) that the Selati River does contribute a fair amount of water to the Olifants River. This is due to the water, which is used by the mines in the Phalaborwa area, being returned to the Selati River. One mine alone discharges approximately 4 to 5 million m^3 per year into the Selati River. Another mine discharges 7 to 11 million m^3 per annum depending on the rainfall. It is evident that the Selati River does contribute substantially to the flow regime of the Olifants River within the KNP.

Water users

The water users in the Selati River catchment area are similar to the water users in the Olifants River as discussed in 7.3.1.2.

7.4.2.2 Pollution

In order to optimally manage water quality requirements for the KNP (i.e. the aquatic environment) it is necessary to have a clear understanding of the present water quality status of the water resource. Factors which have to be taken into account are the reliability of the water supply, the impact of current catchment development and potential point and non-point pollution sources.

Point sources

The Phalaborwa area has many point sources of pollution ranging from sewage treatment works to mining and industrial effluents. In most cases the origin, concentration and volume of pollutants can be quantified.

Sewage treatment plants are point sources of nutrients i.e. nitrates and phosphates, total dissolved salts, free and saline ammonia, increased chemical oxygen demand, suspended solids, pH changes, residual chlorine and faecal coliform counts. The municipalities of two towns in the developing area of the study catchment, Lulekani and Namakgale, use biological sewage filter systems. Approximately one million m^3 of effluent is discharged into the Selati River per annum and some problems were experienced with pollution. Phalaborwa operates an activated sludge sewage treatment plant and about 1 to 1,5 million m^3 of treated effluent is discharged into the Selati River per annum.

The mines in the area are also point sources of pollution. The water used in the mining processes is obtained from the Phalaborwa Barrage in the Olifants River and returned from the mining operations to the Selati River. Between 11 and 17 million m^3 of mining effluent is discharged into the Selati River per annum. Special permission has been granted by the DWAF to discharge excess water from tailing dams into the Selati River during the rainy season (mostly December and January). The mining pollution contains a number of chemical constituents such as fluorides, sulphates, potassium, chlorides, phosphates and metals.

The industries in the area do not have permits to discharge effluents into any river and all their effluent is disposed of through the municipal sewage system.

Non-point sources of pollution

The volume and concentrations of effluent cannot be quantified and the origin of discharge is diffuse. Non-point sources of pollution in the Selati River catchment are agriculture, atmospheric deposition, mining and industries and urban and rural runoff.

Atmospheric deposition has been found to be an insignificant source of pollution in the Selati River catchment. Surface runoff from the urban areas of the towns in the catchment area could reach the river system via storm-water drains and in doing so, pollute the water. Due to poor sanitation facilities, the informal rural settlements are non-point sources of nutrients and bacterial pollution.

All of the mines in the vicinity of Phalaborwa are potential polluters of the Selati and Olifants Rivers. Mining effluents used to irrigate fields, leakage from evaporation ponds, leaching from mining areas, seepage from tailings dams, sand dumps and rock dumps are potential sources of pollution.

Polluted ground water seeping to surface streams is a sub-surface diffuse source of water pollution. In some instances it has been noted that sub-surface seepage has left salt depositions on the surface within the boundaries of the KNP (Wepener, personal observations).

Increased sediment loads due to erosion caused by over-grazing is also named as a diffuse source of pollution. However, no signs of sediment loads have been observed in the Selati River during the course of the current monitoring programme. It is therefore unlikely that sediments from the Selati River would cause the water quality of the Olifants River to deteriorate.

7.4.2.3 Other factors affecting water quality management

Since the Selati River is one of the tributaries of the Olifants River and lies within the Olifants River catchment, the political, socio-economic, technological and legislative factors affecting the Olifants River (See 7.3.1.4 - 7.3.1.7) would be similar to the factors having an affect on the Selati River.

7.5 Sampling localities

The sampling stations were chosen to represent the different river reaches in the lower catchment of the Olifants River in the KNP as well as the possible effect of mining activities on water quality of the Selati River on the western border just outside the KNP (Fig. 7.2). The following description of the reaches was abridged from the description of the river reaches of the perennial rivers in the KNP (Venter 1991).

Reach 1

The first reach, which includes localities 4 & 5, stretches for 62 km from the western boundary of the KNP to approximately one km west of Nwamanzi. The gradient is 1,2 m/km and the channel is single, between 100-300 m wide, with a flat river bed and shallow stream. The river bed is made up of sand and gravel with alternate rocky patches (mostly passages) and is mostly exposed. Short rapids occur over solid rock passages or rounded rocks and end in single deep pools. The river valley has a wide wave-like land form ending in steep river banks (5-15 m) with clearly distinguishable terraces (up to 3). The river bank is narrow (10-100 m) consisting of alluvial red and brown sand, silt or weathered rock. The riparian vegetation is dense, consisting of *Ficus sycomorus, Trichilia emetica, Lonchocarpus capassa, Acacia robusta* and *Diospyros mespiliformes*. Reeds (*Phragmites* spp.) are isolated in small groups on rocky areas of the river bed. Two sampling stations are found within this reach of the Olifants River; the first (station E) is a few hundred meters downstream of Mamba weir and the second is a few kilometers downstream of the Nhlaralumi confluence (station D).

Reach 2

The second river section is from Nwamanzi to Olifants Rest Camp and is 18 km in length. The river gradient is 1,5 m/km and the channel width is between 100 and 250 m. The channel is irregular and branches to form deep channels (5-10 m) forming islands between them. The river bed has irregular sedimentary sand and silt on solid rock or islands. The river valley is wide ending on the river bank with a gradual gradient (2-5 m). The river bank is narrow (10-50 m) with alluvial sedimentations of brown calcareous silt and sand or weathered rock. Open to semi-dense riparian vegetation occurs. The predominant species are *F. sycomorus, T. emetica, L. capassa, A. robusta, D. mespiliformes* and *Colophospermum mopane*.

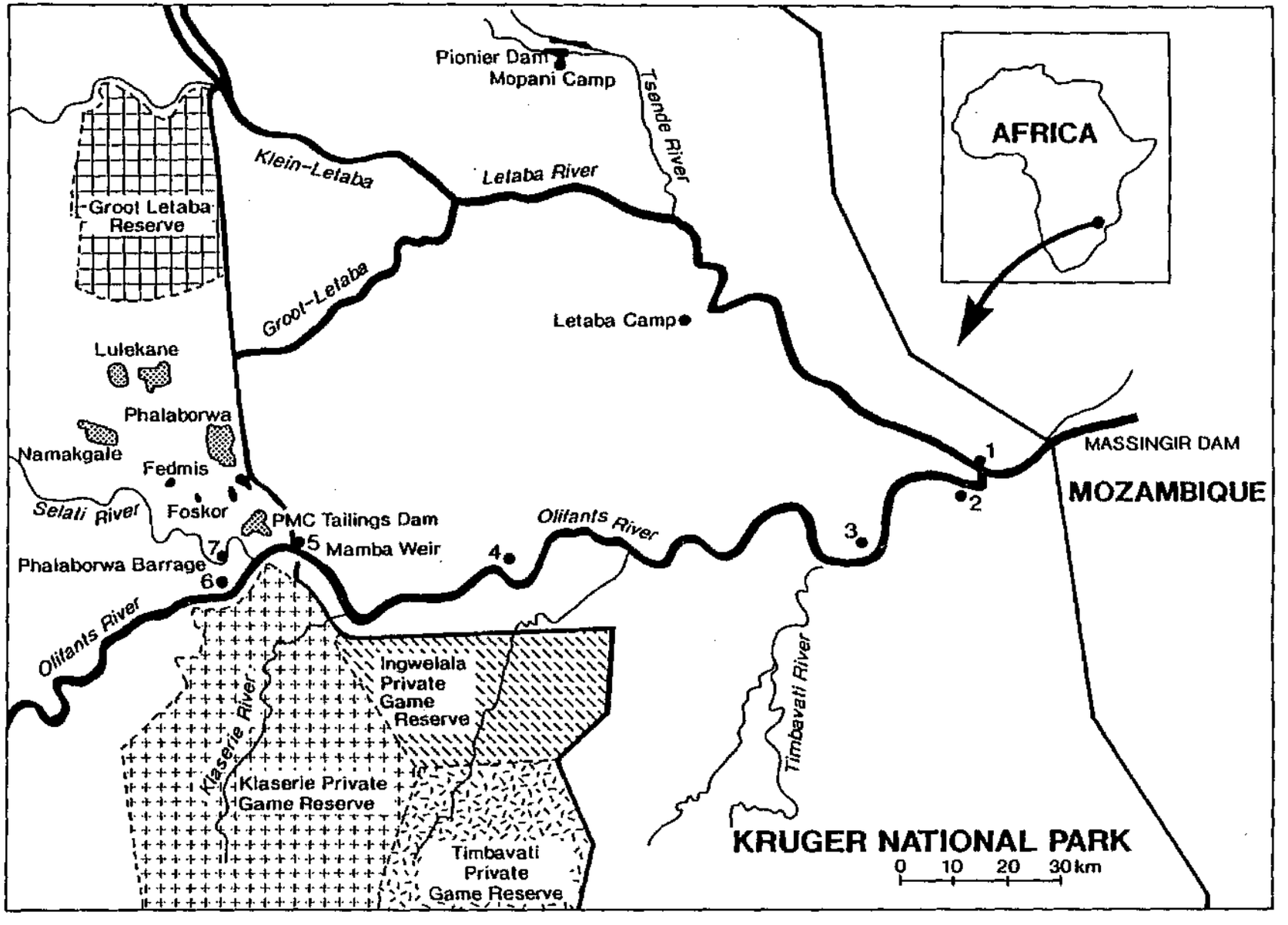

Figure 7.2

The study area in the Lower Olifants River Catchment, indicating the sampling localities 1 to 7

Dense reeds beds (*Phragmites* spp.) and trees like *F. sycomorus* and *Breonadia salicina* occur on islands. Sampling station C is situated approximately 1 km downstream of Balule Rest Camp. Locality 3 is situated in this reach.

Reach 3

This section of river reach which includes locality 2, stretches for 12 km from the Olifants Rest Camp to the Letaba River confluence. There is a very steep gradient (4 m/km) and the channel is between 20 and 100 m in width. The channel has a single V- to U-shaped form with deep pools and short rapids. In the vicinity of the hiking trail camp there are many small water falls and narrow gorges. The river bed consists of bedrock with sedimentary red silt in pools and loose round stones in the rapids. The river valley is relatively wide and the river bank is steep (15-25 m) with definite terraces. The bank is narrow (10-50 m) and consists of solid rock or alluvial sedimentary sand or silt. The predominant riparian vegetation includes: *F. sycomorus, L. capassa, A. robusta, A. zanthophloea* and overhanging reeds (*Phragmites* spp.). The river bed is without vegetation and reeds are isolated to rocky patches or alluvial islands. Sampling station A is situated in the Letaba River, a few meters from the confluence with the Olifants River, and station B is situated on the Olifants River, a few meters upstream from the confluence with the Letaba River.

Reach 4

There are no sampling stations in this section of the Olifants River. This is due to the narrow and deep gorge through the Lebombo Mountains. This section of river is only eight km in length and stretches from the Letaba River confluence to the eastern border with Mosambique. The channel is narrow (20-40 m) with deep pools and few rapids. The river bed consists of bedrock with deep sedimentations of red silt in the pools. The river bank is solid rock with small isolated sand banks and the riparian vegetation is absent.

Localities 6 and 7 were selected in order to study the possible effect that the Selati River might have on metal pollution in the Olifants River. Locality 6 was located below the Phalaborwa Barrage, which is before the Selati-Olifants confluence, while locality 7 was located in the Selati River (Fig. 7.2). The channel of the Selati River is single, with large, deep pools and small rapids occurring there. Black odoriferous silt depositions cover the rocky river bed. Trees such as *Phoenix reclinata, Trichelia emetica* and *Ficus sycamorus* grow on the river banks, while hanging reeds (*Phragmites* spp.) occur in dense spots along the banks.

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8.0 TOXICOLOGY

8.1 Monitoring

8.1.1 *Water Quality*

8.1.1.1 Introduction

In South Africa, which is a developing country, it can be expected that large-scale development will take place. Unfortunately, increasing mining, industrial, agricultural and domestic activities may lead to water pollution unless certain precautions are taken. These precautions can, however, be very costly and are therefore not always enforced. This is partly why the water quality of many South African rivers (eg. the Olifants River, Kruger National Park) has been deteriorating over the last few years.

Pollutants (eg. metals, organic compounds, anions, acids, alkalis) can have a direct or indirect effect on aquatic species, for instance a reduction in the survival, growth and reproduction of the species, an unacceptable level of avoidance behaviour towards the pollutant and an unacceptable percentage of gross deformities or visible tumours in organisms (Stephan, 1986). It is, however, very difficult to relate specific effects to specific pollutants, for the stage of the organism's development, the physical and chemical quality of the environment (eg. temperature, pH, water hardness), the chemical species and complexes present, and the interactions between pollutants all play a role in the toxicity of a substance (Hellawell, 1986). Furthermore, interactions between pollutants can be additive (a combined effect), antagonistic (interfering with one another) or synergistic (the overall effect is greater than when each one acts alone).

Bearing in mind that the toxicity of a pollutant to an organism is not always the same due to the above-mentioned external influencing factors, one can understand that there are some difficulties in the establishment of water quality guidelines and, eventually, water quality standards. A water quality standard is defined as that concentration, level or value of a particular water quality variable that has been promulgated as a standards only apply to effluents discharged into river courses. A water quality guideline, on the other hand, is that concentration, level or value of a particular water quality variable that meets the needs of all water users in a specified river reach (Moore *et al.*, 1991) and has no legal connotations. Water quality guidelines in South African are presently being developed, and are based primarily on values from overseas literature, as well as on the limited data available in South Africa.

The Olifants River is regarded as the most mineralised river flowing through the KNP (Moore *et al.*, 1991). Some of the major problems effecting water quality in the Olifants River have been identified as high silt loads, high salinity and pollutant levels (caused by poor farming, industrial and mining effluent discharges and possibly mine seepage). The transportation of contaminated dust (eg. copper) generated by mining activities to the river by rain-water has also been mentioned as a possible source of pollution (Venter and Deacon, 1992).

In this regard a water quality monitoring programme for the aquatic environment was initiated towards the end of 1983 by the Department of Water Affairs and Forestry (DWAF), National Parks Board and other interested parties (Walmsley and Davies, 1991). Twenty four sampling stations were selected for the six main rivers flowing through the KNP.

The assessment of the water quality status and tendencies of the rivers were limited to certain chemical water quality variables (Moor *et al.*, 1991). The lack of data on metal concentrations led research to determine the extent of metal contamination in the biotic and abiotic component of the Olifants River (Van Vuren and Du Preez, 1991). In this section of the study the metal concentrations (Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn) in water as well as the physical and charateristic of the water, were investigated.

8.1.1.2 Materials and Methods

Water were sampled bimonthly from April 1990 to 1992 at six sampling sites (Fig. 7.2) along the Lower Olifants River and one (locality 7) in the Selati River. During the period April 1992 to February 1993 water samples were only collected quarterly at selected localities (3, 5, 6 and 7).

In February 1992 and April 1992 it was also performed at Pionier and Hlanganini Dams, respectively. These dams were used as natural reference points because they are situated in rivers that originate inside the Kruger National Park and therefore receive no effluent from outside the Park.

The following variables of surface water were determined on site at each locality: pH (ORION, Model SA250), water temperature (WTW microprocessor, Model OXT 96), dissolved and percentage saturation oxygen (WTW microprocessor, Model OXT 96), turbidity (Secci-disc) and conductivity (Jenway, Model 4070). During the first year these variables were determined once a day in the afternoon. However, in order to determine whether there would be any difference between readings taken in the morning and readings taken in the afternoon, these parameters were determined twice a day at localities 3, 4, 5 and 7 during the second and third years. Readings were taken between 7:00 and 9:00 in the morning and between 11:00 and 17:00 in the afternoon. At localities 1, 2 and 6, as well as the two Dams, the variables were only determined once a day between 11:00 and 17:00.

Two surface water samples were collected at each locality. One sample was preserved with mercuric chloride ($HgCl_2$) and was refrigerated until the Hydrological Research Institute analysed it for sodium (Na), magnesium (Mg), calcium (Ca), fluoride (F^-), chloride (Cl^-), nitrate and nitrite (NO_3+NO_2-N), sulphates (SO_4), phosphates (PO_4-P), total alkalinity (as $CaCO_3$), silicon (Si), potassium (K), ammonia (NH_4-N) and total dissolved salts (TDS) concentrations. The other sample was frozen, until it could be subjected to metal concentration analysis in the laboratory.

After the water samples were thawed in the laboratory, 50 ml of well-mixed river water was measured into a 100 ml Erlenmeyer flask. Ten ml concentrated nitric acid (55%) and five ml concentrated perchloric acid (70%) were added and the mixture was evaporated to 2 to 5 ml on a hot plate until clear (Standard Methods, 1989). Each samples was then made up to 50 ml with doubly distilled water and stored in clean storage glass bottles for metal analyses. Prior to use, all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24h, rinsed in doubly distilled water, acid-washed in 1M HCl for 24h and rinsed again in doubly distilled water (Giesy and Wiener, 1977).

A Varian atomic absorption spectrophotometer (Spectra AA-10) was used to determine the total metal concentrations (dissolved plus suspended) of selected metals in the river water. Analytical standards for Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn were prepared from Holpro stock solutions. For the

analysis of strontium, 0.5 ml of a 2.682M potassium chloride (KCl) solution (200g KCl per litre distilled water) was added to the 50ml sample in order to suppress ionisation of strontium (Varian, 1989).

The metal concentrations in the river water were calculated as follows:

Metal concentration (ug/l) = AAS reading (ug/ml) x 1000

8.1.1.3 Results

Reference will be made to results of the first year, results of the second year and results of the third year. The first year refers to the period April 1990 to February 1991, the second year refers to the period April 1991 to February 1992, while the third year refers to the period April 1992 to February 1993. These periods include the seasons autumn (March, April), winter (June, August), spring (October) and summer (December to February).

In general, the readings were found to be slightly higher during the afternoon, except for the conductivity, which was slightly lower. The pH of localities 1 to 6 ranged from 8.3 to 8.7 on average over the three year period, while the pH of locality 7 (in the Selati River) were slightly lower, namely 7.8 to 7.9. The pH of the Pionier Dam (February, 1992) and the Hlanganini Dam (April 1992) were 8.1 and 8.6 respectively. As can be expected, the temperatures were the lowest during winter time (on average 19.2°C±1.4°C in the afternoon for the first year, 20.4°C±2.2°C in the afternoon for the second year and 17.0°C±1°C in the afternoon for the third year and the highest during spring and summer (on average 26.7°C±2.3°C in the afternoon for the first year, 30.6°C±1.5°C in the afternoon for the second year and 27.4±0.9°C in the third year. The overall temperatures were higher in the second than in the first and third (Tables 8.1 - 8.3) as a result of the low flow during the drought. The Olifants River, the Pionier Dam and the Hlanganini Dam seemed to be very well oxygenated with the dissolved oxygen concentrations ranging from 7.7±1.1 mg/l to 12.0±1.9 mg/l on average over the three year period. Locality 7, however, had a low dissolved oxygen concentration of 5.6±1.5 to 5.7±0.3 mg/l (Tables 8.1 - 8.3). Turbidity was not always an easy parameter to determine because of the secci-disc. At times, especially during the drier second year, the river was too shallow in order to take a measurement. In winter the water seemed to be the least turbid, while the highest turbidity occurred in summer, for example in December 1990, when values of 1 to 3 cm were measured. During this month, heavy rainfall occurred and the entire length of the river flowing through the Park was flooded. Due to this, locality 4 was inaccessible, and no readings could be taken for pH, temperature, oxygen and turbidity. Conductivity shows some differences in pattern for each year but in each case locality 7 had the highest conductivity which was therefore also higher than the conductivity of the water released from the Phalaborwa Barrage in the Olifants River. The conductivity of the water generally decreased as the river flows eastwards. After the Selati-Olifants confluence, but an increase to 95.9±64.2 mSm was recorded at locality 3 (near Balule) in the first year. This can mainly be attributed to the high value of 230 mSm recorded during December 1990. It is, however, clear that the water from the Selati River has an significant impact on the conductivity of the Olifants river as it enters the Kruger National Park (see values at locality 5; Tables 8.1 - 8.3).

The variables Na, Mg, Ca, F, Cl, SO_4 and K as well as the total alkalinity and TDS were the highest in concentration at locality 7 (in the Selati river)

and the lowest at locality 6 (located before the Selati-Olifants confluence) for the three years (Tables 8.1 - 8.3). Although no values were available for locality 1 in the first year, the general trends seemed to follow the same pattern as for the second year. The concentrations of these variables decreased from localities 7 and 1 (excluding locality 6). However, during the first year, the concentrations of Na, Mg, F, Cl, SO_4 and K slightly increased at locality 3 (near Balule) and Ca, along with the total alkalinity, increased slightly at locality 4. In the second year the total alkalinity also increased slightly at localities 3 and 4. During the third year (April 1992 - February 1993) the concentrations of Na, Mg, Ca, Cl and SO_4 slightly increased at locality 3, while F and K decreased when compared to locality 5 (Mamba weir) near the western border of the Kruger National Park. Noticeably was the low sulphate concentrations at the Pionier Dam (7 mg/l) and Hlanganini Dam (11 mg/l) in comparison with localities 2 to 7 (range 29 mg/l to 969 mg/l), during February 1992 and localities 2, 5, 6 and 7 (range 35 mg/l to 876 mg/l) during April 1992. For the first year, the general seasonal pattern observed for these variables indicated that the highest concentrations occurred during August and October at localities 2 to 6, and the lowest in February. At locality 7, two peaks of high concentrations were recorded in June/August and February, with the lowest concentrations in April. In the second year, the highest concentrations occurred in October, and another peak was formed in February. The lowest concentrations occurred in April (which is two months later than was the case for the first year). For locality 7 the highest concentrations were recorded in January/February with the lowest being in June. During the third year the highest concentrations of these variables were during April or June while the highest concentrations at locality 6 were recorded during October. In general, the concentrations of these values at locality 7 were very similar with no clear trend. Comparing the first two years, the concentrations were higher in the second than in the first year, with the exception of fluoride at localities 2 and 7, and sulphate at locality 2. The mean concentrations at localities 3 and 5 for the above variables were generally lower during the second year compared to the third year. At locality 7, the opposite trend was observed while at locality 6 some were higher (Na, Mg, Ca, Cl) and the other lower (Tables 8.2; 8.3).

Nitrite, nitrate and ammonia concentrations were determined as nitrogen. The highest concentrations occurred at locality 7 and the lowest varied between localities 2, 3 and 4 (Tables 8.1 - 8.3). In the first year, the concentrations decreased from localities 7 to 2, with a slight increase in concentration at locality 4. This increase, especially of nitrite and nitrate, can be attributed mainly to the concentration of 1.19 mg/l NO_3+NO_2-N recorded in December 1990. In the second year, the concentrations also decreased from localities 7 to 1, but nitrite and nitrate increased slightly at locality 1 (due to 0.66 mg/l recorded in February 1992), while ammonia increased slightly at locality 3 (due to 0.62 mg/l recorded in October 1991). During the third year the NO_3+NO_2-N and ammonium concentrations were the lowest at locality 3 (Table 8.3). Seasonal variations were not clear. The concentrations of the second year were generally higher than those of the first year, with the exception of nitrite and nitrate at locality 4. In the third year the nitrite and nitrate concentrations at localities 3 and 5 were higher, but at locality 7 lower when compared to the second year (Tables 8.2; 8.3). The ammonia concentrations were lower during the third year at localities 3 and 7 but higher at localities 5 and 6.

The phosphate concentrations (PO_4-P) ranged from 0.052 ± 0.038 mg/l at locality 7 to 0.009 ± 0.002 mg/l at locality 2 in the first year, and from 0.136 ± 0.167 mg/l at locality 7 to 0.022 ± 0.007 mg/l at localities 3 and 4 in

TABLE 8.1: MEAN VALUES (±SE*) OF SELECTED VARIABLES FROM THE OLIFANTS RIVER (APR 1990 - FEB 1991) COMPARED TO GUIDELINE VALUES FOR THE ENVIRONMENT BY KEMPSTER et al (1982), KÜHN (1991) AND CANADA (1987)

Variables	Locality							Guideline values			
	1	2	3	4	5	6	7	Kempster et al.		Kühn	Canada
								(min-max)	median		
*pH	8.3±0.3	8.4±0.3	8.3±0.4	8.6±0.3	8.3±0.03	8.4±0.2	7.8±0.1	6.0-9.0	6.5-9.0		6.5-9.0
Temperature (°C)	24.3±4.1	24.5±4.3	23.1±3.7	23.1±4.2	24.1±3.4	22.3±3.7	23.3±2.5		a	b	
Dissolved O_2 (mg/l)	9.2±2.1	8.2±1.8	8.9±2.1	10.4±1.1	12.0±1.9	9.8±0.6	5.7±0.3	>4>5.8	>5		>5
O_2 saturation (%)	114.5±17.8	105.8±19.7	111.3±25.6	119.3±14.8	115.5±24.4	109.3±15.3	66.5±2.9				
Turbidity (cm)	19.4±7.4	18.6±21.6	27.8±20.4	37.4±24.4	33.6±25.7	24.2±13.8	34.8±18.4				
Conductivity (mS/m)	32.5±9.3	61.8±23.7	95.0±64.2	64.0±27.5	69.0±29.9	44.4±10.6	224.8±39.5		a		
Na(mg/l)	N/A	48.8±18.8	49.8±19.9	45.2±22.7	54.8±25.2	44.0±9.2	160.0±22.1		500	100	
Mg(mg/l)	N/A	35.6±16.8	35.8±17.9	34.8±18.4	36.2±22.9	22.6±6.1	155.6±31.9		1500		
Ca(mg/l)	N/A	33.8±7.8	33.6±7.5	35.7±8.5	33.9±8.4	26.2±1.8	86.0±19.8		1000		
F(mg/l)	N/A	0.81±0.34	0.74±0.31	0.69±0.32	0.70±0.34	0.33±0.04	4.49±1.56	1.5-1.5	1.5	1.5	
Cl(mg/l)	N/A	57.1±22.9	60.4±24.5	53.0±29.1	63.2±31.7	50.7±11.1	187.7±28.7	50-400		100	
$NO_3(NO)_2$-N(mg/l)	N/A	0.05±0.07	0.06±0.08	0.25±0.43	0.10±0.09	0.19±0.17	0.72±0.23			c6	[0.06
SO_4-P(mg/l)	N/A	115.0±54.4	117.7±52.9	108.2±82.4	129.6±92.9	20.2±7.3	797.0±160.2		1400	250	
PO_4-P(mg/l)	N/A	0.009±0.002	0.012±0.007	0.018±0.010	0.016±0.004	0.011±0.003	0.052±0.038		0.1		
Alkalinity($CaCO_3$)(mg/l)	N/A	142.7±48.0	151.1±41.1	154.3±40.4	152.0±43.0	160.2±30.8	213.5±23.3	>20±20	>20		
Silica(mg/l)	N/A	6.18±1.10	6.29±1.09	6.46±1.78	7.53±1.72	7.57±1.67	14.56±2.13		50		
K(mg/l)	N/A	9.90±5.36	10.31±5.26	9.66±6.68	10.83±8.15	2.26±0.20	72.87±9.99		50	50	
NH_4-N(mg/l)	N/A	0.03±0.01	0.03±0.01	0.05±0.03	0.04±0.02	0.05±0.01	0.89±0.05	0.016-1.24	0.016	d001*	d/g 1.37-2.2
TDS(mg/l)	N/A	492±180	499±188	483±190	526±214	376±70	1600±282			800	
Chromium(µg/l)	298±214	393±258	572±339	508±228	398±221	518±239	478±145	10-100	50		2
Copper(µg/l)	43±22	62±29	73±55	56±29	48±28	55±36	97±43	5-200	5	50	h2-4
Iron(µg/l)	1506±403	6908±10230	26795±4668	5950±5480	2660±1799	18973±37989	3443±4966	200-1000	200	300	300
Manganese(µg/l)	34±23	208±264	703±1261	108±90	80±71	3160±6023	123±62	100-1000		50	
Nickel(µg/l)	153±50	177±55	278±200	226±79	212±100	262±136	228±170	25-50	50	50	h25-150
Lead(µg/l)	196±121	263±145	272±147	294±167	262±186	275±180	340±254	20-100	30	2	h1-7
Strontium(µg/l)	116±20	333±74	387±69	340±78	291±59	260±158	2387±1265		200000	e10000	
Zinc(µg/l)	405±404	750±611	607±567	556±463.	813±1360	658±689	983±1263	30-100	100	50	30

* Standard Error ◆-log[H^+]; N/A - Not available; a Depend on local conditions and life species present; b Within 5°C of background temperature (99.9% of the time)
c Nitrate; d Depend on pH [Ca^{2+}] and DO; e 90Sr; f Nitrite; g Ammonia; h Dependent on hardness;

TABLE 8.2: MEAN VALUES (±SE*) OF SELECTED VARIABLES FROM THE OLIFANTS RIVER (APRIL 1991 - FEBRUARY 1992) COMPARED TO GUIDELINE VALUES THE ENVIRONMENT FOR THE ENVIRONMENT BY KEMPSTER *et al* (1982), KÜHN (1991) AND CANADA (1987)

Variables	Locality								Guideline values			
	1	2	3	4	5	6	7	*Pionier Dam	Kempster *et al.*		Kühn	Canada
									(min-max)	median		
pH	8.5±0.2	8.7±0.1	8.6±0.1	8.5±0.3	8.7±0.2	8.6±0.1	7.9±0.1	8.1	6.0-9.0	6.5-9.0		6.5-9.0
Temperature (°C)	26.8±3.7	27.3±4.5	27.4±5.3	27.3±4.8	25.5±5.0	24.1±5.5	24.9±4.4	32.3		a	b	
Dissolved O_2 (mg/l)	8.4±2.0	9.4±1.2	8.9±0.7	8.5±1.2	9.1±1.4	10.0±2.5	5.6±1.5	9.3	>4>5.8	>5		>5
O_2 Saturation (%)	106.8±21.2	118.3±12.2	116.2±15.9	110.3±16.1	113.0±12.7	115.3±15.0	71.8±21.1	133				
Turbidity(cm)	18.0±1.0	36.0±11.1	49.7±19.6	51.7±29.1	50.5±33.5	46.2±12.8	73.3±20.6	29				
Conductivity (mS/m)	37.3±11.5	73.2±26.3	81.0±32.1	83.7±34.3	94.3±42.0	51.0±13.3	230.3±11.7	82		a		
Na(mg/l)	36.0±13.6	63.7±23.7	66.7±28.2	67.5±31.3	74.3±35.2	47.0±13.4	170.5±16.7	120		500	100	
Mg(mg/l)	16.8±7.0	37.0±16.3	46.3±22.3	49.0±26.1	57.7±32.1	27.4±7.9	176.7±9.0	26		1500		
Ca(mg/l)	23.6±3.8	33.7±6.5	38.5±9.1	38.0±7.6	41.2±13.3	30.0±2.9	88.3±9.8	22		1000		
F(mg/l)	0.32±0.12	0.65±0.36	0.77±0.37	0.97±0.53	1.18±0.68	0.46±0.15	4.10±0.41	0.8	1.5-2.5	1.5	1.5	
Cl(mg/l)	80.4±15.1	72.7±29.0	76.8±35.4	76.5±36.7	86.5±41.2	51.0±16.1	202.7±9.0	84	50-400		100	
NO_3+NO_2-N(mg/l)	0.16±0.25	0.07±0.04	0.10±0.09	0.12±0.11	0.16±0.16	0.17±0.15	0.81±0.43	0.04			≤6	f0.06
So_4(mg/l)	15.2±20.4	99.2±68.0	130.2±93.1	156.0±102.1	222.3±154.8	28.8±9.0	837.3±74.4	7		1400	250	
PO_4P(mg/l)	0.031±0.016	0.026±0.011	0.022±0.007	0.022±0.007	0.036±0.025	0.027±0.016	0.136±0.167	0.033		0.1		
Alkalinity($CaCO_3$) (mg/l)	140.2±22.8	186.0±39.6	195.0±55.3	182.2±43.7	176.2±40.9	189.6±38.1	234.8±20.3	330	>20->20	>20		
Silica(mg/l)	9.50±0.96	7.33±1.46	7.77±1.91	7.50±1.96	76.3±8.85	9.04±1.08	14.93±0.58	6.4		50		
K(mg/l)	7.42±2.05	11.27±5.84	12.70±7.56	14.25±9.31	19.53±13.08	2.74±0.84	80.48±2.69	21.8		50	50	
NH_4-N(mg/l)	0.05±0.01	0.06±0.03	0.14±0.21	0.04±0.00	0.06±0.03	0.05±0.01	0.27±0.18	0.04	0.016-124	0.016	d0.01e	d/g1.37 -2.2
TDS(mg/l)	310±78	545±187	617±247	624±262	711±339	419±90	180±133	603			800	
Chromium(µg/l)	147±120	76±71	114±76	112±91	73±65	95±76	78±63	53	10-100	50		2
Copper(µg/l)	49±25	36±25	39±13	25±11	28±12	29±13	36±11	53	5-200	5	50	h2-4
Iron(µg/l)	3671±3334	1755±943	18045±35157	8090±16115	15626±32733	12023±23892	1743±1376	1710	200-1000	200	300	300
Manganese(µg/l)	59±29	46±30	175±252	25±110	124±209	101±144	293±212	43	100-1000		50	
Nickel(µg/l)	62±46	53±40	58±41	47±40	58±35	53±42	63±49	30	25-50	50	50	h25-150
Lead(µg/l)	128±37	108±39	130±55	131±55	119±30	102±30	156±42	74	20-100	30	2	h1-7
Strontium(µg/l)	280±199	326±94	465±231	503±389	384±153	143±47	2730±659	N/A		200000	e10000	
Zinc(µg/l)	174±166	182±295	155±87	105±69	45±21	50±43	44±19	57	30-100	100	50	30

*Standard Error; *Only one value available; e -log(H+); N/A - Not available; a Depend on local conditions and life species present; b Within 5°C of back ground temperature (99.9% of the time) c Nitrate; d Depend on pH (Ca^{+2}) and DO; e 90Sr; f Nitrite; g Ammonia; h Dependent on hardness

TABLE 8.3 MEAN VALUES (SE ± •) OF SELECTED VARIABLES FROM THE OLIFANTS RIVER (APR 1992 - FEB 1993) COMPARED TO GUIDELINE VALUES FOR THE ENVIRONMENT OF KEMPSTER *et al.* (1982), KÜHN (1991) AND CANADA (1987)

Variables	Locality							Guideline values				
	1	2	3	4	5	6	7	Hlangan Int Dam	Kempster *et al*		Kühn	Canada
									(min-max)	max/ta g		
ϕpH	N/A	N/A	8.3±0.1	N/A	8.4±0.2	8.0±0.1	8.4±0.3	8.6	60-90	65-90		65-90
Temperature (°C)	N/A	N/A	23.9±4.5	N/A	25.0±3.5	23.1±4.0	23.7±5.3	28.1		a	b	
Dissolved O_2 (mg/l)	N/A	N/A	7.7±1.1	N/A	7.8±1.0	5.6±0.6	9.0±1.2	10.3	>4>58	>5		>5
O_2 saturation (%)	N/A	N/A	90.3±16.5	N/A	90.6±5.6	74.5±14.6	107.5±14	122.0				
Turbidity(cm)	N/A	10	29.0±10.5	N/A	21.9±15.7	45.5±14.1	22.6±11.3	21				
Conductivity (mSm)	N/A	N/A	147.6±94.5	N/A	153.5±110.4	49.1±19.5	2432±153.7	39		a		
Na(mg/l)	N/A	N/A	122.3±79.0	N/A	81.0±56.2	39.6±23.5	169.5±27.6	25		500	100	
Mg(mg/l)	N/A	N/A	96.1±72.6	N/A	715±61.9	20.5±7.7	170.0±43.0	10		1500		
Ca(mg/l)	N/A	N/A	45.5±44.6	N/A	37.2±14.0	298±9.8	81.5±10.3	26		1000		
F(mg/l)	N/A	N/A	1.8±1.3	N/A	2.1±1.7	0.85±2.6	3.8±1.1	0.02	15-15	15	15	
Cl(mg/l)	N/A	N/A	123.0±80.6	N/A	101.0±72.5	43.5±28.9	175.5±22.8	19	50-400		100	
NO_3+NO_2-N(mg/l)	N/A	N/A	0.12±0.15	N/A	0.10±0.16	0.33±45	0.54±0.31	0.04			c6	f0.06
SO_4(mg/l)	N/A	N/A	430.5±394.0	N/A	395.0±333.8	40.5±25.5	821.3±2250	11		1400	250	
PO_4-P(mg/l)	N/A	N/A	0.08±0.11	N/A	0.037±0.023	0.038±0.035	0.10±0.08	0.06		01		
Alkalinity($CaCO_3$) (mg/l)	N/A	N/A	213.3±88.3	N/A	180±69	145.5±50.6	241.0±22.5	150	>20>20	>20		
Silica(mg/l)	N/A	N/A	6.5±1.3	N/A	6.5±4.0	6.9±1.1	13.9±2.0	9		50		
K(mg/l)	N/A	N/A	32.0±25.2	N/A	38.2±33.3	3.6±2.1	74.7±22.2	14.0		50	50	
NH_3-N(mg/l)	N/A	N/A	0.095±0.054	N/A	0.13±0.07	0.1±0.08	0.14±0.07	0.23	0.016-124	0.016	d001	dg13722
TDS(mg/l)	N/A	N/A	1118±759	N/A	1076±766	376±146	1790±365	296			800	
Chromium(µg/l)	N/A	N/A	19±14*	N/A	15**	37**	28±7*	N/A	10-100	50		2
Copper(µg/l)	N/A	N/A	15±6	N/A	13**	12**	28±3*	N/A	5-200	5	50	h2-4
Iron*µg/l)	N/A	N/A	1299±130*	N/A	770**	2330**	566±99*	N/A	200-1000	200	300	300
Manganese(µg/l)	N/A	N/A	145±10*	N/A	30**	64**	368±8*	N/A	100-1000		50	
Nickel(µg/l)	N/A	N/A	49±13	N/A	40**	30**	63±33*	N/A	25-50	50	50	h25-150
Lead(µg/l)	N/A	N/A	150±32**	N/A	43**	99**	177±36*	N/A	20-100	30	2	h1-7
Zinc(µg/l)	N/A	N/A	89±14	N/A	55**	67**	147±45*	N/A	30-100	100	50	30

ϕ-LOG(h+); N/A - not available; a Depend on local conditions and life species present; b Within 5°C of back ground temperature (99.9% of the time); c Nitrate; d Depend on pH (Ca^{+2}) and DO; e 90Sr; f Nitrite; g Ammonia; h Dependent on hardness;

* Mean values for June and April 1992
** Values for June 1992 only
· Standard Error

the second year (Tables 8.1; 8.2). During the third year similar range of concentrations were recorded (Table 8.3). The silicon concentrations ranged from 6.18±1.10 mg/l at locality 2 to 14.56±2.13 mg/l at locality 7 in the first year. In the second year the concentrations decreased from 14.93±0.58 mg/l at locality 7 to 7.33±1.46 mg/l at locality 2, whereafter it increased to 9.50±0.96 mg/l at locality 1 (Tables 8.1 and 8.2). During the third year the silicon concentration were also the highest in the Selati River (locality 7) with much lower mean concentrations at the other localities (Table 8.3).

Pronounced variations in the metal concentrations precluded unambiguous interpretation of the results (Tables 8.1 - 8.3). In the first year Cr, Fe and Ni had the highest concentrations at locality 3; Cu, Pb, Sr and Zn at locality 7 and Mn at locality 6. All the metals were the lowest in concentration at locality 1. The iron concentration seemed to increase tremendously at most localities during December 1990 after the heavy rainfalls. These increased concentrations varied from 5680 ug/l at locality 5 to 129240 ug/l at locality 3. In the second year the highest concentrations of Cr and Cu were recorded at locality 1, and the lowest at localities 5 and 4 respectively. In October 1991 very low concentrations of chromium were recorded failing below the minimum detection limit of 6 ug/l. The iron concentrations ranged from 1743.3±1376.1 ug/l at locality 7 (which is similar to the concentration found in Pionier Dam) to 18045.0±35156.5 ug/l at locality 3, while the zinc concentrations ranged from 44.0±19.1 ug/l at locality 7 to 181.8±295.3 ug/l at locality 2. The concentrations of nickel, lead, strontium and manganese were the highest at locality 7 and the lowest at localities 4, 6, 6 and 2 for each metal respectively (Table 8.2). In October 1991 and January 1992 the nickel concentrations were below the minimum detection limit which is 10 ug/l. The concentrations of iron (1710 ug/l), manganese (43 ug/l) and lead (74 ug/l) in the Pionier Dam were lower than the concentrations recorded at the other localities during February 1992. In general, the metal concentrations were lower in the second than in the first year, except for copper at locality 1; iron at localities 1, 4 and 5; manganese at localities 2, 5 and 7; and strontium at localities 1 to 5 and 7. The trends regarding strontium should, however, be treated with caution, as there is insufficient data for this metal. The metal concentrations presented in Table 8.3 do present the data for all the sampling months and can therefore not be used for realistic comparisons. This data would be available in the next few months making comparisons possible. Nevertheless, it appears that the concentrations would be in the same range as for the previous two years.

8.1.1.4 Discussion

In evaluating the water quality of the study area, three sets of guidelines were used: those of Kempster *et al.* (1982), those proposed by Kühn (1991) specifically for the Olifants River and the Canadian guidelines (Environment Canada, 1987). According to these guidelines, there were chemical constituents in the water of the study area that exceeded the guideline limits (Table 8.1 - 8.3), especially in the Selati River (a tributary of the Olifants River). Variables of special concern are sodium, fluoride, chloride, sulphate, potassium, the total dissolved salts and the metal concentrations (except strontium). This situation would render the Selati River at locality 7 unfit for aquatic life and might be one of the reasons why some fish species, eg. *B. marequensis*, was only occasionally captured there. Furthermore, the Selati River had a negative influence on the water quality of the Olifants River after their confluence. The concentrations of most parameters detected at localities 2 to 5 were higher than the concentrations

detected at locality 6 (located before the Selati-Olifants confluence). In most cases (except for the metal concentrations), the concentrations of the variables decreased from the western side of the KNP to the eastern side. This phenomenon can be attributed to the dilution of the water, caused by the tributaries of the Olifants river. At locality 3 (near Balule) an increase in concentration could sometimes be detected, especially during the first year. The explanation for this might lie in the frequent occurrence of reed beds in that part of the river. Reed beds are known for their cumulative capacity of chemical substances or toxicants (like metals), but during a flood reeds may get deposited on the bottom of the river, from where the toxicants (the metals) may eventually be released again into the river water during the decay process (De Wet *et al.*, 1990). The toxicant concentration in the river water will therefore increase again.

The mean sodium, fluoride, sulphate, chloride, potassium and total dissolved salt concentrations detected at Mamba during April 1990 to February 1991 were compared to the mean concentrations detected in the previous six years (October 1983 - October 1989) and a decrease in concentrations was found. On the other hand, an increase in the mean concentrations was detected during April 1991 to February 1992 and April 1992 to February 1993, when compared to the existing six-year record of Van Veelen (1990). The most probable explanation for the decrease and increase of the mean concentrations in the first year is the difference in rainfall pattern of the three years. In the first year the floods contributed to the dilution of the chemical constituent concentrations, but because of the drought in the second year, and to a serious extent in the third year, no dilution could take place and the concentrations have therefore increased.

Conductivity has an influence on the growth rate and life expectancy of fish, depending on the species sensitivity and conductivity level present (Hellawell, 1986). The effects that TDS concentrations have on aquatic species are, however, due to sudden changes in the concentrations, rather than absolute values of the determinants. Some macrophytes sensitive to changes will, for instance, be replaced by less sensitive species at high TDS concentrations of 1500 - 3000 mg/l (Theron *et al.*, 1991). Such high concentrations were detected at locality 7 (1679 ± 282 mg/l, 1808 ± 133 mg/l and 1798 ± 365 mg/l from years 1, 2 and 3 respectively), exceeding the guideline limits of 800 mg/l (Kühn, 1991) and 350-550 mg/l TDS (Department of Water Affairs, 1986) by far. Therefore the macrophyte species status in the Selati River needs further investigation.

At the Pionier Dam and Hlanganini Dam, the recorded TDS concentrations were 683 mg/l and 276 mg/l respectively. The fairly high TDS concentrations at the Pionier Dam is higher than the recommended limit of 350-550 mg/l TDS (Department of Water Affairs, 1986). One of the reasons might be evaporation, leading to increased concentrations of dissolved mineral salts (Department of Water Affairs, 1986). The ionic composition seemed to be dominated by sodium, chloride, potassium, carbonate and bicarbonate. The mean TDS concentrations at localities 2 to 5 ranged from 545 ± 187 mg/l to 710 ± 339 mg/l in the second year (April 1991 - February 1992), which were slightly higher than the TDS concentrations recorded for 1983 to 1989 in the Olifants River (Van Veelen, 1990). As already mentioned, this increase can be attributed to the fact that April 1991 to February 1992 was a very dry period. During dry periods, which is also case in winter time, the lower flows recorded at the barrage, combined with the almost continuous effluent flow in the Selati River, result in poorer water quality in the Lower Olifants River (CSIR, 1990). The major sources responsible for the high TDS concentrations are the effluents

(1660 mg/l) and seepage (1660 mg/l) from a phosphorus extraction mining company (CSIR, 1990). Moderate TDS loads are contributed by the storm water overflow of a copper extraction mining company *via* Loole Creek (1250 mg/l) and seepage from a magnetite tailing dam (1200 mg/l). Upstream inflow also contributes heavily to the daily TDS load in the Lower Selati River (1280 mg/l). The impact of these loads on the TDS load of the Olifants River, inside the Kruger National Park is also clearly shown by the data of the third year (Table 8.3).

Sulphate is the anionic component mainly responsible for the high TDS concentrations in the Olifants River (Moore *et al.*, 1991). The sulphate concentrations recorded at locality 7 exceeded one of the proposed guideline values, namely 250 mg/l by Kühn (1991). As the concentrations were above 600 mg/l, the water should be considered unfit for household purposes. Sulphates may give rise to gastro-intestinal irritation (Department of Water Affairs, 1986). The mean sulphate concentrations at localities 1 to 5 were fortunately well below 600 mg/l required for the main uses of the Lower Olifants River after entering the KNP (game watering, aquatic ecosystem maintenance and the supply of domestic water to the Olifants, Satara and Balule rest camps). Further downstream, the Massingir Dam inside Mozambique also supplies some water for domestic use and game watering (CSIR, 1990). High sulphate concentrations have a definite effect on fish (Burnham and Peterka, 1975). The sulphate concentrations have a definite effect on fish (Burnham & Peterka, 1975). The increased mortality of fathead minnows (*Pimephales promelas*) was attributed to water being high in sodium and sulphate concentrations. This might be one of the reasons why only a few fish species were captured in the Lower Selati River.

The mean fluoride concentrations at locality 7 (4.5 ± 1.6 mg/l, 4.1 ± 0.4 mg/l and 3.8 ± 1.1 mg/l for years 1, 2 and 3 respectively) were much higher than the concentrations recorded at the other localities and exceeded the limit of 1.5 mg/l. Studies on the ecological significance of exposure of aquatic animals to fluoride are limited (Rose and Marier, 1977). However, when fry of *Catla catla* were exposed to different fluoride concentrations for 96 hours, protein synthesis was inhibited from 1.2 mg/l fluoride upwards, glycogen and iron decreased from 4.3 mg/l fluoride upwards and the lipid metabolism was altered from 7.2 mg/l fluoride upwards (Pillai and Mane, 1984). Fluoride toxicity is influenced, however, by water hardness. High calcium concentrations suppress fluoride concentrations by precipitating insoluble calcium fluoride (Smith *et al.*, 1985). LC_{50} values (96-hour) for fluoride toxicity do exist, ranging from 51 to 460 mg/l - depending on the species and conditions (Smith *et al.*, 1985). However, the available data suggests that a consensus about the maximum safe level of fluoride concentration for fish in natural waters of varying hardness has not yet been achieved. It is not known if the continuous exposure of hippopotami to these high fluoride concentrations will result in dental fluorisis (mottling) in these animals, but must be considered.

Chlorine (a gas) is a highly toxic substance and is more toxic than the chloride ion. Chlorine gas forms hypochlorous acid (HOCl) or its conjugated base (OCl^-) in water, which are commonly called "free chlorine" (Heath, 1987). In the presence of ammonia, some or all of the free chlorine is converted into monochloramine (NH_2Cl) which is known as "combined chlorine". Free chlorine is more toxic, but combined chlorine is more stable and therefore remains active longer (Heath, 1987). The toxicity of chlorine depends on the total amount of chlorine present whether complexed or not (Merkens, 1958). Chlorine causes the epithelium of fish

gills to slough off, which leads to mucus production and the eventual clogging of the gill lamellae (Cairns *et al.*, 1975).

However, chlorides occur in all natural soil and water. As salinity increases, the chloride concentrations also increase (Hahne and Kroontje, 1973). At all the localities the chloride concentrations were above 35 mg/l which means that the MCl^+ species of Zn(II), Cs(II) and Pb(II) will then appear (Hahne and Kroontje, 1973). However, at a pH of 8.5 (which is the case at some localities), competition between the hydroxyl and chloride complexes will arise, depending on the chloride concentrations. Therefore, in order to determine exact distributions of metals, all other reactions such as organic complexes, carbonate formations and pH ranges should be considered. Chloride can, however, be regarded as one of the most mobile and persistent complexing agents with regard to metals and may, under certain circumstances, be of great significance in determining metal distribution in the environment (Hahne and Kroontje, 1973).

Although the sodium and potassium concentrations at locality 7 were higher than the guideline values and were also fairly high at Pionier Dam, the lack of sufficient research data on the effects of elevated sodium and potassium concentrations on aquatic life precludes discussion thereof. However, fish mortalities in the Olifants River have previously been associated with high levels of K, Cl, SO_4, Mg and Na. Elevated potassium levels are thought to have been the actual cause of death (Moore, 1990). Potassium and sodium seemed to follow the same trend: at localities 2 to 6, a sudden increase in concentration was detected during October - especially in the second year. At locality 7, however, no sudden increase in concentration could be detected; the changes were more gradual throughout the year. These findings might be explained by the fact that 1991 was a very dry year and only in October 1991 did the first rains fall in the catchment area. The result was an increase in flow during that time, accompanied by the leaching of salts from areas adjacent to the catchment into the river water. Except for potassium and sodium, magnesium, chloride, sulphate, alkalinity and TDS also showed a similar trend.

Ammonia is produced as a metabolite from the natural degradation of nitrogenous organic material present in all surface waters (Ellis, 1989). However, high levels reach waters as fertiliser components and through effluents from industries and sewage works. Ammonia can exist in two forms in water namely as the ammonium cation (NH_4+) or as free ammonia (NH_3). The equilibrium existing between the ammonium cation and ammonia (NH_4- + OH- < > NH_3 + H_20) depend on pH and temperature (Boyd, 1982). The less toxic ammonium ion (NH_4+) exists at lower pH values, while the more toxic ammonia (NH_3) is present in more alkaline conditions. Therefore as the temperature and pH increase, the percentage toxic free ammonia increases. Even a small increase in pH, from 7 to 8, will increase the toxicity of ammonia approximately 10 fold. In order to obtain the free ammonia concentration, the percentage free ammonia for the specific temperature and pH (Table 2.12 in Boyd, 1982) are multiplied nitrogen concentration. In the study area, the pH tended to be more alkaline and the temperatures were high. Therefore the ammonia concentrations should be carefully monitored. In addition to its toxicity, ammonia may also impose an additional oxygen demand on the receiving stream as a result of its potential to be oxidised by autotrophic bacteria to nitrite and then to nitrate (Ellis, 1989).

Freshwater plants are more resistant to ammonia than are invertebrates and invertebrates are in turn more resistant than fish. Fish exposed to sublethal

ammonia concentrations experience reduction in growth rate and morphological development, pathological changes in the tissue of kidneys, livers and gills and reduction in the proportion of successful hatchings (Ellis, 1989). A more notable effect is a diuretic response whereby the fish increases its urine production as a result of its increased permeability, in other words, more water permeates the body (Lloyd and Orr, 1969). An indication of sublethal concentrations might be 0.006 - 0.34 mg/l NH_3, for Smith and Piper (1975) detected histological effects at these concentrations. This means that the calculated concentration of NH_3 at locality 7 might have been sublethal. However, in addition to pH and temperature, there are other factors affecting the toxicity of ammonia. A decrease in dissolved oxygen will increase the toxicity of ammonia, but an increase in [$C0_2$] in water up to a level of approximately 30 mg/l appears to decrease the toxicity (Ellis, 1989). Copper salts apparently combine additively with ammonia in their toxic effects (Herbert and Van Dyke, 1964), while calcium reduces the toxicity of ammonia.

Nitrite ($N0_2^-$) and nitrate ($N0_3^-$) are two forms of total nitrogen (TON). An imbalance in the nitrification reaction can lead to the accumulation of nitrite. However, organisms that oxidise ammonia to nitrite and those that oxidise nitrite to nitrate coexist; nitrite therefore does not accumulate in natural environments as a result of nitrification (Boyd, 1982). The reduction of nitrate by bacteria in anaerobic sediments or water can also produce nitrite. In addition to nitrates being present as a result of nitrification, it can also be present in treated effluents being discharge into the river, or in the run-off from agricultural land containing fertiliser (Ellis, 1989). According to Ellis (1989) the concentrations of TON in drinking waters should be restricted to less than 11.0 mg/l (as N). In the study area TON concentrations were less than 1.0 mg/l (as N), and therefore comply with the acceptable standards for drinking water. The low TON values can be ascribed to the abundance of phytoplankton occurring in the river during the course of this study (Seymore, *pers. obs.*). Phytoplankton represents the main factor responsible for a decrease in nitrate and nitrite concentrations (Saad, 1987).

Nitrite poisoning in fish is referred to as "brown blood disease" for nitrite absorbed by fish reacts with haemoglobin to form methaemoglobin (a brown substance). This disease can lead to hypoxia and cyanosis, since methaemoglobin is not an effective oxygen carrier (Boyd, 1982). The toxicity of nitrite to fish can be reduced by the addition of calcium (Wedemeyer and Yasutake, 1978) and chloride (Perrone and Meade, 1977; Tomasso *et al.*, 1979). These substances do occur in moderate to high concentrations in the study area, with the result that nitrite toxicity will be reduced if elevated nitrite levels should occur.

Phosphorus in surface water will mostly be present either as orthophosphates or as polyphosphates. All polyphosphates in water will, however, revert in time to orthophosphates (Ellis, 1989). The phosphate levels in the Lower Olifants River were generally around 0.02 mg/l. Only at locality 7 (in the Selati River) higher levels of 0.136 ± 0.167 mg/l and 0.10 ± 0.08 mg/l in average were detected in the second and third year, respectively. Although phosphates are non-toxic, they are indicative of pollution from detergents, fertilisers, sewage, etc (Kempster *et al.*, 1982). According to a survey done by the CSIR (1990), orthophosphate ($P0_4$-P) concentrations in the seepage and effluent discharged into the Selati River by a phosphorus extraction mining company were sufficiently high to cause moderate eutrophication problems. This statement can be confirmed by personal

observations, for during the course of the study the aquatic plants and algae seemed to increase, especially at localities 5 (Mamba weir) and 4.

Calcium is an integral part of bone and is non-toxic (Kempster *et al.*, 1982). It is relevant to this study because of the influence it has on metal toxicity. Calcium reduces the toxicity of metals to fish by hindering their adsorption. According to Mason (1991), calcium is antagonistic to lead, zinc and aluminium. The calcium ion competes with other metal cations for binding sites on the gill surface, thereby decreasing the direct uptake of cationic metals by fish. In contradiction to this, Giesy and Alberts (1984) pointed out that although $Ca^{2}+$ may occupy sites on the organic ligand, the binding strengths are low compared to transition metals. Therefore, $Ca^{2}+$ is not capable of blocking sites in the presence of other metal ions and will be exchanged for by the other metals on the organic ligands.

Alkalinity in water represents its ability to neutralise strong acids. It is caused mainly by the presence of bicarbonates, carbonates and hydroxyl ions which are formed as a result of the interaction of carbon dioxide in water with basic materials such as the calcium carbonate of chalk or limestone in soils and rocks (Ellis, 1989).

The buffering capacity of the study area seemed to be fairly good, as the alkalinity ranged between 140 ± 23 and 235 ± 20 mg/l $CaC0_3$. The alkalinity of natural water is rarely more than 500 mg/l as $CaC0_3$ (Kempster *et al.*, 1982). Total alkalinity is sometimes confused with total hardness. Total hardness refers to the concentration of divalent metal ions in water, expressed as milligrams per litre of equivalent calcium carbonate (Boyd, 1982). Fortunately, total hardness and total alkalinity have similar concentrations in most waters (Boyd, 1982). The water of the Lower Olifants River would be considered hard and most metals are less toxic in hard water than in soft water (Hellawell, 1986).

Temperature changes can have a major impact on fish life. One example is the low temperature discharges from impoundments that may trigger spawning (Theron *et al.*, 1991). According to the guidelines proposed by Kühn (1991), the temperature of the water being discharged into the Olifants River at Phalaborwa Barrage, for instance, should be within 5°C of the background water temperature. Another example of fish being affected by temperature changes, happened on the 25th of October 1989, when a hail storm caused a sudden decline in temperature. This incident was thought to have been the actual reason for fish mortalities in the Olifants River (Deacon, pers. comm.). It is therefore not the temperature itself that causes concern, but the rate of change of water temperature. Although a sudden temperature change was detected in the study area from August to October, it is of no value, since information like this should be recorded on a daily basis.

The effect of temperature on toxicity is complex. Elevated temperatures do not always increase toxicity of substances. The toxicity of some is increased and that of others decreased by an increase in temperature (Alabaster *et al.*, 1972). Temperature influences the rate of metabolic processes, including the uptake, metabolism and excretion of poisons. Increased temperature will increase the oxygen requirements of aquatic organisms, while decreasing the solubility of oxygen in water. The properties of the poison itself may also be directly influenced by temperature (Abel, 1989). In the literature contradictory results are reported to toxicity effects, especially on the effect temperature has on zinc toxicity. It would

therefore be presumptuous to draw conclusions about temperature effects on toxicity.

Dissolved oxygen (DO) is essential to all aquatic life. For warm water species the target guideline value is >5 mg/l (Kempster *et al.*, 1982). At locality 7 the mean DO concentration was just above 5 mg/l, namely 5.7±0.3 mg/l and 5.6±1.5 mg/l and 5.6±0.6 mg/l for the three sampling periods. However, as temperature increases, the DO decreases. This effect could clearly be seen at locality 7 in August 1991 and October 1991 when the DO decreased from 3.9 mg/l to 1.8 mg/l in the morning, with an increase in temperature from 19.0°C to 25.5°C. Although 3.9 and 1.8 mg/l DO concentrations are very low, time is the deciding factor in the survival of fish species. Warm water species would survive 3 to 5 mg/l DO if they are not exposed to it for more than eight hours out of any 24-hour period, and some species would survive 1 to 3 mg/l DO if they are not exposed to it for more than a few hours (Train, 1979). Species not able to resist low DO concentrations would therefore not occur in the Selati River at locality 7, which might be another reason why only a few fish species were detected there. The mean DO concentrations of the other localities ranged from 8 to 12 mg/l. According to Ellis (1989) it is rare to find more than 8 to 10 mg/l of oxygen, even under optimum conditions, since the amount of oxygen dissolved from the air into water is small. Higher oxygen concentrations can, however, occur, due to photosynthetic oxygen produced under the influence of sunlight by algae and other aquatic plants, as was observed for the locality at Mamba.

The effects of dissolved oxygen on toxicity have been less widely investigated, but in general low dissolved oxygen concentrations appear to cause an increase in the toxicity of poisons (Abel, 1989). For instance, the American Petroleum Institute (1983) established that chromium concentrations increased in the gills and kidneys of the bluegill sunfish (*Lepomis macrochiris*) as the dissolved oxygen decreased. The growth of fish is extremely sensitive to reduced oxygen levels and fish eggs develop more slowly with the lowering of oxygen concentrations (Sprague, 1971).

The pH of the water in the study area seemed to be very stable and well within the target guideline range of 6 to 9. A slight decrease in pH was observed in December 1990. The reason is that under high rainfall conditions, leaching is more pronounced and systems usually have lower pH values (Hahne and Kroontje, 1973). Aqueous pH can greatly influence the toxicity and bioavailability of cationic metals to fish. At low pH, hydrogen ion can compete for metal binding sites on particle surfaces and solution ligands (thereby increasing metal bioavailability) and on biological membranes such as the gill surface (potentially reducing metal uptake and toxicity). Hydrogen ion can also act as a stress factor, depleting gill calcium and causing ionoregulatory stress (Spry and Wiener, 1991). The toxic action of hydrogen ions on goldfish has been ascribed by several authors to the precipitation of mucus on the gill epithelium causing death by suffocation, or by precipitation of proteins within the epithelial cells (Ellis, 1937; Westfall, 1945). If waters are more acidic than pH 6.5 or more alkaline than pH 9 to 9.5 for long periods, reproduction and growth of fish will diminish (Swingle, 1961; Mount, 1973).

Mining and industrial effluents are the general sources of elevated metal concentrations in river water. It is usually the ionic forms that produce the immediate fish mortalities, while complexed metal compounds tend to act by accumulation in the body tissue over a considerably longer period (Ellis, 1989). The approximate order of the toxicity of metals, which is based on

published data, is given in Table 8.4 (Hellawell, 1986). Several factors can influence their toxicity, for instance their concentration in the water, the form in which they are present (ionic, complexed or organic), the difference in species sensitivity and life stage sensitivity to toxicants, the type and concentration of other toxicants present (the effect being additive, antagonistic or synergistic) or the conditions and quality of the water itself (factors such as dissolved oxygen, water hardness, temperature and pH). Generally toxicity increases with decreasing dissolved oxygen and pH and declines with increasing hardness (Ellis, 1989). There are, however, a few exceptions, like zinc, for which the effects of certain parameters are uncertain. The effects that elevated metal concentrations have on fish will be discussed in the following chapters.

Table 8.4 TENTATIVE TABLE OF THE APPROXIMATE ORDER OF TOXICITY OF METALS. (FROM HELLAWELL, 1986)

Highly toxic					Decreasing toxicity							
Hg												
	Cu	Cd Zn	Au?	Ag?	Pt?							
			Sn	Al Ni	Fe^{3+} Fe^{2+}							
						Ba						
							Mn Co	Li K	Ca	Sr Mg	Na	

If the factors influencing metal toxicity are excluded for the moment, it is clear from Table 8.1, 8.2 and 8.3 that the metal concentrations of the selected metals in the water of the study area are mostly higher than the recommended guideline values (except for strontium). The assumption was made that the authors of the guidelines refer to total metal concentrations and not bioavailable or soluble metal concentrations. The specific effects on aquatic biota of the metals studied will be dealt with in the section on bioaccumulation.

There is a continuous interaction between the water and the sediment columns, depending on factors such as the water pH. When the pH is alkaline, in other words more hydroxyl ions (OH^-) are present than hydrogen ions (H^+), insoluble metal hydroxyl complexes will form. However, when rainfall occurs, as was the case in December 1990, the hydrogen ion concentration will increase. The solubility of the metals will increase slightly and an increase in the water metal concentrations may be detected. The iron concentrations in the water increased considerably in December 1990 but increasing solubility was not the only reason for this phenomenon. Weathering of underlying rock formations, especially basalt, will produce iron (Dury, 1981). As locality 3 (near Balule) is underlain by basalt, the highest iron concentrations were detected there. Iron is also a highly abundant element and therefore, of all the metals investigated, iron was found to occur in the highest concentrations. The copper and strontium concentrations in the Selati River, were much higher than the concentrations in the Olifants River. This indicates that these two metals originate from a local source which is not connected to the Kruger National Park.

A factor playing a major role in metal distribution is, as mentioned earlier, rainfall. A noticeable difference could be seen between the wetter first year and the drier second year. In the first year peaks of the metal concentrations in the water

occurred at localities 7 and 3. Peaks at locality 7 can mainly be attributed to mining and industrial effluents, while peaks at locality 3 might be attributed to the frequent occurrence of reed beds, accumulating the metals and releasing them again when decaying. In the second year, peaks also occurred at localities 7 and 3, but with the addition of locality 1 (in the Letaba River). It might be that because of the drought, the river flow in the Olifants River was very low and therefore the carrying capacity of the water volume for metals decreased. By contrast, the Letaba River might have had a stronger flow, thus rendering higher solubility and concentrations of metals.

8.1.1.5 Conclusions

The mining and industrial activities in the Phalaborwa complex definitely have an influence on the water quality of the lower Selati River. The sodium, fluoride, chloride, sulphate, potassium, TDS and metal concentrations (except for strontium) were higher than the guideline values of Kempster *et al.* (1982), Kühn (1991) and Canada (Environment Canada, 1987). The water quality of the Lower Olifants River after the Selati-Olifants confluence was also influenced by activities upstream of the Selati River, especially localities 5 (Mamba weir) and 3 (near Balule). At Mamba the mean TDS, potassium, chloride, sulphate fluoride adn sodium concentrations reported for 1991/1992 were very similar or slightly higher but generally higher for the 1992/1993 period than the mean concentrations reported for 1983 to 1989 by Van Veelen (1990). However, dilution caused by smaller tributaries decreased the toxicant concentrations to levels that, with the exception of the metal concentrations, comply with the recommended guideline values. The mean metal concentrations (excluding strontium) were higher than the guideline values at all the localities. The large variance detected in the metal concentrations of the water points to the need for more frequent monitoring of this area.

It is recommended that a more intensive study should be undertaken specifically on the water and of the study area. The metal levels in particular should be studied, as well as the effect thereof on aquatic life. It will be necessary to combine the field study with experimental work, in order to determine the effects of the physical and chemical environment on the metal toxicity. This is very important, for the water in the Lower Olifants River is hard and alkaline and will definitely have an influence on the metal toxicity. Monitoring can be limited to localities 2, 3, 5, 6 and 7. Special attention should be given to locality 3, in order to determine the role of the reed beds. The interaction between water and sediment with regard to metal distribution should be investigated, as well as seasonal effects on toxicity and metal distribution.

For future management it is recommended that drastic measurements should be taken in order to reduce the impact of mining activities on the water quality of the Selati River, because it is not only the water quality of the Selati River that is being influenced, but also the water quality of the Lower Olifants River (especially during low flow periods). If, for some or other reason, the water quality of the Selati River cannot be improved, it should at least be maintained at its present status. A further degradation in water quality cannot be afforded.

8.1.1.6 References

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8.1.2 *Development of an aquatic toxicity index to assist in the management of water quality*

8.1.2.1 Introduction

When one considers the demand for water by the Kruger National Park, it is evident that water is required for more than one use, i.e. for potable use, game watering and ecosystem (aquatic and riparian) maintenance (Moore 1990a). In order to facilitate proper water quality management, it is necessary to develop a comprehensive water quality management plan for the river. According to Van der Merwe and Grobler (1990) the development of such a plan requires a thorough understanding of the fate and effects of pollutants in the environment. It is furthermore important to have reliable information on the trends and status of important water quality determinants in these systems.

In the field of water management, scientists, engineers and managers are often confronted with a large array of data which can be totally overwhelming. In order to convey the interpretation of the data in a simplified manner, a set of numbers or a single number may be devised to integrate the data pool (Smith, 1990). This number is known as a water quality index. The use of a water quality index (WQI) would make it possible to bridge the gap between the extremes of water quality monitoring and reporting (House, 1989).

It is therefore clear that the development of a WQI, or more specifically, an aquatic toxicity index could be used as an additional tool in the operational management of the water quality in the Olifants River within the Kruger National Park boundaries. With the development of such a WQI emphasis was placed on the development of an index reflecting the toxicological effects of selected water quality variables on the aquatic environment. An aquatic toxicity index (ATI) was therefore developed to reflect the effect of the water quality on a specific water use i.e. the aquatic environment.

8.1.2.2 Theoretical basis for the ATI development

The term water quality is seldom defined and in many instances the water quality index produced is an attempt to pool several aspects of water uses, including pollution (House and Ellis 1980), the reason for this being that different water users have different water quality requirements. In the development of the current index the toxic effects of the water quality on aquatic organisms, specifically fish, were taken into account. The index was only developed for fish because of the extensive toxicity data base available for fish. We envisage incorporating the toxic effect of the chosen water quality variables on aquatic invertebrates into the ATI in the future.

The question that was addressed when developing the ATI was, "how suitable is the water for the continued existence of the fish populations in the Olifants River?". The three stages in the development of a water quality index (House, 1989) that were followed during the development of the current ATI were: determinant selection; determinant transformation; determinant aggregation.

Determinant selection

The determinants that were chosen comprised some of the most commonly measured attributes of natural water as measured by the Department of Water Affairs and Forestry (DAWF). The determinants chosen included physical, chemical and potentially hazardous trace and heavy metals. Each determinant had to conform to three basic requirements, i.e. (a) was the determinant data readily available at frequent intervals, (b) are the determinants considered important indicators of water quality change and (c) were there maximum permissible water quality criteria available for the determinants.

The physical water properties selected were pH, dissolved oxygen and turbidity. The chemical determinant included ammonium, total dissolved salts, fluoride, potassium and orthophosphates while the potentially hazardous metals chosen were total zinc, manganese, chromium, copper, lead and nickel concentrations. All the above-mentioned variables conformed to the requirements set and from a management point of view these determinants could be limited in effluents in receiving waters.

Determinant transformation

The most efficient method of transforming information on individual determinant concentrations is to select or obtain rating curves for each determinant (Walski and Parker, 1974; House, 1989; Smith, 1990). Before developing a rating curve it was necessary to define the scale on which the ATI was to operate.

a) Defining an ATI scale

An ATI scale similar to the WQI scale proposed by Smith (1990) for salmonid spawning was used. A scale of 10 to 100 was selected with a score of 10 reflecting water which is totally unsuitable for fish life and that of 100 akin to pristine conditions. The interpretation and classification for the aquatic toxicity WQI scale is given in Table 8.5.

Table 8.5: THE INTERPRETATION AND CLASSIFICATION OF THE ATI SCALE

ATI Scale	Interpretation
60-100	Indicates water of suitable quality for all fish life
51-59	Indicates quality of water suitable only for hardy fish species e.g. adult *Oreochromis mossambicus* and adult *Clarias gariepinus*
0-50	Indicates water quality which is totally unsuitable for normal fish life

b) Selecting ATI rating curves

Three different methods were followed when selecting the ATI rating curves:

(i) Use of existing WQI rating curves

The existing curves used were those for dissolved oxygen (Smith 1990) total dissolved salts turbidity (Moore 1990b), orthophosphates (Walski and Parker 1974) and potassium (Workshop: Sensitive fish species 1991). Details of these curves are presented by Wepener *et al.* (1992a).

(ii) Modification of existing WQI rating curves

Existing curves for pH and ammoniacal nitrogen were modified in order to take into consideration the highly mineralised conditions that occur in the Olifants River (Van Veelen, 1991). In existing curves the 100 rating score for pH was at a pH of 7.2 whereas the median pH for the Olifants River for the period 1983-1992 was 8.0 (Van Veelen, 1991). The modified pH rating curve showed a steep gradient. This is because the Olifants River is a fairly well buffered river sytem. In the unlikely event of the pH differing with more than one pH unit the resultant altered toxicity of ammonium and heavy metals due to speciation changes, will be taken into consideration. The NH_4^+ rating curve was adapted from NH_3 toxicity data to reflect the effect of a higher pH value (pH 8-9) and temperature (25-30°C) on the toxicity of NH_4^+ to fish (Thurston *et al.* 1983; Hellawell, 1986). The graphical presentations of these modified curves are given by Wepener *et al.* (1992a).

(iii) Development of ATI rating curves

The rating curves were obtained as follow: Blank graph formats were taken on which the y-axis represented the suitability-for-use (index rating score) and ranged from 0-100. The x-axis represented the range of determinant concentrations or values likely to have an effect on fish. In all the cases the curves were to be plotted through a fixed point, i.e. the index rating score of 60 and the x-axis value representing the water quality standard for that determinant; this corresponds with the lowest value in the "suitable for all fish life" category (see Table 8.5). The rest of the concentrations of the specific determinants and their corresponding rating scores were plotted on the graph by employing current toxicity data in the form of LC50 and no observed effect concentration (NOEC) values.

Rating curves for fluoride, zinc, manganese, copper, chromium and nickel were obtained for the above-mentioned method. All the LC50 and NOEC values were obtained from existing toxicological data in literature (Smith *et al.*, 1985; Hellawell, 1986; Mance, 1987; Van de Meent *et al.*, 1990). Only toxicological data corresponding with the basic water properties of the Olifants River were used, i.e. pH between 7.8 and 9.0 water hardness 120 mg/l as $CaC0_3$, and temperature between 20°C and 30°C. In most of the cases the toxicological data used were obtained using the same fish species. These fish species included *Lepomis macrochirus* (bluegill), *Pimephales promelas* (fathead minnow), *Oncorhynchus mykiss* (rainbow trout), *Ictalurus punctatus* (channel catfish) and fish species found in Southern Africa e.g. *Oreochromis mossambicus* (Mozambique tilapia). The concentration on the x-axis for the index rating score of 60 (y-axis value) for each determinant was derived by combining and aggregating the water quality standards of the United Kingdom (Gardiner and Zabel 1989), Netherlands (Van de Meent *et al.*, 1990), Australia (Hart, 1974), Canada (Environment Canada, 1987), European Inland Fisheries Advisory Commission (EIFAC) (1980) and South African guidelines (Kempster *et al.*, 1980), as well as water quality guidelines for the Olifants River (Moore *et al.*, 1991; Workshop: Sensitive fish species 1991). The rating score of 60 is regarded as the minimum ATI value where the water quality is suitable for all fish species. When a water quality standard was above or below available toxicity data, that specific standard was not taken into consideration when deriving the x-axis value.

To illustrate the development of an index rating curve, the rating curve of zinc is described. All the LC_{50} and NOEC values were plotted on the x-axis. In order to determine the NOEC value, the LC_{50} value was multiplied by a factor of 0.01 (United States Environmental Protection Agency, 1984). The lowest NOEC value, in this case 16 μg/l, was given an index rating of 100 (Mance, 1987; Van Meent *et al.*, 1990). The median water quality standard or guideline for zinc as set by the above-mentioned countries was taken as 200 .μug/l and the rating score of 60 was attained. The zero rating was taken as the lowest LC50 i.e. 1 400 .μug/l (Mance, 1987). The rest of the NOEC values were awarded corresponding rating scores of between 61 and 99 according to the toxicity of zinc to the aforementioned fish species. The plotted points were connected with a curved line. When the curve did not join all the points smoothly, a trend line was drawn resembling an inverted LC50 curve. The index rating curves described here is graphically presented in the paper by Wepener *et al.*, (1992a).

Determinant aggregation

The values obtained from the rating curves had to be aggregated in some way to produce a final index score. For the ATI the aggregation technique employed was the Solway Modified Unweighted Additive Aggregation function (House & Ellis 1980):

$$I = 1/100.(1/n\sum_{i=1}^{n} q_i)^2 \qquad (1)$$

where I is the final index score, q_i is the quality of the ith parameter (a value between zero and 100) and n is the number of determinants in the indexing system. This function is similar to the aggregation technique developed by the Scottish Development Department: (SDD) (1976) and which is being advocated for use in England (Tyson and House, 1989). The only difference between the technique employed in this study and the technique used by the SDD is that the latter has a weighted value (an indication of preceived importance) attached to each determinant. No weighted system was applied to the determinants in the current study as the authors felt that too little is known about the importance of one determinant compared to another under local conditions. Furthermore, it is not possible to compare factors which have a direct and interactive effect on one another, e.g. pH and heavy metals, and apply a weighted system whereby it is implied that the relative importance of one parameter over another is known. It will, however, be possible to develop a weighted system when more information concerning the chemistry of the system as a whole (including synergistic/antagonistic effects) becomes available.

It is, however, a problem when deriving a final index score through an aggregation technique, because it tends to conceal the most valuable piece of information, namely the identity of the determinant which limits the water's "suitability-for-use" and the degree to which this occurs (Smith, 1990). It was therefore decided to use an aggregation function which would not conceal valuable information in addition to the use of function (1). This function uses the lowest determinant index score to produce the final index score and it is known as the "minimum operator" function:

$$I = \text{minimum } (q1, q2 \ldots\ldots, qi) \quad (2)$$

In order to minimise the laborious task ofdetermining the index score of each determinant from an index rating curve and then to calculate the final ATI score, a computer software package (WATER 2) was developed. The curve of each determinant was plotted and fitted and an equation for each curve was determined by means of numerical calculations and Pascal (see Table 8.6). All the equations were incorporated into a computer program which was written in Pascal with "Turbo Pascal Version 6". The program is able to compute both the additive and the minimum operator final index values. In addition to computing the final index values, the program provides valuable information on the harmful effects that the different determinants would have on fish should the "suitable-for-use" concentration limit be exceeded. This was done by incorporating an expert system into the software program which certain sets of rules to specific concentration levels of variables.

Table 8.6: EQUATIONS FOR THE DETERMINATION OF INDEX RATING VALUES

Determinant	Index Rating Equation
Dissolved Oxygen	$0 < DO < 5$; $y = 10(DO)$ $5 < DO < 6$; $y = 20(DO)-50$ $6 < DO < 9$; $y = 10(DO) + 10$ $DO > 9$; $y = 100$
pH	$y = 98 \exp [-(pH-8.16)^2(0.4)]$ $+17 \exp [-(pH-5.2)^2(0.5)]$ $+15 \exp [-(pH-11)^2(0.72)] + 2$
Manganese	$y = a \exp^{-c} \exp^{(Mn)b} + d$ $a=0.115$; $b=0.0013$; $c=0.05$; $d=5$
Nickel	$y = -c \ln (a(Ni+b)) + d$ $a=1$; $b=-10$; $c=28$; $d=211$
Fluoride	$y = -c \ln(a(F+b)) + d$ $a=0.001$; $b=2.5$; $c=71$: $d=-235$
Chromium	$y = -c \ln(a(F+b)) + d$ $a=0.1$; $b=150$; $c=40$; $d=210$
Lead	$y = -c (\ln(a(Pb+b)) + d$ $a=0.1$; $b=-30$; $c=27$; $d=148$
Ammonium	$0.02 < 0\ NH_4^+$; $y = 100$ $0.02 < NH4^+ < 0.062$; $y = 500(NH_4^+) + 110$ $0.062 < NH_4^+ < 0.5$; $y = 40/(NH_4^+ + 0.65)^2$ $NH4^+ > 0.5$; $y = -5.8(NH_4^+) + 32.5$
Copper	$y = -c \ln(a(Cu+b)) + d$ $a=1$; $b=18$; $c=26$; $d=180$
Zinc	$y = -c \ln(a(Zn+b)) + d$ $a=0.001$; $b=-20$; $c=22$; $d=16$
Orthophosphates	$y = a \exp (P)b$ $a=100$; $b=-2.4$
Potassium	$y = a \exp^{-b(K)} + c$ $a=150$; $b=0.02$; $c=-8$
Turbidity	$y = -c \ln(a(NTU)+b) + d$ $a=0.001$; $b=30$; $c=220$; $d=-689$
Total dissolved salts	$y = a \exp^{-b(TDS)} + d$ $a=117$; $b=0.00068$; $d=-7$

8.1.2.3 The Water 2.1 computer software program to derive the ATi

In order to facilitate the use of the Water 2 Computer Software program to derive the ATI, a reference manual was computed. In this manual both the use and the ruler-based expert system are explained.

8.1.1.3.1 The Water 2.1 Reference Manual

TABLE OF CONTENTS

A. INTRODUCING WATER2

WATER2 is a DOS based software package designed by the Department of Zoology and Research Unit for Aquatic and Terrestrial Ecosystems, Rand Afrikaans University for use in water quality interpretation and subsequent operational management of water quality.

This program incorporates an aquatic toxicity index which manipulates water quality data to produce a "suitability-for-use" value and an expert system which indicates the consequences of different water quality variables.

The first section of the program calculates an index value for the water quality variables and allows the operator to compare these values on the suitability-for-use graph. The second section will allow the operator to run the water quality index values through a rule base to predict the effects of the aforementioned variables on fish.

The following water quality variables are represented to WATER2.

*Dissolved oxygen (mg/l)
*pH
*Turbidity (NTU)
*Ammonia (NH_4^+-N in mg/l)
*Orthophosphates (mg/l)
*Fluorides (mg/l)
*Potassium (mg/l)
*Manganese (/g/l)
*Nickel (/g/l)
*Copper (/g/l)
*Chromium (/g/l)
*Lead (/g/l)
*Zinc (/g/l)
*Total dissolved salts (mg/l)

It must be stressed that this program was specifically designed for hard water bodies (> 120 mg/l aO $Cac0_3$), pH > 7.8 and water temperatures above 20°C.

B. STARTING WATER2

You run WATER2 by typing a single command. If you are using a dual floppy disk system, be sure that the default disk drive is A: If you are using a hard disk system; make WATER your current directory (type cd\WATER) then:

TYPE: WATER2

WATER2 displays an opening menu with two options:

Afrikaans or English

These options allow the operator to receive instructions in either Afrikaans or English. Currently the expert system is in English and therefore this manual will refer to the English option only.

TYPE: E

The main menu screen appears with four options:

Graphs
Calculations for final index
Expert system
Quit system

B.1 CALCULATING THE FINAL INDEX VALUE

Using the cursor keys, select the CALCULATIONS FOR FINAL INDEX VALUES option and :

PRESS: RETURN

A new screen appears and lists the different water quality variables which are to be used in calculating the final index scores.

B.2 OPENING A NEW FILE

PRESS the F3 key

The cursor will flash in the highlighted field of the first variable. Type the value or concentration or the first variable and:

PRESS: ENTER

The value or concentration of the first variable has now been entered. Use the cursor key to move to the next variable and:

PRESS: F3

You may now enter the value or concentration for the corresponding variable. Repeat the procedure for the remaining variables. If there is no concentration or value available for the variable:

TYPE 0 (zero)

In the highlighted variable field. The program will automatically enter the missing value and award an index score of 61 (akin to the "suitable-for-use" function). This is done so that final index scores calculated will not be misleading due to missing values due to missing values.

B.3 CALCULATING THE INDEX SCORE

After all the values for the variables have been entered:

PRESS: F4

Index values for all the variable concentrations will appear on the screen. Two index scores are calculated i.e. the minimum operator score and the modified mean score. They appear at the bottom of the screen. A WARNING notice will appear next to any variable index value below the suitability-for-use index value. (For a detailed explanation of the "suitability-for-use"- value, refer to Wepener *et al.*, 1992a).

To continue:

PRESS: ENTER

B.4 SAVING VALUES INTO A FILE

To save the values or concentrations of the different variables into a file:

PRESS F2

A field now appears on the screen prompting the operator to type in the file name. WATER2 allows you to save the values in a file or directory of your choice.

Saving onto the A: Type into the FILE NAME or MASK field:

a:\file name

and

PRESS : ENTER

The file has now been created and the values have been saved into the given file on the A: drive. The file name on the A: drive will have *.dat extension.

If no drive is specified and only a file name is entered, the data will be saved onto the default drive in the default directory. In this case data will be saved in the specified file in the WATER directory on the hard disk (C: drive).

B.5 RETRIEVING A FILE

A file which has been created and saved can be retrieved from the directory or drive in which it was saved by:

PRESSING: F5

A file name or mask field appears on the screen. In order to retrieve a file from the A: drive:

TYPE: a:\file name and PRESS ENTER

In order to obtain a director of all the *.dat files on the A: drive:

TYPE: a:*.dat and PRESS ENTER

All the WATER2 files on the A: drive will be listed in the top of the screen. Select the file you want to retrieve with the cursor keys and:

PRESS: ENTER

If the data was stored on the hard disk and you wish to retrieve it:

PRESS: F5

The file name or mask field appears. By pressing ENTER all the WATER2 DATA FILES IN THE water directory is displayed in the top section of the screen. Select the file you would like to retrieve by using the cursor keys and:

PRESS : ENTER

The file you have selected will now appear on the screen.

If the data files have been saved in another directory other than the default directory:

PRESS : F5

The file or mask field appears, then:

PRESS : ENTER

The *.dat files in the WATER directory appear on the screen. Select the ...\ option with the cursor keys and:

PRESS : ENTER

All the directories on the hard disk appear. Select the directory with the data files by using the cursor keys and:

PRESS : ENTER

the WATER2 data file will now appear on the screen

B.6 EDITING A FILE

Retrieve the file as described in section B.5. Use the cursor keys to select the variable that has to be edited and:

PRESS : F3

Type the new value or concentration and:

PRESS : ENTER

If a mistake was made whilst typing the value or concentration and the variable has not been entered, you can cancel the value by:

PRESS : DELETE

Retype the correct value or concentration and:

PRESS : ENTER

C. GRAPHS.

In order for the graph section to be executed by WATER2 it is necessary to have a TURBO PASCAL graphics file (corresponding to your specific monitor and graphics setup) e.g. EGAVGA.BGI in the WATER directory.

To select the graphs option the operator must return to the main menu by:

PRESS : F10

Select the graphs option with the cursor keys and:

PRESS : ENTER

A (GRAPH SCALE FACTOR) field appears. The following scale factors and their corresponding variables are available in WATER2:

*Dissolved oxygen - 50
*pH - 40
*Fluoride - 100
*Ammonia - 150
*Orthophosphates - 200
*Turbidity - 100
*Potassium - 1
*Copper - 1
*Chromium - 1
*Lead - 1
*Manganese - 1
*Nickel - 1
*Zinc - 1
*Total dissolved salts - 1

Type the corresponding scale factor into the field and

PRESS : ENTER

A menu appears with all the index variables. Select the chosen variable with the cursor keys and:

PRESS : ENTER

The graph appears on the screen. This is the toxicity curve from which the index value is derived. The y-axis represents the suitability for use or the index value and the x-axis represents the concentration of the chosen variable. To return to the GRAPH menu:

PRESS : ESCAPE

The main menu appears and the operator must select the GRAPHS option and proceed by entering a new scale factor.

D. EXPERT : SYSTEM

The *rules-based* expert system allows the operator to predict the effects that the different water quality variables might have on fish, should the "suitability-for-use" value be exceeded. The *rule base* is based on *if*, and then statements. The *if* part of the rule is referred to as the condition, while the *"then"* part is the action. This program asks the user to enter concentrations or values for different variables and then reduces from the *rules* which possible cause could be incurred. This allows for the direct inspection of the knowledge base by the user, and allows the system to explain its operation in terms of the rules applied. If you are familiar with the "rules" continue directly to page ..... If you are not familiar with the rules, first read the following paragraph.

----OoO----

The *initial rules* set were obtained from toxicological and physiological data available in the literature:

(I). Dissolved oxygen (DO) [mg/l]

If 0 mg/l less than (DO) less than or equal to 5.5 mg/l then reduce survival as well as reduced growth, reproduction, hatching of eggs, larval survival, fecundity and behaviour, dissolved oxygen should not be lower than 4 mg/l for more than 8h out of any 24h period.

If (DO) greater than 20 mg/l then supersaturation may cause mortalities.

(II). pH

If 0 less than (pH) less than or equal to 5, then lethal to most fish species although some species may survive for a short period of time in the upper end of the range, lethal to eggs.

If 5 less than or equal to (pH) less than or equal to 6.5 then the lower end of the range reduces growth while no reproduction takes place, prevents hatching of eggs, the higher end of the range is not harmful unless the water contains salts and carbon dioxide greater than 20 mg/l.

If 6.9 less than or equal to (pH) less than 9.3 then recommend guideline value; a sudden change in pH within the recommended levels may alter the toxicity of heavy metals.

If (pH) greater than 9.3 then lethal to most fish species if exposed for a prolonged period to the lower end of the range, while lethal at pH above 10.

(III). Manganese (Mn µg/l)

If 500 µg/l less than (Mn) less than 300 mg/l, then gill damage resulting in internal hypoxia and reduced oxygen utilization, impaired osmoregulation, altered metabolic processes, high fish egg mortalities.

If 300 mg/l less than (Mn) less than 1500 mg/l, then massive haemorrhaging and loss of balance found within the first 96 hours.

If (Mn) greater than 1500 mg/l, fish die within 96 hours.

If 7.5 less than (pH) less than 9.0, [Mn] more toxic because of oxidised [MnO_2]-form, but toxicity decreases when there is an increase in chelators, eg. humic acid and bicarbonates.

(IV). Nickel (Ni µg/l)

If 20 µg/l less than [Ni] less than 250 µg/l, then avoidance response in soft water (80 mg/l [$CaCO_3$]) occurs.

If 250 µg/l less than [Ni] less than 500 µg/l then gill lesions will result, inhibits energy forming processes, increases blood glucose levels.

If [Ni] greater than 500 µg/l, fish die within 96 hours.

If (pH) less than 6.5, then toxicity of (Ni) increases, but toxicity decreases when water hardness increases.

(V). Fluoride (F mg/l)

If 1.9 mg/l greater than or equal to [F] greater than or equal to 1 mg/l then the international standard for fluoride is 1 mg/l, concentrations above this level have accumulated in the teeth of hippopotami and could cause degradation of the teeth.

If 2.0 mg/l greater than or equal to [F] greater than or equal to 1.9 mg/l then fluoride is accumulated by fish but no adverse effects could be observed.

If [F] greater than 2.0 mg/l then LC_{50} values reported from 40 mg/l.

If [F] greater than 1.9 mg/l, then fluoride toxicity is decreased by an increased water hardness and [F] is precipitated as insoluble calcium fluoride.

(VI). Chromium (Cr µg/l)

If 100 µg/l less than [Cr] less than or equal to 27 µg/l, then anaemic conditions occur resulting in decreased oxygen utilization and hypoxia, osmoregulation is influenced and metabolism is decreased.

If 1000 µg/l greater than or equal to [Cr] greater than 275 µg/l, then hexavelant chromium is more toxic than the trivalent form, the hypoxic conditions are aggrevated by secondary infection resulting in decreased feeding and reproduction.

If [Cr] greater than 1000 µg/l then chromium concentrations reach the LC_{50} concentrations which may lead to mortalities.

If [pH] less than 6.9 then toxicity of chromium increases due to the increased amount of toxic monovalent oxo-anions.

(VII). Lead (Pb µg/l)

If 75 µg/l less than [Pb] less than 230 µg/l then anaemia due to inhibition of haemoglobin synthesis, ALA-D inhibition points out lead poisoning, lowering of blood sugar due to damage of kidney tubules or depression of gluconeogensis in liver, stimulation of alkaline phosphatase but inhibition of some enzymes involved in energy metabolism.

If (pH) less than 6.9, then a threefold increase in [Pb] uptake, whereas if water hardness decreases, toxicity increases.

If 230 µg/l less than [Pb] less than 500 µg/l, then histopathological changes in gonads resulting in decreased reproduction rate, heme synthesis adversely inhibited, causing chronic tissue hypoxia.

If [Pb] greater than 500 µg/l, then swimming performance affected, lower concentrations will result in death of sensitive fish species.

(VIII). *Ammonium ($NH_4^{\pm}$ mg/l)*

If 0.3 mg/l greater than [Nh_4^+] greater than 0.165 mg/l then reduced growth rate and adverse physiological and histopathological effects may occur.

If 2 mg/l greater than [NH_4^+] greater than 0.3 mg/l then lethal to most species but reduced growth rate for catfish.

If [NH_4^+] greater than 2 mg/l then lethal to catfish.

(IX). *Copper (Cu µg/l)*

If 38 µg/l less than [Cu] less than 85 µg/l, then increased red and white blood cell production due to greater oxygen demand, increase in oxygen consumption of fish eggs which may lead to an increased uptake of pollutants.

If 85 µg/l less than [Cu] less than 200 µg/l, then decreased survival and growth in mature fish as well as reduced egg viability and hatchability, altered osmoregulation due to damaged gill surface, impaired feeding regime and increased coughing frequency, locomotor activity affected.

If [Cu] greater than 200 µg/l then whole body oxygen consumption decreases, hypoxia due to the swelling of red blood cells, impaired immune response.

If (pH) less than 6.9 then toxicity of copper is increased four fold, whereas a decrease in water hardness will lead to an increase in toxicity.

(IX). *Zinc (Zn µg/l)*

If 50 µg/l less than [Zn] less than 155 µg/l, then avoidance response (water hardness 112 mg/l), atagonistic behaviours by dominant individuals increases, egg production reduced.

If 155 µg/l less than [Zn] less than 400 µg/l, then hypoxia because of gill damage, impaired osmoregulation, impaired carbohydrate metabolism because of inhibited glucose production.

If 400 µg/l less than [Zn] less than 1500 µg/l, then adverse effect of growth, reproduction, spawning and egg production.

If [Zn] greater than 1500 µg/l, then it may lead to mortalities.

If (pH) less than 5, then increased [Zn] toxicity due to action oflow (Ph).

If 5 less than (pH) less than 6.9, then [Zn] not very toxic due to the ionic form [$ZnOH^+$], however, increased water hardness causes decreased (Zn) toxicity.

(X). *Orthophosphates (P mg/l)*

If [P] greater than 0.21 mg/l then organic enrichment of the water may be found to untreated sewage effluent entering the river, phosphates may be due to high quantities of organophosphorous compounds.

(XI). *Potassium (K mg/l)*

If [K] greater than or equal to 52.2 mg/l then [K] ions influences the osmoregulation of fish; should an excess [K^+] concentration occur in the extra cellular fluids then it could lead to an abnormal increase in heart rate.

If [K] less than 52.2 mg/l, then water hardness has an effect on the availability of free ions.

(XII). *Turbidity (NTU)*

If O less than (NTU) less than 25, no effects but turbidity increase should not be greater than 5-25 NTU's above the natural. In South Africa 1 mg/l is roughly equivalent to 1 NTU.

If 25 less than (NTU) less than 400, then it may decrease production.

If 400 less than (NTU) less than 1000 then sublethal effects on respiration, feeding and behaviour.

If 1000 less than (NTU) then mortalities or larvae and mortalities of adult fish may occur.

(XIII). *Total dissolved solids (TDS mg/l)*

If (TDS) greater than or equal to 820 mg/l, then it is uncertain what the effect of high (TDS) values have on fish but any increase in free ion concentrations in the external environment as well as concentrations of other stressors would lead to osmoregulation inbalances; should the concentrations of the ions increase in plasma, then adverse effects on the heart rate and nerve excitability will occur.

If (TDS) less than 820 mg/l, then water hardness has an effect on the availability of free ions.

If 6.9 less than (pH) then toxicity of the free ions may increase.

Select the EXPERT SYSTEM option by using the cursor keys. A file or mask field appears on the screen. Enter the name of the file which you want to run through the expert system (refer to section B.5 to retrieve a file):

TYPE : file name.dat

A second file name or mask field appears on the screen prompting you to enter the name of the file into which the information generated by the expert system is to be written (refer to section B.4 to save a file):

TYPE : file name dat

HINT: Provide the same file name to the expert system file as was given to the original data file:

PRESS : ENTER

The program accesses the expert system and runs the data through the rule base. When this is completed the program returns to the main menu. The expert system files have been written into the specified files.

In order to generate an expert system report, the operator must exist WATER2.

E. EXITING WATER2

Select the QUIT SYSTEM option on the main menu by using the cursor keys and:

PRESS : ENTER

The operator has now returned to the WATER directory on the hard disk (C: drive).

F. GENERATING A REPORT

Run any word processing program on the hard disk. Retrieve the expert system report file (*.RPT) from the directory into which it was saved. The operator is now able to use the work processing program to prepare a report which outlines the possible physiological effects the water quality variables may have on fish.

8.1.2.4 Application of the ATI : A case study on the Olifants River, Kruger National Park

As an example of how the WATER2 can be applied to obtain an ATI score for a specific river, a case study on the lower Olifants River, Eastern Transvaal, was initiated.

Material and Methods

Bi-monthly sampling was conducted for the period February 1990 to June 1992. Five sampling stations were situated within the borders of the Kruger National Park (four stations in the Olifants River and one in the Letaba River) whereas two stations were located outside the Park on the western boundary (Figure 8.1). Station A is situated in the Letaba River, close to the confluence with the Olifants River (a few kilometres downstream from DWAF station B8M30 at Mingerhout Dam). Station B is situated in the Olifants River close to the confluence with the Letaba River (± 2 km downstream of DWAF station B7M18 at the Olifants hiking trail base camp). The locations of stations C and D correspond to DWAF stations B7M17 (Balule rest camp) and B7M16 (Nhlaralumi confluence) whereas station E is situated close to Mamba weir (DWAF station B7M15). Station F is below Phalabarwa barrage (DWAF station B7M15) and station G is situated in the Selati River close to the low water bridge. The stations chosen for this study and those of the DWAF were not necessarily in the same locations, but in close enough proximity to compare the water quality results obtained.

Two reference stations were selected inside the KNP in order to compare with the results obtained from the previously mentioned stations with results from theoretically upolluted stations. These unpolluted reference stations (Hlanganini Dam - Station H and Pionier Dam - Station I) only receive water from catchment areas within the borders of the KNP. The locations of stations H and I are shown in Figure 8.2. Water samples from stations H and I were collected during February 1992 and April 1992 respectively.

Two sets of unfiltered water samples were collected (± 5cm below the surface) at the different sampling stations. All water samples and physical measurements were obtained between 14:00 and 16:00. The following physical variables were measured: dissolved oxygen (WTW Microprocessor, Model OXT 96), pH (Orion, Model SA 250) and turbidity (Novisina analite 152 nephelometer). A set of water samples was preserved with mercuric chloride and stored at 4°C until further analysis. These samples were analysed by the Hydrological Research Institute of the DWAF for ammonium, nitrates and nitrites, fluoride, total alkalinity, sodium, magnesium, silica, phosphates, sulphates, chloride, potassium, calcium, conductivity and total dissolved salts.

A second set of water samples was acidified (pH2) with 55% HNO_3 (AR) and evaporated on a hot plate to 5ml. Each sample was made up to 50ml with double distilled water.

All metal analyses were completed with a "Varian Spectra AA-10" atomic absorption spectrophotometer with an impact bead to increase sensitivity. The metals analysed and corresponding detection limits were chromium (0.006 mg/l), copper (0.003 mg/l), iron (0.006 mg/l), lead (0.01 mg/l), manganese (0.002 mg/l), nickel (0.1 mg/l) and zinc (0.001 mg/l). Standard

curves were constructed for the different metals using commercially available certified stock solutions (Saarchem-Chemicals).

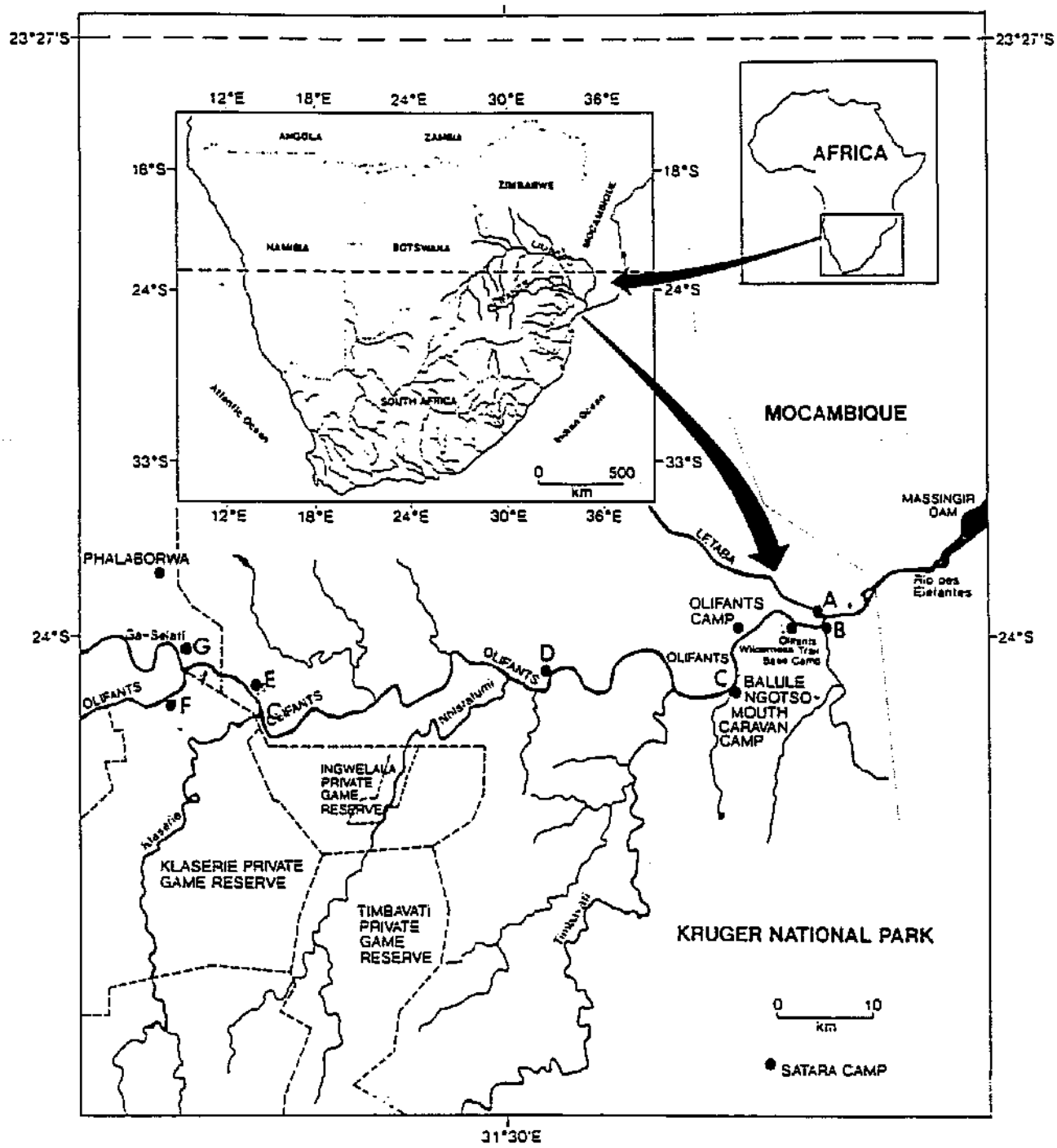

FIGURE 8.1
Sampling stations along the Olifants, Selati, and Letaba Rivers

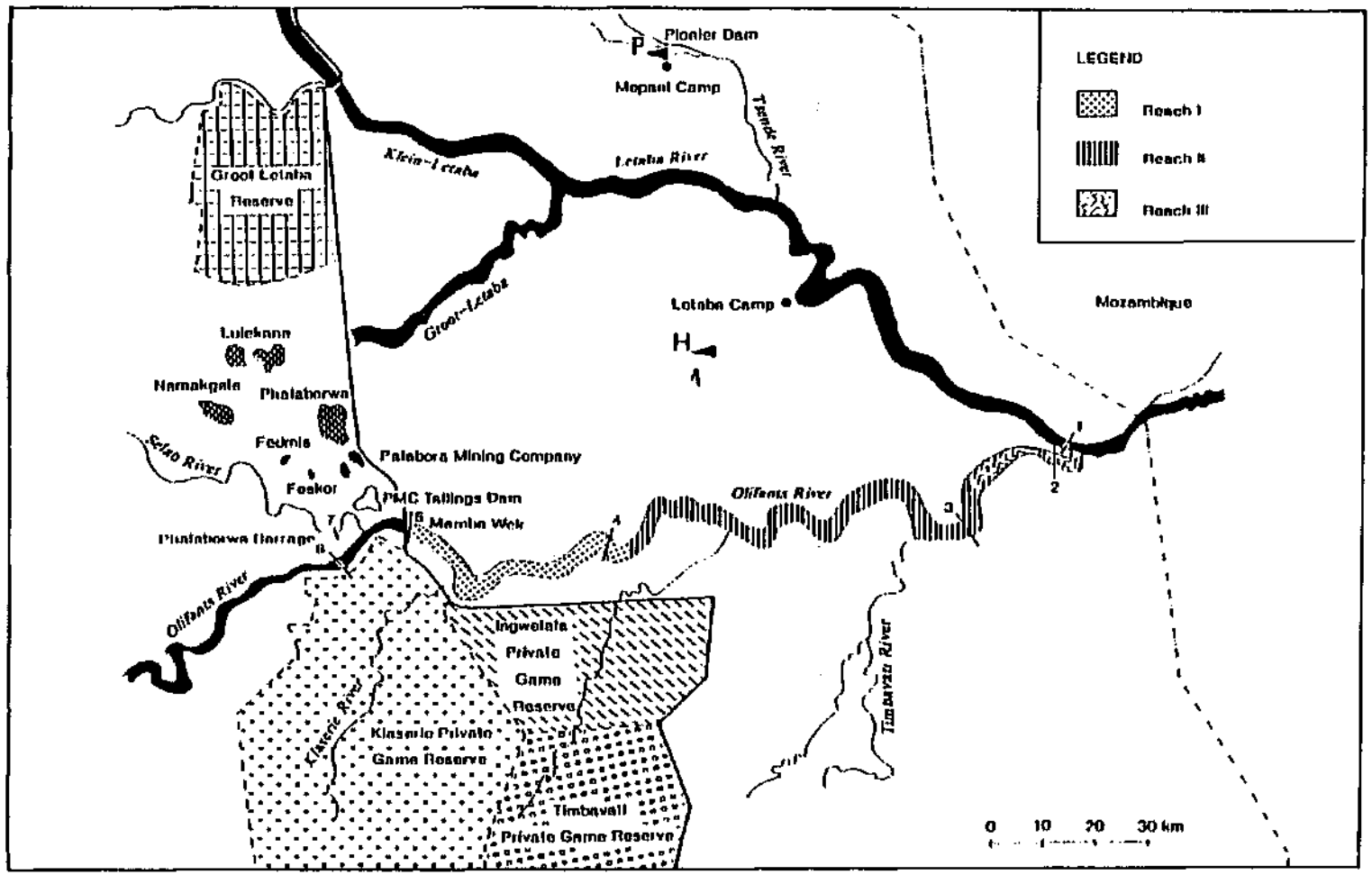

FIGURE 8.2
Sampling stations in the Pioneer Dam (P) and Hlanganini Dam (H)

Aquatic toxicity index scores for bi-monthly samples were calculated for each station. The water quality variables used to derive an index score from, were: dissolved oxygen (DO), pH, ammonium (NH_4^+), total dissolved salts (TDS), potassium (K), phosphates ($P0_4$), turbidity (NTU), fluoride (F), manganese (Mn), nickel (Ni), copper (Cu), chromium (Cr), lead (Pb) and zinc (Zn). A final index score was produced for all the above-mentioned variables by employing the computer base software program WATER2. The final index score is presented as a "suitable for use" value between 0 and 100. A score of 10 and less reflecting water quality totally unsuitable for sustaining fish life and that of 100 presenting unimpacted water conditions.

Results

The water quality at the different sampling stations in the Olifants River, Selati River and Letaba River are represented as aquatic toxicity index scores ranging between 0 and 100. The results obtained are presented graphically in Figs. 8.3 - 8.5. The individual scores at each sampling station and corresponding lowest index scores are presented in Tables 8.7 - 8.9.

It is evident from Figs. 8.3 - 8.5 that the water quality exhibited certain seasonal and cyclic trends. The water quality decreased at virtually all the stations during the winter of 1990 (June-August) and increased during the summer of 1991 (December-February) due to heavy rainfall in the catchment areas of the rivers. Stations C and B had low index scores (21.78 and 46.23 respectively) during December 1990 due to high suspended solids and metal concentrations. The water quality gradually decreased from autumn to spring in 1991 (April-October) and increased slightly in December 1991 due to the dilution effect of the summer rains. The 1991/1992 summer rainfall was, however, below average and the water quality at all the stations were lower and continued to decline.

From the results it was clear that the water quality in the Selati River was much lower with an average score of 42.48, compared to the water quality in the Olifants and Letaba Rivers. This was due mainly to high TDS and metal concentrations. The quality of the Letaba River was slightly better (average score of 62.65) when compared to the stations in the Olifants River. It was interesting to note that the water quality of the Olifants River at the stations towards the eastern boundary of the KNP (stations B and C) was lower (60.34 and 62.64 respectively) than the stations on the western boundary (66.52 at station D, 65.18 at station E and 67.01 at station F). It is evident that the water quality of the Olifants River, within the KNP boundaries, is influenced by certain determinants before the water flows into the Park.

The ATI scores of Hlanganini Dam and Pionier Dam were 74.6 and 73.3 respectively. The average ATI score for Station B for the period 1990-1992 ws 66.81 compared to the average ATI score of 71.45 for the period 1983-1989. The average ATI score at Station E was slightly lower (62.67) during 1983-1989 period than the period from 1990-1992 (65.91).

Discussion

The implementation of an aquatic toxicity index on water quality data from the Olifants River, Letaba River and Selati River has indicated that definite trends and cyclic patterns could be observed. The general trend at the stations investigated, was the improvement of water quality during the rainy

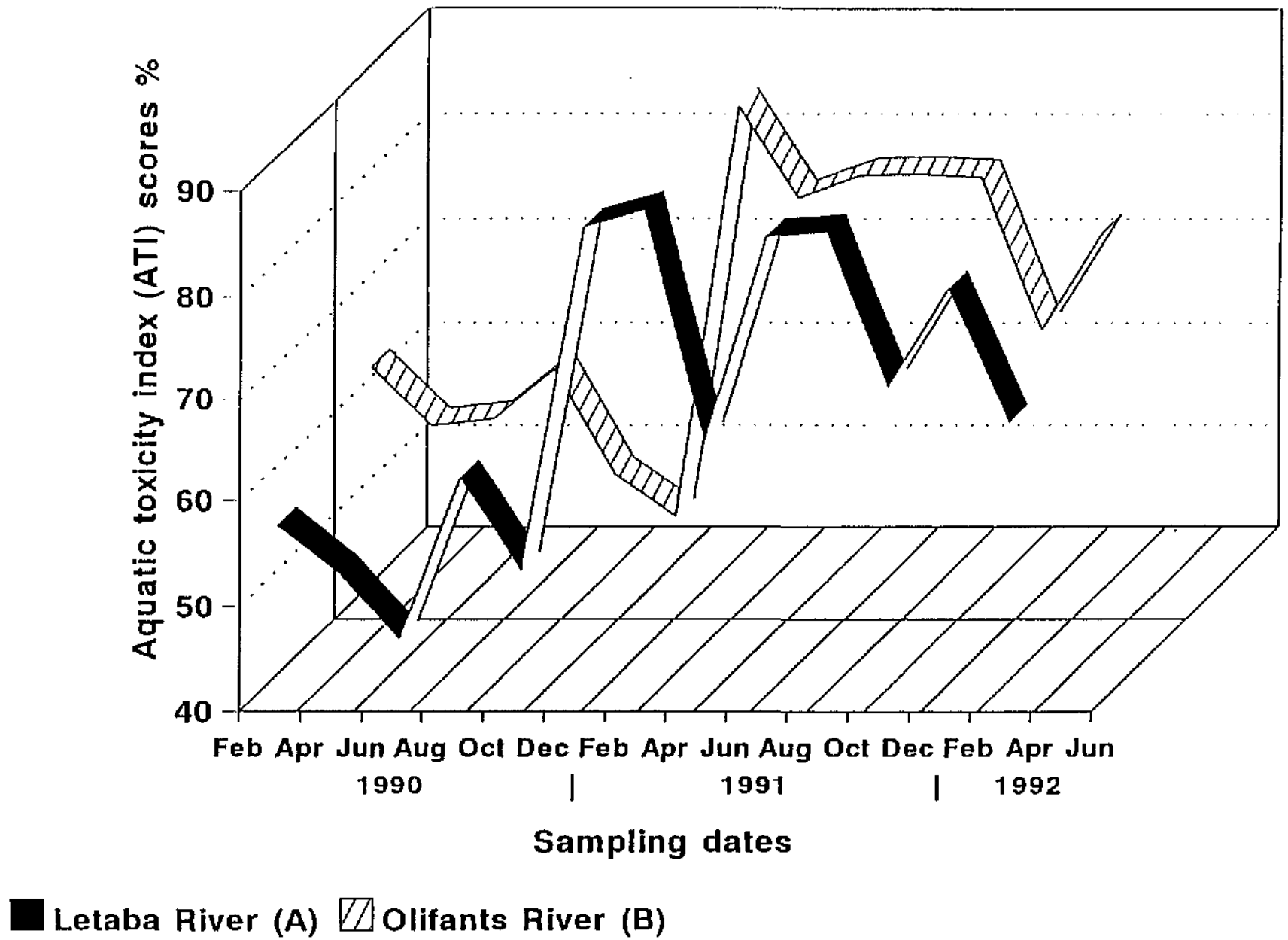

FIGURE 8.3
Aquatic toxicity index (ATI) for the Letaba river (A) and Olifants River (B) from February 1990 to June 1992.

FIGURE 8.4

Aquatic toxicity index (ATI) for Balule (C) and Nhlaralumi (D) from February 1990 to June 1992.

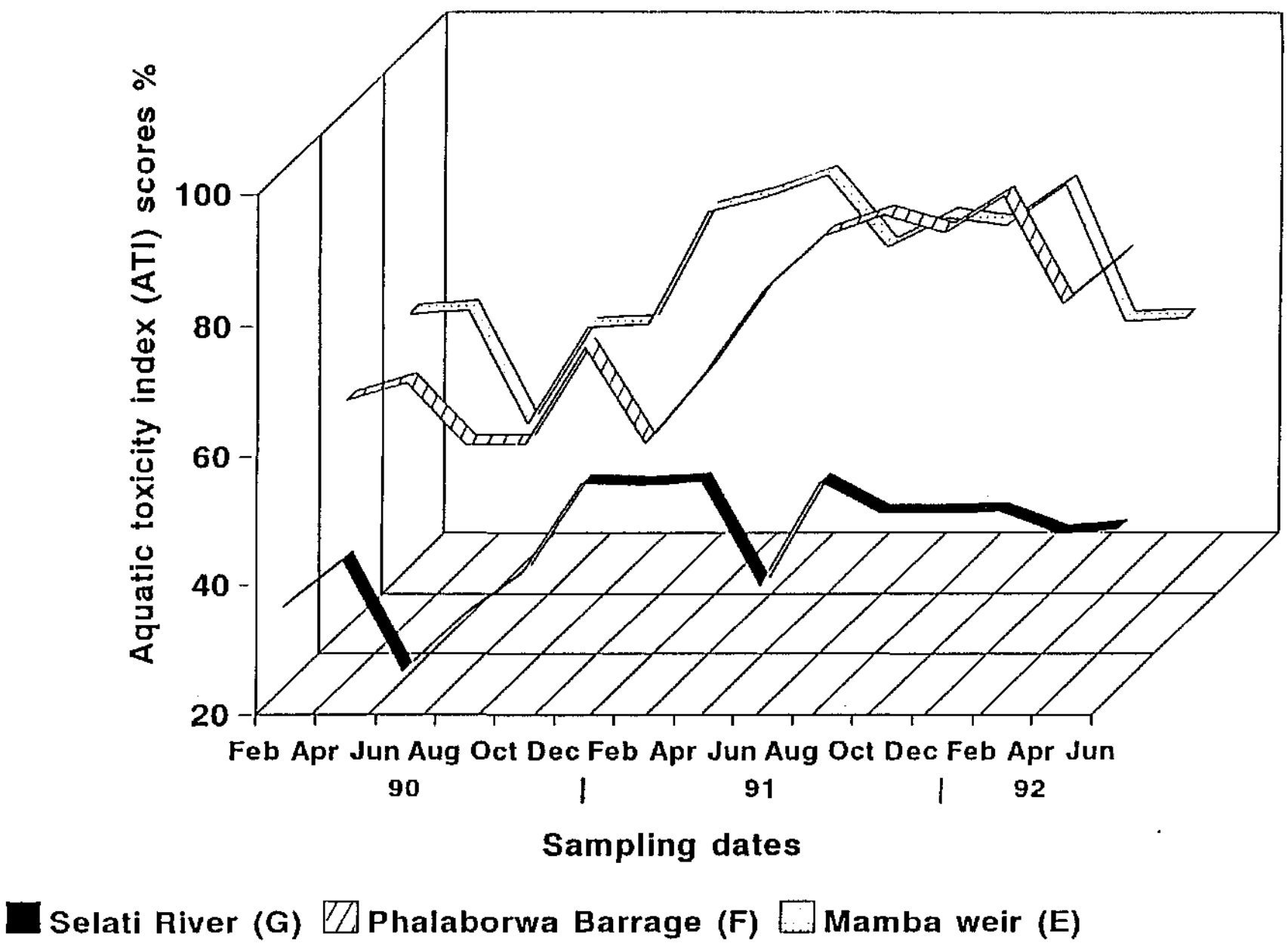

FIGURE 8.5

Aquatic toxicity index (ATI) for the Selati River (E) Phalaborwa Barrage (F) and Mamba Weir (E) from February 1990 to June 1992.

TABLE8.7 INDIVIDUALATI SCORES AND CORRESPONDING LOWEST SCORE RATINGS

Period	Letaba River (A)		Hiking trail (B)	
	Index Score	Lowest rating	Index score	Lowest rating
February 1990	54.12	Zn (43.23)	60.87	Zn (3.69)
April 1990	49.56	Zn (18.81)	55.18	Zn (4.07)
June 1990	43.38	Zn (16)	55.83	Zn (11.99)
August 1990	50.53	Cr (40.64)	55.78	Cr (36.25)
October 1990	49.94	Cr (36.77)	50.33	Zn (17.13)
December 1990	83.18	Cr (78)	46.23	Cr (48.99)
February 1991	84.75	Pb (80.91)	85.92	Pb (78.71)
April 1991	62.54	Cu (58.27)	77.15	Cr (65.56)
June 1991	82.29	Cr (71.47)	79.29	Zn (71.57)
August 1991	82.68	Cr (70.69)	79.40	TDS (70.69)
October 1991	67.77	Zn (34.21)	79.14	TDS (57.67)
December 1991	77.36	Pb (68.50)	64.62	Zn (20.42)
February 1992	65.24	Zn (41.07)	73.93	NTU (60.57)
April 1992	N.S.	N.S.	N.S.	N.S.
June 1992	N.S.	N.S.	N.S.	N.S.

N.S. No samples collected

TABLE 8.8 INDIVIDUAL ATI SCORES AND CORRESPONDING LOWEST SCORE RATINGS

Period	Balule (C)	a	Nhlaralumi confluence (D)		Mambe weir (E)	
	Index score	Lowest rating	Index score	Lowest rating	Index score	Lowest rating
February 1990	44.58	Zn (12.83)	57.92	Zn (14.93)	59.46	Zn (38.48)
April 1990	55.61	Zn (16.79)	57.69	Zn (27.98)	60.06	Zn (37.29)
June 1990	71.11	Zn (49.31)	46.81	Zn (8.6)	42.55	Zn (9.85)
August 1990	60.65	Cr (35.73)	60.57	Cr (32.77)	47.41	Cr (33.25)
October 1990	57.84	Cr (27.43)	55.01	Cr (27.84)	57.87	Zn (46.49)
December 1990	21.78	Cr (16.23)	N.S.	N.S.	74.89	NTU (50.54)
February 1991	86.31	NTU (61)	74.35	Cr (60.49)	77.18	NTU (49.87)
April 1991	73.47	Cr (66.66)	74.29	Zn (59.25)	80.43	NTU (66.89)
June 1991	71.11	Zn (49.31)	81.33	Zn (50.33)	69.68	Cr (67.79)
August 1991	74.71	Zn (44.64)	76.88	TDS (66.93)	74.06	TDS (64.61)
October 1991	67.18	NH_4^+ (28.91)	74.02	TDS (49.59)	72.94	TDS (47.74)
December 1991	73.15	Zn (58.94)	77.73	Pb (67.94)	78.98	F (76.54)
February 1992	66.84	Mn (47.02)	61.96	TDS (59.95)	66.84	TDS (45.23)
April 1992	62.89	TDS (40.07)	N.S.	N.S.	62.91	TDS (30.11)
June 1992	52.43	TDS (22.36)	N.S.	N.S.	52.43	TDS (20.32)

N.S. - No samples collected

TABLE 8.9 INDIVIDUAL ATI SCORES AND CORRESPONDING LOWEST SCORE RATINGS

Period	Phalaborwa Barrage (F)		Selati River (G)	
	Index score	Lowest rating	Index score	Lowest rating
February 1990	55.51	Zn (8.57)	32.66	Zn (10.92)
April 1990	58.19	Zn (11.31)	39.74	Zn (8.13)
June 1990	48.74	Zn (12.73)	22.63	Zn (2.06)
August 1990	63.64	Zn (12.74)	31.09	Cr (20.91)
October 1990	48.85	Cr (44.91)	37.77	TDS (27.99)
December 1990	59.81	Mn (10.02)	51.86	TDS (36.01)
February 1990	72.54	Mn (15.23)	51.47	F (27.29)
April 1991	80.66	Zn (64.56)	52.09	F (30.41)
June 1991	83.79	Cr (71.37)	35.78	NH_4^+ (28.96)
August 1991	81.18	TDS (78.81)	52.03	TDS (28.31)
October 1991	86.67	Pb (68.93)	47.11	TDS (25.65)
December 1991	70.38	NH_4^+ (80.03)	47.12	TDS (22.28)
February 1992	77.82	NTU (58.39)	47.38	TDS (22.06)
April 1992	58.42	NH_4^+ (55.32)	43.84	TDS (23.96)
June 1992	58.81	TDS (45.36)	44.62	TDS (23.46)

NS. No samples collected

season (October to February) and a deterioration in water quality, as observed in the lower ATI scores, during the dry season (March to August).

From the ATI scores in Table 8.9 and Fig. 8.5 it is clear that the water quality of the Selati River was the lowest of all the stations monitored. The water quality is influenced mainly by high TDS concentrations (mean of 1765 mg/L and maximum concentration measured 2064 mg/L). The high TDS concentrations in the Selati River is caused by elevated levels of anions and cations in the surface water. The major cationic and anionic constituents were found to be sodium (166 mg/L) and sulphate (822 mg/L).

Although the regional geology of the catchment area does contribute to the natural mineralisation of the surface and ground water (Theron *et al.*, 1991), it is unlikely that the excessive TDS concentrations could be attributed to the regional geology. The lower catchment of the Olifants River falls within the same granitic geological region as the Selati River. The TDS concentrations at station F (360 mg/l) in the Olifants River (approximately two kilometres upstream from the confluence with the Selati River) was much lower when compared to the TDS levels in the Selati River. It could be inferred that the geogenic effects are of lesser importance than the anthropogenic effects.

It is obvious that the source of TDS concentrations can not be attributed to natural weathering of geological formations. The point sources of TDS concentrations can, therefore, be attributed to effluents, seepage and storm water overflow from mining activities in the vicinity of Phalaborwa.

These mining operations are dependent on water abstractin from the Olifants and Selati Rivers. The combined effect of the anions and cations, as reflected in the low ATI scores, is the most probable cause of the low fish species diversity found in the Selati River during this survey.

The extremely high sulphate (1765 mg.L) and fluoride (4 mg.L) concentrations measured in the Selati River gives rise to concern as these determinants seem to be the main factors lowering the water quality and affecting aquatic biota at this sampling station (See Fig. 8.5 and Table 8.9). Research by Burnham and Peterka (1975) and Smith *et al.*, (1985) have shown that high sulphate and fluoride concentrations are responsible for mortalities in larval fathead minnows (*Pimephales promelas*). These constituents most certainly have had an effect on the aquatic fauna in the Selati River.

From the results, it was evident that the Selati River has a major influence on the TDS levels in the Olifants River within the Kruger National Park boundaries. The TDS levels increase in the Olifants River after the confluence with the Selati River and shows a slight improvement at the eastern most sampling station. This resulted in TDS concentrations affecting the ATI score at Mamba weir and to a lesser extent at the other sampling stations in the Olifants River.

It was noted that during this sampling survey the water quality of the Selati River was not influenced by high turbidity levels. The water quality of the Olifants River is, however, influenced by increased turbidity. Erosion of land surfaces in catchment areas by rain and wind is a continuous and historically natural process. In the case of the Olifants River land use practices such as overgrazing, non-contour ploughing and the removal of riparian vegetation has accelerated the process of erosion and resulted in increased suspended solids in the river.

Most suspended solids from the upper-catchment of the Olifants River are deposited in Phalaborwa Barrage. This has led to a 46% reduction in the capacity of the Barrage (Theron *et al.*, 1991). During periods of high flow regimes the Barrage is scoured, releasing extremely high concentrations of suspended solids into the Park. Suspended solids concentrations of 24 000 mg.L to 77 000 mg.L have resulted in massive fish kills in the Olifants River (Venter and Deacon, 1992). The high suspended solids concentrations measured in the Olifants River during December 1990 to April 1991 were partly responsible for the low ATI scores at sampling stations, B, C and F during December 1990.

Some of the effects that increased turbidity may have had on aquatic organisms in the Olifants River include effects on predator-prey interactions and physiological impairments such as gill function, reduced foraging efficiency, growth and destruction of spawning habitats. Increased suspended solids concentrations together with metalic elements have been cited as reasons for the decreased distribution of four fish species and the disappearance of six fish species from the Olifants River (Russel pers. comm.). Protection of aquatic organisms from avian and piscine predators, due to increased turbidity, has lead to decreased distribution of scarce species such as Pel's Fishing Owl (*Scotopeli peli*) and tigerfish (*Hydrocynus vittatus*) (Moore *et al.*, 1991).

Nutrients, metals and other xenobiotics adsorb to suspensoids and are transported in this form. During periods of low flow (June 1990 to October 1990 and December 1991 to June 1992), the levels of metals increased at most stations. Although the flow during December 1990 increased, 175.7 million m^3 compared to 2.4 million m^3 in September 1990 (Workshop: Sensitive fish species, 1991), the total metal concentrations increased instead of decreasing due to a dilution effect. This can be attributed to increased turbidity, resulting in increased metal adsorption to the silt particles as well as liberation of deposited metals from the sediments. With the subsequent increased flow experienced from January 1991 to April 1991, the total metal concentrations and TDS concentrations decreased in the water column due to the diluting effect of fresh water.

In most natural water systems trace metal concentrations are low, and any increase could lead to contamination of the ecoystem, resulting in adverse effects. From Tables 8.7 to 8.9 it is clear that metals (specifically chromium, manganese, lead and zinc) were important factors in decreasing the suitability-for-use of the water at all the sampling stations. The mean metal concentrations at all the stations monitored, clearly show that the Selati River is the main source of copper and manganese, whereas the main sources of the other metals were in the upper catchment of the Olifants River.

Increased ammonium concentrations at Stations F and G (See Table 8.9) and increased orthophosphate concentrations at Station G can be attributed to industrial pollution and possibly sewage effluent from the towns in close proximity to the Olifants and Selati Rivers. High levels of these nutrients may lead to eutrofication problems at these stations. The high concentrations of NH_4^+ at Station C during the low flow period in October 1991 can be attributed to the excretory products of hippopotami in densely populated pools.

Experimental research has shown that the measured levels of total metal concentrations in the Olifants and Selati Rivers (zinc - 1.5 to 3.6 mg/l and

manganese - 2.3 mg/l) have negative effects on the physiology of fish (Wepener *et al.*, 1992b; Wepener *et al.*, 1992c; Wepener *et al.*, 1992d). Increased metal concentrations together with high TDS and turbidity levels are most probably the cause of decreased fish biodiversity in the Olifants River and Selati River.

The average ATI score of below 50 for the Selati River, indicated its inability to sustain normal physiological processess in fish. This is supported by the virtual absence of "pollution sensitive" fish species in the Selati River (e.g. Cyprinidae species). The background ATI scores at the reference stations (Stations H and I) clearly indicated that the water quality of the Olifants River was influenced by anthropgenic activities in the upper-catchment rather than geogenic factors. The water quality in the Olifants River did not change very much from 1983 to 1992 at Station E but the water quality at Station B did decrease slightly over the same period. Although the ATI scores of the Olifants River were indicative of pollution, the average ATI values were above 60 for most of the time. The Olifants River would therefore sustain normal fish life but periodic pollution events have resulted in fish kills and possibly reduction in biotic diversity. The ATI scores obtained from the Station A in the Letaba River indicated that the water quality would sustain fish life to a better degree than the water quality of the Olifants River.

8.1.2.5 Discussion

In the past, water quality indices were criticised for the loss of information due to the aggregation of a number of determinants. This was overcome by the implementation of a minimum operator function in deriving a final ATI value. The ATI can further provide information on the determinant concentrations in terms of quality criteria legislation (when implemented in South Africa) for aquatic life as the standard for the different determinants were used in the development of the individual determinant rating curves.

The ATI is an efficient method to monitor trends in water quality since it enables the reduction of large amounts of data to a single index value in a highly reproducible way. It is, however, important to keep into consideration that should the number of determinants included in each index application vary, the results obtained may be misleading.

In order to implement an ATI successfully, it is necessary to have access to the different determinants in the index at frequent intervals. The National Parks Board is in the fortunate position that it has access to frequently sampled chemical data on 22 localities in the six main rivers from which water is supplied to the Kruger National Park (Van Veelen, 1991). Physical determinants are obtainable by employing portable field instruments which are easy to handle. The trace metal concentrations in the water can be determined by means of portable diagnostic kits which are freely available. The trace metal concentrations obtained in this manner are not necessarily one hundred percent accurate, but they enable the researcher to obtain a very good idea of the concentration range and also if there are any changes in concentrations. Should a drastic change in trace metal concentrations be found, the water sample could be sent away for very accurate and detailed atomic absorption spectrometry analyses.

The data obtained by the researcher would then be fed into the WATER computer program and an ATI value for the specific locality could be produced. The computerised handling and interpretation of data enabled the ATI to produce valuable management information in a timely and efficient

manner. This procedure was successfully applied to the data sets from Hlanganini Dam, Pionier Dam, the Olifants, Letaba and Selati Rivers and has demonstrated that the water quality indexing system is an efficient method of representing and condensing water quality data since it reduces large amounts of data to a single index value. The index value produced gives an indication of the river's suitability for a specific beneficial use (i.e. maintenance of the aquatic ecosystem).

The advantages of using a computerised ATI in the operational management of surface river water may be vested in the fact that a number of determinants can be interpreted immediately and the resultant index value will enable the game ranger or researcher to pinpoint deteriorating water quality and even the possible source of the decreased water quality. A further advantage of this ATI is the information which is provided concerning the toxicological effects of the determinants on aquatic life. This would aid water management by the Parks Board when establishing water quality guidelines for upstream users. It is also possible to update the program, should recent and more relevant toxicological data become available.

House (1989) summarises the advantages of a WQI as follows:

a) it is able to demonstrate annual cycles and trends in water quality, even at low concentrations, in an efficient and wise manner;

b) it can be applied to unpolluted as well as polluted rivers and enables managers to place rivers into ranked order, thus indicating spatial variation in water quality;

c) it indicates possible water use in terms of guideline values (or legally adopted water quality standards contained within legislative directives to be implemented in the future); and

e) it assists in the evaluation of benefits which may accrue form investment capital schemes.

We would like to stress the fact that this aquatic index is designed specifically for fish in a water system with a hardness greater than 120 mg/l as $CaC0_3$ and pH greater than 7.8 such as the Olifants River in the Kruger National Park.

8.1.2.6 Conclusions

We conclude that the Aquatic Toxicity Index can be applied in an objective manner and the results obtained would be reproducible and repeatable both temporarily and spatially. The present WATER2 program should be expanded in the future to include a wider range of variables as well as more information regarding level variable. This would make the present ATI more widely applicable. It is proposed that the relationship between results generated by the ATI and information produced by a biotic index such as SASS3 be investigated. This could lead to greater certainty when making management decisions concerned with water quality control. Furthermore, the integration of water resources management models, expert systems and geographical information systems provides environmental researchers with exciting and promising tools for water resource management.

8.1.*2.7* References

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8.1.3 *Sediment*

8.1.3.1 Introduction

The Kruger National Park (KNP) in South Africa is committed to the conservation of ecosystems. Rivers are longitudinal ecosystems which cannot be subdivided into isolated sections and, therefore, all the rivers which flow through the Park are inexorably connected to the KNP. Management objectives are thus aimed at maintaining natural conditions thereby ensuring ecological functioning both within and outside the river channels as well as maintaining species and genetic diversity (Gertenbach, 1991). The Olifants River catchment (Fig. 7.1) is the largest of all the rivers running through the KNP and by virtue of the KNP's position on the eastern border of South Africa, water entering the KNP is polluted by mining, industrial, urban and agricultural effluents from the catchment areas (Wepener *et al.*, 1992). The water quality status of the rivers entering the Park is therefore a management dilemma for the Parks Board.

Metallic elements are important causes of environmental pollution and the Olifants River is no exception. Metals are not biodegradable and undergo a global eco-biological cycle in which natural waters are the main pathway. The cycling of materials in flowing waters is probably much less than in static fresh waters, but pollutants associated with sediments, for example, metals, may be settled, resuspended in higher flows and resettled once more downstream. Sediments are composed of numerous individual layers, each of which corresponds to a distinct condition of water flow. Coarse layers represent bed load depositions during strong currents, while fine layers consist mainly of suspended load deposited during weaker currents. Fine grained sediments, build up of a large number of individual layers, should therefore represent an average value for certain contaminants over a long period of time (Förstner and Müller, 1973). This is especially true for metals which are strongly absorbed by the clay minerals and the organic fraction of the sediment.

Sediments act as carriers and thus possible sources sof pollution because metals are not permanently fixed by them. The metals found in sediments present no direct danger as long as they are bound to the sediment (Bowen, 1966). The potential danger, however, lies in the possibility that, under certain circumstances, metals can be released back into the water by changes in environmental conditions such as pH, redox potential or the presence of organic chelators (Förstner and Solomons, 1984). Furthermore, sediment bound metals are assimilated by aquatic organisms in close proximity to sediments (Luoma, 1983).

The primary objective of this section of the study was to document the concentrations of selected sediment bound metals in the Olifants River at different sampling stations and to compare it with other data available for South Africa.

Materials and Methods

Sediment were sampled bimonthly from April 1990 to 1992 at six sampling sites (Fig. 7.2) along the Lower Olifants River and one (locality 7) in the Selati River. During the period April 1992 to February 1993 samples were only collected quaterly at selected localities (3, 5, 6 and 7).

In February 1992 and April 1992 it was also performed at Pionier and Hlanganini Dams, respectively. These dams were used as natural reference

points because they are situated in rivers that originate inside the Kruger National Park and therefore receive no effluent or runoff from outside the Park.

Sediment samples were taken with a pole-operated Ekman grab. The samples were frozen until further metal analysis in the laboratory. In the laboratory the samples were thawed and dried in an oven at 90°C for a period of 48 hours. After cooling, one gram of sediment was weighed into a 100 ml Erlenmeyer flask. Concentrated nitric acid (10ml; 55%) and 5ml concentrated perchloric acid (70%) were added, after which digestion was performed on a hot plate (200-250°C) for at least four hours, until the solutions were clear. Each solution was then filtered using an acid resistant 0.45 um paper filter and a vacuum pump. After filtration the filter system was rinsed with doubly distilled water and the sample was made up to 50 ml with doubly distilled water. The samples were then stored in clean acid-washed glass bottles for the analysis of the different metals. The sample procedure was followed as for the water metal analysis. The metal concentrations in the sediment were calculated as follows:

$$\text{Metal concentration } (\mu g/g) = \frac{\text{AAS reading } (\mu g/ml)}{\text{Sample mass (g)}} \times \text{Sample volume } (ml)$$

Results

Reference will be made to results of the first year, results of the second year and results of the third year. The first year refers to the period April 1990 to February 1991, while the second year refers to the period April 1991 to February 1992. These years include the seasons autumn (month April), winter (months June and August), spring (month October) and summer (months December-February).

In the first year chromium and manganese were the highest in concentration at locality 5, while copper, nickel and strontium were the highest in concentration at locality 7. The highest mean concentration of iron was recorded at locality 6 (24069 ± 17088 $\mu g/g$), that of lead at locality 3 (32 ± 12 $\mu g/g$) and that of zinc at locality 1 (249 ± 448 $\mu g/g$). All the metals, except for zinc, were the lowest in concentration at locality 1. In the second year, manganese and zinc were the highest in concentration at locality 3, copper and strontium at locality 7 and chromium and lead at locality 6. The manganese concentration recorded at Pionier Dam (54 $\mu g/g$) was much lower than the concentrations recorded at the other localities.

The metal concentration in the sediment of the Pionier Dam (Table 8.10) was generally lower than that of the Hlanganini Dam. However, the iron concentration was apparently twice as high (Table 8.10). The highest mean concentrations of nickel and iron were recorded at localities 5 (58 ± 34 $\mu g/g$) and 4 (27723 ± 8597 $\mu g/g$) respectively (Table 8.10). The lowest mean concentrations of all the metals were recorded at locality 1. In general, the metal concentrations were lower in the second year than in the first, except for chromium at localities 4 and 6; copper at localities 1 and 3 to 6; iron at localities 2 to 4; manganese at localities 2 to 4 and 6 to 7; nickel at localities 4 and 6; and strontium at localities 1 to 4 and 6 to 7. It should be mentioned that the trends found for strontium should be treated with caution, as there is insufficient data for this metal. The concentrations recorded

ILE 8.10: MEAN VALUES OF SELECTED METALS IN THE SEDIMENT FROM THE OLIFANTS RIVER COMPARED TO MAXIMUM LEVELS FOR SOIL AND VALUES FOR OTHER AQUATIC SYSTEMS IN SOUTH AFRICA

ariable	Locality															
	1	2	3	4	5	6	7	*Pionier Dam	Klangenlani Iron	Max levels of soil	Cowles Dam§	Nigel Dam§	Germiston Lake§	Natalspruit River§	Bronkhorst Spruit§	Hoottpadacht Dam§
year: April 1990 - February 1991																
ium (µg/g)	45±7	54±20	131±124	77±38	239±301	200±286	1264±54	N/A	N/A		64-381	93-364	108±32	138±96	214±200	29±2
per (µg/g)	10±2.3	14±3	28±10	13±6	22±7	14±5	209±187	N/A	N/A	100	25-2605	11-106	227±211	103±111	46±32	13±2
n (µg/g)	10071±5559	15622±10916	21459±17138	15656±1867	22030±8625	24069±1208	165686±13484	N/A	N/A		8512-58310	2168-45307	21117±7349	45531±33206	21650±21703	37921±29842
anese (µg/g)	88±39	127±38	252±149	160±75	300±112	250±71	214±152	N/A	N/A		222-3805	96-9502	511±258	6704±6897	1283±1160	674±305	
ckel (µg/g)	26±9	37±11	36±40	43±14	71±24	50±19	79±45	N/A	N/A	15	96-6372	20-232	160±97	217±194	57±36	24±8
ad (µg/g)	21±12	27±13	32±12	26±8	25±13	21±9	30±18	N/A	N/A	56	24-290	15-50	356±475	56±124	17±10	24±5
nium (µg/g)	12±5	25±12	36±31	32±23	62±26	39±3	114±94	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
c (µg/g)	247±446	29±15	49±25	38±15	93±27	65±24	42±33	N/A	N/	165	66-1643	254-770	307±412	307±337	37±18	49±10
d year April 1991 - February 1992																
ium (µg/g)	25±9	41±26	115±176	230±371	84±61	320±510	68±26	17	N/A		64-381	93-364	108±32	138±96	214±200	29±2
per (µg/g)	10±4	11±3	29±20	20±10	24±16	20±23	203±236	14	N/A	100	25-2605	11-106	227±211	103±111	46±32	13±2
n (µg/g)	8045±3340	19724±8045	25036±20764	23724±8593	14961±4807	19422±1328	155381±5902	19785	N/A		8512-58310	2168-45307	21117±7349	45531±33206	21650±21703	37921±29842
anese (µg/g)	864±46	174±50	351±356	224±88	253±151	275±142	300±176	54	N/A		222-3805	96-9502	511±258	6704±6897	1283±1160	674±305
ckel (µg/g)	11±7	27±9	50±28	51±22	58±24	54±26	43±11	24	N/A	15	96-6372	20-232	160±97	217±194	57±36	24±8
ad (µg/g)	9±4	9±5	13±10	12±7	11±0	18±12	90±4	9	N/A	56	24-290	15-50	356±475	56±124	17±10	24±5
nium (µg/g)	14±6	25±5	51±23	40±14	57±27	42±12	211±214	N/A			N/A	N/A	N/A	N/A	N/A	N/A
c (µg/g)	15±15	20±0	40±13	36±19	17±8	37±32	25±12	41		165	66-1643	259-770	307±412	307±337	37±18	49±10
d year April 1992 - February 1993																
ium (µg/g)	N/A	N/A	132±23*	N/A	71**	109**	75±70	N/A	39		64-381	93-364	108±32	138±96	214±200	29±2
per (µg/g)	N/A	N/A	50±8*	N/A	23**	9**	37±18*	N/A	16	100	25-2605	11-106	227±211	103±111	46±32	13±2
n (µg/g)	N/A	N/A	21946±8915*	N/A	12096**	22965**	7463±2429*	N/A	10530		8512-58310	2168-4307	21117±7349	45531±31206	21650±21703	37921±29842
anese (µg/g)	N/A	N/A	197±207*	N/A	254**	249**	497±401*	N/A	394		222-3805	96-9502	511±258	6704±6893	1283±1160	674±305
ckel (µg/g)	N/A	N/A	89±15*	N/A	46**	26**	70±14*	N/A	27	15	96-6372	20-232	160±97	217±194	57±36	24±8
ad (µg/g)	N/A	N/A	7±9*	N/A	6**	5**	11±2*	N/A	16	56	24-290	15-50	356±475	56±124	17±10	24±5
nium (µg/g)	N/A	N/A	N/A*	N/A	N/A	N/A**	N/A	N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A
c (µg/g)	N/A	N/A	78±7	N/A	70**	42**	28±10*	N/A	43	165	66-1643	259-770	307±412	307±337	37±18	49±10

N/A Data not available

an April 1992 and June 1992
ata for April 1992
a Met, E.P.D. 1990
a Met, I.W. 1990
openhagen, 1992

during the third year were generally in the same range as the previous two years. It must, however, be stressed that the data of the third year is still not complete making comparisons reliable.

8.1.3.3 Discussion

Metal accumulations in sediments, may have various sources, they are either directly induced by sewage and industrial effluent or indirectly brought into rivers from atmospheric emission, precipitation or by surface run off. The particular threat of metals lies in the fact that they are not decomposed by microbiological activity. On the contrary, metals can be enriched by organisms and the type of bonding can be converted to more poisonous metal organic complexes.

The major mechanisms for accumulation of metals in sediments lead to the existence of five categories (Salomon and Förstner, 1980): (1) adsorptive and exchangeable, (2) bound to carbonate phases, (3) bound to reducible phases, (4) bound to organic matter and sulphides and (5) detrital metals. These categories have different behaviours with respect to remobilization under changing environmental conditions, like a change in redox potential or water pH (Förstner, 1982), so knowledge of the total content of metals in a sediment, does not give a complete picture of the environmental situation. The environmental impact of the five speciation categories depends upon remobililzation. The first three, whcih can release their metal loads by lowering the pH (adsorptive and exchangeable and bound to carbonate) or redox potential (bound to reducible phases) are highly dangerous. From the preceding it is therefore clear that bottom sediment play an important role in the distribution of metals in the aquatic environment. They can act as resorvoirs and release metals into the water through resuspension or leaching (Salomons, 1985; Salomons *et al.*, 1987).

In the present study, much higher concentrations were detected in the sediment (ug/g x 1000) than in the water (ug/l), due to the adsorption of metals on sediment particles. It is also an indication of the chronic nature of pollution in the area (Dallinger and Kautzky, 1985; Mac and Schmitt, 1992). There is, however, a continuous interaction between the water and the sediment columns (Fig. 8.6) depending on factors such as the water pH. When the pH is alkaline, in other words more hydroxyl ions (OH^-) are present than hydrogen ions (H^+), insoluble metal hydroxyl complexes will form. However, when rainfall occurs, as was the case in December 1990, the hydrogen ion concentration will increase. The solubility of the metals will increase slightly and an increase in the water metal concentrations may be detected. The iron concentrations in the water increased considerably in December 1990, but increasing solubility was not the only reason for this phenomenon. Weathering of underlying rock formations, especially basalt, will produce iron (Dury, 1981). As locality 3 (near Balule) is underlain by basalt, the highest iron concentrations were detected there. Iron is also a highly abundant element and therefore, of all the metals investigated, iron was found to occur in the highest concentrations. The copper and strontium concentrations in the Selati River, especially in the sediment, were much higher than the concentrations in the Olifants River, indicating that these two metals originated from a local source in the Selati River catchment.

At present any evaluation of the obtained sediment metal concentrations can only be speculative. This can be attributed to the fact that no sediment quality guidelines for Southern Africa or background (natural) sediment metal concentrations for the Olifants and Selati River catchment are available. Furthermore, no attempt was made to distinguish whether the

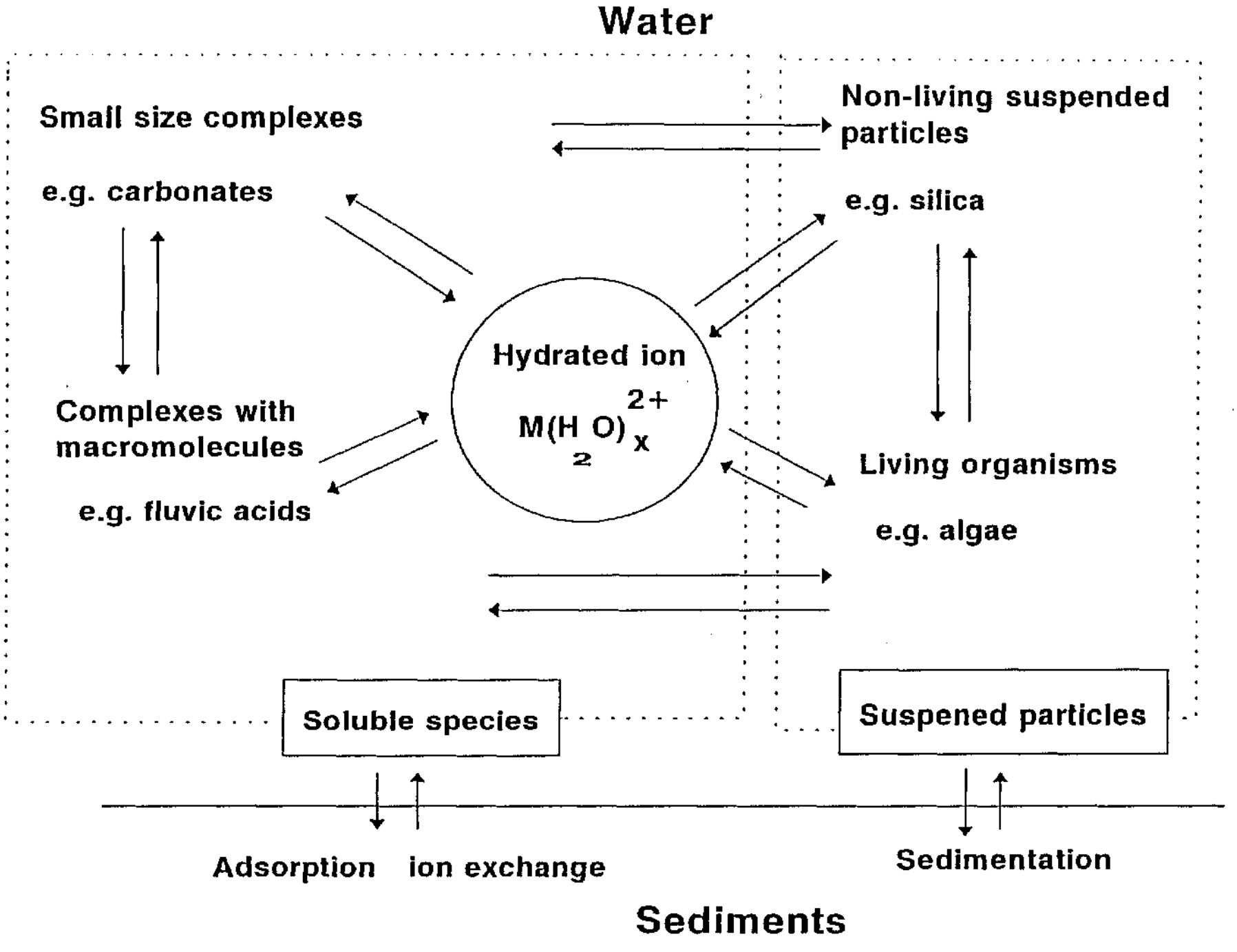

FIGURE 8.6

Distribution of metals (M) between the different abiotic and biotic compartments of the aquatic ecosystem (After Brezonic etal., 1991)

trace metals in the sediment are in a readily available form which is very important when evaluate the pollution potential of the sediment (Van der Merwe *et al.*, 1994). Nevertheless, from Table 8.10, it is evident that the mean sediment copper concentrations of locality 7 (Selati River) and the mean nickel concentration at all the localities exceeded the maximum permissible content of these metals in soil (Vivier *et al.*, 1988). The lead and zinc concentrations at the localities were however, lower than the maximum levels for soil (Table 8.10). The need for background trace metal concentrations in sediment for a specific system is clearly demonstrated by the fact that although the nickel concentrations of the two reference sites (Pionier Dam and Hlanganini Dam which receives no effluents or runoff from outside the Kruger National Park) were higher than the proposed maximum levels for soil, these levels were generally lower than the nickel concentrations at the other localities.

If it is assumed that the natural background metal levels of the sediment from the localities are in the same range as Germiston Lake or the Natalspruit River (which are known to be subjected to metal pollution), it appears that the sediment at localities 3 to 7 are contaminated by chromium (Table 8.10). Furthermore, copper contamination may be occuring at locality 7 while localities 3, 5 and 6 are subjected to iron contamination. The lead, manganese and nickel concentration at the localities were much lower than the levels in sediment from Germiston Lake and the Natalspruit River (Table 8.10) and generally in the same range as levels in the sediment from the Nooitgedacht Dam which is much less contaminated. It, therefore, appears that the sediment of the Selati River and Olifants River below the Phalaborwa Barrage do not contain abnormal high levels of these (Mn, Ni, Pb) metals. The levels for copper in the sediment of localties 1 to 6 were also in the same range (but not locality 7) as the concentration at the Nooitgedacht Dam, therefore also indicating relatively low levels of contamination. It must, however, be stressed that information regarding natural background levels as well as some indication of sediment quality guidelines must be obtained before conclusive evaluation of possible sediment contamination in the lower Olifants River can be made.

8.1.3.4 Conclusions

The data indicated that variable concentrations of metals in the sediment at the lower Olifants river and the Selati River were detected. It appears that the sediment of some of the localities in the Olifants River may be subjected to chromium contamination. The data also suggests the contamination of the Selati River sediment by copper and chromium. It must be stressed that this evaluating is based on the assumption that the natural background levels are in the same range as data from the literature (Table 8.10). There are an urgent need for more information regarding natural background metal concentrations and sediment quality guidelines. Furthermore, since metals can be released to the water column, it is also important to know whether trace metals in the sediment are in a readily available form (Van der Merwe *et al.*, 1994).

8.1.3.5 References

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8.1.4 *Bioaccumulation*

8.1.4.1 Introduction

The urgency to propose a set of standard water quality criteria for aqautic life in South Africa has brought about the need of finding adequate indicator systems for various pollutants. Biological systems consist of a vast diversity of habitat and organization and yet it thrives within relative narrow physical and chemical limits. Metals play an important role in these systems, but there are carefully circumscribed boundaries. Those metals essential to life processes, pose the joint threat of a deficiency disease at excessively low concentrations and toxic reactions at excessively high ones (Weiss, 1978). Toxic substances are not easily defined, since the effect of exposure to any potentially poisonous material, depends on its concentration, the duration of exposure and environmental conditions, such as temperature, pH, hardness and alkalinity. Susceptibility to poison will also vary, not only with species, but also within some species e.g. male, female, differences and juvenile, adult differences.

An important property of living organisms is their ability to bioaccumulate organic and inorganic substances in their tissue. Thus, substances which occur at such low levels in water and food as to pose no direct threat through toxicity may, if absorbed, be accumulated in food chains and affect predators. Bioconcentration is the process of accumulation of water-borne chemical substances by fish, through the gills and other membranes and exclude accumulation via dietary routes (Veith, *et al.*, Barron, 1990). A proportional constant relating the concentration of a chemical in the water (Cw) or Sediment (Cs) to its concentration in an aquatic organism at steady state equilibrium, is the bioconcentration factor (BCF). The BCF is the estimate of a chemical's propensity to accumulate in an aquatic animal, however, it is not an absolute value for any metal or organ, as a given tissue changes in concentration over time of exposure to a specific toxicant. The following equation represents the bioconcentration factor in relation to the concentration in the water (Cw) and the sediment (Cs) (Veith, *et al.*, 1979; Barron, 1990):

$$Cf = Cw \times BCF > \text{ or } < Cf = Cs \times BCF$$

Biomagnification is the term used for the process where an organism accumulates a toxic substance via dietary routes, by being at a higher trophic level (Heath, 1987). Bioaccumulation is the term used for the accumulation of a toxicant from the ambient water as well as from the food, thus bioconcentration and biomagnification. Bioaccumulation is one of the most important environmental transports and partitioning processes (Clark *et al.*, 1990).

The four possible routes for a toxic substance to enter a fish are via the gills (Matthiessen and Brafield, 1977; Heath, 1987), food (Waldichuk, 1974; Baudin, 1987), drinking water (Eddy, 1981) and the skin (Heath, 1987). In the natural environment, it is difficult to establish whether toxic chemical substances enter the fish through dietary routes, through membranes or both. Metal accumulation via the gills may be correlated with a mass specific ration, where smaller fish accumulate metals at a higher rate irrespective of the species (Anderson and Spear, 1980; Marks *et al.*, 1980). The relative importance of each of these routes varies, but the most significant factor may be the availability of the toxic substance for bioaccumulation (Heath, 1987).

The accumulation of metals in fish can be considered as a function of uptake and excretion rates (Heath, 1987). Uptake is contemplated to be passive and involves diffusion down gradients created by adsorption or binding of the metal to the tissue and cell surface. The gills of teleost are a likely site for metal uptake from the ambient water, due to the large surface area and the close proximity of the internal and external environment (Stagg and Shuttleworth, 1982; Heath, 1987). From the gills, metals are transported by blood bound to a protein in most cases, while some metals bind the amino acids (Stagg and Shuttleworth, 1982). Copper- and zinc-binding proteins have already been found in fish and it may be suspected that there are a different protein for each essential trace metal (Fletcher and Fletcher, 1980). Within the body, the degree of accumulation in various tissues will be dependent on the binding of metals to specific ligands. It is expected that organs such as liver and kidney, which secrete specific metal binding proteins and organs which are targets of toxicant action, will accumulate metals to significant levels (Marafante, 1976). The liver is known to be an organ of considerable importance in the storage and uptake of metals and it is also known to be the site of a number of detoxification functions (Klaassen, 1976).

The advantageous position of the liver for clearing the blood of substances entering the circulation from the gastro-intestinal tract is eminent. Since the blood from the gastrointestinal tract passes through the liver before reaching the systemic circulation, theoretically the liver can remove toxicants from the blood, biotransform it or excrete it into the bile and thus prevent its distribution to other parts of the body (Klaassen, 1976). The contents of the bile is however emptied into the gastro-intestinal tract, which make re-absorption of metals via the gut possible. The metals in the bile may be bounded by organic material or detoxifying enzymes (metallothioneins), which will result in the metal being unavailable for re-absorption. There is evidence that proteins (metallothioneins) in the liver and kidney bind metals and thereby reduce their toxicity (Buhler *et al.*, 1977; Stagg and Shuttleworth, 1982). Metallothioneins are small, low molecular weight proteins, unique in their high cysteine and metal content, and are present in low concentrations in the liver under normal conditions (Webb, 1982). Metal exposure drastically increase the concentration by stimulation metallothionein synthesis in the liver (Noël-Lambot *et al.*, 1978). The hypothesis is that metallothioneins act to sequester metals and thus reduce their toxicity (Klaassen, 1976).

After a toxicant has entered the bloodstream of a fish it may be biotransformed, stored or excreted (Fig. 8.7). The three existing pathways that metals follow after entering the bloodstream, are therefore biotransformation by either the liver, kidney or certain metabolites, storage by either the liver, kidney, muscle or fat tissue and excretion by the liver, skin, gills or kidney (Fig. 8.7).

CHEMICAL	
BIOTRANSFORMATION	STORAGE
	FAT
LIVER	LIVER
KIDNEY	KIDNEY
METABOLITES	MUSCLE
EXCRETION	
MUCOUS	LIVER-BILE-INTESTINE
GILLS	KIDNEY-URINE

FIGURE 8.7: Schematic diagram of the possible routes for a toxicant after entering the bloodstream of a fish (Heath, 1987).

The process of toxicant accumulation could be influenced by factors with included environmental conditions (eg. temperature, salinity, organic) material), physiochemical properties, biotransformation and intra- and interspecies variation, to mention only a few. Temperature affects the physiology as well as the biochemistry of aquatic animals to a large degree. In general increasing temperature tends to increase rates of uptake and excretion and will thus affect the rate of bioconcentration (Heath, 1987). The uptake of water-borne metals by aquatic organisms is inversely related to the salinity of the medium (Somero *et al.*, 1977). A partial explanation for the reduced toxicity of metals in hard water is the decreasing solubility due to the formation of complexes between metals and anions present in the water. There is a possibility that the concentration of environmental calcium may be a vital factor in influencing metal uptake and toxicity (Varanasiu and Gmur, 1978). A considerable amount of organic material or suspended solids, may reduce the actual amount of dissolved metal available for the fish to absorb. This tendency to form complexes varies with the metal. A considerable amount of organic material or suspended solids, may reduce the actual amount of dissolved metal available for the fish to absorb. This tendency to form complexes varies with the metal. The physiochemical properties of a metal determines its biological activity. This leads to the fact that only the bioavailable fraction of an organic chemical in water can be accumulated by an aquatic animal (Baron, 1990).

Biotransformation of organic chemicals act to decrease the equilibrium level of the chemical an aquatic animal accumulates by increasing the rate of elimination. Inhibition of metabolism has been shown to increase the bioconcentration of a variety of organic chemicals (Baron, 1990). Animal size is of recognized importance in determining the rate of physiological processes and the allometry of tissue growth. The uptake of DDT in fish also show size dependence related to a decrease in uptake per unit weight with increasing body size.

In this section of the study the extent of metal bioaccumulation in the tissue of the benthic feeder, *Barbus marequensis, Labeo rosae* and omnivorous fish

specie, *Clarias gariepinus* from the Olifants River (Kruger National Park), the Selati River and the Pionier Dam (Kruger National Park) was determined as well as the tissue that accumulated the highest and lowest metal levels respectively. As a result of the large volume of data collected, the results and discussion section will only deal with the data collected for *B. marequences*. By adopting this strategy the general trends in the bioaccumulation of metals by fish in the study area would be adequately defined.

8.1.4.2 Materials and methods

Field sampling

Large scaled yellowfish (*Barbus marequensis*) were sampled with gill nets (70 - 120 mm stretched mesh size) and throw nets every alternative month from April 1990 to February 1992 at localities 3, 4 and 5 in the Olifants River and at locality 7 in the Selati River (Fig. 7.2).

In February 1992 ten fish were also collected at Pionier Dam (Kruger National Park), the natural reference point used in this study. After capture, the weight and fork length of each fish were recorded. Fish scales were collected for age determination and blood samples were drawn for metal analysis. The fish were then dissected on a polyethylene work-surface, using stainless steel tools (Heit and Klusek, 1982) and wearing surgical gloves. The gut contents, as well as the following organs and tissues were romoved for metal analysis: skin, axial muscle, gills, gonads, fat, liver, kidney, gut (fore and hind separately), bile and vertebrae. During the first year (April 1990 - February 1991) only selected tissues were used which were subsequently expanded during the second year (April 1991 - April 1992). All the samples were kept frozen, until they could be subjected to metal concentration analysis in the laboratory.

Laboratory procedures

After the tissue samples were thawed, all the organs and tissues (except for the bile and blood) were dried in an oven at 60°C for a period of 48 hours. The wet and dry weights of the samples were recorded in order to calculate the percentage of moisture of each sample. Ten ml concenrated nitric acid (55%) and 5 ml perchloric acid (70%) were added to one gram dry tissue in a 100 ml Erlenmeyer flask. Digestion was performed on a hot plate (200 tot 250 °C) for at least four hours, until the solutions were clear (Van Loon, 1980). The bile was digested in a similar manner, except that it had not been dried. For the blood digestion, 5 ml each of concentrated nitric (55%) and perchloric acid (70%) were added to 0.5 ml blood in a 100 ml Erlenmeyer flask and digestion similar to the other samples was then performed.

After digestion each solution was filtered using an acid-resistant 0.45 μm filter paper and a vacuum pump. The filter system was then rinsed with doubly distilled water, whereafter the samples were made up to 50 ml each with doubly distilled water. The samples were stored in clean glass bottles, until the zinc concentrations could be determined. Prior to use, all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 hours, rinsed in doublty distilled water,a cid-washed in 1M HCl for 24 hours and rinsed again in doubly distilled water (Giesy and Wiener, 1977).

A Varian atomic absorption spectrophotometer (Spectra AA-10) was used to determine the zinc concentrations in the tissue samples of the fish.

Analytical standards were prepared from Holpro stock solutions. The metal concentrations in the tissue samples were calculated as follows:

$$\text{Metal concentration } (\mu g/g) = \frac{\text{AAS reading } (\mu g/ml)}{\text{Sample mass}} \text{ X Sample volume (50ml)}$$

Bioconcentration factors between the fish tissues and the water (BFw) and sediment (BFs) were determined, using only the mean zinc concentration in each organ. The formula (Wiener & Giesy, 1979) is:

$$\text{BFw or BFs} = \frac{\text{[Zn] in organ } (\mu g/g \text{ dry wt.})}{\text{[Zn] in water } (\mu g/ml) \text{ or sediment } (\mu g/g)}$$

Age determination

The scales were washed with warm water and soap and were placed between two objective slides which were then tightened with masking tape. The circuli were counted under a microprojector (Nielsen and Johnson, 1983).

Statistical procedures

Statistical differences between the different organs and tissues were determined by grouping together the localities inside the KNP (3, 4 and 5). Comparisons were made for winter 1991 (June and August 1991), spring 1991 (October 1991) and summer 1992 (January and February 1992) by means of the Scheffe statistical test. The significant level was $p \leq 0.05$.

Variation in capture success limited the statistical comparisons of the localities. Only a few organs, sampled in months when the number of fish caught at each locality was three or more, were used. For the first year, localities 3 to 5 were compared using the zinc concentrations in the gill, liver and muscle tisues of October 1990. In the second year, the zinc concentrations in the fat, muscle, vertebrae and blood were used to compare localities 3, 4, 5 and 7 in January 1992 and localities 3 to 5 in June 1991, October 1991, January 1992 and February 1992. Pionier Dam was also compared to localities 3 to 5 in February 1992. The Hotelling T^2 and Scheffe test of the BMDP 2V statistical program were used ($p \leq 0.05$).

Seasonal differences were determined for males and females, as well as for the sexes combined. The data collected at localities 3, 4 and 5 were grouped into seasons as follows: autumn 1990 (April 1990), winter 1990 (June and August 1990), spring 1990 (October 1990), summer 1990/92 (December 1990 and February 1991), autumn 1991 (April 1991), winter 1991 (June and August 1991), spring 1991 (October 1991) and summer 1992 (January and February 1992). The seasons were statistically compared using the zinc concentrations in the muscle, gill (excluding autumn 1991), liver (excluding autumn 1990 and summer 1990/91), blood (excluding the seasons of the first year), skin and vertebrae (excluding the seasons of the first year, as well as autumn 1991). The Scheffe and Hotelling T^2 tests (BMDP 2V program) were used ($p \leq 0.05$).

Using the Hotelling T^2 test (BMDP 2V program), the first year (April 1990 - February 1991) and second year (April 1991 - February 1992) were statistically compared with respect to the gill, gonad, liver and muscle zinc

concentrations. In order to obtain a reliable comparison with a large N value, the data of localities 3 to 5 were grouped together.

8.1.4.3 Results

Fish size, age and tissue moisture content.

The mean weight and length of the fish (*B. marequensis*) that were caught at the different localities for each month are presented in Table 8.11. In general, the female yellow fish (*B. marequensis*) were larger than the male fish at each locality (Table 8.11). The largest yellow fish were usually caught at locality 5 (Mamba weir), except in February 1992 when the largest fish were caught at Pionier Dam. The breeding season stretches from October to April and it was noted that the largest fish were caught during the month of October.

The age determination was difficult due to unclear circuli which were formed during the dry periods and also because no sharp difference in water temperature had occurred between the different seasons. Nevertheless, the data indicated that the yellow fish were 1 to 2 years of age at a forklength and 4 to 6 years at 34 to 40 cm forklength.

The moisture content in the tissues of *B. marenquensis* differed, with the mean percentage of moisture being 79 ± 2% in the gut, 77 ± 2% in the muscle, 74 ± 3% in the gills, 69 ± 5% in the kidney, 67 ± 5% in the liver, 62 ± 3% in the skin, 42 ± 2% in the vertebrae and 10 ± 8% in the fat.

Zinc bioaccumulation in the tissues of B. marequensis

Large variation was detected between the tissue zinc concentrations of indiciduals at the same locality, e.g. zinc concentrations in the female gonads ranged from 107 ug/g to 483 ug/g Zn at locality 4 in October 1991. Variation was also detected between the zinc concentrations of the different tissues, but the bioaccumulation pattern of zinc in *B. marequensis* was determined to be: skin > gonads (F) > liver > hindgut contents > vertebrae > gills > kidney > hindgut ≈ foregut > gonads (M) > foregut contents > muscle > blood > fat > bile. Zinc concentrations in the skin and female gonads differed significantly ($p \leq 0.05$) from the zinc concentrations in all the other organs and tissues. However, no significant difference ($p > 0.05$) was detected between the various zinc concentrations in the muscle, blood, fat and bile (Table 8.12).

The bioconcentration factors between the tissues and water were mostly very high, ranging from 2.2 for the bile (June 1991) to 15 760 for the skin (August 1991). The BFs between the tissues and sediment, on the other hand, were much lower and ranged from 0.02 for the bile (June 1991) to 32.7 for the skin (February 1992).

In the first year (October 1990), locality 3 differed significantly from both localities 4 (with respect to liver and muscle zinc concentrations) and 5 (with respect to gill, liver and muscle zinc concentrations). In the second year, locality 3 differed significantly from locality 4 in January 1992 (with respect to the blood zinc concentrations) and February 1992 (with respect to the muscle, blood, fat and vertebrae zinc concentrations). Localities 3 and 5 differed significantly only in February 1992 with respect to the muscle, fat and vertebrae concentrations, while localities 3 and 7 differed significantly in January 1992 with respect to the blood zinc concentrations. No

TABLE 8.11: LENGTHS AND WEIGHTS OF *BARBUS MAREQUENSIS* CAUGHT IN THE OLIFANTS RIVER, (KRUGER NATIONAL PARK) DURING THE PERIOD APRIL 1990 - FEBRUARY 1992

			Weight (g)		Length (cm)					Weight (g)		Length (cm)	
Month	Locality	N	Range	X ± SD	Range	X ± SD	Month	Locality	N	Range	X ± SD	Range	X ± SD
April 1990	3	1 F/f	800		35.5		April 1991	3	4 F/f	205 - 470	304.4 ± 94.2	23.6 - 29.0	25.9 ± 1.9
									2 M*	125 - 193	159.0 ± 48.1	20.0 - 22.4	21.1 ± 1.7
	4	7 F	186 - 701	353.1 ± 169.3	23.8 - 36.5	28.8 ± 3.9		4	9 F	33 - 470	161.4 ± 154.7	13.5 - 30.4	20.1 ± 6.3
		2 M*	201 - 199	150.5 ± 68.6	19.1 - 23.9	21.5 ± 3.4			1 M	220		24.9	
	5	0	-	-	-	-		5	7 F	205 - 900	452.1 ± 255.1	23.0 - 38.5	29.0 ± 5.5
	7	10 F/M+	46 - 134	64.8 ± 27.5	15.0 - 21.4	16.4 ± 2.0			3 F/M+	93 - 135	116.3 ± 21.4	17.0 - 20.0	18.7 ± 1.5
								7	1 M	240		25.0	
									1 F/M	45		14.5	
June 1990	3	2 F	216 - 222	219.0 ± 4.2	26.5 - 28.0	27.3 ± 1.1	June 1991	3	5 F	215 - 720	319.4 ± 154.6	23.0 - 33.3	25.7 ± 3.1
	4	0	-	-	-	-		4	1 F	457		28.7	
									6 M	230 - 360	309.2 ± 52.7	23.1 - 27.9	25.6 ± 2.0
	5	0	-	-	-	-		5	3M	200 - 330	261.7 ± 65.3	22.7 - 26.3	24.5 ± 1.8
	7	0	-	-	-	-		7	0	-	-		
August 1990	3	4 F	81 - 509	254.8 ± 181.4	17.3 - 32.5	24.8 ± 6.2	August 1991	3	1 F	400		27.2	
		3 M	50 - 126	115.0 ± 63.3	15.1 - 23.8	19.6 ± 4.4							
		2 F/M	61 - 182	121.5 ± 85.6	17.3 - 22.5	19.9 ± 3.7							
	4	6 F	116 - 352	244.0 ± 91.9	24.3 - 30.5	26.7 ± 2.6		4	8 F	290 - 550	404.8 ± 76.6	24.5 - 31.1	28.0 ± 1.9
		3 M	391 - 592	507.7 ± 104.3	30.5 - 35.5	33.0 ± 2.5							
	5	2 F	262 - 356	309.0 ± 66.5	26.0 - 28.5	27.3 ± 1.8		5	11 F	610 - 1110	872.7 ± 178.5	30.6 - 40.0	35.4 ± 3.0
		1 M	533		32.0				1 M	510		27.5	
		3 F/M	227 - 246	237.0 ± 9.5	25.0 - 25.9	25.5 ± 0.5							
		5 F/M	24 - 44	34.2 ± 9.5	12.5 - 15.2	14.0 ± 1.3		7	1 F/M	120		20.6	
	7												
October 1990	3	2 F	383 - 545	464.0 ± 114.6	28.3 - 30.9	29.6 ± 1.8	October 1991	3	4 F	390 - 793	540.5 ± 177.0	28.0 - 34.6	30.8 ± 2.8
		1 M	1000		35.9				2 M	269 - 400	334.5 ± 92.6	25.0 - 27.2	26.1 ± 1.6
		4 F/M	222 - 483	282.0 ± 140.9	19.4 - 28.0	24.7 ± 3.6							
	4	3 F	392 - 600	480.7 ± 107.3	21.1 - 29.5	24.7 ± 4.3		4	9 F	155 - 889	603.9 ± 206.3	21.0 - 36.0	31.1 ± 4.4
		2 M	550 - 800	636.3 ± 98.0	27.5 - 33.7	30.2 ± 2.5			2 M	400 - 459	429.5 ± 41.7	26.8 - 28.5	27.7 ± 1.2
	5	1 F	592.0		31.2			5	12 F	474 - 800	655.9 ± 114.8	27.9 - 34.0	31.0 ± 1.9
		6 M	166 - 1050	477.3 ± 323.8	20.3 - 38.7	30.6 ± 6.9			3 M	400 - 617	502.3 ± 109.0	28.5 - 31.0	29.7 ± 1.3
		1 F/M	169 - 272	235.7 ± 57.8	21.5 - 23.5	22.8 ± 1.1							
	7	1 F	900		34.0			7	1 F	188		23.0	
December 1990	3	4 F	171 - 549	382.0 ± 175.0	22.0 - 32.3	26.0 ± 4.7	January 1992	3	4 F	451 - 641	567.3 ± 84.2	29.1 - 32.0	30.7 ± 1.3
		1 M	254		24.2				2 F/f	117 - 148	132.5 ± 21.9	19.3 - 20.8	20.1 ± 1.1
		2 F/M	70 - 80	75.0 ± 7.1	16.6 - 18.6	17.6 ± 1.4							
	4	0	-	-	-	-		4	7 F	98 - 965	386.6 ± 315.8	17.9 - 38.0	26.5 ± 7.3
									1 M	120		18.6	
									3 F/M	99 - 150	118.3 ± 27.6	17.8 - 21.0	19.0 ± 1.7
	5	1 F/M	80		17			5	8 F	439 - 944	695.5 ± 163.9	29.2 - 34.7	32.4 ± 1.7
									4 M	368 - 520	456.0 ± 63.6	29.2 - 34.7	28.7 ± 1.3
	7	0	-	-	-	-		7	8 F/M	46 - 245	90.1 ± 69.1	27.0 - 29.9	17.8 ± 3.8
February 1991	3	0	-	-	-	-	February 1992	3	4 F	135 - 216	184.5 ± 35.5	22.3 - 23.0	22.7 ± 0.34
									1 M	108		22.4	
									1 F/M	151		20.4	
	4	0	-	-	-	-		4	6 F	138 - 1108	583.8 ± 343.4	20.8 - 40.5	33.4 ± 7.0
									4 M	190 - 410	286.5 ± 99.9	23.8 - 28.2	25.2 ± 2.0
	5	1 M	220	125.0 ± 69.8	24.0	19.5 ± 3.0		5	10 F	399 - 1211	659.9 ± 242.6	29.0 - 40.5	33.1 ± 3.6
		4 F/M	71 - 225		16.9 - 23.5				0	-	-	-	-
	7							7	5 F	1035 - 1679	1408 ± 300.7	35.4 - 43.5	40.2 ± 3.5
								Pionier Dam	5 M	710 - 845	806.6 ± 55.9	33.3 - 34.6	34.2 ± 0.5

f Female * Male + Female or male (fish immature)

TABLE 8.12

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE ZINC CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	SP2, S2												
Gonad (Males)		SP2, S2											
Fat	W2, SP2, S2	W2, SP2, S2	W2										
Liver	S2	W2, SP2	SP2, S2	W2, SP2, S2									
Muscle	W2, SP2, S2	W2, SP2, S2	W2		W2, SP2, S2								
Skin	W2, SP2, S2	SP2, S2	SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2							
Gut		W2, SP2		W2, SP2, S2		W2, SP2	W2, SP2, S2						
Gut cont.	SP2	SP2		W2, SP2	SP2, S2	W2	SP2, S2						
Vertebrae		SP2, S2		W2, SP2, S2	S2	W2, SP2, S2	W2, SP2, S2		SP2				
Kidney		SP2					S2						
Bile	SP2, S2	SP2, S2			SP2, S2		SP2, S2	SP2, S2	S2	S2	S2		
Blood	W2, SP2, S2	W2, SP2, S2	W2		W2, SP2, S2		W2, SP2, S2	W2, SP2	W2, SP2	W2, SP2, S2			

differences occurred in June 1991, while in October 1991 only localities 4 and 5 differed significantly with respect to the muscle zinc concentrations. Localities 4 and 5 also differed in January 1992 with respect to the muscle and fat zinc concentrations. In February 1992, Pionier Dam differed significantly from all three localities: from locality 3 with respect to the muscle, from locality 4 with respect to the blood and from locality 5 with respect to the fat, muscle and vertebrae zinc concentrations.

Generally, significant seasonal differences were detected (Table 8.13), but it was not always the same organs that indicated these seasonal differences. For instance, winter 1990 differed from summer 1992 with respect to the muscle, gill and liver zinc concentrations, while spring 1991 and summer 1992 only differed with respect to the liver zinc concentrations. No differences occurred, however, between spring 1990 and summer 1990/91, as well as between autumn 1991 and each of winter 1990, summer 1990/91 and winter 1991. Comparing the zinc concentrations in the organs of the males and females seasonally, a difference was noticed in some organs. The females had higher zinc concentrations than the males in the gonads, liver, hindgut, kidney and bile, while the males had higher zinc concentrations in the vertebrae. The zinc concentrations in the skin were higher in the females in winter 1991 and summer 1992, but in spring 1991 the males had a concentration of 295 μg/g Zn (dry weight) compared to the 213 μg/g Zn (dry weight) of the females.

Not all the organs and tissues were sampled during the first year, but by comparing the mean zinc concentrations in the organs and tissues, as well as in the gut contents, of the second year (Fig. 8.8), the bioaccumulation pattern was as follows: skin > gonads (F) > liver > gills > vertebrae > gonads (M) > hindgut > foregut contents > kidney ≈ foregut > hindgut contents > muscle > blood > fat > bile.

This pattern is slightly different from the one already mentioned (based on the monthly data), but the skin, female gonads and liver still accumulated the highest zinc concentrations, while the muscle, blood, fat and bile accumulated the lowest. The first and second year differed significantly with respect to the zinc concentrations in the gills, gonads and muscle, but not with respect to the liver zinc concentrations (Fig. 8.8).

Copper and Iron bioaccumulation in tissue of B. marequensis

The order of bioaccumulation of copper and iron in the different organs and tissues of *B. marequensis* differed slightly, but both metals accumulated mostly in the liver, kidney and gut. High copper and iron concentrations were also detected in the gut contents. The general order of bioaccumulation for copper was: liver > hindgut contents > foregut contents > hindgut > foregut > kidney > gill > bile > female gonads > vertebrae > blood > male gonads > skin > muscle > fat. The largest variation in copper accumultaion was detected in the liver concentrations, but the overall variation in copper concentration was much less than the variation in iron concentration. Statistically the copper concentrations in the liver differed significantly ($p \leq 0.05$) from the copper concentrations in all the other organs. Additionally the gut contents differed significantly from the gills, fat, muscle, skin, vertebrae and blood with respect to the accumulated copper concentrations, but only during the winter of 1991 (Table 8.14). The general order of bioaccumulation for iron was: hindgut contents > foregut contents > hindgut > liver ≈ kidney > blood > foregut > gill > skin ≈ female gonads > male gonads > bile ≈ muscle > fat > vertebrae. From April 1990 to August 1991, however, the gills accumulated higher

TABLE 8.13

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN ZINC CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *BARBUS MAREQUENSIS* FOR THE SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →	M	M	M	M	M, G	M, G	M, G
	Male →							
Winter 1990	M*	Female →	L			M, G	M, G	M, G, L
		Male →				M	M	M
Spring 1990	M*	M*	Female →			M, G, L	M, G	M, G
			Male →			M, G	M	M
Summer 1990/91	M*	G*		Female →		G	G	G
				Male →				
Autumn 1991	M*		M*		Female →		M	M
					Male →			
Winter 1991	M*,G*	M*,G*	M*,G*	M*,G*		Female →	S, V	L, S, V
						Male →	S	S
Spring 1991	M*,G*	M*,G*	M*,G*	M*,G*	M*,G*	S*,V*	Female →	L
							Male →	
Summer 1992	M*,G*	M*,G*,L*	M*,G*	M*,G*	M*,G*	L*,S*,V*	L*	

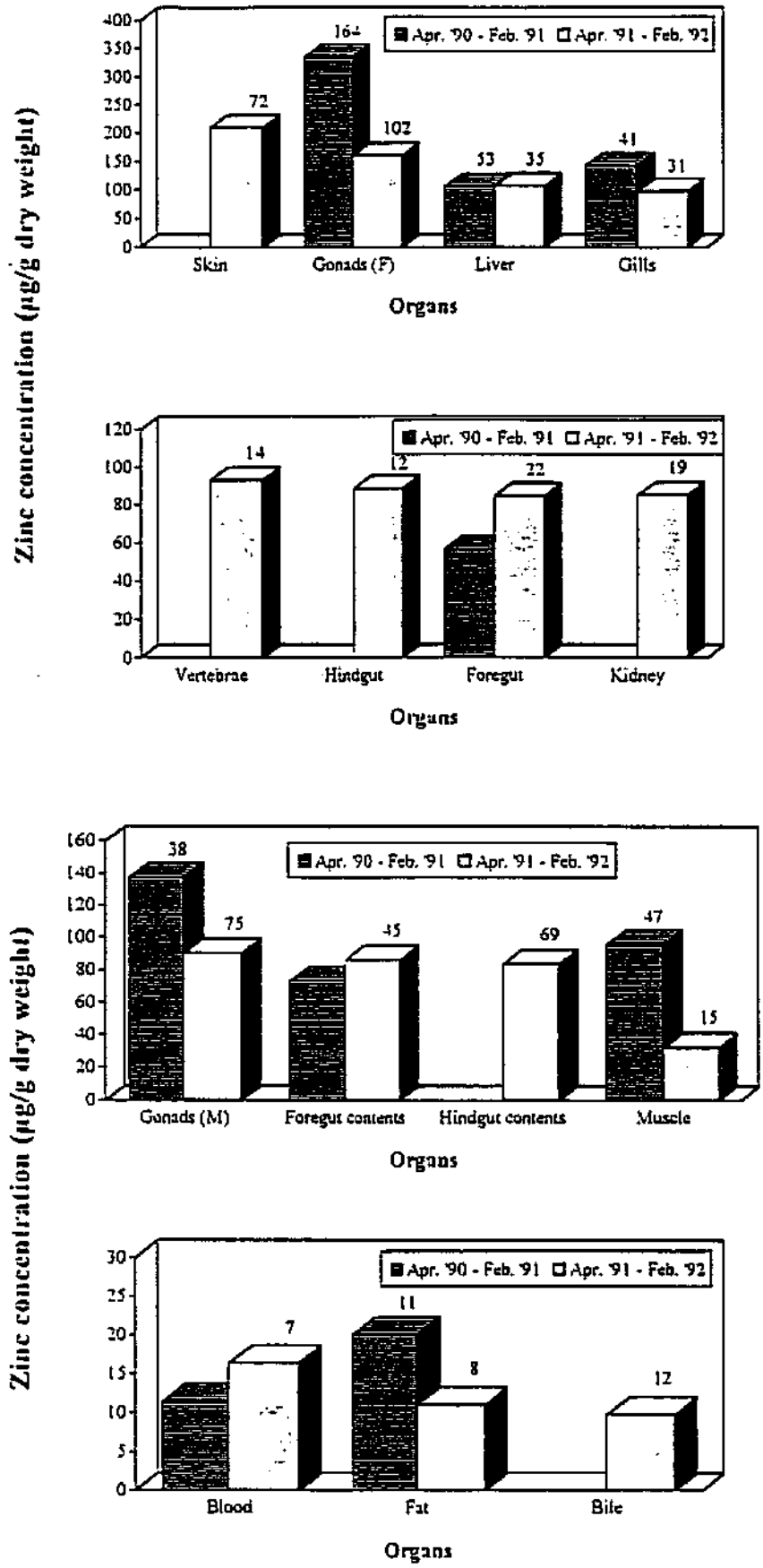

Figure 8.8

Mean zinc concentrations (µg/g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

iron concentrations than the liver. A large variation in iron concentration was detected between the different organs and tissues, as well as between individuals, especially with regard to the gut contents. The concentrations in the gut contents differed significantly from the iron concentraions in all the other organs (Table 8.15). The iron concentrations in the gills, liver, skin and blood also differed significantly from the concentrations in a few other organs, but only during the summer of 1992 (Table 8.15).

The copper and iron bioconcentration factors between the water and the organs (BFw) were much higher than the bioconcentration factors between the sediment and the organs (BFs). The copper BFw values ranged from 10 (calculated for fat tissue in December 1990) to 18 090 (Calculated for the liver in August 1991), while the BFs values ranged from 0.01 (calculated for tissues in January 1992) to 10.27 (calculated for the liver in October 1991). In the case of iron, the BFw values ranged from 0.2 (calculated for bile in February 1992) to 49 705 (calculated for the gindgut in Janaury 1992), while the BFs values ranged from 0.001 (calculated for tissues from August 1991 to February 1992) to 1.11 (calculated for the gill in December 1990).

No locality differences had occurred in the first year (October 1990) with respect to the copper concentrations in the gill, liver and muscle tissues. In the second year locality 3 differed significantly ($p \leq 0.05$) from locality 4 during the months of June 1991 (with respect to the vertebrae copper concentrations) and February 1992 (with respect to the fat muscle and vertebrae copper concentrations). Localities 3 and 5 differed significantly in October 1992 (with respect to the muscle, fat and vertebrae copper concentrations), while localities 4 and 5 only differed significantly with respect to the vertebrae copper concentrations in October 1991 and also the blood copper concentrations in January 1992. In general most of the organs collected at locality 7 had accumulated higher copper concentrations than the collected organs at the other localities. In January 1992 locality 7 differed significantly from localities 3 (with respect to the fat, muscle and vertebrae concentrations), 4 (with respect to the fat and vertebrae concentrations) and 5 (with respect to the fat, muscle and vertebrae concentrations). Most of the organs collected at Pionier Dam in February 1992 had accumulated lower copper concentrations than the collected organs at the other localities, and differed significantly from localities 3 (with respect to the fat, muscle, vertebrae and blood concentrations), 4 (with respect to the muscle and vertebrae concentrations) and 5 (with respect to the vertebrae concentrations).

In the case of iron, locality 4 differed significantly from both localities 3 (with respect to the gill and liver iron concentrations) and 5 (with respect to the gill, liver, muscle and fat iron concentations) in October 1990, the first year of this study. In June 1991, the second year, locality 3 differed significantly from localities 4 (with respect to the muscle and vertebrae iron concentrations) and 5 (with respect to the muscle, vertebrae and blood iron concentrations), but in January 1992 it only differed significantly from locality 4 (with respect to the blood iron concentrations). Locality 4 differed significantly from locality 5 in June 1991 (with respect to the blood iron concentrations), in October 1991 (with respect to the fat iron concentrations) and in February 1992 (with respect to the muscle iron concentrations). In January 1992 locality 7 differed significantly from all the other localities (3, 4 and 5) with respect to the iron concentrations in the fat and vertebrae. Pionier Dam differed significantly from localities 3 (with respect to the blood iron concentrations) and 5 (with respect to the fat iron concentrations).

TABLE 8.14

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE COPPER CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)													
Gonad (Males)													
Fat													
Liver	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2									
Muscle					W2, SP2, S2								
Skin					W2, SP2, S2								
Gut					W2, SP2, S2								
Gut cont.	W2			W2	SP2, S2	W2	W2						
Vertebrae					W2, SP2, S2				W2				
Kidney					SP2, S2								
Bile					SP2, S2								
Blood					W2, SP2, S2				W2				

TABLE 8.15

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE IRON CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	S2												
Gonad (Males)	S2												
Fat	S2												
Liver	S2	S2	S2	S2									
Muscle	S2				S2								
Skin	S2				S2	S2							
Gut													
Gut cont.	W2, SP2, S2	W2, SP2	W2, SP2	W2, SP2, S2	W2, SP2, S2	W2, SP2	W2, SP2, S2	W2, SP2, S2					
Vertebrae	S2				S2		S2		W2, SP2, S2				
Kidney									SP2, S2				
Bile	S2				S2				SP2, S2				
Blood	S2	S2	S2	S2	S2	S2	S2		W2, SP2	S2		S2	

Significant seasonal differences ($p \leq 0.05$) were detected, but it was not always the same organs that indicated these differences (Tables 8.16 and 8.17). Using the data for both sexes combined, the summer of 1990/91 differed significantly from all the other seasons (except autumn 1990 and autumn 1991) with respect to the copper and iron concentrations in the gills. This trend was also found for the females, but to a lesser extent than for the males (except in the case of iron). The iron concentrations in the muscle also indicated significant differences between the summer of 1990/91 and the seasons of the second year, except autumn 1991 (Table 8.17). Autumn and winter of 1990 differed significantly from most of the other seasons (especially in the case of the female fish), but only with respect to the iron concentrations in the organs (Table 8.17) and not with respect to the copper concentration (Table 8.16). Furthermore, all the seasons in the second year differed significantly from one another with respect to the copper and iron concentrations in various organs (Table 8.16 and 8.17).

Comparing the metal concentrations in the organs and tissues of the males and females seasonally, a difference was noticed in some organs. The copper concentrations in the gonads, muscle, vertebrae and fat of the males were mostly higher than that of the females, while the females had higher copper concentrations in the blood and bile. The iron concentrations were mostly higher in the gills, gonads, muscle and vertebrae of the males, while the females had higher hindgut, liver, skin and bile iron concentrations.

The first and second year differed significantly with respect to the gill, liver, muscle and male gonad copper concentrations (Fig. 8.9) and also with respect to the iron concentrations in the gill, muscle and gonads of both sexes (Fig. 8.10). The mean metal concentrations in the organs and gut contents during the second year (Figs 8.9 and 8.10) were also used to determine the order of bioaccumulation and it differed slightly from the order based on the monthly data. For copper it was: liver > foregut contents > hindgut contents > hindgut > foregut > kidney > gill > bile ≈ female gonads > male gonads > vertebrae ≈ muscle > skin > blood > fat; and for iron, hindgut contents > foregut contents > hindgut > foregut > kidney > liver > blood > gill > male gonads > female gonads > skin > muscle > fat > bile > vertebrae.

Chromium and nickel bioaccumulation in the tissues of B. marequensis.

The order of bioaccumulation of chromium and nickel in the different organs and tissues of *B. marequensis* was not clear to distinguish, but the highest concentrations of both metals were detected in the gut contents and blood of the fish, as well as in the vertebrae in the case of nickel. Variation in metal concentration, especially in chromium concentration, was mostly detected in the gut contents. The general order of bioaccumulation for chromium was: hindgut contents > foregut contents > blood > bile > vertebrae > hindgut > gill > foregut ≈ kidney > liver > male gonads ≈ fat > female gonads ≈ muscle > skin. Statistically the gut contents differed significantly ($p \leq 0.05$) from all the organs with respect to the accumulated chromium concentrations. In addition, the blood and vertebrae differed significantly from most of the other organs with respect to the accumulated chromium concentrations, but only in the summer of 1992 (Table 8.18). In the case of nickel, the general order of bioaccumulation was: hindgut contents > foregut contents ≈ blood > vertebrae > gill > hindgut > bile > kidney > foregut > liver > muscle ≈ female gonads > male gonads ≈ skin > fat. Statistically the gills, blood and vertebrae differed significantly from most of the other organs with respect to the accumulated nickel concentrations, while the gut contents differed significantly from all

TABLE 8.16

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN COPPER CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *B. MAREQUENSIS* FOR SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →							
	Male →							
Winter 1990		Female →		G				
		Male →						
Spring 1990			Female →	G				
			Male →					
Summer 1990/91		G*	G*	Female →		G	G, M	G, M
				Male →				
Autumn 1991					Female →	B	B, M	B, M
					Male →		B	B
Winter 1991				G*	B*	Female →	M, V, S	M, B, S
						Male →	V, B	V, B
Spring 1991			M*	G*	B*	V*, S*, B*	Female →	V
							Male →	
Summer 1992			M*	G*	M*, B*	V*, S*, B*	V*	

TABLE 8.17

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN IRON CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *B. MAREQUENSIS* FOR SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →	G	G			G, M	G, M	G, M
	Male →							
Winter 1990	G*	Female →		G		M, L	G, M	G, M
		Male →		G		L		
Spring 1990	G*		Female →	G				M
			Male →			M	M	M
Summer 1990/91		G*	G*	Female →		G	G, M	G, M
				Male →		G, M	G, M	G, M
Autumn 1991					Female →	M	M, B	M
					Male →		M	M
Winter 1991	G*, M*	M*, L*	M*	G*, M*	M*	Female →	V	V, L
						Male →	V	V
Spring 1991	G*, M*	G*, M*	M*	G*, M*	G*, M*, B*	V*	Female →	
							Male →	
Summer 1992	G*, M*	M*	M*	G*, M*	G*, M*	V*, L*	B*, L*	

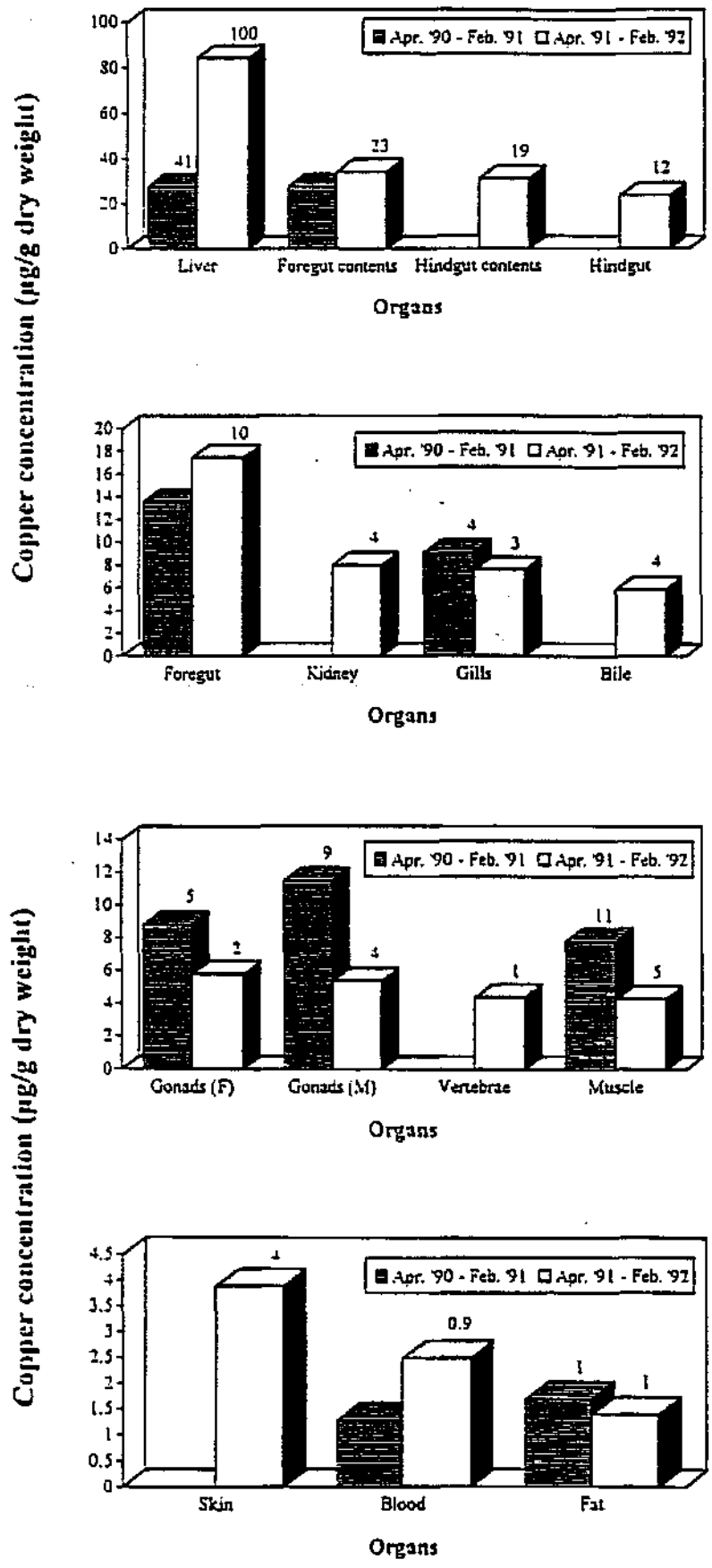

Figure 8.9

Mean copper concentrations (µg·g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

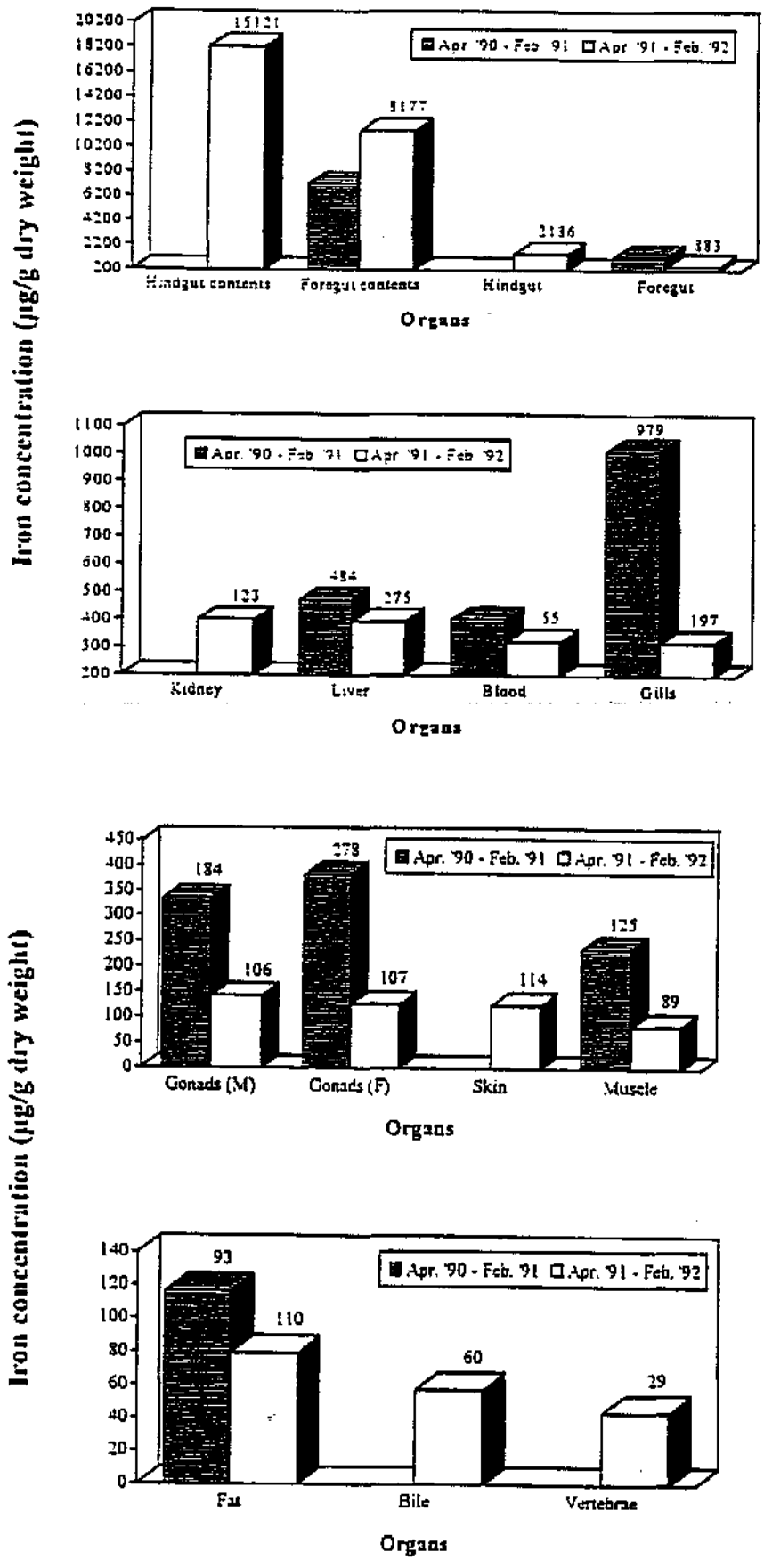

Figure 8.10

Mean iron concentrations (µg/g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

the organs with respect to the accumulated nickel concentrations (Table 8.19).

The calculated bioconcentration factors between the water and the organs (BFw) were higher than the bioconcentration factors between the sediment and the organs (BFs). The chromium BFw values ranged from 4.6 (calculated for female gonads in February 1992) to 2314.3 (calculated for blood in January 1992), while the BFs values ranged from 0.001 (calculated from various tissues in August 1991) to 3.45 (calculated for the gills in December 1990). Nickel BFs values ranged from 2.9 calculated for fat tissue in August 1991) to 1090 (calculated for blood in February 1992), while the BFs values ranged from 0.01 (calculated for various tissues in August 1991 and February 1992) to 1.69 (calculated for blood in October 1991).

Although the chromium and nickel concentrations in the fish organs were in the same range at each locality, significant differences ($p \leq 0.05$) between the localities did occur. In the fisrt year (October 1990) locality 3 differed significantly from localities 4 (with respect to the muscle and vertebrae chromium concentrations) and 5 (with respect to the muscle chromium concentrations), but in October 1991 it only differed significantly from locality 5 (with respect to the fat and muscle chromium concentrations). Locality 7 differed significantly from localities 3 (with respect to the fat chromium concentrations) and 5 (with respect to the fat and vertebrae chromium concentrations) in January 1992 and in February 1992. Pionier Dam differed significantly from localities 3 (with respect to the fat, muscle and blood chromium concentrations), 4 (with respect to the fat and blood chromium concentrations) and 5 (with respect to the blood chromium concentrations). The chromium concentrations in the blood of the fish at Pionier Dam were lower than at the other localities, but in the other organs the chromium concentrations were higher.

Nickel concentrations detected at locality 7 differed significantly from concentrations detected at localities 3 and 4 (with respect to the muscle), as well as from concentrations detected at locality 5 (with respect to the liver) in October 1990. Locality 3 differed significantly from localities 4 (with respect to the fat, muscle and vertebrae nickel concentrations) and 5 (with respect to the muscle nickel concentrations) in June 1991, but only from locality 5 (with respect to the blood nickel concentrations) in October 1991. In January 1992 locality 7 differed significantly from localities 5 and 4 (with resepct to the vertebrae nickel concentrations), as well as from locality 3 (with respect to the vertebrae and fat nickel concentrations). Pionier Dam differed significantly from localities 3 and 4 with respect to the fat and muscle nickel concentrations, for more nickel accumulated in the fish tissues at Pionier Dam than it did at the other localities.

Significant seasonal differences ($p \leq 0.05$) with regard to the mean chromium and nickel concentrations in various organs were detected. Chromium in the summer of 1990/91 and winter of 1991 differed significantly from all the other seasons, as indicated in Table 8.20. The spring of 1991 and summer of 1992 also differed significantly from the other seasons, but not from each other (Table 8.20). As for the nickel concentrations, the winter of 1990 and summer of 1990/91 differed significantly from all the seasons, but the autumn periods, while the winter of 1991 differed significantly from all the seasons, but the spring of 1990 (Table 8.21). In addition, the spring of 1991 and summer of 1992 differed significantly from all the other seasons (Table 8.21).

TABLE 8.18

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE CHROMIUM CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)													
Gonad (Males)													
Fat													
Liver													
Muscle	S2												
Skin													
Gut													
Gut cont.	W2, SP2, S2	W2, SP2	W2	W2, SP2	W2, SP2, S2	W2, SP2	W2, SP2, S2	W2, SP2, S2					
Vertebrae		S2		S2	S2	S2	S2		W2, SP2				
Kidney									SP2, S2				
Bile									SP2, S2	S2			
Blood	S2	S2	S2	S2	S2	S2	S2		W2, SP2	S2		S2	

TABLE 8.19

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE NICKEL CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	S2												
Gonad (Males)													
Fat	SP2, S2												
Liver	S2												
Muscle	SP2, S2												
Skin	SP2, S2												
Gut				W2									
Gut cont.	W2, SP2, S2	W2, SP2	W2, SP2	W2, SP2, S2	W2, SP2, S2	W2, SP2	W2, SP2, S2	W2, SP2, S2					
Vertebrae	S2	SP2, S2	S2	W2, SP2, S2	SP2, S2	SP2, S2	SP2, S2		W2, SP2				
Kidney									SP2, S2				
Bile	S2								SP2, S2	SP2, S2			
Blood	SP2, S2	W2, SP2, S2	SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	SP2, S2	SP2	W2	SP2, S2		SP2, S2	

Comparing the seasonal chromium and nickel concentrations in the organs, tissues and gut contents of the males and females separately, a difference was noticed in the gut contents and some organs. The chromium concentrations in the gut contents of the females were higher than that of the males, while the males generally had higher chromium concentrations in the bile, vertebrae, hindgut, skin and gonads. Differences were not so obvious for nickel, but the males did have higher nickel concentrations in the foregut contents and gonads than the females did.

The first and second year differed significantly with respect to the chromium concentrations in the gill, liver, muscle and male and female gonads (Fig. 8.11), and also with respect to the nickel concentrations in the gill, liver, muscle and ovaries (Fig. 8.12). The mean metal concentrations in the fish organs during the second year (Fig. 8.11 and 8.12) were also used to determine the order of bioaccumulation, which differed slightly from the order based on the monthly data. For chromium it was: hindgut contents > foregut contents > hindgut = blood > foregut > male gonads > vertebrae = gills > bile > liver > skin > muscle > kidney = fat > female gonads; and for nickel, hindgut contents > foregut contents > blood > vertebrae > hindgut > gills > foregut > male gonads > bile > liver > skin > muscle > kidney > female gonads > fat.

Manganese, lead and strontium bioaccumulation in the tissues of B. marequensis.

Manganese, lead and strontium accumulated mostly in the vertebrae and gills of *B. marequensis*. High metal concentrations were also detected in the gut contents of the fish. Variation in the metal concentrations of individuals was detected, but it was more pronounced in manganese and strontium than in lead. The largest variation in manganese concentration was detected in the gut contents (e.g. 977 - 4575 μg/g Mn at locality 5 in October 1991) and, in the first year, also in the gills (e.g. 23- 123 μg/g Mn at locality 4 in April 1990). For strontium, the largest variation was detected in the vertebrae (e.g. 1403 - 3925 μg/g Sr at locality 7 in January 1992), gills (e.g. 600 - 2115 μg/g Sr at locality 5 in January 1992) and gut contents (e.g. 132 -1326 μg/g Sr at locality 4 in August 1991). The general order of bioaccumulation for manganese was: hindgut contents > foregut contents > gills > vertebrae > hindgut > foregut > liver > kidney > blood > female gonads > fat = bile > skin > muscle > male gonads. For lead the order was: foregut contents > hindgut contents = vertebrae > hindgut > gills > foregut > blood > bile > male gonads > kidney = liver > fat > female gonads > skin > muscle and for strontium it was vertebrae > gills > foregut contents > hindgut contents > hindgut > muscle > foregut > liver > female gonads > bile > kidney > male gonads > skin > blood > fat. Statistically the gut contents, vertebrae and gills differed significantly ($p \leq 0.05$) from the other organs with respect to the manganese, lead and strontium concentrations. (Tables 8.23 - 8.24). In addition, the liver and blood differed significantly from some organs with respect to the manganese and lead concentrations respectively (Tables 8.22 and 8.24), but only during the summer of 1992.

The calculated bioconcentration factors between water and organs (BFw) were higher than the bioconcentration factors between sediment and organs (BFs). Manganese BFw values ranged from 0.7 (calculated for bile in February 1992) to 3593.3 (calculated for the hindgut in April 1991), while the BFs values ranged from 0.001 (calculated for bile in February 1992) to 1.51 (calculated for the gills in December 1990). Lead BFw values ranged from 10.8 (calculated for fat in October 1990) to 2610.0 (calculated for bile

TABLE 8.20

SUMMARY OF STATISTICAL DIFFERENCES (P ≤ 0.05) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN CHROMIUM CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *B. MAREQUENSIS* FOR SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →			G		G, M	G, M	G, M
	Male →			M	M			
Winter 1990		Female →		G		G, M	G, M	G, M
		Male →		G, M		M, L	M, L	M
Spring 1990			Female →	G			G	G
			Male →	G, M	M	M, L	M, L	M
Summer 1990/91	M*,G*	M*,G*	M*,G*	Female →		G, M	G, M	G, M
				Male →		G, M	G, M	G, M
Autumn 1991				M*	Female →	M	M, L, B	M, L
					Male →	M	M	M
Winter 1991	M*,G*	M*,G*	M*,G*	M*,G*	M*	Female →	S	S
						Male →	S, V	S, V
Spring 1991	M*,G*	M*,G*,L*	M*,G*,L*	M*,G*	M*,G*, L*,B*	B*,S*	Female →	
							Male →	
Summer 1992	M*,G*	M*,G*,L*	M*,G*,L*	M*,G*	M*,G*,L*	L*,S*		

TABLE 8.21

SUMMARY OF STATISTICAL DIFFERENCES (P ≤ 0.05) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN NICKEL CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *B. MAREQUENSIS* FOR SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →					M	G, M	G, M
	Male →							
Winter 1990		Female →	G, M			G, M	G, M, L	G, M
		Male →				M	M	M
Spring 1990		G*,M*	Female →	G				
			Male →	G			M	M
Summer 1990/91	G*	G*	G*	Female →		G	G	G
				Male →		G, M	G, M	G, M
Autumn 1991					Female →	M	M, L, B	M, L, B
					Male →		M, B	M
Winter 1991	M*	G*,M*		G*,M*	M*,L*	Female →	B, S	B, S, V
						Male →	B, S, V	V
Spring 1991	G*,M*	G*,M*,L*	M*,L*	G*,M*	G*,M*, L*,B*	L*,B*,S*	Female →	B, V
							Male →	
Summer 1992	G*,M*	G*,M*,L*	M*	G*,M*	G*,M*, L*,B*	B*,S*,V*	B*,V*	

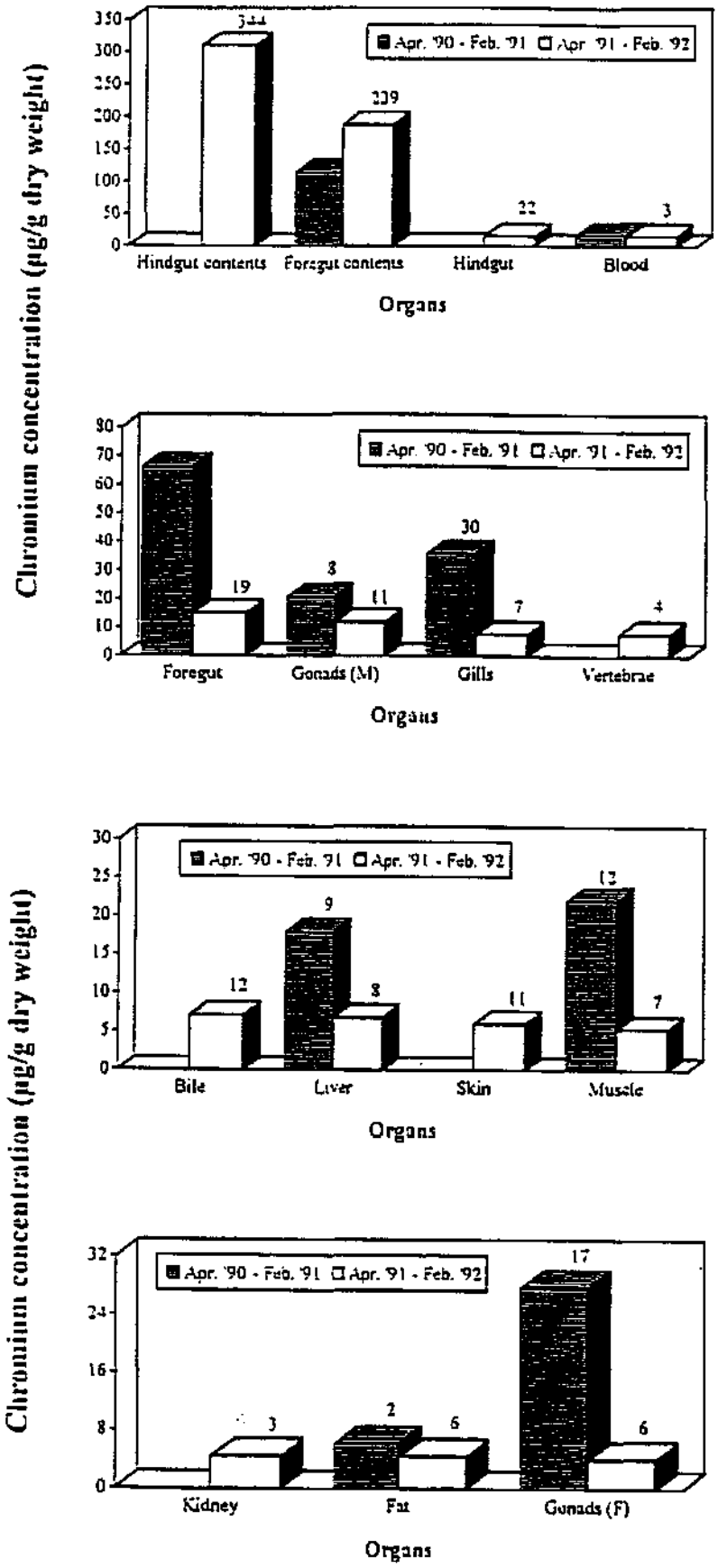

Figure 8.11

Mean chromium concentrations (ug·g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

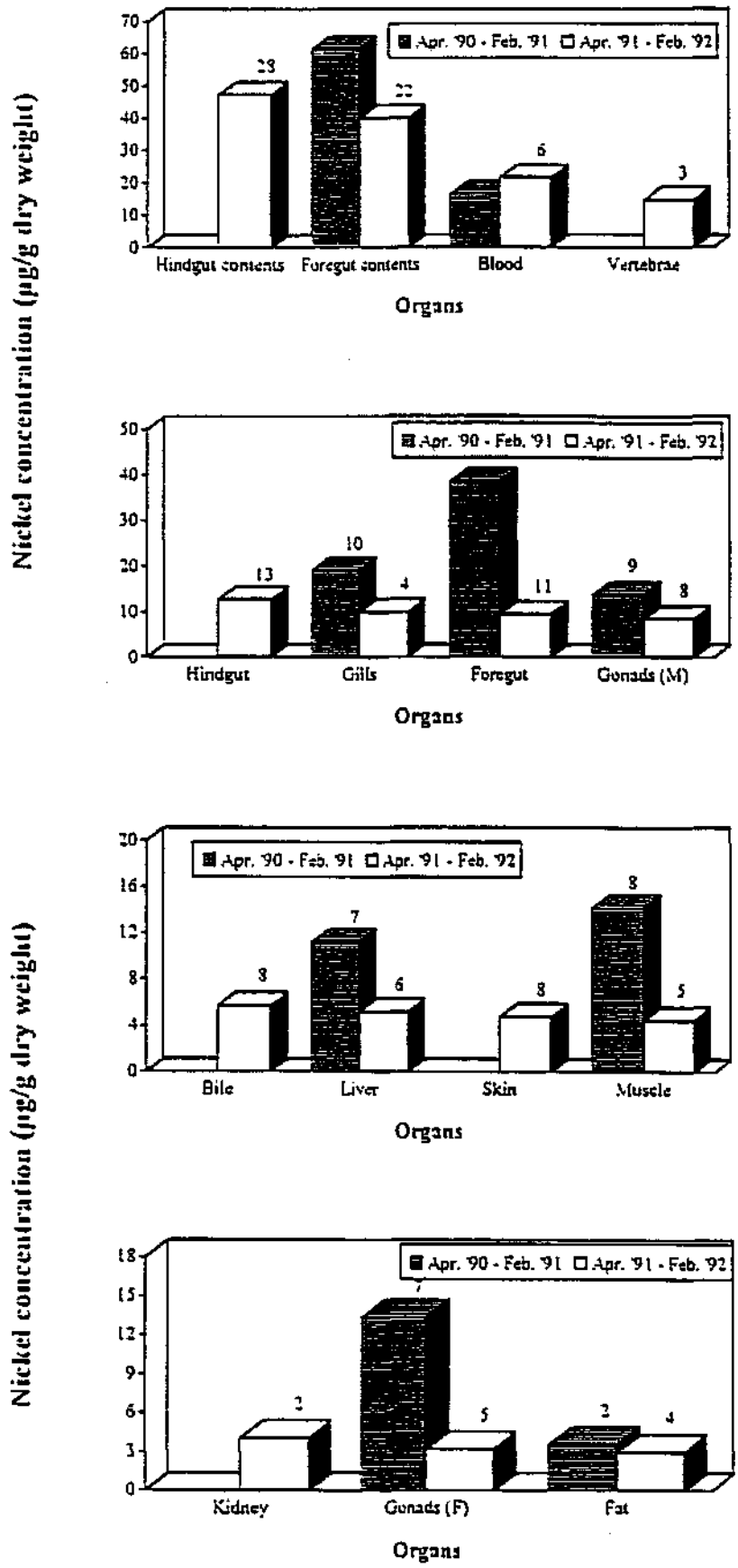

Figure 8.12

Mean nickel concentrations (µg/g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

in June 1991), while the BFs values ranged from 0.08 (calculated for fat in December 1990) to 12.47 (calculated for the gills in October 1991). Strontium BFw values ranged from 1.4 (calculated for blood in January 1992) to 255533.3 (calculated for the vertebrae in June 1991). While the BFs ranged from 0.005 (calculated for blood in January 1992) to 70.98 (calculated for the gills in December 1990).

Although the manganese, lead and strontium concentrations in the fish organs were mo-stly in the same range at each locality, significan-t differences ($p \leq 0.05$) did occur between localities. Higher manganese and strontium concentrations seemed to occur in the fish tissues at locality 7 than at the other localities, while lower strontium concentrations occured in the fish tissues at Pionier Dam.

In October 1990 (the first year) locality 7 differed significantly from localities 3 (with respect to the gill, liver and muscle manganese concentrations), 4 (with respect to the muscle manganese concentrations) and 5 (with respect to the gill and muscle manganese concentrations). Lead concentrations detected at locality 5 differed significantly from those at localities 3 (with respect to the liver) and 4 (with respect to the gills, liver and muscle), while strontium concentrations detected at locality 3 differed significantly from those at localities 4 (with respect to the liver) and 5 (with respect to the muscle and liver) in October 1990. In June 1991 (the second year) locality 4 differed significantly from localities 3 and 5 with respect to the manganese concentrations in the muscle tissue, as well as the lead concentrations in the muscle and fat tissues. Locality 5 differed significantly from locality 3 in October 1991 with respect to the lead concentrations in the fat and the strontium concentrations in the vertebrae. In January 1992 locality 7 differed significantly from localities 3, 4 and 5 with respect to the strontium concentrations in the blood and vertebrae and the manganese concentrations in the fat, but it only differed significantly from locality 5 with respect to the lead concentrations in the vertebrae. Locality 5 differed significantly from locality 4 with respect to the muscle and vertebrae lead concentrations in January 1992, the muscle and blood manganese concentrations in February 1992 and the blood strontium concentrations in January and February 1992. Furthermore in February 1992, locality 3 differed significantly from localities 4 (with respect to the lead and strontium concentrations in the vertebrae and blood respectively) and 5 (with respect to the lead and strontium concentrations in the vertebrae). The Pionier Dam differed significantly from locality 3 with respect to the lead concentrations in the vertebrae, as well as the strontium concentrations in the fat, muscle, vertebrae and blood. It also differed significantly from locality 4 with respect to the manganese concentrations in the muscle and blood, as well as the strontium concentrations in the muscle and vertebrae, and from locality 5 with respect to the manganese concentrations in the fat, vertebrae and blood, as well as the strontium concentrations in the muscle, vertebrae and blood.

Significant seasonal differences ($p \leq 0.05$) with regard to the mean manganese, lead and strontium concentrations in various organs were detected, but no distinguished trend could be established. In the case of manganese, the summer of 1990/91 and winter of 1991 differed significantly from all the other seasons. Additional seasonal differences regarding the mean manganese concentrations are indicated in Table 8.25. Nearly all the seasons differed from each other with respect to the mean lead concentrations detected in various organs (Table 8.26), but not with respect to the mean strontium concentrations. The seasonal differences regarding the mean strontium concentrations are indicated in Table 8.27.

TABLE 8.22

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE MANGANESE CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	S2												
Gonad (Males)	S2												
Fat	S2												
Liver	S2			S2									
Muscle	S2				S2								
Skin	S2				S2								
Gut													
Gut cont.	W2, SP2, S2	W2, SP2	W2	W2, SP2, S2	W2, SP2, S2	W2, SP2	W2, SP2, S2	W2, SP2, S2					
Vertebrae	S2	S2	S2	S2	S2	S2	S2		W2, SP2, S2				
Kidney									SP2, S2				
Bile	S2				S2				SP2, S2	S2			
Blood	S2				S2				W2, SP2	S2			

TABLE 8.23

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE LEAD CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	SP2, S2												
Gonad (Males)													
Fat	SP2, S2												
Liver	S2												
Muscle	SP2, S2												
Skin	SP2, S2												
Gut													
Gut cont.	W2, SP2	W2, SP2	SP2	W2, SP2, S2	W2, SP2, S2	W2, SP2	W2, SP2, S2	W2, SP2, S2					
Vertebrae	SP2, S2	W2, SP2, S2	SP2, S2	W2, SP2, S2	SP2, S2	SP2, S2	SP2, S2	SP2	W2, SP2				
Kidney									SP2, S2	S2			
Bile	S2								SP2, S2	SP2, S2			
Blood	S2	S2				S2	S2		W2, SP2	SP2, S2			

TABLE 8.24

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE STRONTIUM CONCENTRATIONS IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* DURING THE SEASONS WINTER 1991 (W2), SPRING 1991 (SP2) AND SUMMER 1992 (S2). (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Gill	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	W2, SP2, S2												
Gonad (Males)	W2, SP2, S2												
Fat	W2, SP2, S2												
Liver	W2, SP2, S2												
Muscle	W2, SP2, S2												
Skin	W2, SP2, S2												
Gut	W2, SP2, S2												
Gut cont.	W2, S2	W2, SP2	W2, SP2	W2, SP2, S2	W2, SP2, S2	W2, SP2	W2, SP2, S2	W2, SP2, S2					
Vertebrae	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2				
Kidney	S2								SP2, S2	S2			
Bile	SP2, S2								SP2, S2	SP2, S2			
Blood	W2, SP2, S2								W2, SP2	W2, SP2, S2			

There were no clear-cut and continuous differences in metal accumulation between the two genders. The males did, however, have higher manganese and lead concentrations in their gut contents than the females.

The first and second year differed significantly ($p \leq 0.05$) with respect to the manganese concentrations in the gills, muscle and gonads (Fig. 8.13); and also with respect to the lead and strontium concentrations in the gills, liver, muscle and gonads (Figs. 8.14 and 8.15). Using the mean manganese, lead and strontium concentrations detected in the fish organs during the second year (Figs 8.13 - 8.15), the order of metal accumulation in *B. marequensis* was determined and it differed slightly from the order based on the monthly data. For manganese it was: hindgut contents > forgut contents > gills > hindgut > vertebrae > foregut > liver > kidney > female gonads = male gonads > blood = muscle > skin = fat > bile; for lead, foregut contents > vertebrae > hindgut contents > hindgut > male gonads > gills > foregut > blood = kidney > bile > skin > liver > fat > muscle = female gonads; and for strontium, vertebrae > gills > hindgut contents > foregut contents > hindgut > foregut = muscle > male gonads > > liver = kidney > bile = female gonads > skin > fat > blood.

TABLE 8.25

SUMMARY OF STATISTICAL DIFFERENCES (P ≤ 0.05) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN MANGANESE CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *B. MAREQUENSIS* FOR SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →	G, M	G, M	G	M	G, M	G, M	G, M
	Male →							
Winter 1990		Female →		G		M	M	M
		Male →		G				
Spring 1990			Female →	G		M	M	M
			Male →	G		M	M	M
Summer 1990/91	G*	G*	G*	Female →	M	G, M	G, M	G, M
				Male →		G	G	G
Autumn 1991	M*		M*	M*	Female →	B	M	M
					Male →			
Winter 1991	G*,M*	M*	G*,M*	G*,M*	B*	Female →	B	B, S
						Male →		
Spring 1991	G*,M*	M*	M*	G*,M*		S*,B*	Female →	
							Male →	
Summer 1992	G*,M*	M*	G*,M*	G*,M*		S*,B*	B*	

TABLE 8.26

SUMMARY OF STATISTICAL DIFFERENCES (P ≤ 0.05) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN LEAD CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *B. MAREQUENSIS* FOR SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →		M		M	G, M	G, M	G, M
	Male →					M	M	G, M
Winter 1990		Female →	G, M	G, M	M, L	G, M, L	G, M, L	G, M, L
		Male →	G, M	M	M	G, M, L	G, M, L	G, M
Spring 1990	M*	G*,M*,L*	Female →		M, L	G, M, L	G, M, L	G, M, L
			Male →			G, M, L	G, M	G, M
Summer 1990/91	M*	G*,M*		Female →	M	G, M	G, M	G, M
				Male →				G
Autumn 1991	M*	M*,L*	M*,L*	M*	Female →	B		
					Male →			
Winter 1991	G*,M*	G*,M*,L*	G*,M*,L*	G*,M*	B*	Female →	V, S	V, B, S
						Male →		
Spring 1991	G*,M*	G*,M*,L*	G*,M*,L*	G*,M*	G*,B*	V*,S*	Female →	
							Male →	
Summer 1992	G*,M*	G*,M*,L*	G*,M*,L*	G*,M*	G*	V*,S*,B*		

TABLE 8.27

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN STRONTIUM CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *B. MAREQUENSIS* FOR SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →							
	Male →							
Winter 1990		Female →	L		M	G, M, L	M, L	M, L
		Male →				M, L	M, L	G, M
Spring 1990			Female →		M, L	M, L	M, L	M, L
			Male →			M, L	M	G, M
Summer 1990/91			M*	Female →	M	G, M	M	M
				Male →				
Autumn 1991		M*,L*	M*,L*		Female →	B, L	B	B, L
					Male →		B	B
Winter 1991		G*,M*,L*	G*,M*,L*	G*,M*	B*	Female →	G	G, V
						Male →	S	G, S
Spring 1991		M*,L*	M*,L*	M*	B*	S*	Female →	V
							Male →	G
Summer 1992		M*,L*	M*,L*	M*	G*,B*	V*,S*,G*	G*	

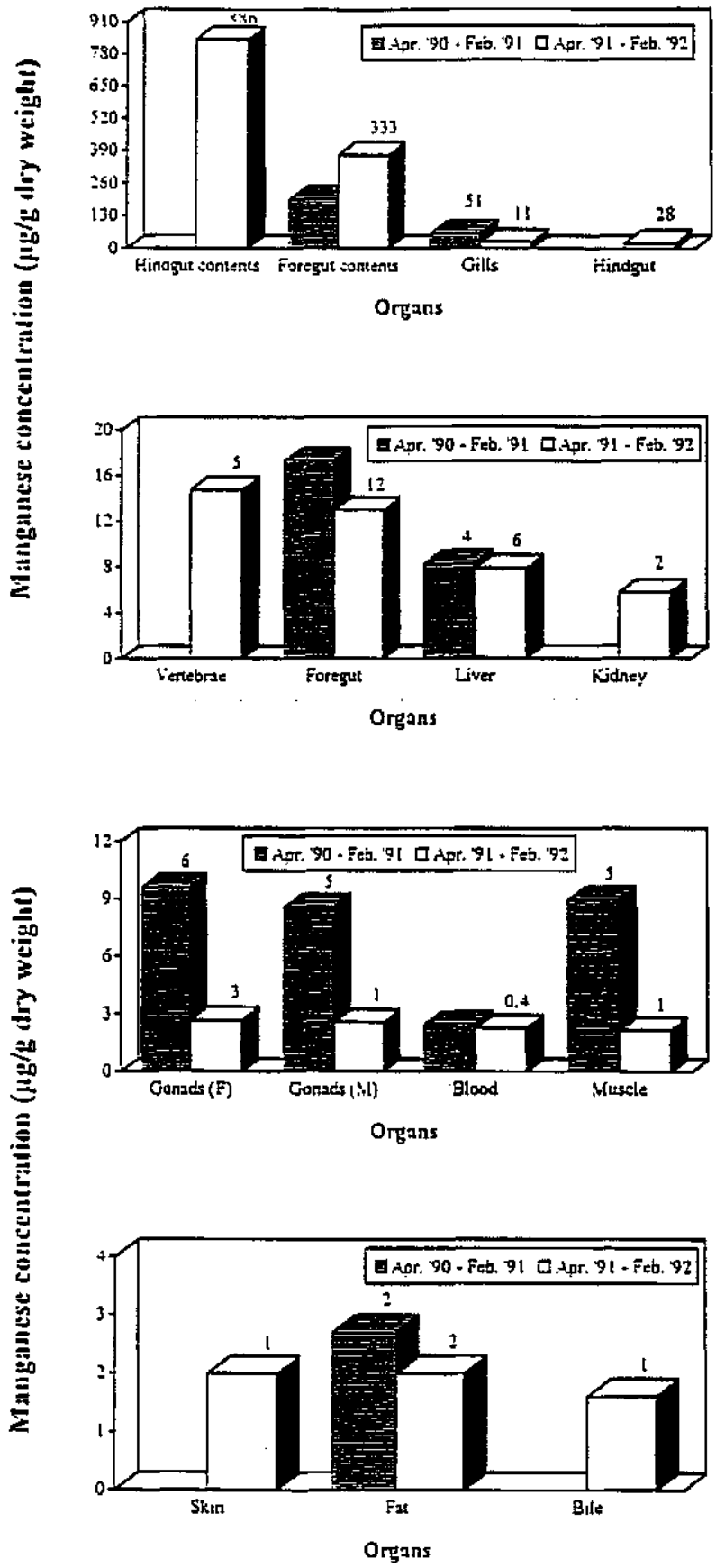

Figure 8.13

Mean manganese concentrations (µg g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

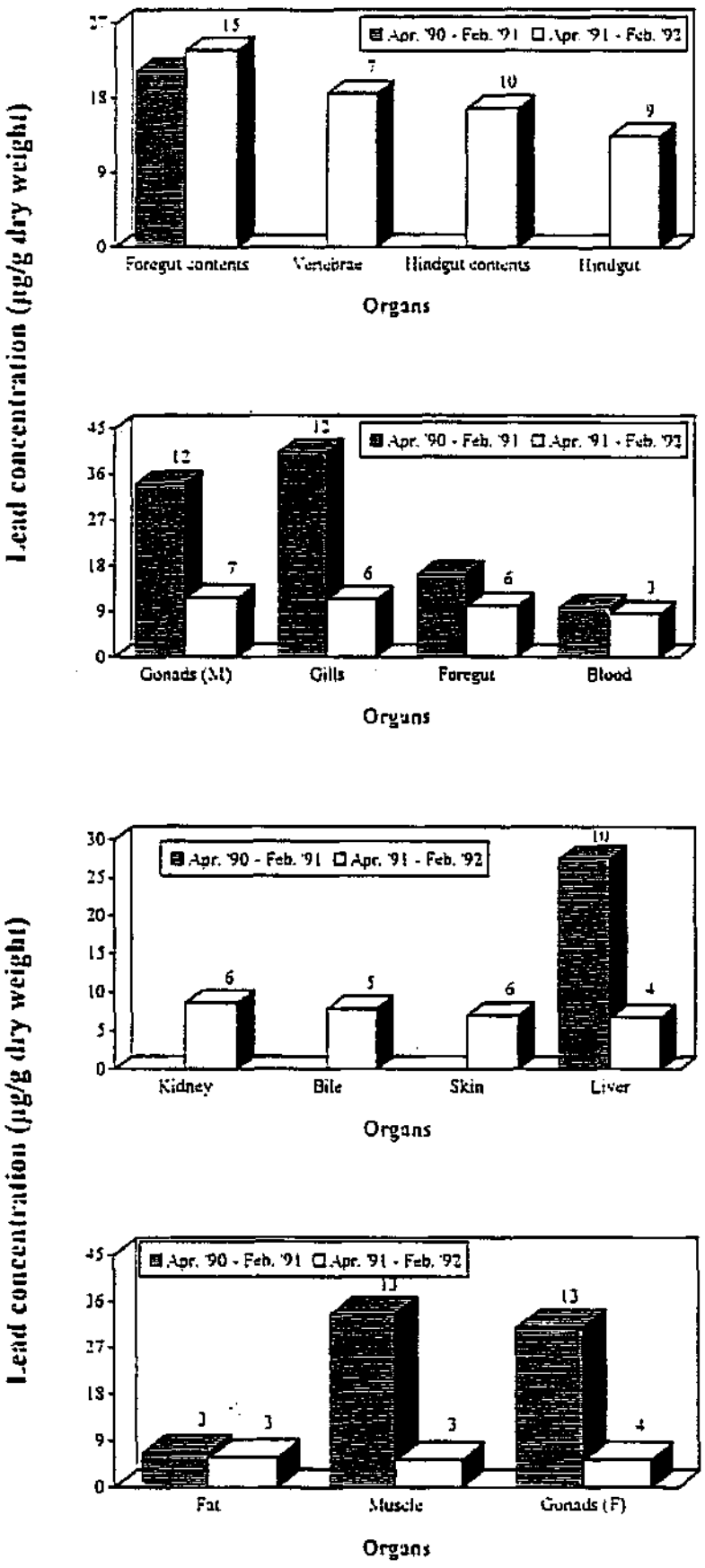

Figure 8.14

Mean lead concentrations (µg g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

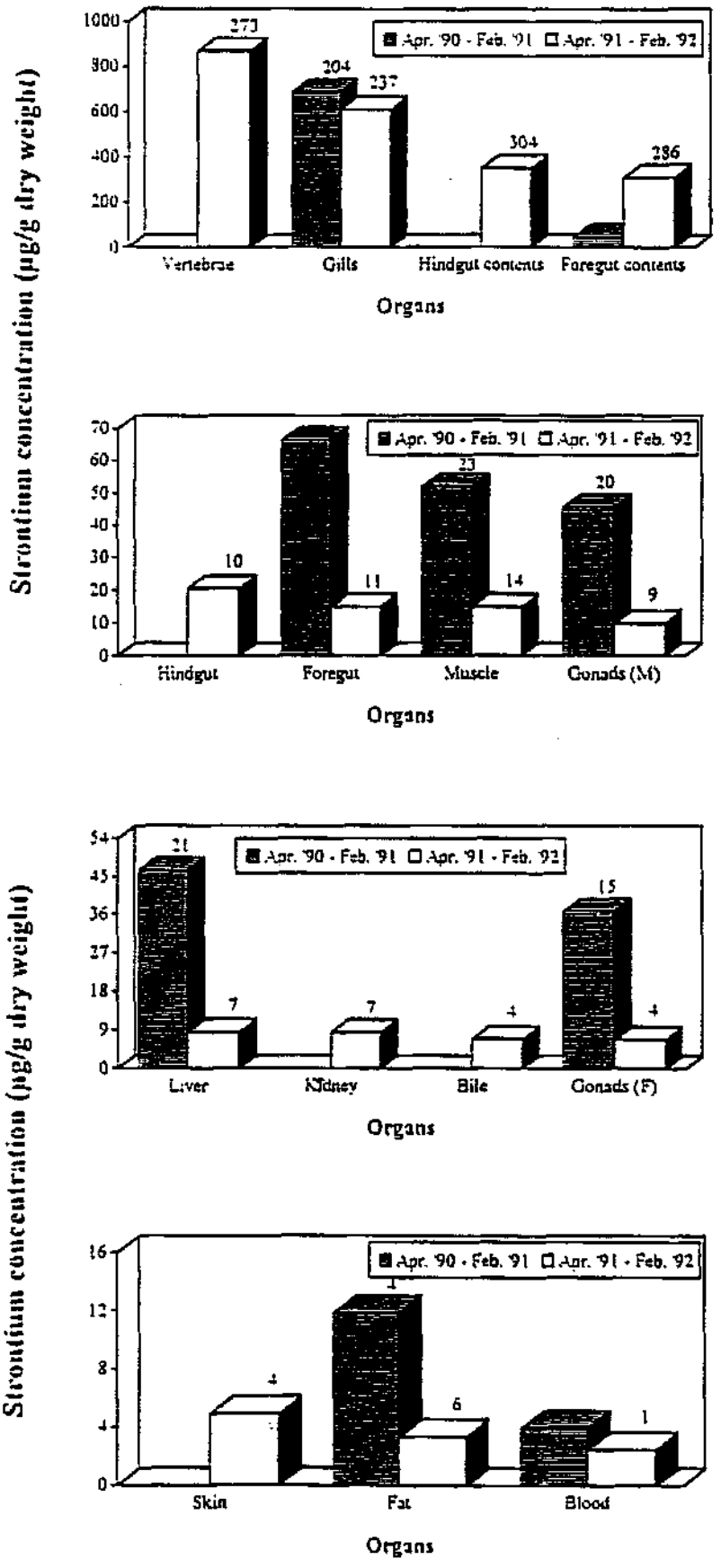

Figure 8.15

Mean strontium concentrations (µg g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

8.1.4.3 Discussion

Zinc bioaccumulation in the tissues of B. marequensis.

The large variation in zinc concentration between the individual fish suggests that the number of fish sampled at each locality should be increased to at least 20 to 30 individuals, but in order to still conserve the fish species, the number of sampling sites will need to be decreased. The size of the fish is also important, for one gram of dried tissue is necessary for accurate and reliable metal analysis with the atomic absorption spectrophotometer. The removal of external "surface" water from wet tissues will affect the determination of the actual metal concentration in the tissue, thereby increasing the experimental error. Furthermore, since the moisture contents of individual tissues differ from one another, as well as from one individual to the next, it is suggested that working on a dry weight basis instead of a wet weight basis would decrease variation.

The zinc concentrations in the tissues of *B. marequensis* (recorded in summer 1992 for the Olifants River, KNP) were generally lower than the summer 1988/89 zinc concentrations in the tissues of *Clarias gariepinus*, recorded by Bezuidenhout *et al.* (1990) for the industrial and mine polluted Germiston lake in the Transvaal. The only tissues of *B. marequensis* that had similar or higher zinc concentrations than *C. gariepinus* had, were the liver, gonads and vertebrae. Ignoring for a moment species differences, it seems that *C. gariepinus* was exposed to higher zinc levels than *B. marequensis*, although the higher liver and vertebrae zinc concentrations of *B. marequensis* might suggest chronic zinc exposure at a lower level.

Bioconcentration factors are not readily available in literature, making it difficult to compare data on the basis. Saltes and Bailey (1984) did, however, record factors of 9708X and 3835X for the gill and liver tissues respectively, which is higher than or similar to the factors determined in this study. On the other hand, the BFs recorded by Du Preez and Steyn (1992) were lower than a hundred, which are much lower than the BFs determined in this study. However, the BFs recorded by Du Preez and Steyn (1992) were based on wet tissue zinc concentrations and not dry tissue zinc concentrations. The high water bioconcentration factors (BFw) determined in this study suggest a high degree of zinc bioavailability to the fish. But these factors only represent the ratio of the metal concentration in the fish to the total (not bioavailable) concentration in the water. In hard water systems, as in the case of the Olifants River, metals will be less available for uptake by the fish. This aspect, as well as the fact that zinc is being regulated in the fish and therefore mostly independent of concentrations in the water (Wiener and Giesy, 1979), are not taken into consideration in the BF formula. Therefore, in this discussion more emphasis will be placed on the actual concentrations in the organs than on the BFs.

Zinc is primarily taken up by the intestine of the fish *via* the food (Pentreath, 1973; Willis and Sunda, 1984). Because not all the fish feed on the same food at the same time in nature, a high standard deviation can be expected for the zinc concentrations in the gut contents. However, when the dietary supply of zinc is low (Spry *et al.*, 1988) and/or the zinc levels in the water are elevated, as was the case in the study area, zinc can also be taken up through the gills and maybe even the skin (Skidmore, 1964; Handy and Eddy, 1990; Hogstrand and Haux, 1991; Heath, 1987). In the first year, the mean zinc concentration in the river water was higher than the mean zinc concentration in the water of the second year. One would therefore expect that the zinc concentrations in the gills would be higher than the zinc

concentrations in the gut for the first year. Unfortunately, only a pilot study was conducted in the first year (sampling only the basic organs) in order to determine whether considerable zinc levels would be detected in the fish. No gut tissue was therefore sampled until the second year, when the study was expanded. In February 1991, however, the one fish that was caught at locality 5 did show the expected trend. From April 1991 to August 1991 the gills still seemed to be the main route of uptake, but as the mean zinc concentration in the water decreased, the gills as an uptake route became less pronounced, until in January and February 1992 the gut was the main route of uptake as usual. Zinc uptake was mostly higher in the hindgut than in the foregut, but at times it was also the reverse.

After absorption, zinc is distributed *via* the blood to accumulate in both the soft (skin, liver, kidney, muscle and fat) and skeletal tissues (scales and vertebrae). The data showed that high zinc concentrations occured in the skin, which is similar to the findings of Mount (1964) and Khalaf *et al.* (1985). This may suggest that zinc is primarily distributed to this tissue (Hogstrand and Haux, 1991). It can also be that the skin plays a role in the uptake and/or excretion of zinc. The liver is also a site of high zinc bioaccumulation, reflecting it's multifunctional role in the detoxification (through metallothionein binding) and storage processes (Carpenè *et al.*, 1990). The exact role of the kidney in the regulation of zinc is not yet known, especially because zinc excretion through the kidneys is minimal (Romanenko *et al.*, 1985; Klaassen, 1976). Good regulation takes place in the muscle and therefore low zinc concentrations were detected in this tissue. The muscle zinc concentrations were well below the set standard for food by the National Health and Medical Research Council, which is 1000 230g/g Zn wet weight or in this case 4000 μg/g Zn dry weight (Anon, 1974). Scales and bone are regarded as significant storage sites (Sauer and Watabe, 1984) and therefore a substantial amount of zinc accumulated in the vertebrae. It appears that the zinc content of fish scales is closely correlated to the concentration of zinc in environmental water (Sauer and Watabe, 1984), making it a sensitive environmental indicator when zinc levels increase. In future monitoring programmes, scales should therefore be included in the tissues that are being sampled for zinc analysis.

Zinc is necessary for gonad development and, consequently, the concentrations in the gonads will increase until the fish are sexually mature. Dietary zinc sources are, however, not adequate during this time and therefore internal sources, such as the liver, skin, muscle, vertebrae and scales are utilised (Fletcher and King, 1978; O'Grady, 1981). It was noted in this study, that when the zinc concentrations in the gonads (especially female gonads) decreased in spring 1990 and summer 1992, the zinc concentrations increased in the internal zinc sources (e.g. liver, skin, vertebrae) and vice versa. The breeding season stretches from October to April (Bell-Cross and Minshull, 1988) and in the first year the female gonads were fully developed by winter, but in the second year it was developed only later in spring, probably due to the prolonged drought period. The standard deviations of the zinc concentrations in the gonads were very high, because the gonads of individuals were in different stages of development at the same time. The males were sexually mature by winter in the second year, which is one season earlier than the females were. Because growth is retarded by sexual development (Love, 1980), the male fish were smaller than the female fish of the same age. Presumably, the zinc deposited in the gonads during their development was lost from the fish at spawning (spring 1990 to autumn 1991). This suggests that female fish would require greater amounts of zinc each year than the male fish would (Fletcher and King, 1978). This might possibly be a reason why the zinc

concentrations in the male vertebrae were higher than the zinc concentrations in the female vertebrae. Females need to utilise all possible sources for gonad development, but this is not the case with males, and skeletal sources would most likely be utilised after the soft tissue sources have been utilised.

After storage and transformation processes in the different soft and skeletal tissues have taken place, excessive zinc is excreted. The major excretion route for zinc is faecal, with little being excreted by the kidneys and gills. The bile may (Romanenko *et al.*, 1985) or may not (Klaassen, 1976) play a role in zinc elimination; however, the low zinc concentrations detected in the bile of *B. marequensis* support the findings of Klaassen (1976). The role of the skin in zinc excretion has not yet been ascertained.

The differences in localities did not seem to be correlated to the different zinc concentrations in the water of each locality (Section 8.1.1). This may be attributed to the fact that too few water samples were taken, so that no realistic and reliable correlation could be obtained. It is possible that the differences in localities were related rather to the type of food taken by the fish at each locality. Sometimes the fish caught at locality 3 bioaccumulated the highest zinc levels (e.g. October 1990, October 1991 and February 1992), while in June 1991 it was the fish at locality 5 and in January 1992 the fish at locality 7.

An aspect to consider is whether regulating organs (e.g. the muscle and liver) are reliable for use in statistical comparisons. Zinc levels in these organs will be regulated to a physiological acceptable level which is similar in all fish of the same species. As the zinc concentration in the environmental water increases, regulation will take place on a higher level than it does normally. In other words, if there is no distinct difference between the uptake of zinc concentrations by the fish at the different localities, the zinc concentrations in the fish will be similar at all the localities. Therefore, it might be better to use a storage organ, such as the vertebrae, where transformation and regulation are slow.

The seasons in the first year differed significantly from most of the seasons in the second year with the zinc concentrations in the gill and muscle tissues being higher in the first than in the second year. These findings may be attributed to the difference in climatic conditions between the two years. Autumn 1990 also differed significantly from the other seasons in the first year with respect to the zinc concentrations in the muscle. This might not have been realistic, however, due to the fact that a value of 252.2 μg/g Zn at locality 3 in April 1990 increased the mean muscle zinc concentration in autumn 1990 to a value of 151 μg/g Zn.

Females showed greater seasonal differences in zinc concentrations than males. This can be attributed to female gonad development, for no significant differences were recorded in the males with respect to liver and vertebrae zinc concentrations. However, differences were detected in the females. As mentioned before, the two years did differ significantly, mostly due to the rain and flood in the first year compared to the continuous drought in the second year. No significant difference was, however, recorded between the zinc concentrations in the liver tissues of the two years indicating the good regulation and detoxification of zinc that takes place in this organ.

Bioaccumulation of copper and iron in tissues of B. marequensis.

Copper and iron were found to have accumulated in all the tissues and organs of *B. marequensis*. Of all the organs, the liver accumulated the highest copper concentrations, thereby confirming the view that the liver of freshwater fishes is a copper storage organ. Elevated copper levels in the liver can be ascribed to the binding of copper to metallothionein (MT), which serves as a detoxification mechanism (Hogstrand and Haux, 1991). Copper is also part of the liver proteins hemocuprein and hepatocuprein (Voynar, 1960) and several oxidative enzymes. The activity of the liver enzyme, xanthine oxidase, can be used as an indicator of sub-lethal copper exposure, because copper increases the activity in exposed fish whereas lead, mercury, silver and cadmium inhibit it (Jackim *et al.*, 1970). A large variation was detected in the liver copper concentrations which might be partly due to the rate of erythrocyte maturation differing from individual to individual. Copper is essential for this maturation process (Vorob'yev and Zaytsev, 1975). The liver of *B. marequensis* also accumulated significant levels of iron. These elevated iron levels can be ascribed to the ferritin content (Vorob'yev and Zaytsev, 1975), iron-containing enzymes (Voynar, 1960) and the extensive vascular system of the liver. The haemoglobin in the blood binds approximately three-quarters of the iron present in the body (Voynar, 1960), explaining the accumulation of iron by the liver and kidneys. Copper is required for the synthesis of haemoglobin (Heath, 1987), but it is transported in the blood by the protein ceruloplasmin, which is believed to be the link between copper and iron in the vertebrates (Moore and Ramamoorthy, 1984).

Food seems to be a more important source of copper than water to fish (Moore and Ramamoorthy, 1984). Presumably this is also the case for iron, because higher iron concentrations were detected in the gut than in the gills. The large variation in iron concentration that was detected in the gut contents of *B. marequensis* can largely be explained by its feeding habits. *B. marequensis* is a benthic feeder and, in addition to the benthic organisms, sediment rich in iron, the amount of which will differ from individual to individual, will be ingested by the fish. Furthermore, the mouth form of *B. marequensis* is highly variable (Pienaar, 1978), resulting in varied foraging habits in a population. As manifested by the order of bioaccumultion, the gut wall was a major site of deposition. Increased metal levels, especially of iron, in the hindgut suggested, however, that much of the ingested copper and iron was not assimilated (Vidal, 1978).

Accumulation in the gills is related to the copper concentration in the water (Benedetti *et al.*, 1989) and presumably also the iron concentration in the water. This was illustrated in December 1990 when elevated copper and iron levels in the water, mainly caused by the floods, led to significant accumulation of these metals in the gills. Elevated copper and iron concentrations in the gills could be due to the metals complexing with the mucus (Heath, 1987), while the extensive vascular network in the gill would have ensured that the blood-borne metals were in intimate contact with the gill tissue (Laurent and Dunel, 1980). Gills have been shown to produce a Cu-binding MT, but in contrast to the liver MT, gill MT only binds very small amounts of copper (Noël-Lambot *et al.*, 1978).

The female gonads accumulated less copper and iron than the male gonads did, except in summer 1990/91, spring 1991 and summer 1992. It was noted that the copper and iron concentrations in the gonads followed a seasonal trend that differed from the trend regarding the zinc concentrations in the gonads. The highest copper concentration in the female gonads

occurred in summer 1990/91 whereas the highest zinc concentration occurred in winter 1990. The specific role , if any, of copper and iron in gonad development is not certain, but from this study it seemed that if copper and iron were required for certain stages of gonad development, zinc was required for others.

Unlike copper, iron accumulated more in the skin than in the vertebrae. The low copper levels in the muscle tissues were well below the set standard for food by the National Health and Medical Research Council, which is 30 ug/g Cu wet weight (Anon., 1972) or in this case 120 ug/g Cu dry weight (the moisture percentage of the muscle was 75%). No comparable standard was available for the iron concentration in the muscle. The copper concentration in the muscle did, however, exceed 4 μg/g Cu dry weight (or 1 μg/g Cu wet weight) from April 1990 to August 1991, which is seldom the level of concentration in fish from polluted fresh water (Moore and Ramamoorthy, 1984). Metals that tend to concentrate in the liver may be excreted by the bile (Heath, 1987), which might be the case for copper but not for iron. Little is known about excretion routes in teleosts, but excretion of iron is presumably faecal and/or urinary. The main route for excretion of copper in mammals is *via* the faeces (Klaassen, 1976) and it might also be the case in fish. There are, however, indications that at least some urinary and biliary excretion of copper occurs (Dixon and Sprague, 1981; Heath, 1987).

The low calculated bioconcentration factors (BFs) between the fish organs and the sediment, indicated that very little to no copper and iron in the sediment were bioavailable to the fish for uptake. The higher BFs that were calculated between the fish organs and the water suggested a higher degree of metal bioavailability to the fish through the water, although factors such as the water chemistry and regulating processes of copper and iron in the fish should also be considered in determining the actual degree of metal bioavailability to the fish. The BFs recorded for *B. marequensis* in October 1990 at locality 3 in this study, were generally higher than the BFs recorded for *Hydrocynus vittatus* in October 1990 at the same locality (Du Preez and Steyn, 1992), which were generally lower than a hundred. It was only the BFs regarding the copper concentrations in the fat and liver, as well as the iron cncentrations in the liver of *B. marequensis* that were lower than the BFs recorded for *H. vittatus* as mentioned earlier. The BFs recorded by Du Preez & Steyn (1992) were, however, calculated on a wet weight basis and not a dry weight basis, making direct comparisons between the two studies difficult.

The concentrations of copper and iron in the organs and tissues of *B. marequensis* (recorded in summer 1992 in the Olifants River, KNP) were generally lower than the recorded concentrations in the organs and tissues of *Clarias gariepinus* (summer 1988/89) from the industrial and mine polluted Germiston lake in the Transvaal (De Wet, 1990). Although the copper concentration in the water of Germiston lake was higher than that in the Olifants River, the liver of *B. marequensis* accumulated more copper than the liver of *C. gariepinus*. It therefore appears that the detected copper concentration in the liver of *B. marequensis* was still below the toxic level and was thus accumulated rather than regulated. The iron concentration in the water of Germiston lake was either lower or higher than that of in the Olifants river, depending on the locality. The higher accumulation of iron by *C. gariepinus* suggested, however, that iron was more available for uptake in Germiston lake than it was in the Olifants River, except at locality 7 in the Selati River (a tributary of the Olifants River), where the vertebrae, fat and gut of *B. marequensis* accumulated more iron than did the same

organs of *C. gariepinus*. The gut of *B. marequensis* must therefore have been a more important uptake route of iron that it was for *C. gariepinus*.

No definite trend as to where the highest bioaccumulation had occurred could be established, especially with regard to copper. In general, the fish at locality 7 did, however, accumulate more copper than the fish at the other localities, while the fish at Pionier Dam accumulated the least. The highest iron concentrations were accumulated by the fish at localities 3 and 4, as well as at Pionier Dam and this is probably due to underlying rock formations that produce iron through weathering processes.

The differences that occured between the localities with regard to the accumulated copper and iron concentration in the fish organs, did not seem to be correlated to the copper and iron concentrations in the water but rather to the concentrations in the food. In October 1991 for instance, the fish at locality 5 biomagnified more copper than the fish at the other localities did. In the first year (April 1990 to February 1992), however, there was a correlation between the iron concentrations in the water at each locality and the iron concentrations in the gills of the fish at each locality. This might be due to the fact that the fish were exposed to higher iron concentrations in the first year, because the stronger river flow caused more iron to be available from the underlying substratum through weathering processes.

The summer of 1990/91 differed from the other seasons with respect to the copper and iron concentrations in the fish gills. This was due to the higher metal levels in the water after the heavy rainfall in December 1990. The other fish organs did not necessarily accumulate the highest copper and iron concentrations in December 1990, because these metals are biomagnified (accumulated through food) by the fish rather than bioconcentrated (accumulated through water). No definite seasonal trend could therefore be established for most of the organs.

The gonads accumulated the highest copper and iron concentrations in summer 1990/91, but high iron concentrations were also accumulated in autumn 1990. It is not sure what role, if any, copper and iron played in the gonad development, but there dit not seem to be a relationship between the concentrations in the gonads and the concentrations in the liver to prove that these metals were actually being taken from the liver for gonad development, as was the case with zinc. Instead it was noted that the seasonal trend in the muscle copper and iron concentrations were similar to the trend in the gonad concentrations, being more pronounced for the copper concentrations. It is not certain why the sexual differences in accumulation had occurred, but these differences are similar to the findings of Vorob'yev and Zaytsev (1975) and De Wet (1990).

The iron concentrations in the organs of *B. marequensis* were higher in the first year that in the second year. More iron was therefore taken up by the fish in the first year, as was illustrated by the foregut iron concentrations. The high accumulation of iron by the gills in the first year, compared to the accumulation in the second year, occurred because the fish were exposed to high iron concentrations in the summer of 1990/91 as a result of the heavy rainfall during that time (See 8.1.3.3, p88).

The copper concentrations in the fish organs also seemed to be higher in the first than in the seond year, suggesting that more copper must have been ingested by the fish in the first year. Although the foregut showed lower instead of higher copper accumulation in the first year, it was based on only one sample, which was collected during February 1991. It can therefore be

assumed that the copper concentrations in the foregut were actually higher in the first than in the second year. Contradicting this was the low copper concentrations in the blood and liver tissues in the first year compared to that in the second year. A reason for this might be that the fish were actually confronted with higher copper levels in the first year and therefore regulation and detoxification had to take place in the liver. In the second year, however, the copper levels to accumulate were much lower and copper could accordingly be stored in the liver instead of being regulated or detoxified. Low copper levels in the liver could therefore actually indicate high copper intake by the fish. The lower blood copper concentrations in the first year can be explained by the findings of Grobler-Van Heerden *et al.* (1991), where a decreased bioconcentration in the blood occurred with an increased exposure concentration. Fish, therefore, have a mechanism to prevent excess bioconcentration of copper in the blood.

Bioaccumulation of chromium and nickel in the tissues of B. marequensis.

Limited research has been undertaken on the uptake, distribution and excretion of chromium and nickel by freshwater fish. The role of each fish organ in these processes has therefore not yet been ascertained. From this study it seemed, according to the order of chromium and nickel bioaccumulation in the organs and tissues, that these metals were taken up by the gills and/or the gut *via* the gut contents. More chromium and nickel would probably have concentrated in the gills, however, if the water pH were more acidic. It is important to note that the high metal levels in the gut contents were not necessarily due to the accumulated metal levels in the food, but rather to the metal rich bottom sediments associated with the food (Wren *et al.*, 1983). A large variation in the chromium and nickel concentrations of the gut contents can be expected, because of the differing foraging habits of *B. marequensis*. Excretion was mainly biliary, especially in the case of chromium. It has been suggested by Flos *et al.* (1983), who experimented with chromium accumulation in goldfish (*Carassius auratus*), that biliary excretion was more important in small than in the large fish. *Barbus marequensis*, therefore, probably also excreted chromium and nickel through the gills, kidneys and in the faeces.

The blood of *B. marequensis* accumulated chromium and nickel levels that were higher than the levels in the surrounding water. It was also noticed that the chromium and nickel cncentrations (especially nickel) increased in the blood when the primary uptake route of these metals was through the gills, which was the case in August 1991 and October 1991. A relationship between the gill uptake of chromium and nickel and the consequent concentrations of these metals in the blood is therefore suggested. This suggestion, as well as an observation made by Van der Putte *et al.* (1981) that hydrochromate and chromate ions caused common effects in the blood of *Oncorhynchus mykiss* when acutely exposed, may render blood a good indicator of chromium and nickel poisoning in fish. Furthermore, sublethal concentrations of hexavalent chromium (0.098 mg/l) at different pH values have been shown to alter the haematology of *Tilapia sparmanii* in such a way that they were potentially hazardous (Wepener *et al.*, 1992). Hexavalent chromium did, for instance, decrease the clotting ability of the blood, causing internal bleeding which can ultimately lead to death (Gey van Pittius *et al.*, 1992). Apart from accumulating chromium and nickel, blood also distributes these metals to the different organs and tissues, where they are accumulated to some degree. In this study, chromium and especially nickel were mainly stored in the vertebrae and, other than that, accumulation was preferentially by the kidneys rather than the liver, which is in accordance with previous reports (N.R.C.C., 1981). According to the

concentrations in, for example, the muscle tissue, *B. marequensis* was exposed to higher chromium and nickel levels from April 1990 to June 1991 than from August 1991 to February 1992. The suggested chromium concentrations in the muscle of freshwater fish from industrialised parts is below 0.25 µg/g Cr wet weight (Moore and Ramamoorthy, 1984) or in this case 1 µg/g Cr dry weight (the moisture percentage of the muscle was 75%), which was not the case from April 1990 to June 1991 when the chromium concentrations in the muscle ranged from 5.7 (June 1991) to 43.3 (February 1991) µg/g Cr dry weight. Suggested nickel concentrations in the muscle of freshwater fish were not available. From April 1990 to June 1991 the chromium and nickel concentrations in the water of the study area were higher than the water concentrations from august 1991 to February 1992, which might explain the high accumulation of these metals during the first period.

The nickel BFs recorded for *B. marequensis* in October 1990 at locality 3 in this study, were mostly higher than the nickel BFs recorded for *H. vittatus* in October 1990 at the same locality (Du Preez and Steyn, 1992), which ranged from 17.8 to 54.1. It was only the BFs regarding the nickel concentrations in the fat of *B. marequensis* that were lower than the BFs recorded for *H. vittatus*. The chromium and nickel concentrations in the organs and tissues of *B. marequensis* (recorded in summer 1992 in the Olifants river, KNP) were generally lower than the concentrations in the organs and tissues of *Clarias gariepinus* (summer 1988/89) from the industrial and mine polluted Germiston lake in the Transvaal (De Wet, 1990). *Barbus marequensis* (collected at all the localities in the study area) did, however, accumulate more nickel than *C. gariepinus* in their vertebrae, while only the fish collected at locality 7 accumulated more chromium in their vertebrae than *C. gariepinus* did. This suggests chronic exposure of *B. marequensis* to sublethal concentrations of these metals at the relevant localities. Furthermore, *B. marequensis* collected at Pionier Dam, accumulated more chromium than *C. gariepinus* in their kidneys and gut, while the livers of both species accumulated similar chromium concentrations. More chromium was therefore taken up by the gut of *B. marequensis* than was the case with *C. gariepinus*.

The localities did not differ that much from each other and therefore no definite trend as to where the highest bioaccumulation had occured could be established. In February 1992 the fish at Pionier Dam did, however, accumulate slightly more chromium and nickel in their organs (with the exception of the blood) than the fish at the other localities. Lower chromium and nickel concentrations were detected in the gills of the fish from Pionier Dam than in their gut and, therefore, the gills did not play a major role in the uptake of these metals, which was not the case at the other localities. This might be a reason why less chromium and nickel were detected in the blood of the fish from Pionier Dam than in the blood of the fish from the other localities. The chromium and nickel concentrations in the fish did not seem to be related to the metal concentrations in the water. It must be stressed, however, that water samples were only collected every second month, making comparisons difficult.

The high chromium and nickel concentrations in the gills of *B. marequensis* during the summer of 1990/91 might have been due to the heavy rainfall in December 1990, but the concentrations of these metals in the water were not necessarily higher during that period. Instead, the concentrations in the gills seemed to have been related to the concentrations in the gut, for similar seasonal trends were observed in these tissues, as well as in the liver and muscle tissues. The seasonal trends regarding the chromium and nickel

concentrations in the gonads of *B. marequensis* differed slightly from the trends regarding the zinc, copper and iron concentrations in the gonads and it is therefore not certain what role, if any, chromium and nickel played in the gonad development. The highest nickel concentrations in the gonads did, however, occur in the winter of 1990, which is the period when the gonads were fairly well-developed. No relationship seemed to have existed between the chromium and nickel concentrations in the liver and gonads, although it has been observed by Shearer (1984) that the chromium levels in the liver of *Oncorhynchus mykiss* decreased significantly during sexual maturation, while the levels in the female gonads increased. The observed seasonal sexual differences in accumulation cannot be explained readily, but they might be related to female gonad development, seeing that higher chromium levels were detected in the gonads and vertebrae of the male fish.

Higher chromium and nickel concentrations were detected in the water of the study area in the first year than in the second year, but this is not necessarily the main reason why the organs of *B. marequensis* accumulated higher chromium and nickel concentrations in the first than in the second year. As mentioned before, there was no direct relationship between the monthly water data and the monthly fish data and, therefore, annual differences in accumulation might rather have been related to chromium and nickel uptake through the gut. Unlike the majority of organs and tissues, the blood accumulated less nickel in the first than in the second year. This can be explained by assuming that fish have a mechanism to prevent excess bioconcentration of nickel in blood (Grobler-Van Heerden *et al.*, 1991) as was found with copper.

Bioaccumulation of manganese, lead and strontium in the tissues of B. marequensis.

The uptake and excretion of metals by fish is a subject of interest to many researchers, but little is known about the exact routes of these processes in fish. Existing literature indicates that manganese, lead and strontium can be taken up indirectly from food and ingested sediments *via* the gut, or directly through concentrations of dissolved metals *via* the gills (Bendell-Young and Harvey, 1986; Hodson *et al.*, 1978; Carraca *et al*, 1990 and Wren *et al.*, 1983). The gills, however, seem to be the main route of uptake of these metals, especially in the case of manganese and strontium, for little resorption of these two metals occurs through the gut from the food (Katz *et al.*, 1972). These were also the findings in the present study, because higher manganese and strontium concentrations were detected in the gills than in the gut. It has been demonstrated, though, that water-borne lead was readily taken up by fish resulting in subtle sublethal physiological responses, while dietary lead was not taken up and therefore did not affect the fish (Hodson *et al.*, 1978). If the calcium concentrations of the water were low, however, they would probably have enhanced the dietary uptake of lead by fish due to the more effective uptake of aqueous lead by organisms in the lower trophic levels, leading in turn to a greater dietary absorption by fish (Spry and Wiener, 1991). Lead concentrations were very similar in the gills and in the gut of *B. marequensis*, indicating that both routes must have been utilised to the same extent in the uptake of lead. Apart from being uptake routes of manganese, lead and strontium, the gills and gut have also been suggested to be excretion routes, especially of lead (Klaassen, 1976; Latif *et al.*, 1982). The gills, as well as the skin, have an abundance of mucus and therefore, excretion through these routes would probably involved the sloughing off of mucus (Varanasi and Markey, 1978). Other possible routes of excretion are the urine and bile of the fish. In this

study, the higher manganese concentrations in the kidneys compared to the bile of *B. marequensis* suggested urinary excretion of manganese rather than biliary excretion. On the other hand, excretion of lead and strontium seemed to be biliary and urinary although the biliary excretion of lead has been reported to be quantitatively more important than urinary lead excretion (Klaassen, 1976).

After absorption, metals are distributed to various tissues in the body of the fish. The importance of each tissue in the storage and detoxification of a metal differs from metal to metal. The high manganese, lead and strontium cncentrations in the vertebrae of *B. marequensis* indicated that these metals were primarily distributed to the skeletal tissues. Manganese is a normal constituent of vertebrate skeletal tissues and is thought to be essential to the normal mineralization process (Guggenheim and Gaster, 1973; Love, 1980). Lead and strontium, on the other hand, are not essential for bone formation, but they accumulate in bony tissues due to their resemblances to calcium (Moore and Ramamoorthy, 1984; Phillips and Russo, 1978). The retention of strontium can be sufficiently long, because it interchanges with calcium (Radtke, 1989). Older fish will therefore have higher strontium concentrations in their bony tissues than the younger ones. This might explain the large variation that was detected in the vertebrae strontium concentrations of *B. marequensis* for the age of the first that were caught during the study varied from one to six years. Scales have also been reported to be major storage sites of manganese, lead and strontium (Sauer and Watabe, 1989). Bony tissues of fish (e.g. vertebrae, scales and opercular bone) will therefore be good indicators of sublethal manganese, lead and strontium exposures.

Other tissues of *B. marequensis* also accumulated manganese, lead and strontium, although to a much lesser degree than the skeletal tissues. Blood, the distributor of these metals, is a good indicator of leadl uptake by the fish, for the activity of the erythrocyte enzyme ALA-D is inhibited by the presence of lead. Furthermore, the ALA-D activity is negatively correlated with the lead concentration in the blood (Dwyer *et al.*, 1988). The muscle tissue of *B. marequensis* accumulated relatively high strontium concentrations which would probably render this tissue a good indicator of strontium exposure. Lead concentrations in the muscle differed only slightly from the lead concentrations in some other tissues, such as the liver. This might have reflected the relatively low rate of binding to SH groups and, in addition, the low solubility of lead salts might have restricted movement across cell membranes (Moore and Ramamoorthy, 1984). In the first year the muscle lead concentrations ranged from 13 to 56.6 μg/g Pb dry weight, exceeding the maximum allowable concentration of lead in fish flesh, which is 2 μg/g Pb wet weight or 8 μg/g Pb dry weight (assuming the moisture percentage of the muscle was 75%) (Brown *et al.*, 1984). The fish were therefore exposed to higher lead concentrations in the first year than in the second year and these were probably sublethal concentrations. No "normal" or allowable values are available for manganese and strontium concentrations in fish flesh. The detected concentrations of these two metals in the muscle tissues during the first year were, however, also higher than the muscle concentrations in the second year. Fish were therefore exposed to higher manganese and strontium concentrations in the first year.

The manganese and lead BFs recorded for *Barbus marequensis* in October 1990 at locality 3 in this study, were mostly higher than the manganese and lead BFs recorded for *Hydrocynus vittatus* in October 1990 at the same locality (Du Preez and Steyn, 1992), which ranged from 28.9 to 156.6 and 20.7 to 41.4 respectively. It was only the BFs regarding the manganese

concentrations in the gonads and fat, as well as the lead concentrations in the fat of *B. marequensis* that were lower than the BFs recorded for *H. vittatus*.

The manganese and lead concentrations in the organs and tissues of *B. marequensis* (recorded in summer 1992 in the Olifants River, KNP) were generally lower than the concentrations in the organs and tissues of *Clarias gariepinus* (summer 1988/89) from the industrial and mine-polluted Germiston lake in the Transvaal (De Wet, 1990). The fish caught at locality 7 in the Olifants River (*B. marequensis* did, however, accumulate more manganese in their organs than *C. gariepinus* did and the average water manganese concentration at locality 7 (229.5 ± 2.1 µg/l Mn) was, in fact, higher than the average manganese concentration at Germiston lake (35.6 ± 31.0 µg/l Mn). This proves the Selati River to be more polluted with manganese than Germiston lake. In general, *B. marequensis* accumulated more manganese in their gut than *C. gariepinus* did. This suggests that conditions in the Olifants River were more favourable for manganese to be taken up through the gut of the fish than was the case in Germiston lake.

The localities inside the Kruger National Park (localities 3, 4, 5 and Pionier Dam) did not differ that much from each other and therefore no definite trend as to where the highest bioaccumulation had occurred could be established. The fish at Pionier Dam did, however, accumulate the lowest strontium levels. The highest strontium, as well as manganese levels, were detected in the fish at locality 7 (Selati River). These findings coincided with the manganese and strontium concentrations in the water of the study area, which were also the highest at locality 7. Indications are, therefore, that manganese and strontium originated from a source close to locality 7, which was not connected to the KNP.

The high manganese concentrations in the organs of ***B. marequensis*** during the summer of 1990/91, might have been due to the heavy rainfall in December 1990. Under high rainfall conditions, leaching is more pronounced and systems usually have lower pH values (Hahne and Kroontje, 1973). More hydrogen ions will therefore be available to compete with manganese for binding sites on particle surfaces and solution ligands, thereby increasing the bioavailability of manganese to fish. Lead and strontium accumulation did not, however, seem to be directly by the rainfall, but were rather mediated by the lead and strontium concentrations in the water. The seasonal trend regarding manganese accumualtion in the gonads was similar to that of iron, lead accumulation was similar to that of chromium and strontium accumulation was similar to that of copper and iron in the gonads. It is not certain what role, if any, manganese, lead and strontium played in gonad development, but no relationship seemed to exist between the concentrations in the gonads and the concentrations in the liver (as was the case with zinc). Strontium has, however, been reported to increase in concentration in the ovary of *Oncorhynchus mykiss* throughout maturation, while the manganese concentrations increased only during early maturation before it declined rapidly as the GSI increased (Shearer, 1984). The strontium levels in the liver was observed to decrease significantly during the sexual maturation of *O. mykiss*.

Seasonal differences that occurred between the males and females in the accumulation of manganese, lead and strontium in their organs were such that no definite pattern could be established to relate the differences to processes taking place in the bodies of the fish. The requirements of the two genders regarding manganese, lead and strontium could therefore not be

established, except that there was a difference in metal levels between the two genders at times.

As mentioned before, the accumulation of manganese, lead and strontium in the organs of freshwater fish is related to the concentrations of these metals in the surrounding water. Due to generally higher concentrations of these metals in the water of the study area in the first than in the second year, more manganese, lead and strontium were accumulated by *B. marequensis* in the first year. It was only the gut contents that did not necessarily accumulate higher manganese, lead and strontium levels in the first year, for there would be no direct relation between the gut contents concentrations and the water concentrations.

8.1.4.5 Conclusions

The skin and female gonads of *B. marequensis* accumulated the highest zinc concentrations, while the fat and bile accumulated the lowest. The zinc concentrations detected in all the organs and tissues suggest no serious zinc pollution problem in the study area, although the zinc levels detected in the liver and vertebrae might indicate chronic zinc exposure of the fish, causing possible sublethal effects. However, the latter statement needs to be further investigated in future monitoring programmes and also through experimental work. Suggested organs to sample for analysis of zinc pollution in fish, are: skin, vertebrae, scales, gonads (within in season) and muscle tissue (to test its fitness for human consumption). The gill and liver tissues will only be of value during acute exposures, unless histopathological studies are performed in addition to the zinc analysis.

The liver accumulated the highest copper concentrations, followed by the gut and kidney, while the fat accumulated the lowest. The detected concentrations in the fish organs suggested no serious copper pollution problem in the study area, although, according to the liver concentrations the fish were exposed to higher copper levels in the first year than in the second year. Suggested organs to sample for copper analysis in fish, are: liver, gut, kidney, bile and muscle tissue (to test its fitness for human consumption). The gills can also be of value in the case of acute copper exposure, especially if histopathological studies are performed in addition to the copper analysis.

Iron mainly accumulated in the gut, followed by the kidney and liver, while the lowest iron concentrations occurred in the vertebrae. Very high iron concentrations had occurred in the study area, but it was mostly unavailable for uptake by the fish. Heavy rainfall can, however, increase iron levels in the water, leading to higher accumulation thereof in the gills (See 9/1/3/3. p88). In serious cases the iron can precipitate on the gills, thereby causing a mechanical obstruction that will impair oxygen exchange. Suggested organs to sample for iron analysis in fish, are: the gut, muscle tissue (to test its fitness for human consumption), gills (coupled with histopathological studies) and maybe the skin.

According to the monthly data, the blood accumulated the highest chromium concentrations, followed by the bile and vertebrae, while the skin accumulated the lowest. Nickel mainly accumulated in the blood, followed by the vertebrae and gills, while the lowest nickel concentrations occured in the fat tissue. The detected concentrations in the fish organs suggested no serious chromium and nickel pollution problem in the study area, but the fish did seem to have been exposed to chronic sublethal concentrations, especially from April 1990 to June 1991, which might have caused sublethal

effects. Suggested organs and tissues to sample for chromium and nickel analysis in fish are: blood, vertebrae, the gall-bladder for bile, the gut, gills, kidney, liver and muscle tissue (to test its fitness for human consumption).

Barbus marequensis bioaccumulated the highest manganese, lead and strontium concentrations in its vertebrae and gills. The high strontium concentrations that were detected in the fish organs, especially in the first year, indicated that the fish were exposed to high strontium levels. Sublethal and lethal levels of strontium to fish are, however, not known, because strontium is regarded as a non-toxic metal and, therefore, limited research is being done on this metal. The detected lead and manganese concentrations in the fish organs suggested no serious lead and manganese pollution problem in the study area, although the fish did seem to have been chronically exposed to sublethal lead concentrations in the first year. In addition, the fish at locality 7 might have been exposed to sublethal manganese concentrations. The source of these metals needs to be identified in future monitoring programmes and, if necessary, measures should be taken in order to reduce the levels thereof. Suggested organs and tissues to sample for the analysis of manganese, lead and strontium in fish, are: bony tissues (e.g. scales, vertebrae and opercular bone), gills, liver and muscle tissue (to test its fitness for human consumption). In addition, blood should also be sampled for the analysis of lead, in order to determine the lead concentrations, as well as the ALA-D activity in the erythrocytes.

8.1.4.6 References

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8.2 Experimental

8.2.1 *LC*50

8.2.1.1 Introduction

The growing need to determine critical concentrations for toxicants in rivers and other water bodies have become inevitable in order to maintain the diversity of species. A number of chemical substances in industrial agricultural and domestic effluents, as well as in effluents resulting from mining activities, are likely to contaminate water courses. These toxicants have a profound effect on all aquatic life. It is not known at what concentrations effects will start to occur and to what degree aquatic life will be affected. It is therefore essential to determine the toxicity of a substance to derive water quality guidelines (Seymore, 1994; Van der Merwe *et al.*, 1993). Acute toxicity tests may provide meaningful answers to problems arising due to the pollution of rivers by comparing the lethality of toxicants between organisms and test conditions or toxicants (Buikema *et al.* 1982). Acute toxicity tests can be defined as the severe effects suffered but organisms from short-term exposure to toxic chemicals (Van Leeuwen, 1988). The objective of such a test is to determine the median lethal concentration (LC50) which is defined as the concentration of the test material which will kill or immobilise 50% of the exposed test organisms in a predetermined length of time - 24 to 96 hours (Rand & Petrocelli, 1985). The lack of operculum movement and reaction to gentle prodding are criteria for death of exposed fish (Parish, 1985). The incipient LC50 is the point at which the toxicity curve determined after exposure of fish, becomes asymptotic to the time axis. At this concentration 50% of the test population can live for an indefinite time or it is the lethal concentration for 50% of the test organisms in long-term exposure (Rand & Petrocelli, 1985). The quotient of the incipient LC50 and the LC50 values is used as a safety factor in the determination of "acceptable" toxicant levels in the natural environment (Van Leeuwen, 1990). These application or extrapolation factors determined, can be used to estimate the incipient LC50 value of species A if the value for species B as well as lethal dose for species A is known (Hellawell, 1986).

Toxicity tests can reliably monitor changes in the lethality of metals to an organism but it cannot adequately predict a concentration of toxicant that will be unlikely to harm a population or ecosystem at all times (APHA [American Public Health Association] 1989). Many factors can effect or modify the degree of sensitivity shown by an organism to different toxicants. Some examples are the specific diet, season of the year and water quality variables such as temperature, pH and hardness (Falk & Dunston 1977). The importance of modifying factors cannot be over-estimated, for many accounts of their influence on the variation in toxicity can be found in the literature .

It is therefore important to determine the specific LC50 value for a given combination of abiotic conditions as found in a specific water body. Lethal threshold concentrations may often be similar for similar species, but it is risky to transfer predictions of the effects of modifying factors from one species to another.

The experiments reported here was conducted to determine the LC50 and incipient LC50 values of copper at winter and summer temperatures for *Clarias gariepinus* and juvenile *Oreochromis mossambicus* as well as the LC50 and incipient LC50 values of manganese for juvenile *Oreochromis*

mossambicus at 27±1°C. The ranges of copper and manganese concentrations found in the Olifants river were compared to the LC50 values found during experimental tests, in an effort to predict acceptable guidelines for copper and manganese concentrations in the Olifants River.

8.2.1.2 Materials and Methods

Clarias gariepinus and *Oreochromis mossambicus* specimens were obtained from Blyde River Aquaculture, a commercial fish farm near Hoedspruit in the eastern Transvaal. *Oreochromis mossambicus* were also obtained from a hatchery in the Brits district. At the aquarium fish were kept separately in recirculating systems, each consisting mainly of a 1000 litre reservoir and a biological filter. Borehole water circulated from the reservoir through the biological filter and was pumped back again to the reservoir. The physico-chemical characteristics of the borehole water were determined (Table 8.28). On arrival fish underwent a week long daily infection treatment with coarse salt and Terravit (Seymore, 1994). The healthy fish were allowed to acclimatise in the recirculating system for four weeks to three months and fed daily on commercial trout pellets (Van der Merwe *et al.*, 1993; Seymore 1994).

Fish were transferred to a flow-through system for performing the toxicity test (Fig. 8.16). The system consisted of four series of four glass tanks in which the fish were exposed to the different metal concentrations. The test solutions were added directly to the glass tanks containing the fish, after which a continuous supply of the specified concentrations were maintained by pumping the test solutions from the four 200 litre reservoirs to each of the series of four glass tanks (Van der Merwe *et al.*, 1993; Nussey, 1994; Seymore, 1994). The rate of flow was regulated to supply a constant flow through the experimental tanks for the 96 h exposure period. The reservoirs were filled twice daily to provide enough test solution . The procedures employed, concentrations of metals administered and number as well as size of fish used during exposure are given by Van der Merwe *et al* (1993), Nussey (1994) and Seymore (1994).

Water samples (50 ml) were taken daily in order to determine the real metal concentration present in the test solution. Standard procedures were followed in the water sample preparation for metal determination (Nussey, 1994; Seymore 1994). The LC50 values were obtained by determining dosage - survival and dosage - mortality rates at 24, 48, 72 and 96 hours (Van der Merwe *et al.* 1993; Nussey, 1994; Seymore, 1994). The predicted metal concentrations where 50% mortality will occur were determined and the 95% confidence limits of these concentrations estimate were calculated. Toxicity curves were drawn by plotting the exposure time in hours (y-axis) on the median concentration of the metals (x-axis) (Litchfield & Wilcoxon, 1949; Gopal & Misra, 1988; Zar, 1984).

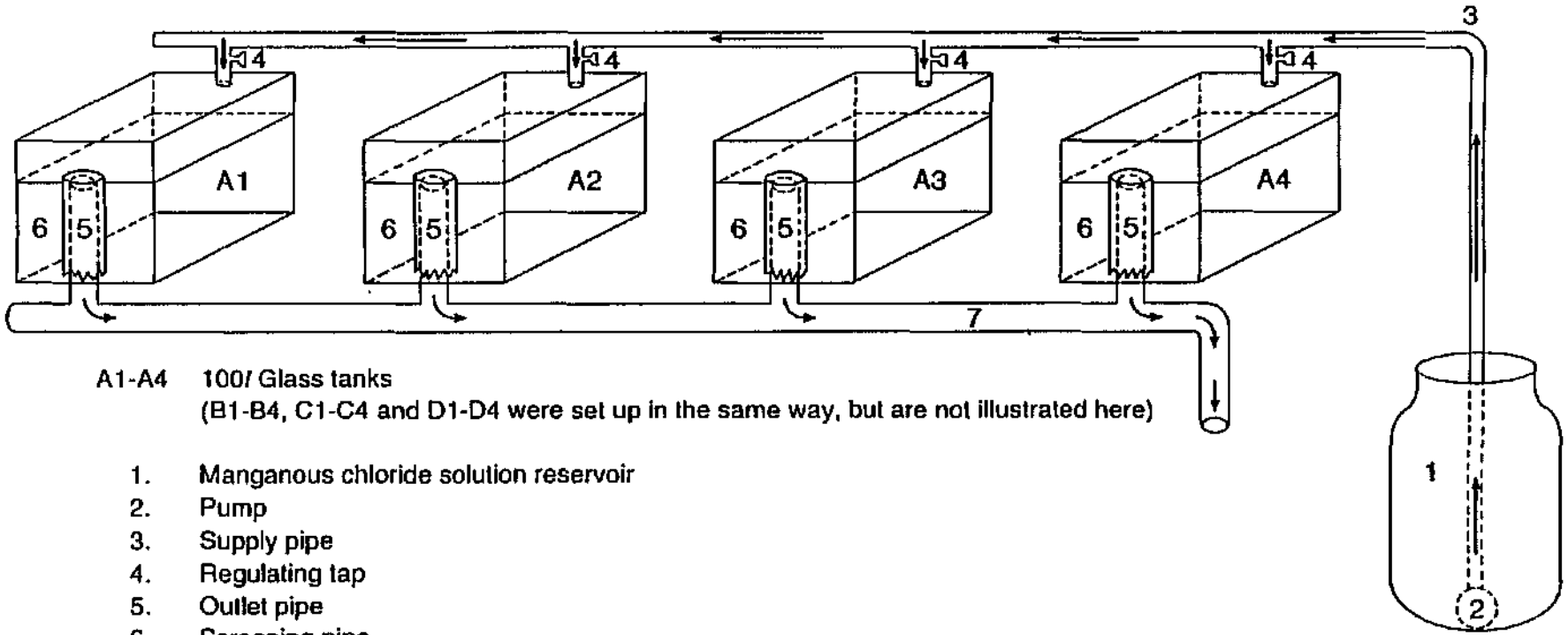
8.2.1.3 Results and Discussion

On the basis of 24, 48, 72 and 96 hours exposure periods, the LC_{50} values were estimated for the species concerned. The regression coefficient was calculated for all the LC50 values. In the case of *C. gariepinus*, it is given in a coefficient percentage. No mortalities were found during the control tests.

In performing a toxicity test, it is essential to determine the actual toxicant concentration present in the water during exposure and to compare it with the toxicant concentration that was originally added to the water. More

TABLE 8.28
SUMMARY OF SELECTED WATER QUALITY VARIABLES
OF BOREHOLE WATER

pH	7.30
Temperature (°C)	26.80
Ammonium (NH_4, mg/l)	0.14
Nitrogen as NO_3+NO_2 (mg/l)	0.67
Fluoride (F, mg/l)	0.20
Total alkalinity as $CaCO_3$ (mg/l)	76.00
Sodium (Na, mg/l)	7.00
Magnesium (Mg, mg/l)	3.00
Silicon (Si, mg/l)	5.40
Phosphate (PO_4, mg/l)	0.01
Sulphate (SO_4, mg/l)	11.00
Bicarbonate (HCO_3, mg/l)	63.00
Chloride (Cl, mg/l)	7.00
Potassium (K, mg/l)	2.20
Calcium (Ca, mg/l)	26.00
Conductivity (Ec, mS/m)	22.20
Total hardness as $CaCO_3$ (mg/l)	79.00
Total dissolved solids (TDS, mg/l)	153.00
Sodium absorption rate (SAR)	0.30



A1-A4 100*l* Glass tanks
(B1-B4, C1-C4 and D1-D4 were set up in the same way, but are not illustrated here)

1. Manganous chloride solution reservoir
2. Pump
3. Supply pipe
4. Regulating tap
5. Outlet pipe
6. Screening pipe
7. Drainage pipe

Figure 8.16
Diagram of the experimental flow-through system used in the toxicity tests

often than not, it is found that on average, the measured toxicant concentration is lower (or even higher) than the original concentration. A decrease in toxicant concentration can be attributed to the apparent adsorption onto the test container material (Sprague, 1969).

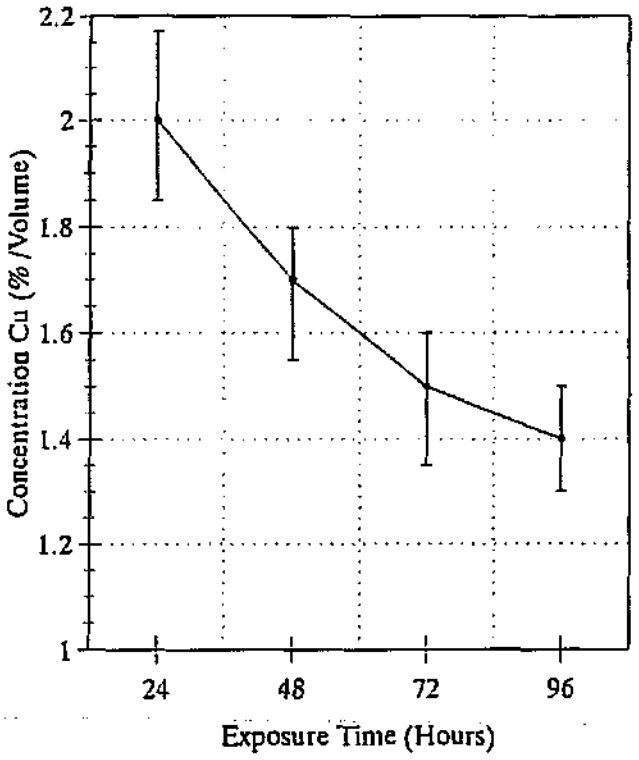
The 24, 48 and 72 and 96-h LC50 value for juvenile *C. gariepinus* at 21 ± 1°C are significantly by higher ($P > 0,05$) than the 24, 48, 72 and 96-h LC50 value for adult *C. gariepinus* (Tables 8.29; 8.30). No statistically significant difference was found between the 96-h LC50 values for adult and juvenile *C. gariepinus* at 28 ± 1°C (Table 8.31; 8.32). The toxicity curves of *C. gariepinus* to various concentrations of copper at the selected temperatures are presented in Fig. 8.17. The curve for juveniles at 21 ± 1°C is almost linear, while it is distinctly curvilinear for the remaining bioassays. A veil-like film which appeared to be excessive coagulated mucus covered the dead fish. A similar film of mucous was found within the operculum on the gills. Other symptoms of toxicosis such as behavioral changes, eg. agitated swimming rates, increased rates of operculum movement, lethargy and loss of rheotaxis were also noted. These reactions were more pronounced in tanks containing the higher levels of copper and less conspicuous at lower concentrations.

The primary goal in the design and use of toxicity tests in biomonitoring is to predict, in combination with other environmental factors, with known accuracy, a concentration of a specific toxicant (metal) that will not harm an entire system, and to make this prediction in a responsible and cost effective manner. In any bioassay, it is important to consider the water quality used in the experimental system. Modifying factors, such as water hardness, pH, alkalinity and temperature, can affect the copper speciation, and thus alter the copper toxicity. The critical factor regarding copper toxicity to fish is not as much as toxic action following copper speciation, but to a larger extent the controlling roles of water hardness, alkalinity and pH (Chakoumakos *et al.*, 1979).

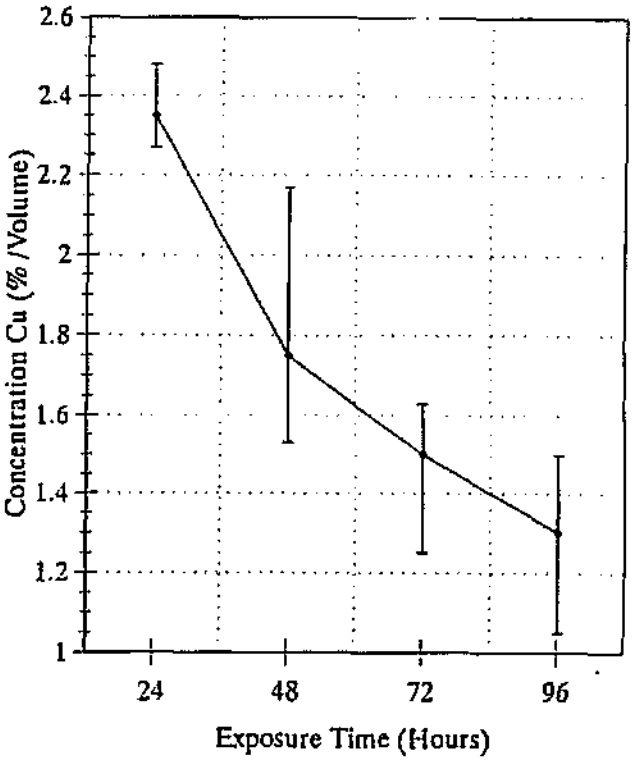
Results indicated that water temperature may alter the lethality of copper to *C. gariepinus*, therefore copper toxicity appears to be enhanced by temperature as a stressor. Several studies have shown that as water temperature rises, waterborne toxicants become lethal to fish at lower concentrations. Heat stress decreased the LC50 values for bluegills exposed to zinc (Burton *et al.*, 1972) and choline exposure on bluegills and rainbow trout (Bass *et al.*, 1977). The difference in LC50 values due to changes in seasonal water temperature may occur due to a transient change in the metabolic rate of the fish. *C. gariepinus* possibly accumulated copper mainly through the gills as suspected in other teleosts (Heath, 1987). The volume of water passing through the gills could control the uptake rate of copper by the test organism. A water temperature of 21 ± 1°C will lead to a decrease in metabolic rate, consequently inducing longer survival by reducing ventilation rate and copper uptake. Similarly, metabolic acceleration due to heat may have shortened survival by accelerating copper accumulation. It has, however, been reported that these changes are not likely to have a large practical importance, for interspecies differences are much greater (Smith and Heath, 1979).

C. gariepinus stayed at the bottom of the tank during stress caused by the toxicant, which forced it to use only the gills for oxygen uptake. The veil-like mucous film, formed on the body and gills of dead fish after copper exposure, confirms the reason for death argued above. Various authors reported the mucous formation after metal exposure (Skidmore and Tovell, 1972). Rapid mouth and operculum movements were also reported for the

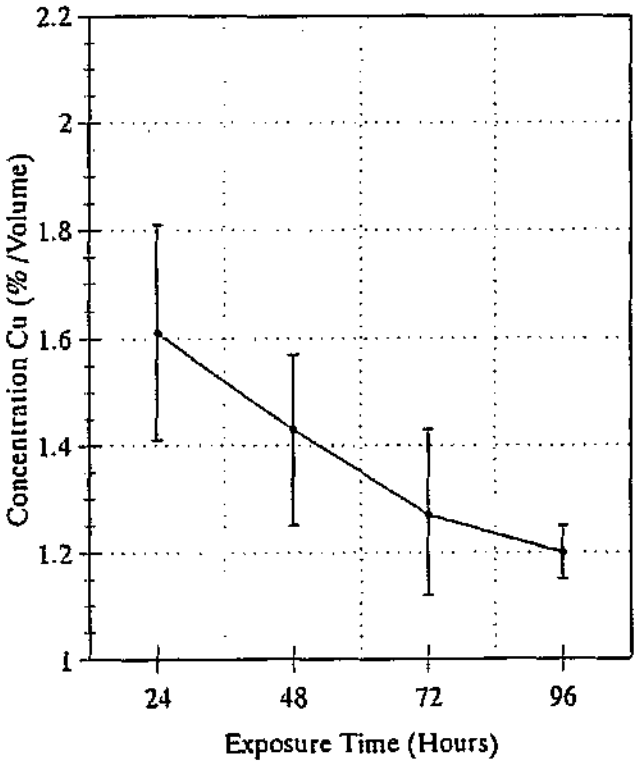
21 °C
A



28 °C
A



21 °C
J



28 °C
J

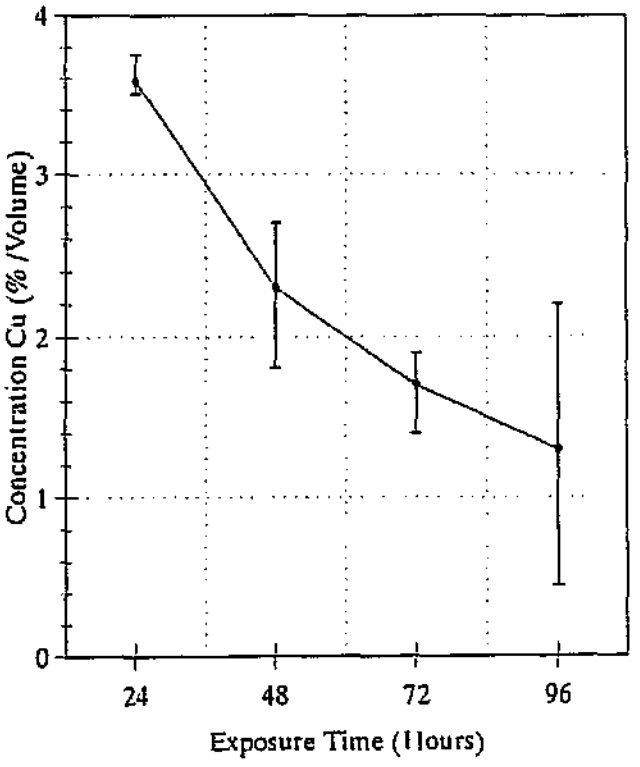


FIGURE 8.17
Toxicity curves for C. gariepinus *after copper exposure. A.: Adults. J: Juveniles*

TABLE 8.29

Experimental data obtained during a toxicity test on adult *C. gariepinus* and copper LC50 values at 21 ± 1 °C after different time intervals

CONCENTRATION OF COPPER % Cu^{2+} /VOLUME	NUMBER OF TEST ORGANISMS	NUMBER OF TEST ORGANISMS DEAD AFTER: 2 H	4 H	6 H	8 H	24 H	48 H	72 H	96 H
2.220	10	0	0	0	2	6	8	9	10
1.744	10	0	0	0	1	4	5	7	7
1.463	10	0	0	0	0	3	4	6	6
1.211	10	0	0	0	0	2	3	3	4
0.949	10	0	0	0	0	1	1	2	3
0.877	10	0	0	0	0	0	0	1	1
0.543	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		-	-	-	-	2.00	1.68	1.48	1.38
95% Confidence limits for Cu^{2+} concentration		-	-	-	-	2.17	1.80	1.59	1.46
		-	-	-	-	1.83	1.56	1.35	1.30
Regression coefficient (%)		-	-	-	-	97.10	96.42	96.98	97.04
Slope of probit line		-	-	-	-	38.26	50.66	58.13	60.79

TABLE 8.30

Experimental data obtained during a toxicity test on juvenile *C. gariepinus* and copper LC50 values at 21 ± 1 °C after different time intervals.

CONCENTRATION OF COPPER % Cu^{2+} /VOLUME	NUMBER OF TEST ORGANISMS	NUMBER OF TEST ORGANISMS DEAD AT: 2 H	4 H	6 H	8 H	24 H	48 H	72 H	96 H
1.629	10	0	0	0	3	6	6	9	10
1.517	10	0	0	0	2	6	5	6	7
1.334	10	0	0	0	0	3	4	5	6
0.968	10	0	0	0	0	1	1	2	2
0.767	10	0	0	0	0	0	0	1	1
0.514	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		-	-	-	-	1.61	1.42	1.28	1.20
95% Confidence limits for Cu^{2+} concentration		-	-	-	-	1.81	1.57	1.43	1.24
		-	-	-	-	1.31	1.27	1.13	1.16
Regression coefficient (%)		-	-	-	-	90.61	93.47	93.73	94.62
Slope of probit line		-	-	-	-	60.38	67.75	75.11	86.52

TABLE 8.31

Experimental data during a toxicity test on adult *C. gariepinus* and copper LC50 values at 28 ± 1 °C at different time intervals

CONCENTRATION OF COPPER % Cu^{2+} /VOLUME	NUMBER OF TEST ORGANISMS	NUMBER OF TEST ORGANISMS DEAD AT: 2 H	4 H	6 H	8 H	24 H	48 H	72 H	96 H
2.263	10	0	0	0	2	4	7	9	10
1.772	10	0	0	0	1	4	5	7	8
1.263	10	0	0	0	0	3	5	6	6
1.071	10	0	0	0	0	2	3	5	5
0.930	10	0	0	0	0	1	1	1	2
0.617	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		-	-	-	-	2.38	1.87	1.48	1.29
95% Confidence limits for Cu^{2+} concentration		-	-	-	-	-	3.16	1.66	1.54
		-	-	-	-	-	1.56	1.20	1.06
Regression coefficient (%)		-	-	-	-	85.27	86.68	84.86	92.14
Slope of probit line		-	-	-	-	25.10	41.29	53.70	59.27

TABLE 8.32

Experimental data obtained during a toxicity test on juvenile *C. gariepinus* and copper LC50 values at 28 ± 1°C at different time intervals.

CONCENTRATION OF COPPER % Cu^{2+} /VOLUME	NUMBER OF TEST ORGANISMS	NUMBER OF TEST ORGANISMS DEAD AT: 2 H	4 H	6 H	8 H	24 H	48 H	72 H	96 H
3.326	10	0	0	0	0	4	7	7	10
1.810	10	0	0	0	0	3	5	7	8
1.398	10	0	0	0	0	3	4	6	6
1.167	10	0	0	0	0	2	2	4	4
0.991	10	0	0	0	0	0	1	1	2
0.790	10	0	0	0	0	0	0	0	0
LC50, estimated by y = a + bx		-	-	-	-	3.62	2.28	1.68	1.30
95% Confidence limits for Cu^{2+} concentration		-	-	-	-	3.72	2.88	1.89	2.20
		-	-	-		3.52	1.88	1.21	0.40
Regression coefficient (%)		-	-	-	-	93.01	84.56	90.07	78.90
Slope of probit line		-	-	-	-	31.53	26.25	73.56	35.94

common guppy *P. reticulata*, for nickel and chromium exposure (Doudorff and Katz, 1953). These findings suggest that the toxic mode of action of copper may be dependent on the toxic effect on the respiratory apparatus of the fish. Many pollutants, irrespective of type, may firstly cause damage to the gills, and this might have an effect on osmoregulation as well as respiration (Wong *et al.*, 1977).

Oreochromis mossambicus

Manganese

The unstable background Mn levels in the borehole water could have contributed to the variation in toxicant concentration. However, the variation could also have been due to the absorption and metabolism of Mn by the fish (Abel, 1989). When the fish were exposed to 0.028 g/l Mn, the mean measured Mn concentration was 0.027 g/l Mn (Table 8.33), indicating good regulation by the fish or perhaps that no absorption took place. At 0.278 and 0.416 g/l Mn exposure, the initial Mn concentration measured lower than expected - possibly indicating absorption - and increased thereafter in the water until it stabilised at a certain level, indicating that a steady state or equilibrium had been reached. Therefore, good regulation took place. From 0.555 g/l Mn to 2.776 g/l Mn exposure, it seemed, however, that the fish had some difficulty in regulating the Mn levels. The Mn concentrations at first measurements were always lower than the original concentrations as made up by dissolving the correct calculated masses of $MnCl_2.4H_20$ per volume of water, thus indicating immediate absorption by the fish. As time progressed, the Mn concentrations increased and reached levels that exceeded the exposure concentrations. This could possibly be attributed to the fish trying to excrete excessive Mn from the inside of their bodies, but failed to do so - indicating that no equilibrium or steady state had been reached.

The effects of pollutants on individuals may range from rapid death through sub-lethal effects to no effects at all (Moriarty, 1990). The most important responses, however, are death, disturbed physiology, reproductive impairment and aberrant behaviour (Hellawell, 1986). The visible sub-lethal effects that Mn had on juvenile *O. mossambicus* were opaque eyes and haemorrhaging at the pectoral fins and nose (0.278 - 2.776 g/l Mn), excessive mucus production (0.694 - 2.776 g/l Mn), white burnt fins (1.666 - 2.776 g/l Mn) and "turnover" (1.388 - 2.776 g/l Mn), which is a common response of fish to toxicants indicating a loss of balance and the inability to control their normal swimming position (Hellawell, 1986). Most of the time the fish remained at the bottom of the tank, but occasionally, at the higher Mn concentrations (1.388 - 2.776 g/l Mn), they would suddenly surge upwards and immediately sink to the bottom again.

Mortalities started to occur at 1.249 g/l Mn (Table 8.33) and continued to occur until 2.776 g/l Mn, at which concentration all the fish were dead. No mortalities occurred in the control tanks. The single deaths that occurred at 0.555 and 1.249 g/l Mn are natural phenomena, since every population has those individuals which are weaker and more susceptible to environmental stress factors or pollutants than others and are thus not truly representative of the whole population. All the fish survived the lower dosage range, while a rapid onset of mortalities occurred at the upper dosage range.

The LC50 values were calculated to be 4.774 g/l Mn at 24 hours, 2.084 g/l Mn at 48 hours, 1.893 g/l Mn at 72 hours and 1.723 g/l Mn at 96 hours (Table 8.33). The 96-hour LC50 value determined in this study (1.723 g/l

TABLE 8.33

EXPERIMENTAL DATA FROM THE Mn TOXICITY TEST ON JUVENILE *OREOCHROMIS MOSSAMBICUS*

[$MnCl_2.4H_2O$] g/l	[Mn] g/l	Measured [Mn] g/l	Total No. of Fish N	% Survival of fish 2hr	6hr	24hr	30hr	48hr	54hr	72hr	78hr	96hr	Time-survival curve: For Y = a + bx a	b	LT50 (hours) estim. from graph	95% Confidence limits
0.0		0.001±0.002	200	100	100	100	100	100	100	100	100	100				
		(0.000-0.011)														
0.1	0.028	0.027±0.006	40	100	100	100	100	100	100	100	100	100				
		(0.020-0.042)														
1.0	0.278	0.265±0.010	40	100	100	100	100	100	100	100	100	100				
		(0.242-0.280)														
1.5	0.416	0.395±0.029	40	100	100	100	100	100	100	100	100	100				
		(0.330-0.432)														
2.0	0.555	0.530±0.037	40	100	100	100	100	100	100	100	100	97.5	100.4	-0.01	5040	
		(0.436-0.574)														
2.5	0.694	0.679±0.021	40	100	100	100	100	100	100	100	100	100				
		(0.630-0.706)														
3.0	0.833	0.818±0.029	40	100	100	100	100	100	100	100	100	100				
		(0.772-0.870)														
4.0	1.110	1.042±0.117	40	100	100	100	100	100	100	100	100	100				
		(0.840-1.202)														
4.5	1.249	1.240±0.050	40	100	100	100	100	100	100	97.5	97.5	97.5	100.6	-0.03	1686.7	1152.5/2541.2
		(1.132-1.316)														
5.0	1.388	1.295±0.050	40	100	100	97.5	97.5	92.5	92.5	85.0	82.5	75.0	103.2	-0.26	204.6	186.2/226.7
		(1.216-1.396)														
5.5	1.527	1.451±0.062	40	100	100	100	97.5	95.0	92.5	85.0	77.5	65.0	105.9	-0.34	164.4	144.0/184.2
		(1.324-1.518)														
5.8	1.610	1.646±0.062	40	100	100	100	100	97.5	95.0	87.5	82.5	55.0	108.0	-0.38	152.6	132.0/178.8
		(1.526-1.726)														
6.0	1.666	1.585±0.047	40	100	100	92.5	90.0	52.5	35.0	17.5	10.0	2.5	110.8	-1.21	50.2	43.7/56.7
		(1.498-1.674)														
10.0	2.776	2.625±0.470	40	100	100	77.5	45.0	7.5	0.0	0.0	0.0	0.0	93.5	-1.25	34.8	26.0/43.7
		(2.038-3.348)														

Dosage-survival curve: For Y = a + bx:							
a	118.6	147.0	174.2	178.3	169.9	165.9	155.6
b	-14.37	-35.23	-59.59	-64.75	-63.33	-62.81	-61.30
LC50 (g/l Mn) estimated from graph	4.774	2.753	2.084	1.981	1.893	1.845	1.723
95% Confidence limits	4.381	2.548	1.904	1.790	1.678	1.620	1.489
	5.198	2.963	2.268	2.183	2.112	2.072	1.957

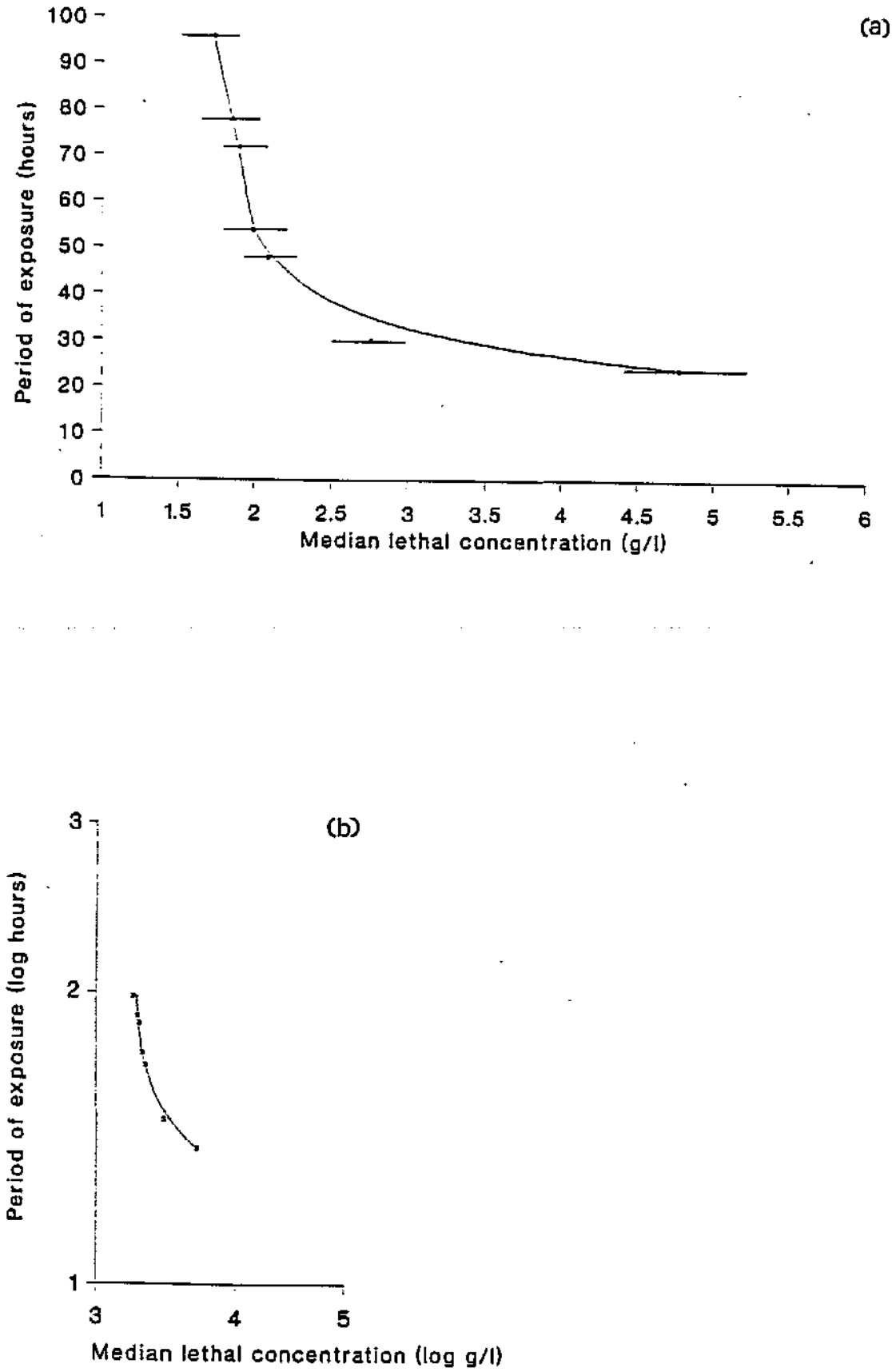


Figure 8.18
Toxicity curve of Mn for juvenile O. mossambicus based on the determined LC50 values: a) Linear scale b) Log scale

Mn), was lower than the 96-hour LC50 value of 3.230 g/l Mn determined by Nath and Kumar (1987). This difference in value can be attributed to several factors. Nath and Kumar (1987) performed a static bioassay, exposing the freshwater perch, *Colisa fasciatus*, to different concentrations of Mn SO_4H_20. The mean weight and length of the adult specimens were 5.74 ± 0.28 g and 6.93 ± 0.28 cm. On average the fish were therefore smaller than the ones used in the performance of this toxicity test, and it was also a different fish species. Furthermore, $MnS0_4$ is known to be less toxic than $MnC1_2$ and a higher LC50 value for $MnS0_4$, can be expected. *Colisa fasciatus* was kept in tap water with a mean temperature of 24.33 ± 1.69°C and a mean hardness of 165.33 ± 6.17 mg/l as $CaC0_3$. On the other hand, *Oreochromis mossambicus* was kept in borehole water with a mean temperature of 26.8 ± 1.3°C and a hardness of 61.0 mg/l as $CaC0_3$. The higher temperature and softer water in the case of *O. mossambicus* could thus have increased the Mn toxicity (Hellawell, 1986).

The incipient LC50 value in this study was calculated to be 1.99 g/l Mn (Fig. 8.18), using LC50. The application factor for Mn (Incipient LC50/96-h LC_{50}) would therefore be 1.155 or 0.847. For certain applications, where the test forms part of a research programme designed to establish water quality standards, it would most probably be preferable to use the concentration-response approach (e.g. Fig. 8.18) rather than the time-response approach (Abel, 1989). Therefore the toxicity curve based on LC50 values would be used. However, Gaddum (1953) estimates that approximately half the information will be lost when using the dosage-response approach, so that twice as many observations will be needed for any given degree of accuracy. Therefore, the reactions of the exposed fish can be monitored carefully with time (Sprague, 1969) and it would depend on the goal set for the toxicity test in order to decide whether the concentration-response or the time-response approach should be used.

Copper

When juvenile *O. mossambicus* was exposed to low copper concentrations, such as 1.0 and 1.7 mg/l at both temperatures, the measured copper concentration indicated good regulation by the fish or that little or no absorption took place. During the 1.8 - 2.8 mg/l exposures, at both temperatures, the first measurement was lower than the initial copper concentration added to the water. This was an indication of the absorption of copper by fish. Thereafter, an increase in the copper concentration in the water, until it stabilised at a certain level, indicated that a steady state had been reached and the fish could still regulate the copper (Lloyd, 1992). However, during the 3.0 - 4.0 mg/l exposures, at both temperatures, it was evident that fish had some difficulty to regulate the copper since the first measurements at these concentrations were higher than original concentrations.

The first water sample taken after the exposure began showed a lower copper concentration than the initial concentration added to the water. This is an indication of the absorption of copper by juvenile *O. mossambicus*. The water samples thereafter showed a steady increase in copper concentration, thus reaching an equilibrium (Lloyd, 1992) and fish could still regulate the copper. This phenomenon confirms the observation after exposure to manganese. Water samples taken after 96 hours, showed an increase in copper concentration. This increase could be an indication that these fish had some difficulty to regulate the copper concentrations, they try to excrete copper from their bodies but failed to do so and thus no equilibrium or steady state had been reached.

Fish respond to pollutants either by a rapid death or sublethal effects, when a pollutant concentration is very low there may even be no visible effects (Moriarty, 1990). During this study dead fish were covered in a veil-like film, which looked like coagulated mucus. The sublethal effects that copper had on juvenile *O. mossambicus* were darkening of skin pigmentation, erratic swimming, increases in coughing action and operculum movement (respiration), there was a marked decrease in activity. Fish also concentrated motionless at the water surface, as close as possible to the point where water jets into the experimental tanks. Haemorrhage at the pectoral fins, operculum and nose was evident and there was an excessive mucus production. In some cases there was a loss of equilibrium (partial or complete), this was noted when fish lost their balance and they were inable to control their swimming position. They swam on their side, skew or even upside down. All the abovementioned reactions were more obvious in the tanks containing the higher copper concentrations, this was the case at both temperatures.

There were no mortalities in the control groups. During the summer toxicity test (29 ± 1°C) the first mortalities were noted at 1.8 mg/l after 96 hours. On the other hand mortalities during the winter toxicity test (19 ± 1°C noted at 2.3 mg/l after 48 hours (Tables 8.34; 8.35). Therefore it can be concluded that juvenile *O. mossambicus* are more susceptible to copper at higher temperatures. The higher the copper concentrations the more mortalities occurred, until 3.0, 3.5 and 4.0 mg/l where all fish were dead, at both temperatures. Both small and larger fish died (7.0 - 11.8 cm), thus smaller fish are not necessarily the weaker individuals in a population.

There were no definite death patterns. Fish survived low concentrations (1.0 and 1.7 mg/l Cu) and then started dying. Death occurred gradually in the sense that the higher the copper concentrations the more fish died (Nussey, 1994). Copper is one of the most studied of all trace metals and a very substantial body of literature exists on the subject (Hellawell, 1986). It is a common phenomenon that metal toxicity varies with the type of test organism. Data reported on 96 hour LC50 values for copper cover a wide range of values from 0.1 mg/l for *Pseudopleuronectes americanus* (EPA, 1980) to 20.0 mg/l for *Fundulus heteroclitus* (La Roche, 1974). It thus seems that copper is species specific and therefore it is important to determine LC50 values for local species which could be used as a standard or guideline for other species under similar environmental conditions.

The summer LC50 values were calculated to be 5.52 mg/l copper after 24 hours, 3.10 mg/l copper after 48 hours, 2.86 mg/l copper after 72 hours and 2.61 mg/l copper after 96 hours (Fig. 8.19; Table 8.35). Winter LC50 values were calculated to be 10.73 mg/l copper after 24 hours, 3.62 mg/l copper after 48 hours, 3.03 mg/l copper after 72 hours and 2.78 mg/l copper after 96 hours (Fig. 8.19; Table 8.34). Results indicate that seasonal variations in water temperature can alter the lethality of copper to juvenile *O. mossambicus*. This corresponds with the results on the lethal concentration levels of copper to juvenile and adult *Clarias gariepinus* at two temperatures (Van der Merwe *et al.* 1993). As mentioned earlier juvenile *O. mossambicus* are more susceptible to copper at the higher temperature. This may be due to the fact that at the higher temperature (29 ± 1°C) the fish have a relative higher metabolic rate (Heath, 1987) and thus more water, and copper, passes through the gills. The excretion rate of copper will also be higher due to the higher metabolic rate (Van der Merwe *et al.* 1993). The higher water turnover in the gills could be an argument for a greater sensitivity to copper (Van der Merwe, 1992) because more water containing copper is transported to the blood. Studies done on LC50

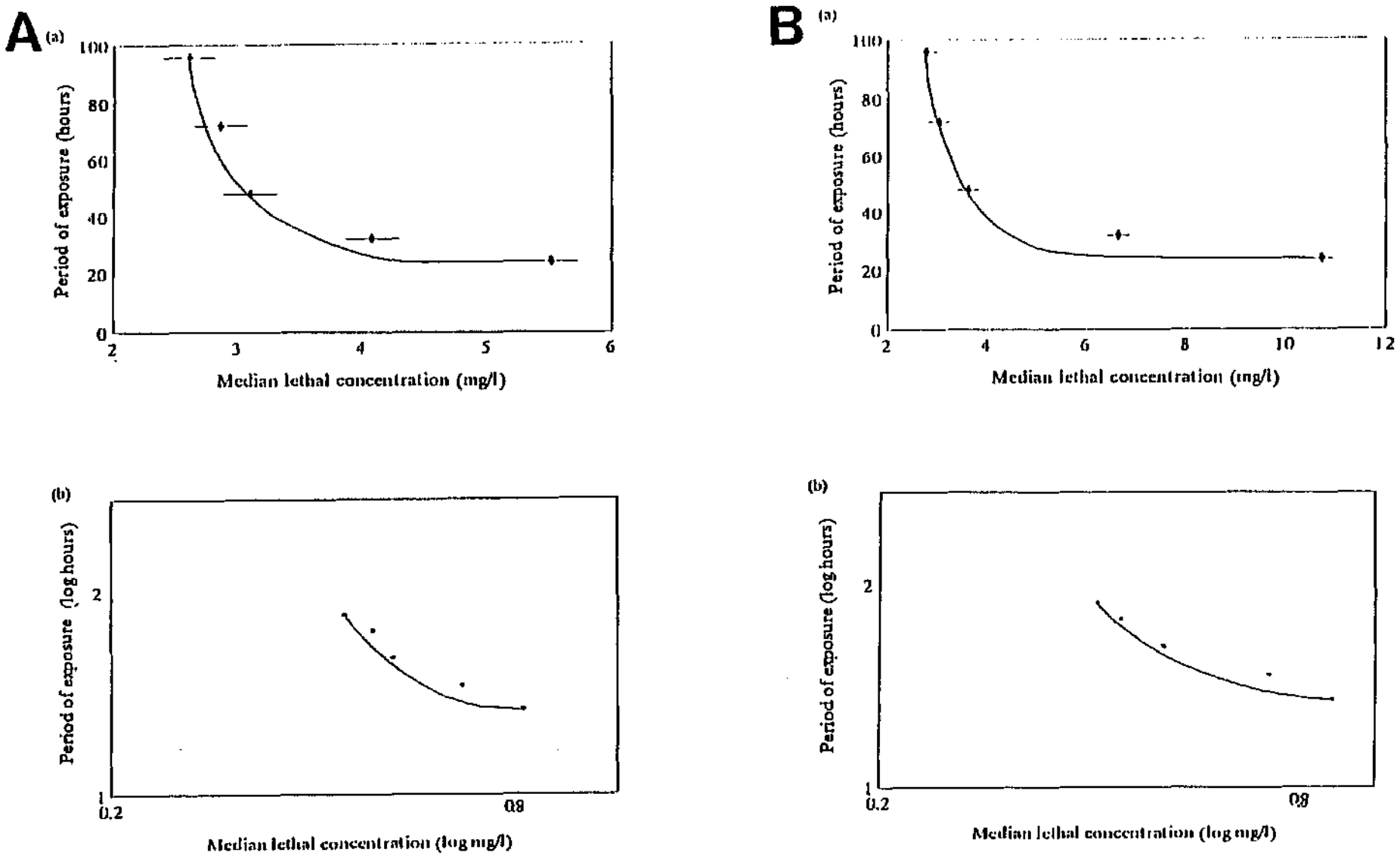


FIGURE 8.19

Toxicity curve of copper for juvenile Oreochromis mossambicus *based on the determined LC50 values.*
A.. 29 + 1 °C, (a) Linear scale and (b) Log scale.
B. 19 + 1 °C, (a) Linear scale and (b) Log scale.

TABLE 8.34
EXPERIMENTAL DATA OF THE COPPER TOXICITY TEST ON JUVENILE *OREOCHROMIS MOSSAMBICUS*, AT 19 ± 1°C.

[Cu] mg/l	[$CuCl_2$.$2H_2O$] mg/l	[Cu] measured in H_2O mg/l	Total number of fish N	% Survival of fish										*Time-survival curve:*				
				2hr	4hr	8hr	12hr	16hr	24hr	32hr	48hr	72hr	96hr	For Y = a + bx a	b	LT50 (hours) estimated from graph	95 % Confidence limits	
0.0		0.002±0.001	40	100	100	100	100	100	100	100	100	100	100					
		(0.001-0.004)																
1.0	2.6828	0.997±0.005	40	100	100	100	100	100	100	100	100	100	100					
		(0.990-1.002)																
1.7	4.5607	1.704±0.030	40	100	100	100	100	100	100	100	100	100	100					
		(1.682-1.748)																
1.8	4.8290	1.822±0.018	40	100	100	100	100	100	100	100	100	100	100					
		(1.811-1.849)																
2.0	5.3656	1.969±0.050	40	100	100	100	100	100	100	100	100	100	100					
		(1.899-2.014)																
2.2	5.9021	2.184±0.060	40	100	100	100	100	100	100	100	100	100	100					
		(2.099-2.239)																
2.3	6.1704	2.272±0.050	40	100	100	100	100	100	100	100	97.5	97.5	82.5	103.19	-0.16	332.44	91.22	99.16
		(2.199-2.301)																
2.4	6.4387	2.410±0.035	40	100	100	100	100	100	100	100	95	85	77.5	104.46	-0.26	209.46	88.42	94.50
		(2.389-2.462)																
2.5	6.7070	2.490±0.010	40	100	100	100	100	100	100	100	95	80	62.5	107.53	-0.40	143.83	81.41	93.66
		(2.477-2.502)																
2.6	6.9752	2.585±0.024	40	100	100	100	100	100	100	100	92.5	70	60	108.21	-0.47	123.85	78.35	91.07
		(2.550-2.603)																
2.7	7.2435	2.740±0.059	40	100	100	100	100	100	100	100	90	67.5	52	109.60	-0.55	108.36	75.11	89.09
		(2.680-2.791)																
2.8	7.5118	2.807±0.040	40	100	100	100	100	100	100	100	85	65	45	110.54	-0.63	96.10	72.09	85.99
		(2.775-2.866)																
3.0	8.0483	2.987±0.063	40	100	100	100	100	100	100	87.5	62.5	40	17.5	110.98	-0.96	63.52	56.73	69.23
		(2.899-3.050)																
3.5	9.3897	3.476±0.063	40	100	100	100	100	100	95	77.5	40	15	0	109.39	-1.21	49.08	39.95	57.83
		(3.399-3.521)																
4.0	10.7311	3.947±0.054	40	100	100	100	100	100	82.5	67.5	17.5	0	0	104.64	-1.28	42.69	24.17	57.11
		(3.899-4.015)																

Dosage-survival curve:

For Y = a + bx:	a	131.67	121.82	156.20	171.72	175.28
	b	-15.75	-10.81	-29.53	-40.12	-45.12
LC50 (mg/l Cu) estimated from graph		10.73	6.64	3.62	3.03	2.78
95% Confidence limits		11.74	5.58	3.83	3.36	3.03
		32.26	12.53	6.94	5.34	4.82

TABLE 8.35

EXPERIMENTAL DATA OF THE COPPER TOXICITY TEST ON JUVENILE *OREOCHROMIS MOSSAMBICUS*, AT 29 ± 1°C.

[Cu] mg/l	[$CuCl_2$.$2H_2O$] mg/l	[Cu] measured in H_2O mg/l	Total number of fish N	% Survival of fish 2hr	4hr	8hr	12hr	16hr	24hr	32hr	48hr	72hr	96hr	Time-survival curve: For Y = a + bx a	b	LT50 (hours) estimated from graph	95 % Confidence limits	
0.0		0.002±0.001	40	100	100	100	100	100	100	100	100	100	100					
		(0.001-0.005)																
1.0	2.6828	0.995±0.011	40	100	100	100	100	100	100	100	100	100	100					
		(0.985-1.011)																
1.7	4.5607	1.678±0.070	40	100	100	100	100	100	100	100	100	100	100					
		(1.623-1.769)																
1.8	4.8290	1.780±0.060	40	100	100	100	100	100	100	100	100	100	95	100.95	-0.04	1273.75	97.49	100.41
		(1.700-1.884)																
2.0	5.3656	1.965±0.042	40	100	100	100	100	100	100	100	100	95	92.5	101.65	-0.08	645.63	95.99	99.31
		(1.923-2.008)																
2.2	5.9021	2.195±0.005	40	100	100	100	100	100	100	100	97.5	95	90	101.88	-0.11	471.64	95.12	97.64
		(2.188-2.202)																
2.3	6.1704	2.297±0.038	40	100	100	100	100	100	100	100	100	92.5	87.5	102.72	-0.14	376.57	95.40	98.04
		(2.258-2.349)																
2.4	6.4387	2.417±0.038	40	100	100	100	100	100	100	100	97.5	95	55	108.53	-0.39	150.08	77.69	100.37
		(2.280-2.469)																
2.5	6.7070	2.489±0.033	40	100	100	100	100	100	100	100	72.5	57.5	52.5	108.21	-0.61	95.43	69.59	85.83
		(2.449-2.530)																
2.6	6.9752	2.581±0.038	40	100	100	100	100	100	100	100	77.5	70	42	110.14	-0.63	95.46	70.52	86.76
		(2.501-2.681)																
2.7	7.2435	2.698±0.010	40	100	100	100	100	100	100	100	72.5	65	40	110.24	-0.68	88.59	68.62	84.25
		(2.676-2.701)																
2.8	7.5118	2.792±0.019	40	100	100	100	100	100	100	100	67.5	65	37.5	110.32	-0.71	84.96	65.62	84.02
		(2.766-2.810)																
3.0	8.0483	2.955±0.053	40	100	100	100	100	100	100	95	45	30	0	116.61	-1.28	52.04	37.39	67.83
		(2.899-3.008)																
3.5	9.3897	3.474±0.055	40	100	100	100	100	100	60	55	17.5	0	0	96.68	-1.20	38.90	19.95	53.41
		(3.399-3.532)																
4.0	10.7311	3.970±0.097	40	100	100	100	100	100	57.5	12.5	0	0	0	85.98	-1.16	31.02	-0.45	56.41
		(3.889-4.096)																

Dosage-survival curve:						
For Y = a + bx: a		129.68	151.41	171.19	176.09	170.27
b		-14.43	-24.85	-39.11	-44.04	-46.15
LC50 (mg/l Cu) estimated from graph		5.52	4.08	3.10	2.86	2.61
95% Confidence limits		5.93	4.08	3.38	2.95	2.83
		18.02	11.38	5.60	5.29	4.69

values by Burton *et al* (1972) and Bass *et al.*, (1977) illustrate that heat stress decreased the LC50 values, therefore as water temperatures rises, waterborne toxicants become lethal to fish at lower concentrations. This explains the lower 96 hour LC50 of copper at 29 ± 1°C (2.61 mg/*l*) in contrast to higher 96 hour LC50 at 19 ± 1°C (2.78 mg/*l*).

Toxicity curves describe the empirical relationship between poison concentration and survival time of the organisms and thus provides information on the biological action of the toxicant (Abel, 1989). These curves were constructed using both LC50 values (Fig. 8.19). A toxicity curve gives an idea of how the test progressed, and it may indicate when acute lethality has ceased - this would be indicated where the curve is asymptotic to the time axis (Rand and Petrocelli, 1985). The incipient LC50 is a useful value in that it enables the comparison of copper toxicity to *O. mossambicus* with those found for *C. gariepinus*. The toxicity curves at 29 ± 1°C and 19 ± 1°C were approximately curvilinear and the incipient LC50 was 2.95 mg/*l* and 2.50 mg/*l* copper at 29 ± 1°C and 3.32 mg/*l* and 2.95 mg/*l* copper at 19 ± 1°C using the LC50 and LT_{50} values respectively. The application factor for copper (incipient LC50/96 hour LC50) is thus 1.13 and 0.96 at 29 ± 1°C, and 1.19 and 1.06 at 19 ± 1°C. It is suggested that the toxicity curve based on the LC50 values (Fig.8.19) will be used in future lethal concentration determinations. This toxicity curve of LC_{50} against observation time differs only in that confidence limits are expressed in terms of concentration rather than time. Abel (1989) suggests that for certain applications this approach has some advantages; e.g., where the test forms part of a research programme designed to establish water quality standards, it is obviously preferable to estimate errors associated with lethal concentrations rather than with survival times.

8.2.1.5 Conclusions

The determined 96-hour LC50 value (1.723 g/*l* Mn) and also the incipient LC50 value (1.46 g/*l* Mn) were much higher than the naturally occurring Mn concentrations in the environment, which rarely exceeds one mg/*l* (Hellawell, 1986). The values were also higher than the Mn concentration of 0.206 g/*l* that was detected in the West Wits Gold Field mine effluent (Whittman and Förstner, 1977). However, 0.206 g/*l* Mn is a concentration level whereby fish might be affected sub-lethally, since in this study visible sub-lethal effects started to occur at a Mn concentration of 0.278 g/*l*. Attention must therefore be given to the performance of chronic Mn toxicity tests in the future, in order to determine the lowest Mn concentration whereby sub-lethal effects will still occur. In this way the existing water quality guideline of one mg/l Mn as a maximum concentration for the protection of aquatic life (Kempster *et al.*, 1982) could be verified.

Copper concentrations in natural water are generally less than a few tenths mg/*l* (Straub, 1989). Thus the LC50 values of 2.61 mg/*l* and 2.78 mg/*l* as well as the incipient LC_{50} values of 2.95 mg/*l* and 3.32 mg/l for copper at 29 ± 1°C and 19 ± 1°C respectively, are much higher than the copper concentrations occurring in the natural environment. Industrial pollution (i.e., mine water) can, however, increase the copper concentration in natural water to as much as several thousand mg/*l* (Straub, 1989), and thus has a negative influence on aquatic life and organisms.

Despite increased sophistication in toxicity testing, increased awareness on the variety of processes operating in a natural system and the integration of information from a system of tests in various hazard evaluation protocols, the ultimate question of what is an acceptable concentration of a chemical

with a known degree of accuracy is still unanswered. In the Olifants River, copper is one of several metals which pose a threat to the conservation status of the river. The LC50 values found during this study, can be used as an indication of the levels at which copper becomes lethal for this specific species. In a larger management-control program like the Kruger National Park, these results must only be seen as the limits within which the concentration of copper and manganese can be regarded as lethal for *C. gariepinus* as well as *O. mossambicus* in the Olifants River. Future research should focus on the development and validation of acceptable prediction strategies. In general, toxicants occur in mixtures in natural waters, therefore the interaction of toxicity is an important factor, which must be taken into account when assessing the hazard of an environmental pollutant to aquatic life and for setting valid water quality standards for diverse use. Additional studies on the effects of toxicants, singly and in mixtures, on biochemical and physiological processes are needed to gain more knowledge of their interactions and toxic effects.

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8.2.2 *Haematology*

8.2.2.1 Introduction

Natural and humanly induced environmental stressors could cause changes in cellular functions, which then alter the physiology of organ systems in individual fish (Heath, 1990). If alterations in organ functions are not compensated for, these alterations may weaken the fish so that it is less able to cope with other stressors. Under these circumstances environmental stressors, although not directly lethal themselves, may reduce the probability of survival of fish stocks by increasing their subceptibility to disease (Wedemeyer, 1970; Snieszko, 1974) and by reducing reproductive success (Pickering, 1989), and may even cause death.

Although some episodes of water pollution cause deoxygenation of the water, and resulting in massive fish-kills due to oxygen shortage, another principal cause of death is contamination by toxic inorganic or organic substances (Mawdesley-Thomas, 1971).

The enormous amount of pollutants that are being released into aquatic environments and their interactions with various elements of these systems make it difficult to estimate and predict the ecological consequence of pollutants. The perplexity of chemical mixtures in aquatic environments and the lack of information on the mode of action of toxicants in biological systems, have made it a difficult task to define acceptable loads of contaminants in receiving water (Van der Merwe, 1992). It is important to note that man's destructive influence on the aquatic environment is in the form of sublethal pollution (Wepener, 1990). This type of pollution results in chronic stress conditions, which have a negative effect on aquatic life.

The toxicity of a pollutant or substance is usually defined by determining the concentration at which organisms die, and is described in terms of the incipient LC50 or lethal threshold concentration (8.2.1) (Sprague, 1969; 1970; 1971). However, normal physiological processes are affected long before the death of an organism and because death is too extreme a criterion for determining whether a substance, is harmful or not, scientists had to search for physiological and biochemical indicators of health and sublethal toxicant effects. Therefore it was suggested that behaviour and biochemical changes, growth rate and oxygen consumption of fish can also be used in determining the toxicity of a pollutant (Fujiya, 1965; Alderdice, 1967; Sprague, 1971; 1976; Wedemeyer and Yasutake, 1977).

A good biological indicator should be able to identify possitive environmental problems before the health of aquatic organisms are seriously altered or compromised (Jimenez and Stegeman, 1990). During the last few decades haematology, the study of blood and the blood forming tissues, has become such a biological indicator for both physiologists and ecologists (Heath, 1987).

It is however important to stress that the interpretation of physiological and biochemical data in terms of well-being of the fish could easily be obscured by environmental factors, such as behaviour, food-supply, predation, seasonal changes and water quality (pH, salinity and temperature), and internal factors, such as age or size, nutritional and reproductive status (Hickey, 1976; Haux *et al.*, 1985; Larsson *et al.*, 1985; Heath, 1987). Physiological disturbances which occur when individual fish is captured, handled and transported in the laboratory also influences haematological variables (Bouck and Ball, 1966; Hickey, 1976; Lowe-Jinde and Niimi,

1983; Haux, *et al.*, 1985; Larsson *et al.*, 1985; Ellsaesser and Clem, 1986). This type of stress might be indicated by a significant decrease or increase in some biochemical variables in the blood or tissue (Thomas, 1990). Changes at these levels of biological organisation can indicate cellular lesions, reflect loss of homeostasis (decreased blood electrolytes), demonstrate a compensatory response due to excitement, fear or pain (elevated blood glucose levels) and indicate altered immune function depressed macrophage activity (Heath, 1990).

Haematological evaluation of fish blood provides valuable facts concerning the physiological response of fish to changes in the external environment (Wedemeyer, 1970). Haematological variables have a well known clinical value in prognosis and diagnosis and the comparative convenience of sampling offered by it (Narain and Srivastava, 1979). It also provides commonly applied techniques to reveal sublethal responses of toxicants such as metals (Larson *et al.*, 1985). Fish haematology is therefore used to diagnose abnormal functioning of physiological mechanisms in fish.

There are a variety of variables that can be employed such as red blood cells, haemaglobin concentration and haematocrit, while blood protein levels are less sensitive to establish the general health status of fish species after stress exposure (Hattingh, 1975; Duthie and Tort, 1985). The dynamics of carbohydrate metabolism are greatly influenced by any stressor. During the stress event chromaffin cells (catecholamines) are activated via the hypothalamus to release adrenalin and noradrenaline which stimulate the conversion of glycogen in the liver to glucose. Hyperglycemia is a classical result of metal pollution as a response to the stimulation of glucocorticoids and catecholamines (Wedemeyer, 1970; Heath, 1987). On the contrary hypoglycemia may result due to either the reduced rate of glucogenolysis or glycogenesis (Nath and Kumar, 1987). Changes in blood glucose levels are therefore regarded a sensitive, reliable indicator of environmental stress in fish (Sibergold, 1974). On the other hand, this feature may limit its applicability since the inevitable capture and handling stress are induced by laboratory procedures (Pickery *et al.*, 1982). Hypoxic conditions inhibit the cellular oxidation of glucose (Van der Putte *et al.*, 1982). Glucose oxydation is regulated by enzymes and the determination of enzyme activities in serum has become an important diagnostic tool in toxicological research. Various factors may be responsible for an alteration in serum enzyme levels, such as altered enzyme activities in fish organs, the leakage of enzymes into the serum after alteration in membrane permeability, an altered removal rate of enzymes from the serum caused by impaired mechanisms, and the release of enzymes from necrotic or damaged cells (Sauer and Haider, 1977). Blood coagulation is an important part of homeostatis, and an organism's survival immediately following injury is largely dependent upon the clotting ability of its blood (McNab and Ronald, 1965). The blood coagulation system has potential as a responsive system, capable of serving as an indicator of environmental stress. An accelerated blood clotting rate after exercise or trauma was reported for fish (Cassilas and Smith, 1977). Heavy delayed mortalities were reported after simulating capture and handling stress (Bouck and Ball, 1966). These deaths were attributed to shock and peripheral clotting after deducing apparent decreasing fibrinogen levels in blood. A variation in the blood coagulation system can occur in response to stress.

One aspect which has largely been neglected is the effects that metals have on blood coagulation and the possible occurrence of disseminated intravascular clotting under such conditions.

8.2.2.2 Materials and Methods

C. gariepinus and *Oreochromis mossambicus* from fish farms and prepared for experimental procedures as described in 8.2.1.2. Bioassays with *C. gariepinus* were conducted for copper at concentrations of 0.055 ± 0.16 and 0.085 ± 0.032 mg/*l*. The copper concentrations used were derived by taking average copper concentrations monitored in the Olifants River, Kruger National Park from February 1990 to February 1991 during summer and winter, respectively. Copper was administered as copper chloride ($CuCl_2.2 H_20$-Mn = 170.48) concentration in 1g copper chloride by taking the molecular mass and water colume to 0.16 ± 0.011 and 0.39 ± 0.19 mg/ copper at $29 \pm 1°C$ as well as $19 \pm 1°C$ to simulate summer and winter temperatures (Table 8.36). These acute exposures were carried out for 96 hours, while untreated fish were kept as control for the same period of time before measurements were taken. Fish were not fed for at least two days before as well as during the acute and control assays. *O. mossambicus* was also exposed under the same conditions at 0.40 mg/*l* copper for 4 weeks as a long term exposure. During the latter, fish were fed except for the last 96 hours when fish were fed every second day (Nussey, 1994).

Blood was sampled after 2h, 6h, 24h, 48h and 96h. Blood was immediately collected from the caudal aorta of unaneasthetized fish with a two millilitre plastic syringe. Plastic syringes were used since contact with glass results in shortened coagulation times (Smith *et al.*, 1952). Ethylene diamine tetra-acetic acid (EDTA) was used as anticoagulant since it produces little adverse effects on the blood within the first 5-6 hours after sampling and mixing (Hattingh, 1975). Blood from *O. mossambicus* was collected in syringes rinsed with 5000 units/ml heparin whilst blood for coagulation studies was sampled with 0,3 ml 3,8% sodium citrate to decalcify the blood (Franz and Coetzee, 1981; Wepener *et al.*, 1992a, b; Nussey, 1994).

Haematological variables analysed were red cell counts (RBC), hemoglobin concentration (Hb), mean carpuscular volume (MCV), mean corpuscular hemoglobin concentration hematocrit (Hct), pH, glucose concentration, lactate concentration, lipid concentration, protein concentration and acetylcholine activity. The hematocrit was determined according to the method described by Korzhuev (1964) and the RBC, WBC and MCV values were obtained by employing a Sysmex CC-120 Microcell Counter. The Hb was determined by using the cyanomet-hemoglobin method (Blaxhall and Daisley, 1973). The glycose lactate, lipid and protein concentrations were determined by means of standard Boehringer Mannheim test kits. A Hithachi 150-20 spectrophotometer was employed to determine the optical density used in the concentration calculations. Delta-aminolevulinic acid activity was determined spectrophotometrically with a Hitachi 150-20 spectrophotometer (Hodson, 1976). Acetyl-choline esterase was determined by measuring the mean change in absorbency per 30 seconds at a wavelength of 405 nm with a Hithachi 150-20 spectrophotometer. A Boehringer-Mannheim test kit was used for this determination. Directly after extraction a tiny drop of blood was also carefully placed on a washed and defatted slide. The slide was defatted by immersing it in an alcohol-ether mixture. To prepare the blood film, the end of a second slide ("spreader slide") was placed against the surface of the slide with the blood drop, at an angle of 45°. By drawing the "spreader slide" up against the drop of blood, it spreaded across the end of the slide by capillary attraction and filled the angle between the two slides. The "spreader slide" was then pushed back along the other slide and the blood drop followed the "spreader slide". The film should be even, uniform and not too thick. This technique is effective to obtain the required blood film.

TABLE 8.36

SUBLETHAL COPPER CONCENTRATIONS ADMINISTERED TO WATER, DURING EXPOSURES.

Exposure groups	Exposure time	29 ± 1°C			19 ± 1°C		
		[$CuCl_2.2H_2O$] mg/l	[Cu] mg/l	[Cu] measured in water mg/l	[$CuCl_2.2H_2O$] mg/l	[Cu] mg/l	[Cu] measured in water mg/l
Control Mean ± Sd Min/Max	14 days (336hr)	*	*	 0.002±0.001 (0.001-0.005)	*	*	 0.002±0.001 (0.001-0.004)
Short-term: A Mean ± Sd Min/Max	4 days (96 hr)	0.4291	0.16	 0.162±0.011 (0.149-0.176)	0.4291	0.16	 0.161±0.006 (0.153-0.169)
Short-term: B Mean ± Sd Min/Max	4 days (96 hr)	1.0731	0.40	 0.394±0.190 (0.377-0.421)	1.0731	0.40	 0.393±0.018 (0.368-0.411)
Long-term Mean ± Sd Min/Max	28 days (672 hr)	1.0731	0.40	 0.391±0.019 (0.356-0.425)	1.0731	0.40	 0.391±0.029 (0.356-0.425)

* $CuCl_2.2H_2O$ not added to controls.

The films were allowed to dry and then immersed in methanol for three minutes for fixation. After fixation the film was allowed to air-dry and therefore stained with Giemsa-Romanowsky stain (Puchkov, 1964). The stained blood film was examined with an Olympus Vanox microscope, under oil immersion (X1000). For the determination of electrolite concentrations blood plasma was diluted with a Rachometer A6241 diluter to determine the plasma sodium concentration as well as the plasma potassium concentration by means of a Radiometer FLM3 flamephotometer. Plasma calcium concentration was determined with a Corning Calcium Analyzer 940. As the quantity chloride that precipitates with silver nitrate in the presence of a combined acid buffer, plasma chloride concentration was determined by using a Corning Chloride Analyzer 925.

A Hellige Thrombelastograph D, which is a mechanically operated optical system that provides a continuous visual and photocymographic observation of blood or plasma during all the phases of coagulation, was used (Van Kaulla and Weiner, 1953; Frans and Coetzee, 1981). This apparatus, that is generally used in coagulation studies, records the process of blood coagulation by measuring the speed of coagulation, the shear modulus of the coagulum and clot retraction *in vitro* (Barham, 1983). The resulting recording is termed a thrombelastogram (TEG). Evaluation of the TEG was done according to described standard techniques (Franz and Coetzee, 1981; Barham, 1983).

Data was processed with a Statgraphics statistical computer program and evaluated for normal distribution as well as summary statistics. Paired one-sample tests were performed to prove probability hypotheses.

8.2.2.3 Results

Clarias gariepinus

After exposure of fish to copper at 21°C, the number of white blood cells increased significantly from the control value (OL) after 2 hours until 48 hours of exposure and remained at a high level after 96 hours ($P < 0.05$). A statistically significant drop in red blood cell numbers was noted after 2 hours of exposure and started to increase again after 48 hours. The haematocrit did not show statistically significant changes throughout the exposure period. The haemoglobin concentration dropped significantly 2-6 hours of exposure ($P < 0.005$) and recover at 48 hours of exposure. The MCV decreased significantly to a low at 24 hours of exposure and were at the same level as the control (OH) after 96 hours ($P < 0.005$). The pH dropped significantly after 6-24 hours of exposure which proved the occurrence of acidosis ($P < 0.025$) (Fig. 8.20). After the exposure to copper at 28°C (summer temperature) the number of white blood cells increased dramatically at 2 hours of exposure and renewed at a high level. The decrease in red blood cell numbers was not of the same magnitude with the lowest value after 24 hours ($P < 0.005$). Haematocrit values fluctuated with the lowest value after 48 hours of exposure. The haemoglobin concentration decreased significantly after 2 hours of exposure but recovered after 24 hours and remained at a relatively stable level. The MCV decreased significantly after 2 hours of exposure and stabilized at a lower level ($P < 0.005$). The pH decreased after 2 hours ($P < 0.005$) after which it increases to higher levels which indicates alkalosis (Fig. 8.22).

After exposure to copper at a temperature of 21°C the glucose concentration increased statistically significant after two hours and was still significantly higher than the control value (Oh) after 96 hours ($P < 0.05$). The lactate

concentration increased significantly after 24 hours of exposure and remained at a significantly high level at the 96 hour measurement ($P < 0.005$). A statistically significant decreased in the lipid concentration was noticed after 6 hours of exposure which recovered slightly after 96 hours ($P < 0.0025$). The protein concentration showed a significant decrease ($P < 0.005$) at 48 hours of exposure whereafter it increased significantly after 96 hours and remained significantly lower than the control concentration (Oh). Acetycholine esterase activity decreased significantly ($P < 0.005$) after 6 and 24 hours and increased significantly from 24 - 96 hours ($P < 0.05$) (Fig. 8.21). After exposure to copper at 28°C, the glucose concentration only increased significantly after 24 and 96 hours. The lactate concentration increased significantly after two hours with a maximum after 24 hours and remained at a significantly high level after 96 hours ($P < 0.005$). A significant decrease in the lipid concentration was evident after 6 hours of exposure ($P < 0.025$) with the lowest concentration after 48 hours and remained at a significantly low level ($P < 0.005$). The protein concentration also decreased significantly after 2 hours of exposure with an overall low compared to the control and remained at that low level after 96 hours ($P < 0.005$). The acetylcholine activity was significantly inhibited after 6 hours of exposure ($P < 0.005$) after which it increased but remained lower than the control ($P < 0.05$) (Fig. 8.23).

Oreochromis mossambicus

The changes in the haematological variables after the exposures to sublethal copper concentrations are presented in Table 8.36. After the "summer" exposure, at 29 ± 1°C, the number of white blood cells (WBC) increased significantly ($P < 0.05$) after the 0.40 mg/*l* exposure and the long-term exposure ($P < 0.005$). After the 0.16 mg/*l* exposure there was only a slight increase in the number of WBC. In contrast, there were significant increases ($P < 0.005$) in the number of white blood cells after all the "winter" exposures, at 19 ± 1°C.

At 29 ± 1°C there was a slight increase in the number of red blood cells (RBC) after the 0.16 mg/*l* exposure and a slight decrease after the long-term exposure, but there was a significant decrease ($P < 0.025$) in these cells after the 0.40 mg/ exposure. Whilst at 19 ± 1°C there was a significant increase ($P < 0.005$) in the number of red blood cells after the 0.16 mg/*l* exposure, but insignificant increases were observed after the 0.40 mg/*l* and the long-term exposures.

The only hemoglobin concentrations that increased significantly were those measured after the short-term ($P < 0.005$) and long-term ($P < 0.05$) exposures to 0,16 mg/*l* copper at 19 ± 1°C. At 29 ± 1°C the mean cell haemoglobin (MCH) increased significantly ($P < 0.025$) after the 0.40 mg/*l* exposure and decreased and increased slightly after the 0.16 mg/*l* and long-term exposures respectively. At 19 ± 1°C the MCH only increased significantly ($P < 0.005$) after the 0.16 mg/*l* exposure and decreased slightly after the 0.40 mg/*l* exposure and increased slightly after the long-term exposure.

The mean cell haemoglobin concentration (MCHC) increased significantly ($P < 0.05$) after the 0.40 mg/*l* exposure and decreased significantly ($P < 0.025$) after the long-term exposure at 19 ± 1°C.

Delta-aminolevulenic acid dehydratase (ALA-D) activity decreased significantly after the 0.16 mg/*l* exposure only.

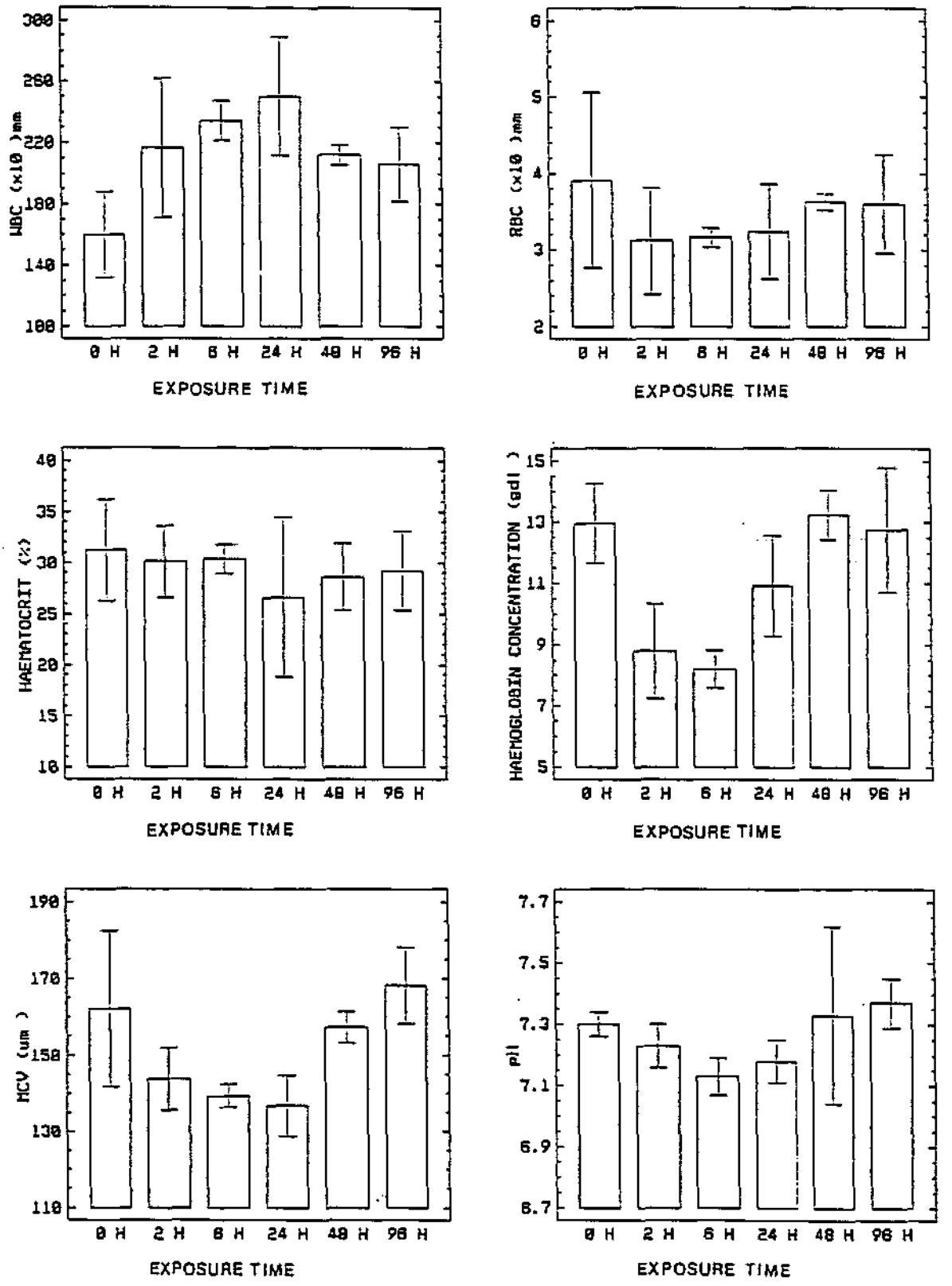


Fig. 8.20. Haematological changes in C. gariepinus *after copper exposure at 21°C (Mean ± SD; n=10)*

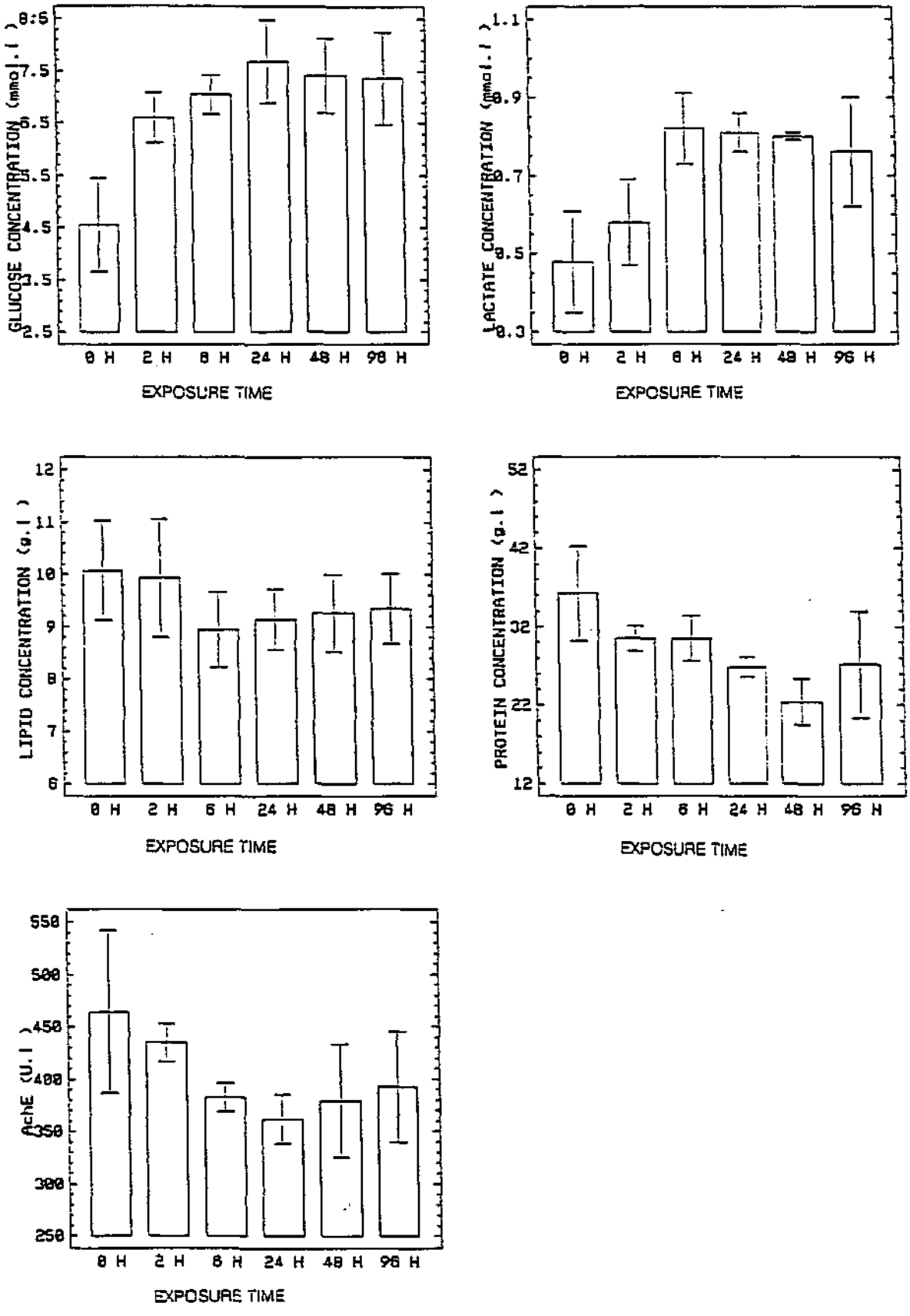


Fig. 8.21 Metabolic changes in C. gariepinus *after copper exposure at 21°C (Mean±SD; n=10)*

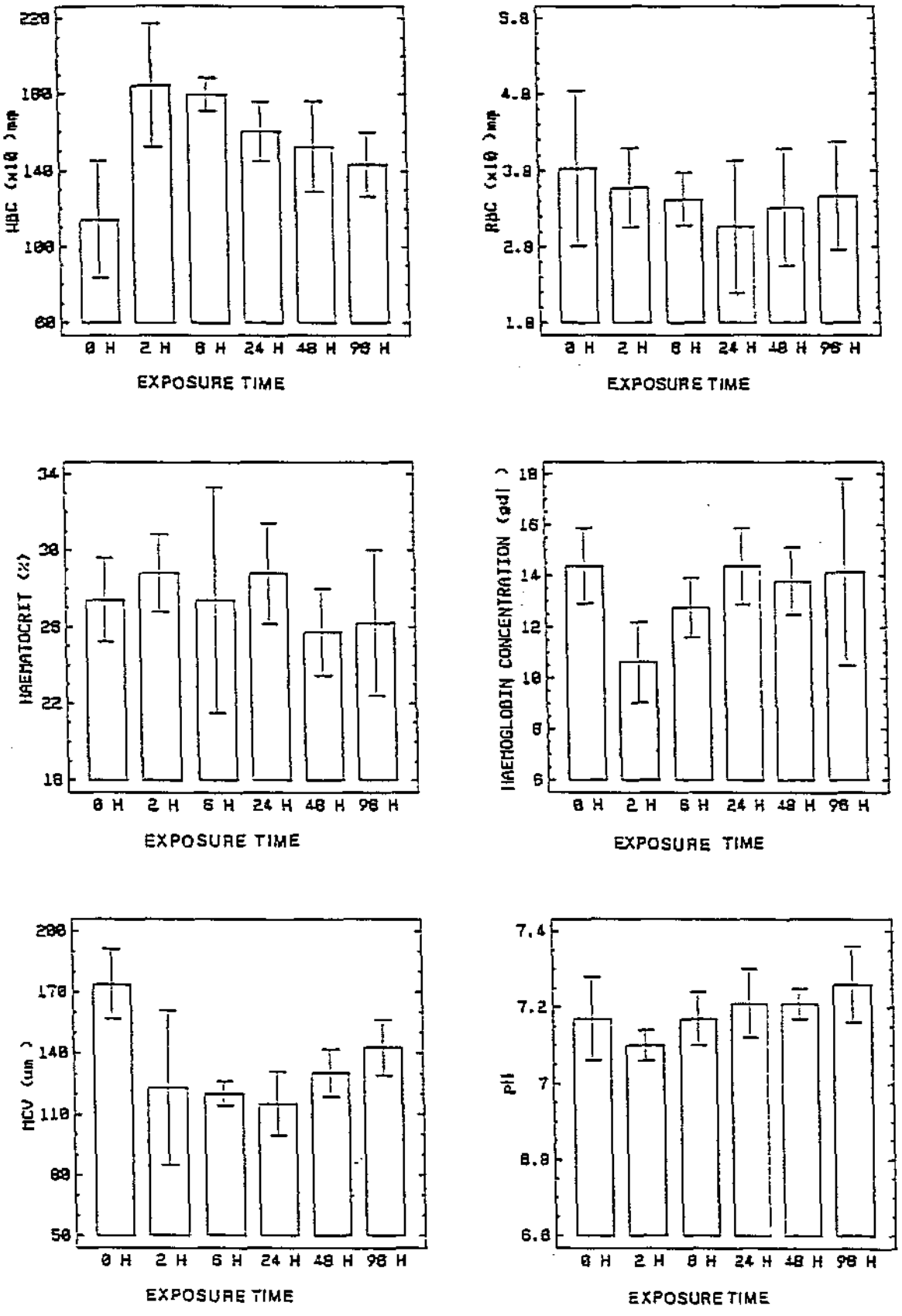
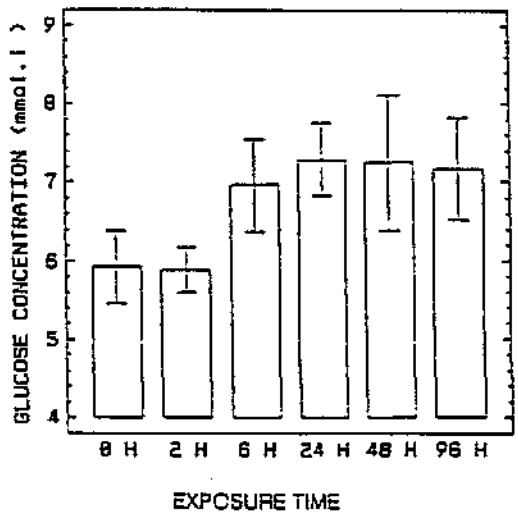
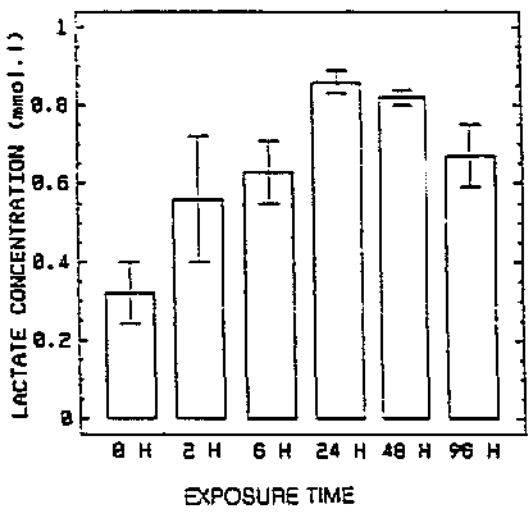
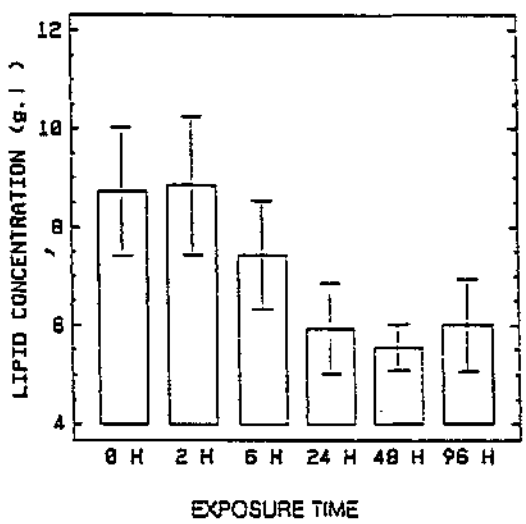
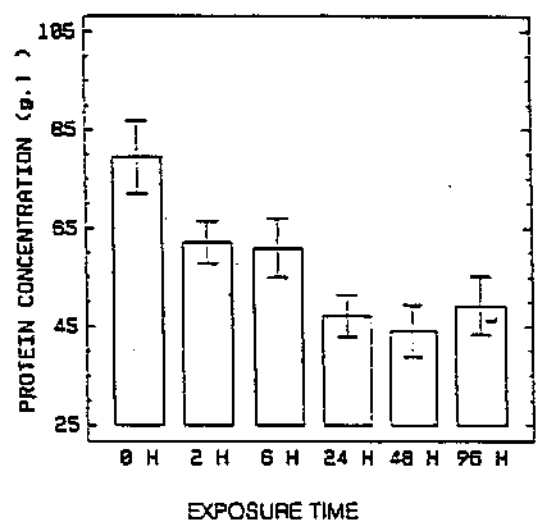


Fig. 8.22 Haematological changes in C. gariepinus *after copper exposure at 28 °C (Mean=SD; n=10)*









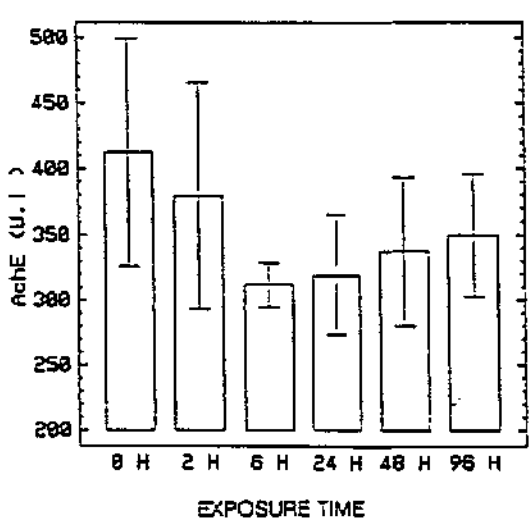


Fig. 8.23 Metabolic changes in C. gariepinus *after exposure at 28.°C (Mean=SD; n=10)*

The osmotic and ionic values obtained after the exposures to copper are presented in Table 8.37. At 19±1°C a statistically significant decrease ($P < 0.005$) was observed in the plasma sodium concentration (Na) at the long-term exposure (Table 8.38). There were significant increases in the plasma potassium (K) concentration ($P < 0.005$) after the 0,40 mg/*l* and long-term exposures at 29±1°C, whilst at 19±1°C a significant increase ($P < 0.005$) was observed only after the 0,40 mg/*l* exposure. No drastic changes were observed in the plasma calcium (Ca) concentrations after any of the exposures. At 29±1°C the plasma chloride (Cl) concentration decreased significantly ($P < 0.005$) after the 0,16 mg/*l* and long-term exposures. At 19±1°C the Cl-concentration decreased significantly after the 0,40 mg/*l* ($P < 0.025$) and the long-term exposures ($P < 0.005$). The osmolarity showed a significant decrease ($P < 0.05$) after the long-term exposure at 29±1°C. At 19±1°C a significant increase ($P < 0.025$) was observed after the 0.40 mg/*l* exposure.

The blood coagulation values of fish, exposed to copper at both temperatures, did differ from the control values. Results are presented in Table 8.39 and Figs. 8.24 & 8.25. After all the exposures to sublethal copper concentrations at 29±1°C as well as 19±1°C, the clotting times increased from the control values. At 29±1°C, there were significant increases in the r- and k-times, r & k-values ($P < 0.005$), maximum amplitude (ma) as well as shear modulus (me) ($P < 0.025$) after short exposure (96h) to 0.16 mg/*l* There were slight increases in the r- and k-times, and the r & k-value after the short-term exposure to 0.16 mg/*l* copper at 19±1°C. After the short-term exposure to 0.40 mg/*l* copper at 29±1°C only the r-time increased significantly ($P < 0.025$). At 19±1°C there were significant increases in the r- and k-times and the r & k-value ($P < 0.005$) after 96-h exposure to 0.40 mg/*l* copper. One major difference between blood coagulation at 29±1°C and 19±1°C was that the blood of *O. mossambicus* exposed at 19±1°C clotted slower than samples taken at 29±1°C. The mechanism responsible for prolonging coagulation at lower temperatures is not known (Steele and Steel, 1987). Evidence of haemophelia and/or thrombocytopenia was supplied by the many purplish blotches displayed on the skin. In some instances, at both temperatures, no clotting was registered which causes internal bleeding. This could cause the death of fish after a long period of time.

The white blood cells of *O. mossambicus* were divided into agranular and granular cells. The leucocytes were of few types, namely, lymphocytes, monocytes, neutrophils and eosinophils and these cells were classified according to their general form and affinity to dyes. No basofils were identified (Nussey, 1994).

The exposure to copper at 29±1°C and 19±1°C resulted in an increase in the leucocyte concentration in the blood of exposed fish. After exposure to copper at 29±1°C significant increases ($P < 0.005$) in lymphocyte numbers were noted after both short and long-term exposures. Monocytes decreased significantly ($P < 0.005$) after all the exposures. After the 0.40 mg/*l* copper short-term exposure, neutrophils decreased significantly ($P < 0.05$) but after the long-term exposure increased significantly ($P < 0.005$). A significant increase ($P < 0.005$) was noted in eosinophil number after the 0.40 mg/*l* exposure as well as the long-term exposure. The total leucocytes increased significantly after 0.40 mg/*l* ($P < 0.05$) and the long-term ($P < 0.005$) exposures. At 19±1°C significant increases were found in the lymphocyte numbers after the short-term exposures of 0,16 mg/*l* ($P < 0.05$) and 0,40 mg/*l* ($P < 0.005$) as well as the long-term exposure ($P < 0.05$). Monocytes decreases significantly ($P < 0.05$) after all the exposures. Neutrophils

TABLE 8.37

MEAN HAEMATOLOGICAL AND OSMOREGULATION VALUES OF *OREOCHROMIS MOSSAMBICUS* AFTER EXPOSURE TO COPPER.

Exposure group	29 ± 1°C				19 ± 1°C			
	Control	Short-term A: [0.16mg/l]	Short-term B: [0.40mg/l]	Long-term [0.40mg/l]	Control	Short-term A: [0.16mg/l]	Short-term B: [0.40mg/l]	Long-term [0.40mg/l]
Exposure period	2 weeks	96 hours	96 hours	4 weeks	2 weeks	96 hours	96 hours	4 weeks
Number of fish	30	10	10	10	30	10	10	7
WBC ($x10^3$)mm^3 Mean ± S_x Min/Max P	46.48±11.37 (32.20-64.10) -	47.73±16.45 (14.40-76.20) *	60.00±12.58 (42.40-83.70) <0.05	111.26±13.51 (85.00-133.30) <0.005	41.93±5.90 (34.50-54.70) -	117.99±34.61 (63.00-179.00) <0.005	60.16±12.75 (36.5-78.70) <0.005	101.61±37.98 (53.10-163.50) <0.005
RBC ($x10^6$)mm^3 Mean ± S_x Min/Max P	1.86±0.47 (1.19-2.74) -	2.01±0.55 (1.16-3.17) *	1.45±0.19 (1.04-1.66) <0.05	1.79±0.08 (1.68-1.90) *	1.43±0.27 (1.07-1.98) -	2.11±0.46 (1.53-2.86) <0.005	1.66±0.33 (1.35-2.49) *	1.47±0.25 (1.02-1.72) *
Hb (g/dl) Mean ± S_x Min/Max P	8.17±1.37 (6.20-10.50) -	7.29±1.20 (5.60-9.00) *	8.16±0.79 (6.9-9.4) *	8.91±0.63 (8.10-9.90) *	7.12±0.55 (6.40-8.00) -	12.40±2.38 (9.60-17.10) <0.005	7.35±0.61 (6.60-8.60) *	8.34±1.68 (5.80-10.40) <0.05
Hct (%) Mean ± S_x Min/Max P	26.89±7.74 (17.60-43.60) -	28.35±7.67 (15.80-44.30) *	18.21±2.57 (12.50-21.30) <0.005	27.07±3.48 (22.20-32.40) *	20.62±4.12 (14.50-29.50) -	34.83±6.57 (26.10-47.20) <0.005	22.57±3.33 (17.00-27.90) *	28.64±5.07 (21.2-35.7) <0.005
MCV (μm^3) Mean ± S_x Min/Max P	143.60±12.30 (120.00-161.00) -	140.20±7.97 (132.00-155.00) *	126.40±5.04 (119.00-132.00) <0.005	150.8±15.02 (128.00-171.00) *	144.60±7.20 (135.00-160.00) -	166.90±11.20 (150.00-189.00) <0.005	142.10±12.32 (126.00-165.00) *	195.86±13.64 (175.00-217.00) <0.005
MCH (pg/cell) Mean ± S_x Min/Max P	43.66±10.87 (30.77-67.23) -	38.03±10.66 (27.62-58.62) *	56.52±8.50 (46.39-76.92) <0.05	50.22±3.40 (44.74-53.76) *	50.93±6.74 (36.87-59.81) -	59.03±4.49 (51.74-63.81) <0.005	45.93±6.61 (30.52-54.82) *	56.95±15.07 (48.32-63.03) *
MCHC (%) Mean ± S_x Min/Max P	32.11±8.41 (20.18-46.78) -	27.05±7.37 (20.32-43.04) *	45.54±6.38 (40.10-62.11) <0.005	33.15±4.55 (26.23-41.89) *	35.38±5.09 (24.75-44.14) -	35.56±2.72 (31.91-38.78) *	33.98±4.79 (27.24-43.53) *	29.21±3.93 (25.09-35.91) <0.05
ALA-D(U/mm^3 RBC) Mean ± S_x Min/Max P	8.76±2.48 (5.54-13.46) -	3.23±2.92 (0.26-7.64) <0.005	8.35±1.43 (6.12-10.89) *	8.73±2.31 (5.28-10.70) *	7.12±3.29 (3.25-12.08) -	5.57±2.01 (3.97-9.76) *	6.83±1.54 (5.33-9.85) *	7.99±1.45 (6.10-9.85) *
[Na] (mmol/l) Mean ± S_x Min/Max P	147.20±5.29 (13.90-158.00) -	148.30±17.92 (105.00-164.00) *	141.10±9.49 (123.00-155.00) *	118.20±13.67 (105.00-140.00) <0.005	148.40±6.90 (135.00-159.00) -	145.30±6.80 (135.00-156.00) *	141.50±8.44 (124.00-150.00) *	129.83±13.14 (112.00-148.00) <0.005
[K] (mmol/l) Mean ± S_x Min/Max P	5.47±1.81 (3.00-8.40) -	5.06±1.57 (3.70-9.00) *	10.94±3.84 (6.10-16.20) <0.005	9.28±2.65 (4.10-12.20) <0.005	5.91±1.32 (4.20-7.70) -	6.15±1.55 (4.50-9.60) *	8.28±3.24 (5.40-13.00) <0.005	4.73±1.33 (3.50-7.20) *
[Ca] (mg%) Mean ± S_x Min/Max P	11.01±2.02 (8.68-14.72) -	10.27±3.57 (4.43-17.94) *	11.84±2.97 (7.00-16.71) *	11.53±2.13 (9.22-15.78) *	10.35±2.71 (7.94-15.53) -	12.64±3.16 (9.56-19.71) *	11.02±2.14 (8.62-15.39) *	10.09±0.86 (9.11-11.49) *
[Cl] (mmol/l) Mean ± S_x Min/Max P	133.20±7.22 (116.00-144.00) -	110.60±11.15 (85.00-125.00) <0.005	127.20±6.29 (117.00-139.00) *	96.56±9.89 (80.00-109.00) <0.005	139.70±11.85 (120.00-159.00) -	131.30±6.33 (123.00-140.00) *	124.50±12.30 (108.00-148.00) <0.05	121.14±14.75 (104.00-140.00) <0.005
Osmo (Osmol/kg) Mean ± S_x Min/Max P	0.30±0.01 (0.28-0.33) -	0.29±0.01 (0.27-0.31) *	0.28±0.01 (0.26-0.30) *	0.28±0.02 (0.25-0.30) <0.05	0.30±0.01 (0.29-0.32) -	0.31±0.01 (0.287-0.322) *	0.29±0.01 (0.26-0.30) <0.05	0.29±0.01 (0.23-0.31) *

* = P is not significant (P > 0.05).

WBC = white blood cell count; RBC = red blood cell count; Hb = haemoglobin; Hct = haematocrit; MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; ALA-D = delta-aminolevulinic acid dehydratase activity; [Na] = plasma sodium concentration; [K] = plasma potassium concentration; [Ca] = plasma calcium concentration; [Cl] = plasma chloride concentration; Osmo = total osmolarity.

TABLE 8.38

MEAN DIFFERENTIAL AND TOTAL LEUCOCYTE COUNTS OF *OREOCHROMIS MOSSAMBICUS* AFTER EXPOSURE TO COPPER.

Exposure group	29 ± 1°C				19 ± 1°C			
	Control	Short-term A: [0.16mg/l]	Short-term B: [0.40mg/l]	Long-term [0.40mg/l]	Control	Short-term A: [0.16mg/l]	Short-term B: [0.40mg/l]	Long-term [0.40mg/l]
Exposure period	2 weeks	96 hours	96 hours	4 weeks	2 weeks	96 hours	96 hours	4 weeks
Number of fish	30	10	10	10	30	10	10	7
Agranulocyte (%)	90.95	90.00	91.70	85.45	90.20	90.20	91.45	85.86
Lymphocyte (%) Mean ± S_d Min/Max P	69.45±1.77 (67.00-72.00) -	78.10±2.33 (73.50-82.50) <0.005	83.85±1.96 (81.00-87.00) <0.005	73.28±3.67 (69.00-79.50) <0.005	67.60±3.41 (62.00-72.50) -	71.30±3.47 (68.00-78.50) <0.05	77.00±2.70 (74.00-82.00) <0.005	71.57±3.32 (66.50-77.00) <0.05
Monocyte (%) Mean ± S_d Min/Max P	21.50±3.32 (17.00-27.00) -	11.90±1.84 (8.50-14.00) <0.005	7.85±1.42 (6.00-10.00) <0.005	12.17±1.62 (9.50-14.50) <0.005	22.60±3.21 (18.00-27.50) -	18.90±2.45 (15.50-22.00) <0.005	14.45±2.54 (9.50-17.0) <0.005	14.29±2.16 (11.00-17.50) <0.005
Granulocyte (%)	9.05	10.00	8.30	14.55	9.80	9.80	8.55	14.14
Neutrophil (%) Mean ± S_d Min/Max P	7.80±1.80 (5.00-10.50) -	8.20±0.82 (7.00-9.50) *	5.80±1.62 (4.00-9.00) <0.05	10.83±1.77 (7.50-13.00) <0.005	8.40±1.73 (6.00-11.50) -	7.95±1.67 (5.00-10.00) *	6.65±1.90 (3.50-9.50) <0.05	10.50±1.80 (8.50-14.00) <0.05
Eosinophil (%) Mean ± S_d Min/Max P	1.25±0.83 (0.50-2.50) -	1.80±1.25 (0.50-4.00) *	2.50±0.62 (1.50-3.50) <0.005	3.72±0.43 (3.50-4.50) <0.005	1.40±0.62 (0.50-2.50) -	1.85±1.11 (0.50-3.50) *	1.90±1.37 (0.50-4.50) *	3.64±0.75 (2.50-4.50) <0.005
WBC (%) Mean ± S_d Min/Max P	46.47±11.37 (32.20-64.10) -	47.73±16.45 (24.40-76.20) *	60.00±12.58 (42.40-83.70) <0.05	111.26±13.51 (85.00-133.30) <0.005	41.93±5.90 (34.50-54.70) -	117.99±34.61 (63.30-179.0) <0.005	60.16±12.75 (36.50-78.70) <0.005	102.61±37.98 (53.10-162.50) <0.005

WBC = Total white blood cell (leucocyte) count (CHAPTER 5).

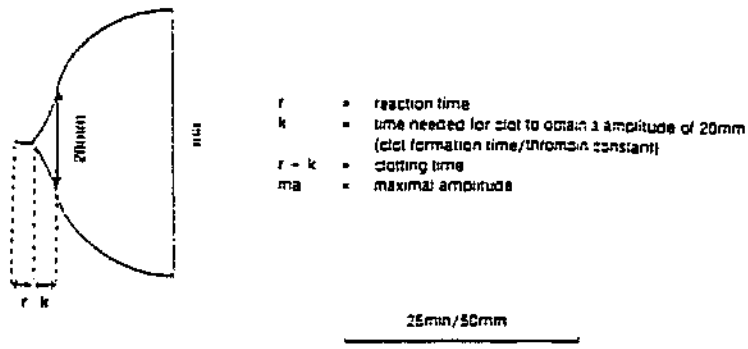
* = P is not significant (P > 0.05).

TABLE 8.39
MEAN THROMBELASTOGRAM (TEG) VALUES OF *OREOCHROMIS MOSSAMBICUS* AFTER EXPOSURE TO COPPER.

Exposure group	29 ± 1°C				19 ± 1°C			
		Short-term		Long-term		Short-term		Long-term
	Control	A: [0.16mg/l]	B: [0.40mg/l]	[0.40mg/l]	Control	A: [0.16mg/l]	B: [0.40mg/l]	[0.40mg/l]
Period	2 weeks	96 hours	96 hours	4 weeks	2 weeks	96 hours	96 hours	4 weeks
Number of fish	30	10	10	10	30	10	10	7
r (min) Mean ± S_d Min/Max P	1.77±1.10 (0.40-3.25) -	3.44±1.35 (1.50-5.00) <0.005	2.78±0.38 (2.00-3.25) <0.05	1.94±0.65 (1.50-3.50) *	1.93±1.03 (0.50-3.75) -	2.58±1.46 (1.00-5.00) *	4.07±1.51 (1.25-5.75) <0.005	2.25±1.28 (0.75-4.50) *
k (min) Mean ± S_d Min/Max P	2.19±1.16 (0.50-4.00) -	4.42±2.04 (1.50-8.75) <0.005	2.56±0.89 (1.50-3.50) *	1.25±0.19 (1.00-1.50) <0.05	1.23±0.78 (0.50-3.00) -	1.53±0.68 (0.75-2.50) *	2.96±1.41 (1.75-5.75) <0.005	2.58±2.44 (0.75-7.00) *
r + k (min) Mean ± S_d Min/Max P	3.91±1.97 (0.90-7.00) -	7.86±1.56 (5.75-10.25) <0.005	5.33±1.05 (4.25-6.75) *	3.19±0.75 (2.75-5.00) *	3.15±1.75 (0.55-1.00) -	4.10±2.01 (1.75-7.25) *	7.04±2.46 (3.25-11.50) <0.005	4.83±3.05 (3.25-9.00) *
ma (min) Mean ± S_d Min/Max P	40.85±12.74 (20.70-58.90) -	27.28±9.14 (10.50-37.00) <0.05	40.17±8.98 (23.00-51.00) *	49.25±5.90 (40.00-56.00) *	44.70±8.49 (34.00-57.00) -	42.20±9.50 (29.00-56.00) *	46.43±10.58 (31.00-61.00) *	35.33±14.83 (16.00-52.00) *
me (min) Mean ± S_d Min/Max P	76.51±39.21 (26.10-143.31) -	39.25±15.66 (11.73-58.73) <0.05	70.37±24.56 (29.80-140.08) *	99.35±22.62 (66.67-127.27) *	85.00±30.36 (51.52-132.56) -	77.38±29.79 (40.85-127.27) *	93.04±38.30 (44.93-156.41) *	63.44±37.13 (19.05-108.33) *
ITP (min) Mean ± S_d Min/Max P	35.29±73.14 (4.81-131.48) -	6.21±5.69 (0.85-19.58) *	14.65±5.66 (7.47-25.34) *	40.81±11.30 (22.22-52.04) *	53.66±43.39 (9.79-127.27) -	36.33±26.27 (9.08-84.85) *	20.23±14.24 (4.68-44.69) *	30.79±31.89 (1.36-72.22) *

* = P is not significant (P > 0.05).

r = reaction time; k = clot formation time; r + k = clotting time; ma = maximal amplitude; me = shear modulus (elasticity); ITP = index of thrombodynamic potential.



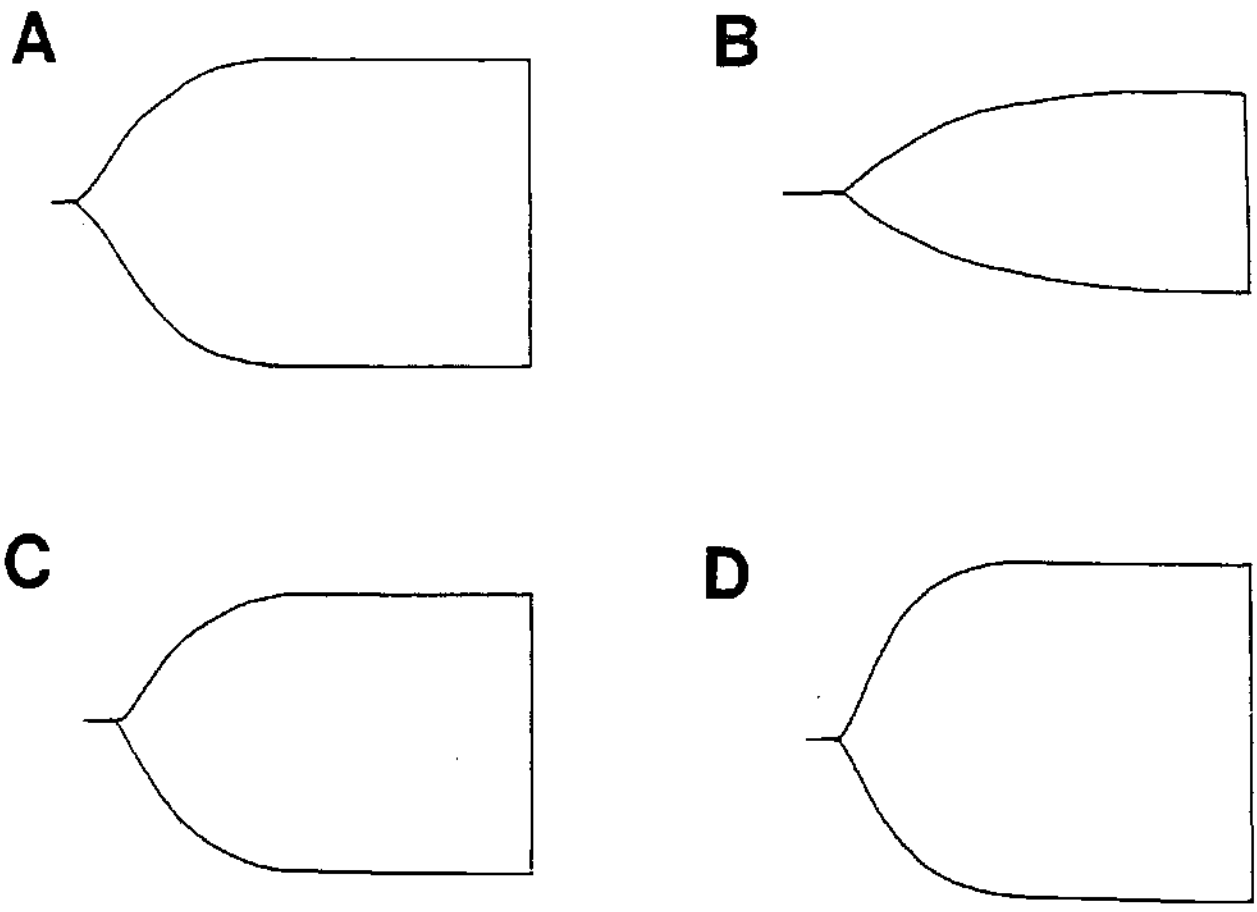


FIGURE 8.24

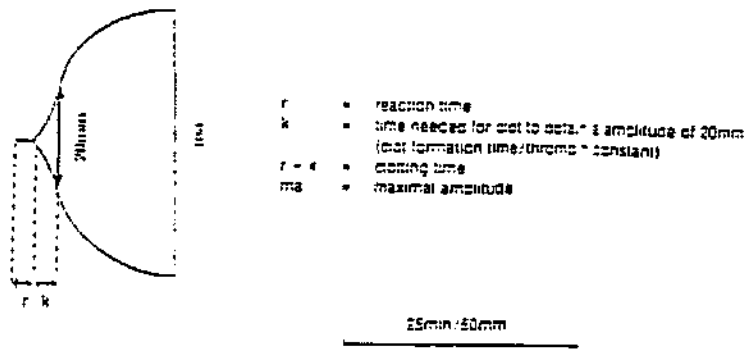
Thrombelastograms drawn from mean values, before and after exposure to copper at 29 ± 1°C

A = Control

B = Short-term A: [0.16mg/l] copper

C = Short-term B: [0.40mg/l] copper

D = Long-term: [0.40mg/l] copper



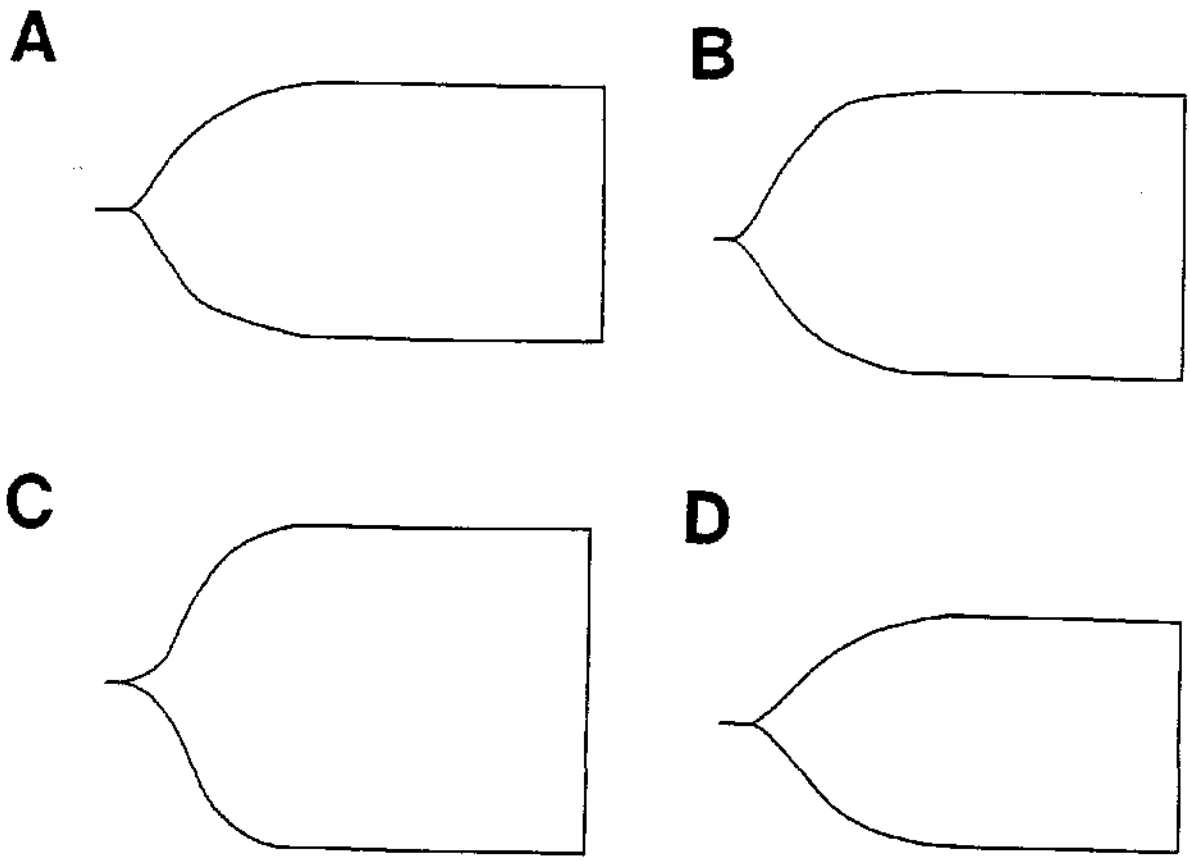


FIGURE 8.25

Thrombelastograms drawn from mean values,
before and after exposure to copper at 19 ± 1°C

A = Control

B = Short-term A: [0.16mg/l] copper

C = Short-term B: [0.40mg/l] copper

D = Long-term: [0.40mg/l] copper

decreased significantly ($P < 0.05$) after the 0.40 mg/l exposure and increased significantly after the long-term exposure. Eosmophils increased significantly ($P < 0.005$) after the long-term exposure. After all the exposures there were significant increases ($P < 0.005$) in the total leucocyte count.

8.2.2.4 Discussion

The changes induced in most of the blood variables measured after 96 hours exposure to copper at 21°C and 28°C show that some degree of chemical stress has been imposed upon *C. gariepinus*. The changes in the variable values decreased with time indicating a strong initial physiological disturbance followed by a desensitization period (Heath, 1987).

White blood cells play a major role in the defence mechanism of fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes (Jurd, 1985). Each type of leucocyte has an unique specific function. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue while lymphocytes produce antibodies (Ellis *et al.*, 1978; Wedemeyer and McLeay, 1981). Thrombocytes have an important function in blood clotting (Wepener, 1990). A phasic leucocytic response is expected during physiological and pathological disturbances (Britton, 1969).

An immediate activiation of the fish immune system is proven by the increase in leucocytes at both temperatures. An initial leucocytosis, which may be directly proportional to the severity of the causative stress condition, may attribute to an increase in leucocyte-mobilization (Srivastava and Narain, 1981). As in man, leucocyte production may be stimulated by the nuclein products of tissue destruction (Britton, 1969). Histological damage to tissue is most likely to occur in the gills, liver and kidneys and the impaired kidney function through necrosis probably results in the inhibition of leucocyte produciton. Furthermore, the persistence of a stress condition will lead to the suppression of the leucopoietic centres with the initial leucocytosis replaced by leucopenia at long term sublethal copper exposures.

The initial decrease in erythrocytes during copper exposure indicate an inhibited production of red blood cells caused by increased erythrocyte destruction (McLeay, 1973). Impaired osmoregulation and gill damage during sublethal copper exposure results in hemodilution. This condition leads to a decrease in erythrocyte numbers through hemolysis and the fish became anemic (Wedemeyer and Yasutake, 1977; Larsson *et al.*, 1985). This described that kidney necrosis also contributes to impaired osmoregulation. Damaged gills are clogged with aggregates of erythrocytes in stressed fish (Narain and Srivastava, 1989). The cell membrane of the red blood cell could also be altered through the hydrolysis of acetylcholine by acetylcholine esterase and contributes further to anemia (Cassilas and Smith, 1977).

The loss in erythrocytes after exposure to copper at both temperatures seemws to trigger a compensatory reaction whereby erythropoiesis increases and ultimately restores the cell numbers. The low mean corpuscular volume indicates a high concentration of immature erythrocytes in circulation. This is probably the result of hyperplasia stimulated by the higher demand for oxygen and carbon dioxide transport as a result of increased metabolic activity or impaired gaseous exchange across gill membranes (Hartman and Leser, 1964; Buckley, 1976; Narain and Srivastawa, 1979). The exposed fish were lying throughout the 96h period

of the bottom of the tank which ruled out the use of the branchial apparatus. The blood cell production in *C. gariepinus* seems to be adequate since there was no statistically significant difference in the numbers at 0h and 96h of exposure.

The haematocrit readings are valuable in determining the effect of stressors on the health of fish (Wedemeyer and Yasutake, 1977; Munkittrick and Leatherland, 1983). It is also used to determine the oxygen carrying capacity of blood (Larsson *et al.*, 1985). A high haematocrit value would imply polycythemia induced by stress and haemoconcentration caused by gill damage and impaired osmoregulation (Wedemeyer and McLeay, 1981). These were not found for *C. gariepinus*. The haemoglobin molecules' most prominent feature is the ability to bind oxygen loosely and reversibly. This phenomenon represents the oxytgen carrying capacity of blood (Guyton, 1982). The decrease in haemoglobin concentrations, with no change in haemotocrit implicates haemodilution aned a resultant increase in cell size. This is accomplished by either cellular swelling or mortality of small immature cells. A replacement by larger cells from the spleen combined with the production and release of erythrocytes from the erythropoietic organs are the probable mechanisms through which it is achieved (Christensen *et al.*, 1972). Haemodilution is regarded as a mechanism through which an irratating factor in the circulatory system is reduced and the swelling of red blood cells in this process is the result of the stimulation of adrenergic receptors by catecholamines (Smith *et al.*, 1979). Increases in erythrocyte sizes are probably stress responses while higher lactate levels and low blood PO_2, which will lead to a low ATP-concentration, increase the oxygen affinity of blood (Soivio and Nikinmaa, 1981). These responses have been noted in other teleosts (Mishra and Srivastava, 1979; El-Domiaty, 1987; Tort *et al.*, 1987). The increase of haemoglobin in *C. gariepinus* after copper exposure may be a mechanism by which more oxygen is absorbed or bound to compensate for the lack of oxygen cuased by impaired gill function (Buckley, 1976). The increased hemoglobin content in metal exposed fish is possibly a process whereby the body produces a high haemoglobin concentration to supplement the denaturised or oxidized haemoglobin formed after exposure to copper. Copper penetrates erythrocytes, inhibits glycolysis, denaturates hemoglobin and oxidises glutathione (Fairbanks, 1967).

The decrease in the mean corpuscular volume (MCV) showed that erythrocytes have either shrunk due to stress and impaired water balance, or a large number of immature erythrocytes have been released from the erythropoietic tissue. The recovery of MCV after 48 and 96h of exposure can be ascribed to the swelling of erythrocytes due to hypoxic conditions setting in.

The copper may damage gill membranes which results in a hipoxic condition (Singh, 1985; Mallat, 1985). Blood acidosis occurs commonly in fish suffering from hypoxic stress (Thomas and Hughes, 1987). An increase in muscle lactate and a redistribution of proteins across the erythrocyte membrane caused by elevated catecholamine levels is probably responsible for the significant decrease in blood pH (Nikinmaa, 1982). The results suggest that hyperventilation occur over longer exposure periods (96h) to neutralise the carbon dioxide concentration in an effort to regain normal pH (Hughes, 1981). On the other hand, blood alkalosis may also be the result of ammonia accumulation, chloride-bicarbonate alterations and electro-neutral sodium-hydrogen exchanges at the gill surface (Spry and Wood, 1984).

The increase in blood glucose levels after copper exposure shows a disruption in carbohydrate metabolism which is probably a hypophysis-adrenal response (Christensen *et al.*, 1972). This can be regarded as a classic stress response of blood sugar increase with the eventual secretion of glucocorticoids and catecholamines (Nath and Kumar, 1987). Catecolamines may deplete glycogen reserves in exposed fish by stimulating glucogenolysis and gluconeogenesis(Heath, 1987). Hyperglycemia may occur at a longer exposure time through the inhibition of hepatic glycolytic enzymes such as insulin (Nath and Kumar, 1987). This may be the result of damage to pancreas -cells. The increase in glucose levels may also provide additional energy to restore the impaired osmotic balance (Wepener, 1990).

The significant increase in lactate concentration implies an increase in anaerobic glycolysis (Heath, 1987). Anaerobic metabolism occurs as a result insufficient oxygen supply from the ambient water to the blood during environmental hypoxia or gill damage. The anaerobic respiration through which lactate is produced, occurs in the muscle and utilises muscle glycogen as energy source (Van Waarde *et al.*, 1983). Swimming fatigue may be experienced with the rise in lactate levels (Soivio and Virtanen, 1980; Larsson *et al.*, 1985). The observale inactive posture on the fish leads one to conclude that hypoxia is causative to high lactate levels. Lipid metabolism is clearly inhibited by the inactivation of hormones regulating lipid biosynthesis (Tandon *et al.*, 1978). The mobilisation of available lipid when glycogenesis is inhibited, may occur and lipolysis of stored fat eventually contributes towards the release of free fatty acids in the blood circulating (Guyton, 1982).

Serum proteins act as transport for the insoluble substances in the blood. A low concentration of serum protein influences the colloid osmotic pressure and is indicative of hemodilution caused by kidney and liver damage, infectious diseases and an impaired water balance (Wedemeyer and Yasutake, 1977; Larsson *et al.*, 1985). The damage caused by copper includes vacuolation and disintegration of renal tubule epithelium, dilatation of glomeruli and internal haemorrhage (Kumar and Pant, 1981). Serum protein circulating enzymes and glucose will therefore be lost via the damaged kidney. This, in turn, will stimulate gluconeogenesis in an effort to maintain or supplement blood glucose levels.

The increase of acetylcholine will be the result of the inhibition of acetylcholine esterase (AchE) activity (Ludke and Ferguson, 1969). Locomotor activity would therefore be reduced to such an extent that the fish remained motionless at the bottom of the tank. This phenomenon was also observed when fish were exposed to a pesticide (Post, 1969). The inhibition of AchE-activity by copper may stimulate an increased secretion of catecholamines in *C. gariepinus* which may result in hyperglycaemia through glycogenolysis (Uppal, 1970).

Oreochromis mossambicus

The changes that occurred in most of the blood variables measured after the exposure to copper, at 29±1°C and 19±1°C, show that some degree of chemical stress had been imposed upon *O. mossambicus*. Stress has been defined by Pickering (1981), as the sum of all the physiological responses by which an organism tries to maintain or compensate for a normal metabolism in the face of a physical or chemical force. From a physiological point of view, the primary reaction of fish under stress, is the increased secretion and release of circulating hypophyseal ACTH and corticosteroids (McLeay,

1973; Haux, *et al.*, 1985), catecholamines (Haux, *et al.*, 1985) as well as increased hypophyseal-interrenal activity (Donaldson, 1981; Mazeaud and Mazeud, 1981). This reaction and the release of the so-called stress hormones takes place while fish attempt to maintain itself during environmental changes, e.g., when copper is added to the water (Wedemeyer and McLeay, 1981).

An increase in the number of white blood cells (leucocytosis) as also shown in *C. gariepinus* in fish is a normal reaction against attacks of foreign substances which can alter their normal physiological processes. The leucocytosis, observed in the present study, at both 29±1°C and 19±1°C, indicates an immediate stimulation of the immune system to protect this species against infections that may have been caused by copper. Similar increases were found when fish were exposed to various pollutants, including metals (Van Vuren, 1986; Flos, *et al.*, 1987; Gill and Pant, 1987; Wepener *et al.*, 1992a).

Increases in the number of red blood cells (erythrocytosis), especially after the short-term exposure to 0.16 mg/*l* copper, can be attributed to several factors. During the exposure of *O. mossambicus* to copper, an oxygen deficiency developed within the fish. This decrease in the availability of dissolved oxygen causes the build-up of oxygen debt, hypoxia, in the fish. As a result of the increased anaerobic respiration, fish subjected to this situation, have a higher carbon dioxide concentration in their blood. During anaerobic respiration latic acid is produced. This, as well as the buffer action of the excess carbon dioxide, causes a rise in the acidity of the blood. This increase in acidity causes swelling of the red blood cells, as reflected by the significant increase in the mean corpuscular volume (MCV) (Soivio, *et al.*, 1974). An increase of erythrocyte size (MCV) has been associated with several factors such as anaesthesia and hypoxia, but it is generally considered as a response to stress (Weber, 1982). Another possible cause for the increase in MCV is beta adrenergic swelling caused by the release of more catecholamines in reacting to acute hypoxic stress (Butler, *et al.*, 1978). Catecholamines thus increase the red blood cell volume as well as the intracellular pH, and then increases the oxygen affinity for haemoglobin (Nikinmaa, 1982; Spry and Wood, 1984). Fish also compensate for a poor oxygen intake in prevailing hypoxic conditions, which may be caused by epithelial lifting of the gill lamellae, by an increase in the number of red blood cells (Wepener, 1990). This increase is due to the adrenergic stimulation of the haemopoietic tissue to contract and release stored erythrocytes into the circulating blood (Nilsson and Grove, 1974). According to O'Connor and Fromm (1975) metal ions are known to stimulate erythropoiesis. Copper-induced increases in red blood cells (erythrocytosis), especially after the short-term exposure to 0.16 mg/l copper, can be attributed to several factors. During the exposure of *O. mossambicus* to copper, an oxygen deficiency developed within the fish. This decrease in the availability of dissolved oxygen causes the build-up of oxygen debt, hypoxia, in the fish. As a result of the increased anaerobic respiration, fish subjected to this situation, have a higher carbon dioxide concentration in their blood. This, as well as the buffer action of the excess carbon dioxide, causes a rise in the acidity of the blood. This increase in acidity causes swelling of the red blood cells, as reflected by the significant increase in the mean corpuscular volume (MCV) (Soivio, *et al.*, 1974). An increase of erythrocyte size (MCV) has been associated with several factors such as anaesthesia and hypoxia, but it is generally considered as a response to stress (Weber, 1982). Another possible cause for the increase in MCV is beta adrenergic swelling caused by the release of more catecholamines in reacting to acute hypoxic stress (Butler, *et al.*, 1978). Catecholamines thus

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A significant decrease in the number of red blood cells after the short-term exposure to 0.40 mg/l at 29±1°C, was an indication of an inhibited production of red blood cells caused by erythrocyte destruction (McLeay, 1973). This was also recorded for *Heteropneustes fossilis* and *C. gariepinus* after exposure to copper (Srivastava and Narain 1985; Van Vuren *et al.*, 1994). The decrease in red blood cells could also be the result of impaired osmoregulation which lead to haemolysis. As a result of this the fish became anemic (Wedemeyer and Yasutake, 1977; Larsson *et al.*, 1985). Internal haemorrage was also caused by the accummulation of copper in the gills, liver and kidneys (Van der Merwe, 1992). Compensation is found over a prolonged period of exposure, as indicated by the number of red blood cells being restored after the long-term exposures to copper.

The most important feature of the haemoglobin molecule is its ability to combine loosely and reversibly with oxygen and thus represent the oxygen carrying capacity of the blood (Guyton, 1982). The significant increases in the haemoglobin concentration at 19±1°C , after the 0.16 mg/l and long-term exposures, were accompanied by increases in the number of red blood cells. As mentioned, fish were subjected to an oxygen tension and the increase in haemoglobin could well have been to elevate the oxygen capacity of the blood in order to supply more oxygen to the tissues (Grobler, 1988). This is thus a mechanism by which the body attempts to absorb more oxygen from the surrounding medium to meet the increased oxygen demand (Cyriac, *et al.*, 1989). The increase in haemoglobin concentration could also be due to cell swelling. The decreases that occurred at 29±1°C after the short-term exposures, is an indication that *O. mossambicus* was in an anaemic condition. An connection exists between the haemoglobin concentration, number of red blood cells and haemotocrit (Peréz *et al.*, 1981). The slight increases that occurred after the long-term exposures at both temperatures, could be an indication that *O. mossambicus* was adapting to the change in the environment. Thus the decrease in haemoglobin concentration signifies that the ability of fish, to provide sufficient oxygen to the tissues, is considerably restricted and will result in a decrease of physical activity (Grobler, 1988; Wepener, 1990).

The haematocrit readings are valuable in termining the effect of stressors on the health of fish (Wedemeyer and Yasutake, 1977; Munkittrick and Leatherland, 1983) and are also used to determine the oxygen carrying capacity of blood (Larsson *et al.*, 1985). Thus a high haematocrit, observed at 19±1°C, would imply polycythemia induced by stress and a haemoconcentration due to gill damage and impaired osmoregulation (Wedemeyer and McLeay, 1981), whereas a low haematocrit, at 29±1°C , would indicate anaemia or oligohaemia (Wepener, 1990).

The mean corpuscular volume (MCV) gives an indication of the status or the size of red blood cells and reflects an abnormal or normal cell division during erythropoiesis (Larsson, *et al.*, 1985). Thus the significant increase in MCV indicates that the erythrocytes have swollen either due to hypoxia, impaired water balance (osmotic stress) or macrocytic anaemia. Whilst a statistically significant decrease in MCV indicates that the erythrocytes have shrunk either due to hypoxia, stress or impaired water balance, microcytic anaemia or a large concentration of immature erythrocytes that have been released from the erythropoietic tissue (Larsson, *et al.*, 1985). The significant increase in MCV after the 0.16 mg/l and long-term exposures, at 19±1°C, can be ascribed to the swelling of erythocytes due to hypoxia setting in (Van der Merwe, 1992).

Mature red blood cells have a greater volume than immature red blood cells (Blaxhall and Daisley, 1973). The MCV, calculated from the number of red blood cells and haemoglobin concentration, showed a significant decrease, after the 0.40 mg/l exposure at 29±1°C (Peréz, *et al.*, 1981). This decrease was the result of the production of large numbers of immature red blood cells. The immature red blood cells are possibly released from the erythropoietic tissue, to counteract the pathological action of copper. Similar decreases were found by Van Vuren (1986) after *Labeo umbratus* was exposed to various pollutants.

Increases in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) clearly indicates that the concentration of haemoglobin in the red blood cells were much higher in the exposed fish than in the control fish. The MCHC is a good indicator of whether red blood cells are experiencing swelling or not, which impair oxygen carrying capacity (Wepener, 1990). The MCHC which is the ratio of the blood haemoglobin concentration as opposed to the haematocrit is not influenced by the blood volume nor the number of cells in the blood, but can be interpreted incorrectly only when new cells, with a different haemoglobin concentration are released into the circulating blood (Soivio and Nikinmaa, 1981). The significant decrease in the MCHC at 19±1°C after the long-term exposure, is probably an indication of red blood cell swelling. Whilst a slight decrease such as the one observed after the 0,16 mg/l exposure, at 29±1°C, could be an indication of haemodilution (Wedemeyer and Yasutake, 1977).

The significant decrease in the ALA-D activity, after the 0.16 mg/l exposure, at 29±1°C, is not demonstrated in the proportionate decrease in the haemoglobin concentration because the haemoglobin only decreased slightly. This confirms the hypothesis that a "safety factor" is involved in the activity of ALA-D to produce haemoglobin (Heath, 1987). The slight changes in ALA-D activity of the red blood cells, after all the other exposures at 29±1°C as well as 19±1°C, emphasize that enought haemoglobin was synthetised for the prevailing oxygen demand (Heath, 1987; Wepener, 1990).

The sodium, potassium and chloride ions are responsible for the preservation of the osmolarity and crystalloid osmotic pressure of the plasma (Nussey, 1994). Copper causes a comparatively large upset in osmoregulation in freshwater fish, even under chronic exposure (Heath, 1987). Various mechanisms such as impairment of active transport mechanisms, changes in membrane permeabilities and changes on cellular level are responsible for this phenomenon. All these changes caused by these mechanisms imply histopathological damage of the gills or necrosis (Van der Merwe, 1992; Nussey, 1994). After short-term exposures, at both temperatures, there

were no drastic changes in the plasma sodium concentration. However, in contrast, to this there were significant decreases in the plasma sodium concentration, at both temperatures, following long-term exposures. These decreases can be ascribed to the nett loss of sodium through damaged gills and kidneys (Wepener, 1990; Van der Merwe, 1992). Structural changes in the gills and kidneys, caused by copper, causes the excretion of sodium and as a result no sodium uptake, which is important for hyperosmotic freshwater fish, takes place from the external environment because the activity of the enzyme Na^+-, K^+-ATPase in the gills prevents the reabsorption of sodium (Heath, 1987). Reabsorption of sodium via the renal tubules is decreased, due to the decrease in Na^+-, K^+- ATPase in the kidney and intestine (Kuhnert *et al.*, 1976). Decreases in plasma sodium concentration were also reported by Wepener (1990) after *Tilapia sparrmanii* was respectively exposed to chromium, iron, manganese and zinc.

The significant increases in the plasma potassium concentration at $20 \pm 1°C$ after the 0.40 mg/l and long-term exposures as well as at $19 \pm 1°C$, after 0.40 mg/l exposure, occurred in all probability to rectify the osmotic differences in the intracellular fluid caused by the decrease in sodium. Similar increases were observed when *Tilapia sparrmanii* were exposed to various metals (Grobler, 1988; Wepener, 1990). The increase in plasma potassium concentration of *O. mossambicus* could be ascribed to osmotic adaptation.

There were no drastic changes in the plasma calcium concentration of *O. mossambicus* at both temperatures. The slight decreases, at both temperatures, could also be due to an impaired tubular reabsorption in the kidney, or an impaired intestinal uptake of calcium (Larsson, *et al.*, 1985). A low calcium concentration may lead to an icnrease in the membrane permeability (Eddy, 1981).

The slight increase in the plasma calcium concentrations, at both temperatures, is probably caused when calcium is displaced from the branchial areas, by divalent cations such as copper (Eddy and Bath, 1979). This is reflected in the decreases of the plasma sodium and chloride concentrations. The secretion of parathormone could also withdraw calcium from bone and thus cause an increase in the plasma concentration (Meyer, 1988).

The decreases in the plasma chloride concentration were due to an excretion of chloride ions via the kidneys. Hypochloremia may be the initial electrolyte imbalance which is a manifestation of both specific and non-specific stress (Selye, 1950).

Metals, such as copper, at low concentrations change the permeability properties of membranes which in turn could alter the chloride equilibria between erythrocytes and plasma (Fromm, 1980). Due to the damaged gills, an impaired active ion uptake will contribute to the hypochloremic state (Larsson, *et al.*, 1985). A disturbed osmotic balance will result in haemodilution.

The decrease in plasma osmolality in freshwater fish exposed to copper is associated with a rise in blood volume (Courtois and Meyerhoff, 1975) and tissue water content (Heath, 1984). These changes suggest the gills of fish in copper have become more permeable to water which is entering the body osmotically (Heath, 1984). Similar decreases were found by Christensen, *et al.* (1972) when *Ictalurus nebulosus* was exposed to sublethal copper concentrations.

Thrombelastograms (TEG) with a typical wine glass-shaped pattern (Hartert, 1971), similar to those found for humans, were observed (Figure 4.24 and 4.25). These were also similar to those found for *O. mossambicus* and *Cyrpinus carpio* (Van Vliet *et al.*, 1985a; 1985b) and *Tilapia sparrmanii* (Gey van Pittius *et al.*, 1992).

The potential of fish blood investigations as a diagnostic tool is well known (Blaxhall, 1972; McCarthy *et al.*, 1973). Thrombelastography is a relatively modern technique devised to detect and diagnose possible abnormalities in human blood coagulation (Hartert, 1971; Franz and Coetzee, 1981) as well as in fish blood coagulation (Van Vliet, 1981; Barham, 1983; Van Vliet *et al.*, 1985a,b; Gey van Pittius *et al.*, 1992).

The prime value of this technique lies with the evaluation of the intrinsic coagulation system, because the intrinsic thromboplastin generation is more complex than that of the extrinsinc system (Biggs, 1976). Evaluation of the role of all the coagulation factors, except factor VII can be done during the intrinsic activity (Dacie and Lewis, 1975; Biggs, 1976). The intrinsic and extrinsic pathways cannot be separated entirely during the process of clotting, thus the thrombelastogram actually represents the total coagulation process (Franz and Coetzee, 1981).

Prolonged coagulation times can be caused as a result of a haemophatic condition called haemophilia. Haemophilia is the most common hereditary disorder of blood coagulation (Hoffbrand and Pettit, 1980) but in this present study on *O. mossambicus* haemophilia was induced. By far the most common cause of haemophilia is the deficiency of factor VIII (Bell, *et al.*, 1972; Guyton, 1982). This defect is an absence or low level of coagulation factor VIII clotting activity (VIII:C). It appears likely that copper causes either defective synthesis of this part of factor VIII or the synthesis of a structurally abnormal molecule (Hoffbrand and Pettit, 1980). A deficiency of factor VIII, antihaemophilic factor (AHF), prevents the activation of factor X, and promotes the adhesion and lysis of thrombocytes after contact with damaged tissue (Grey and Meyer, 1988).

The thrombelastogram of an organism with haemoplia has the following characteristics; increases in the r- and k- time, and an almost normal ma (Gey van Pittius, 1991). This blood disease was evident after *O. mossambicus* was exposed to 0.16 mg/l and 0.40 mg/l copper for a short-term at 29±1°C, and after the 0.40 mg/l short-term exposure at 19±1°C.

Another haemopathic condition observed in this study was thrombocytopenia due to the malfunction of thrombocytes. Thrombocytopenia means the presence of a very low quantity of thrombocytes in the circulatory system as a result of either the underproduction of thrombocytes, or by the increased destruction of thrombocytes in the circulating blood, or the abnormal distribution of thrombocytes (Mammen, 1967; Hoffbrand and Pittit, 1980; Guyton, 1982; Gey van Pittius, *et al.*, 1992). The most common cause of thrombocytopenia is the underproduction of thrombocytes (Hoffbrand and Pettit, 1980). Selective megakaryocyte (precursor of the thrombocyte) depression may result from drug toxicity or from viral infections, and a decrease in the numbers of megakarocytes may be part of general bone marrow failure in humans (Mammen, 1967; Hoffbrand and Pittit, 1980). In this study the copper, as metal pollutant, causes the malfunction of thrombocytes, probably because copper inhibits the production of thrombocytes.

Fish with thrombocytopenia, like haemophiliacs, have a tendency to bleed, except that the bleeding is usually from many small capillaries rather than from larger vessels, as in haemophilia. As a result, small punctate haemorrages occur throughout all the body tissue and the skin of these individuals display many small, purplish blotches (Guyton, 1982). This internal bleeding, together with other physiological disturbances, cause death (Gey van Pittius, 1991).

The thrombelastogram of an organism with thrombocytopenia has the following characteristics; a normal r-time, prolonged k-time and a decrease in ma (Gey van Pittius, 1991). Although changes were not statistically significant, this tendency was evident after *O. mossambicus* was exposed to 0.16 mg/l copper at 19±1°C.

The k-time measures the rapidity of the fibrin build-up or the speed with which a clot of a certain solidity is formed. In individuals suffering from thrombocytopenia this is meaningful because a shortage of thrombocytes will prevent the activation of factor X. Factor X is necessary to activate prothrombin. Thus fibrinogen cannot be converted to fibrin (Gey van Pittius, 1991).

The decrease in ma value indicates that less elastic and less firm blood clots are formed under these circumstances. This can be attributed to the possible counteraction of the fibrinolytic activity in the blood, thus causing the clot to dissolve while still in process of forming, or the decrease in fibrinogen levels (Van Vliet, 1981).

In essence, a longer r-time usually represents an inherited defect in thromboplastin generation (Franz and Coetzee, 1981). This is due to the changes in the generation of plasma thromboplastin components (Gey van Pittius, 1991), or in the factors that control the biological functions of factors VIII, Xi and XII of the intrinsic pathway (Dacie and Lewis, 1975).

The liver of *Tilapia sparrmanii* was severely damaged after exposure to sublethal concentrations of selected metals. After diseases like hepatitis and cerrhosis, severe liver damage are caused which leads to depression of prothrombin, factor VII, IX and X function. The individual then develops a tendency to bleed. Another cause of depressed levels of these substances is vitamin K deficiency (Guyton, 1982; Gey van Pittius *et al.*, 1992).

Due to the insignificant changes after the long-term exposures, at both temperatures, it appears that *O. mossambicus* is adapting to the change in the environment. The blood coagulation system is returning to normal because fish are starting to regulate the copper after exposure to all the different Cu concentrations.

Gey van Pittius (1991) and Gey van Pittius *et al.* (1992) observed that blood coagulation was prolonged after the exposure of *Tilapia sparrmanii* to chromium, manganese, zinc and iron, which coincides with the findings in the present study after *O. mossambicus* were exposed to copper at 29±1°C and 19±1°C, respectively. This it appears that accelerated coagulation is observed in fish subjected to stress, whilst fish exposed to metals, such as copper, chromium, iron, manganese and zinc, have prolonged coagulation times.

The increase in the number of leucocytes (leucocytosis) is a normal reaction of the fish body, against infections of foreign substances, which can alter the normal physiological processes in fish. In the present study there was a

significant increase ($P < 0.05$) in the number of leucocytes after *O. mossambicus* was exposed to copper at 29 ± 1°C and 19 ± 1°C, which can be attributed to the increased production of leucocytes to protect the body against infections that may have been caused by the copper. Metals are known to block the active sites of antibody molecules and disturb the metabolism, ionic balance and cellular division of immunocompetent cells (O'Neil, 1981). Increases in leucocytes have also been noted after the exposure of fish to pollutants and metals (Van Vuren, 1986; Flos *et al.*, 1987; Grobler, 1988; Wepener *et al.*, 1992a,b; Gey van Pittius, 1991). Lymphocytes play a key role in the immune response, especially in the formation of antibodies. Monocytes, neutrophils and eosinophils are mainly responsible for phagocytic action of foreign material (Grey and Meyer, 1988). Significant increases in the number of lymphocytes (lymphocytosis) and eosinophis (eosinophilia), combined with significant decreases in monocytes (monocytopenia) and neutrophils (neutropenia) are indicative of infections that set in after the exposure to metals (Gey van Pittius, 1991). This tendency was evident after the exposures of *O. mossambicus* to copper at 29 ± 1°C and 19 ± 1°C respectively. Lymphocyte and eosinophil numbers in the blood increase as a reaction to foreign proteins and parasitic infections. The same effect is probably caused by copper. It is also possible that an immune reaction occurs after the weakening of the health condition of the fish to combat infections which are secondary to the effect of the pollutant. Lymphocytes in fish are regarded as immunocompetent cells (Ellis, 1976), therefore, it can be concluded from the available results that copper induced stimulation of the immune system which could be responsible for the marked lymphocytosis observed in the present study.

The decreases in the number of monocytes and neutrophils of *O. mossambicus* is a result of elevated phagocytic activity in affected tissues, such as gills, liver and kidneys which were damaged by copper (Wepener, 1990; Gey van Pittius, 1991; Van der Merwe, 1992). The white blood cells leave the circulating blood, to protect the body, by moving (amebic movements) to the infected tissue.

The variation that occurred in the total number of leucocytes of *O. mossambicus*, in the present study, was a result of increases or decreases in the different types of leucocytes. Leucocyte subpopultions are known to fluctuate in response to a variety of environmental stimuli (Johansson-Sjöbeck and Larsson, 1978; 1979).

Fish can adapt to a change in the environment (Ellis, 1981). Although there were significant differences in the number of lymphocytes and neutrophils between the control and long-term groups at both temperatures, it appeared that these leucocytes were starting to return to normal, thus *O. mossambicus* was trying to adapt to the change in the environment. This is due to the fact that the fish were starting to regulate the copper, and in so doing, the kidneys and liver could play an important part in removing copper from the blood (Heath, 1987; Gey van Pittius, 1991). The foregoing decreases did not coincide with the high total leucocyte counts after the long-term exposures to copper. These results suggest that copper caused secondary infections, in the affected tissues (gills, liver and kidneys), and although it seems that *O. mossambicus* had adapted to the change in the environment, these fish only partially recovered from the stress condition.

8.2.2.5 Conclusions

It is, thus, ambient that the present concentration of copper in the Olifants River excerts a physiological effect on *C. gariepinus* at both temperatures (winter and summer) which manifests in a change in blood chemistry like erythrocytopenia, leucocytosis, hyperglycaemia and hyperprotonemia. Although fish were exposed to copper at the level in which it is found in the river, the bioavailability of the copper from the natural environment still remains an unknown factor.

It was clear that the haematology, as well as the osmoregulation of *O. mossambicus*, were altered after the exposure to copper at 29±1°C and 19±1°C, respectively. A physiological effect was excerted *on O. mossambicus*, at both temperatures, by causing changes in the blood biochemistry such as leucocytosis and erythrocytopenia.

It can clearly be seen that copper, at 29±1°C as well as 19±1°C, will induce secondary changes in *O. mossambicus* as illustrated by the number of leucocytes in the blood. The variations in the number of leucocytes is indicative of infections that may occur, or as a protective mechanism to prevent possible infections. Thus the concentration of circulating white blood cell types are important parameters for use in detecting and evaluating the sublethal effects of copper in *O. mossambicus*.

Blood coagulation experiments on *O. mossambicus* proved that copper induced haemophilia at 29±1°C and 19±1°C whilst at 19±1°C it also induced thrombocytopenia. It can therefore be assumed that changes in the blood coagulation system of these fish are sensitive indicators of sublethal copper pollution.

However, it should be noted that although a thrombelastogram may be a handy diagnostic tool, Van Vliet *et al.*, (1985a) have suggested that it should be used discreetly and carefully since Hartert (1971) found large variations in the results obtained by various researchers, when comparing their results. Thus, it is vitally important that a predetermined mode of operation be established before using the thrombelastogram as a diagnostic tool. Chronic stress from copper may reduce the ability of the fish to resist pathogenic organisms, to adjust physiologically or behaviourally to other physical stressors and to reproduce. When chronic stressors are present, the values of haematological and metabolic variables may return to normal with time. The metabolic cost to fish in bringing about compensation and adaptation of this magnitude may be high and does not necessarily reflects normality.

Thus any external stressor, such as copper, even at levels which are considered non-lethal, can have a detrimental effect on aquatic organisms. It is also important to note that the toxicity of copper, which already occur in freshwater ecosystems, can be altered if there is a change in the physical and chemical characteristics of the water.

The results of this section of the project support the believe that the study of fish blood can be used as a reliable indicator of aquatic pollution caused by copper. This haematological and osmoregulation analyses could serve as a rapid and economic method of assessing copper toxicity and possibly other metals and toxicants which affect fish.

8.2.2.6 References

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9. CONCLUSIONS AND RECOMMENDATIONS

The Phalaborwa area has many point sources of pollution from both sewage treament works as well as from mining, industrial and agriculture effluents. Sewage treatment plants are point sources of nutrients, such as nitrates and phosphates, which can lead to eutrophication problems. The mines and industries in the area are point sources of pollution containing constituents, such as calcium fluorides, magnesium, phosphates, potassium, sodium, sulphates and metals (Theron, *et al.*, 1992). The extensive copper-mining activities in this region give rise to the concern that copper and other metals such as Fe, Zn, Mn, Cr, Ni and Pb, may increase to levels in the water which might have an affect on the physiology of fish.

9.1 Water and sediment

The water quality to the Selati River at locality 7 was found to be stressful to the aquatic life due to chemical constituents that exceeded the recommended guideline limits. Variables of special concern were: sodium, fluoride, chloride, sulphate, potassium, the total dissolved salts and the metal concentrations (except strontium). Effluents of the phosphorus extraction mining company and copper extraction mining company in the Phalaborwa area, as well as upstream inflow into the Selati River contributed to the high TDS concentrations in this river. The anionic component mainly responsible for the high TDS concentrations was sulphate. Furthermore, the Selati River had a negative influence on the water quality of the Olifants River after the confluence of the two rivers. This was clearly illustrated by the concentrations of some chemical constituents detected in the water at Mamba Weir. The negative influence of the Selati River was more pronounced during low flow periods (e.g. droughts or winter months) when limited water releases from the Phalaborwa Barrage reduced the dilution effect of the water on chemical constituent levels. However, most of the chemical constituent concentrations (not the metal concentrations) did decrease from the western side of the KNP to the eastern side, due to the dilution of the water through the tributaries of the Olifants River. At locality 3 (near Balule) some chemical constituents increased again in concentration, especially from April 1990 to February 1991 (8.1.1). The frequent occurrence of reed beds in that part of the river was the possible explanation to this. Most of the time, the water quality of the Olifants River in the KNP complied with the recommended guideline limits, except for the metal concentrations at most localities. However, the high metal concentrations in the water did not necessarily indicate conditions toxic to aquatic life. The water of the Olifants River is, amongst other features, hard (as $CaC0_3$), and this decreases the bioavailability of the metals to aquatic life and therefore the toxicity of the metals. Higher metal concentrations were detected in the sediment than in the water, due to the adsorption of metals on sediment particles. This indicated the chronic nature of metal pollution in the area. A large variation was detected in the metal concentrations of the water and sediment, making it difficult to establish the order of metal occurrence in the study area (Fig. 7.1). According to the sediment metal concentrations (which fluctuated less than the water), the general order from April 1990 to February 1991 for localities 1 to 6 was: Fe > Mn > Cr > Ni > Zn > Sr > Pb > Cu. For locality 7 in the Selati River it was Fe > Mn > Sr > Cu > Cr > Ni > Zn > Pb. The sediment At Pionier Dam (control in KNP) had an occurrence pattern of metals similar to that of localities 1 to 6, except that more zinc than chromium was detected in the sediment. In the Selati River (at locality 7) much higher copper and strontium concentrations were detected in the sediment than in the Olifants River (at localities 1 to 6). This indicated that these two metals orgininated from a local source which was not connected to the KNP. The Water 2 water quality index which was developed with water quality data obtained from this project is a useful tool in assessing the water quality of the Olifants River 8.1.2.).

9.2 Bioaccumulation of metals

The accumulated metals (Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn) in the organs and tissues of *Barbus marequensis* gave a good indication of the metal levels to which the fish were exposed, especially when compared with the metal concentrations of a fish species from a polluted system (Germiston Lake). *Barbus marequensis* seemed to have been chronically exposed to zinc, copper, lead and nickel, probably at sub-lethal levels. In addition, the fish at locality 7 seemed to have been chronically exposed to iron, chromium and manganese, also probably sub-lethally.

Metals were usually taken up *via* the gut and/or *via* the gills. The high metal concentrations in the gut contents of *B. marequensis* were not only due to the food ingested by the fish, but also to the metal-rich sediment associated with the food (*B. marequensis* is a benthic feeder). In the summer of 1990/91 the heavy rainfall increased the solubility of the metals and therefore metals could be taken up *via* the gills, and maybe even the skin, more easily, leading to a higher accumulation of metals in the fish. The various metals were distributed differently in the organs and tissues of *B. marequensis* and *Clarias gariepinus*, indicating that it is not necessarily the same organs that should be sampled for the analysis of different metals. It is therefore possible that, in using the wrong organs, an incorrect conclusion can be drawn in the assessment of the extent of metal pollution in an area. The suggested organs and tissues that should be sampled for the analysis of Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn in fish, as well as the organs and tissues of *B. marequensis* that accumulated the highest concentrations of these metals, are indicated in Tabel 3.1. Muscle tissue should always be sampled to test its fitness of human consumption. Apart from this, the gills, liver and bony tissues seem to be good representative organs and tissues in general metal pollution surveys. If, however, surveys are being done on specific metals, organs and tissues as illustrated in Tabel 3.1, should be sampled. Seasonal differences in the bioaccumulation of the metals in the organs and tissues of fish did occur. Zinc is known to be essential for gonad development, especially for females, and therefore displayed a seasonal trend. The role of the other metals in gonad development (if any) is, however, not certain and cannot be related to this process as yet. Moreover, seasonal differences were related to the available metal concentrations that were taken up during a season.

9.3 Acute toxicity

The determined 96-hour LC50 values of copper for juvenile *O. mossambicus* were 2.61 mg/l and 2.78 mg/l, and the incipient LC50 values were 2.95 mg/l and 3.32 mg/l for copper at 29±2°C, respectively. Results also indicated that seasonal variations in water temperature can alter the lethality of copper and fish. *O. mossambicus* was more susceptible to copper at the higher temperature. This may be because the fish had a relatively higher metabolic rate, at 29±1°C, thus more water and copper passed through the gills. The higher water turnover in the gills could thus be an argument for a greater sensitivity to copper at the higher temperature, because more water containing copper was transported to the blood.

The 96-hour LC50 value of copper for adult *C. gariepinus* was 1.38±0.08 mg/l at 21±1°C 1.29±0.25 mg/l at 28±1°C. For juvenile *C. gariepinus* the LC50 value of copper was 1.20±0.04 mg/l at 21±1°C and 1.30±0.9 mg/l at 29±1°C.

Results indicated that water temperature may alter the lethality of copper to *C. gariepinus*. Copper toxicity therefore appears to be enhanced by temperature as stressor.

The difference in LC50 values due to changes in seasonal water temperature may occur due to a transient change in the metabolic rate of the fish.

The 96-hour LC50 value of manganese for juvenile *O. mossambicus* was determined to be 1.723 g/l Mn, while the incipient LC50 value was 1.46 m/l Mn. These concentrations are much higher than in the manganese concentrations occurring in the environment, which rarely exceeds one mg/l. Effluents of mines can, however, contain manganese concentrations that will have sub-lethal effects on fish. The highest manganese concentration detected in the water of the study area was 16.5 mg/l. Attention should therefore be given to the performance of chronic manganese toxicity tests in the future, in order to verify the existing water quality guideline of one mg/l Mn as a maximum concentration for the protection of aquatic life.

Despite increased sophistication in toxicity testing, increased awareness of the variety of processes operating in a natural system and the integration of information from a system of tests in various hazard evaluation protocols, the ultimate question of what is an acceptable concentration if a chemical with a known degree of accuracy is still unanswered. In the Olifants River, copper is one of several metals which pose a threat to the conservation status of the river. The LC50 values found during this study, can be used as an indication of the levels at which copper becomes lethal for this specific species. In a larger management-control program like the Kruger National Park, these results must be seen merely as the limits within which the concentration of copper can be regarded as lethal for *C. gariepinus* in the Olifants River. Future research should focus on the further development and validation of acceptable prediction strategies such as Water 2. In general, toxicants occur in mixtures in natural waters, and therefore the interaction of toxicity is an important factor which must be taken into account when assessing the hazard of an environmental pollutant to aquatic life. Additional studies on the effects of toxicants, singly and in mixtures, on biochemical and physiological processes, are needed to gain more knowledge of their interactions and toxic effects.

9.4 Haematology

After the exposure to sublethal concentrations of copper, several effects have been reported in fish. These include decreased survival, growth and reproduction, tissue damage in the gills as well as a decreased activity of gill Na^+-K^+-ATPase. Fish also accumulate copper in certain organs and tissues, particularly the gills and liver. Copper also affects the whole body energy metabolism, swimming performance, various blood variables and, by binding to proteins, has a detrimental effect on enzymes in various metabolic pathways. Secondary stress responses have also been reported. This happens when fish are affected by various stressors and respond by a series of biochemical and physiological stress reactions.

Exposure of *C. gariepinus* to sublethal copper concentrations, results in certain physiological changes in the blood chemistry and metabolism. Morphological changes in the gills of *C. gariepinus*, although not lethal, had a significant effect on the respiratory and osmoregulatory function of the gills. These morphological changes can be regarded as primary induced changes, which will inevitably lead to further secondary, physiological changes or responses affecting various organ systems. These physiological changes can either be seen as an initial response to the toxicant or as an adaptation reaction to retain a normal condition. Either of these responses will affect the performance of the organism in totality, however, leading to a decreased ability to survive.

The exposure of *O. mossambicus* sublethal copper concentrations over short-term (96 hours) and long-term (4 weeks) periods, at 29±1°C (summer) and 19±1°C (winter), in experimental flow-through systems provides information on the tolerance of this species to sublethal copper concentrations. By determining the physiological and pathological changes in the blood of these fish, the risk and harmfulness of copper pollution were established. Apart from the sublethal

exposures, acute toxicity tests were also conducted in flow-through systems in order to determine the 96-hour LC50 and incipient LC50 values of copper for juvenile *O. mossambicus*, at both temperatures.

During the exposure to sublethal copper concentrations as well as during the acute toxicity tests, at both temperatures, visible sublethal effects were observed, such as darkening of skin pigmentation, erratic swimming, increased coughing action, operculum movement and mucus secretion. Haemorrhaging was evident at the pectoral fins, operculum and the nose of exposed fish. These changes could be ascribed to the effect that copper had on the physiology of the fish. The exposed individuals also showed obvious signs of stress. These stress conditions resulted in an initial increase in the secretion of hormones derived from the adenophypophysis-internal axis. An increase in these so-called stress hormones resulted in changes in the values or blood coagulation, general haematology, osmoregulation and differential white blood cell counts.

There were delays in the blood coagulation times, as well as decreases in the shear modulus (elasticity) of the clots formed, after the exposure of *O. mossambicus* to copper at both temperatures. Copper was found to induce haemophilia at 29±1°C and 19±1°C, whilst at the latter it also induced thrombocytopenia. Haemophilia as well as thrombocytopenia results in haemorrhage in the body tissue. The haemorrhage occurred as a result of a disturbance in the casscade of reactions that leads to the conversion of fibrinogen to fibrin. This could be ascribed to the impaired production of thrombocytes. Although the fibrinogen concentrations were not determined in the present study, the decreases in the elasticity of the clots formed was an indication of a lower fibrinogen level. To determine which coagulation factor(s) is/are influenced by the presence of copper in water, and will thus lead to a defective coagulation process, it would be necessary to test every coagulation factor separately. Since the liver was concerned with the synthesis of some of the coagulation factors, it was presumed that there was damage to the liver. The damage that metals cause to the liver was confirmed by Gey van Pittius (1991).

An increase in the number of leucocytes (leucocytosis) is a normal reaction of the fish body to attacks by foreign substances, such as metals, which can alter the normal physiological function in the fish. Significant increases in the number of lymphocytes (lymphocytosis) and eosinophils (eosinophilia) combined with significant decreases in monocytes (monocytopenia) and neutrophils (neutropenia), are indicative of changes (infections) that set in after short-term (96 hours) exposures to metals. This finding was confirmed by the number of leucocytes of *O. mossambicus* that increased at both temperatures to protect the body against infections that may have been caused by copper.

Exposure of *O. mossambicus* to sublethal copper concentrations gives rise to certain physiological changes in the general haematology. Morphological changes in the gills (Wepener, 1990; Van der Merwe, 1992), as reflected by the decreases in the plasma sodium, potassium, calcium and chloride concentrations, although not lethal, had a significant effect on the respiration and osmoregulatory function of the gills. These morphological changes can be regarded as primary changes, which will inevitably lead to secondary, physiological changes as well as responses that could affect various organ systems. Changes can be seen as an initial response to the toxicant (copper) or as an adaptation reaction to retain a normal condition.

Considering the fact that the copper concentrations (0.16 and 0.40 mg/l), administered to *O. mossambicus*, fall within the existing water quality guideline, as prescribed by Kempster *et al.* (1982) for South African freshwater (0.1-2.0 mg/l), it appears that existing values should be revised. Because although all the fish did not die of these concentrations, there were definite changes in the physiology of these fish. If extrapolated, although, fish in the Olifants River may visually seem

unaffected by metals such as copper, their physiological conditions does not necessarily reflect a state of normality, but rather a state where it had to adapt physiologically to survive the current conditions. Although adaptation to changing environmental conditions in nature is not an uncommon phenomenon, it is essential that developers, economists and even scientists do not use this as an excuse for irresponsible and indiscriminate exploitation of riverine water.

9.5 Recommendations

It is recommended that a more intensive study on the water and sediment quality of the study area should be undertaken. The interaction between the water and the sediment with regard to metal distribution should be investigated, as well as the bioavailability of the metals to the fish. This can best be achieved by combining the field study with experimental work, in order to determine the effects of the physical and chemical environment on the metal toxicity. Water and sediment samples should be increased to at least ten per locality, thereby decreasing the variation in metal concentrations. Monitoring can be limited to localities 2, 3, 5, 6 and 7, giving special attention to locality 3 to determine the role of the reed beds. Sampling should also be performed higher up in the Olifants River catchment, in order to determine the influence of these mining, industrial and agricultural activities in the area. Biological monitoring should not only include a sensitive fish species, but also sensitive plant and invertebrate species. All of the biological species need be sampled only at localities 5, 6 and 7, as well as higher up in the catchment, and only the fish organs suggested in Table 9.1 should be used. The number of fish should be increased to 20-30 individuals, however, the fish size should be large enough for one gram of dried tissue to be made available. Working on a dry weight basis, as well as the large n-value, will decrease the large variation in metal concentrations.

It seems important that methods should be established and adopted because although the results of the present study suggest that blood coagulation, general haematology, osmoregulation and differential white blood cell counts can be used as sensitive indicators in detecting and evaluating the sublethal effects of aquatic pollution caused by copper, it is inevitable that individual variation will hamper the drawing of conclusions. The study of fish blood is of practical importance when conducting experimental pollution studies in a laboratory and does not have the same impact during field studies.

It is important to remember that, in general, pollutants rarely occur singly. Thus, for the purpose of environmental protection, it is necessary to know the lethal and sublethal toxicity to various aquatic species (such as algae and macroinvertebrates) of mixtures under various environmental conditions. Therefore, future studies on the lethal and sublethal effect of copper, on its own and in mixtures, on biochemical and physiological processes under various environmental conditions, are of the utmost importance in gaining more knowledge of its interactions with other metals and its toxic effects.

Laboratory experiments where fish (or other aquatic organisms) are exposed under controlled conditions to different metals, according to toxicity will provide important information on lethal and sublethal levels of the metals. This information is essential for the improvement of water quality guidelines for metals and the "health" status of fish can also be assessed. These experiments are also important in developing improved water quality guidelines for metals which could aid water quality managers, engineers as well as consultants in assessing the impact of metals on the aquatic environment. For further sublethal experimentation and impact assessment, the calculation of LC5, LC10 and LC20, together with LC50 values should be included.

For future management, it is recommended that drastic measures should be taken in order to reduce the impact of mining activities on the water quality of the Selati

River and also, indirectly, the Lower Olifants River (especially during low flow periods). It is important for enough water to be released in the Olifants River from Phalaborwa Barrage in order to dilute the Selati River water, especially during low flow periods (e.g. droughts and winter periods). If the water quality of the Selati River cannot be improved, it should at least be maintained at its present status, for a further degradation in water quality cannot be afforded.

TABLE 9.1
SUMMARY OF FISH ORGANS IMPORTANT IN METAL POLLUTION SURVEYS

	Zn	Cu	Fe	Cr	Ni	Mn	Pb	Sr
Bile		*		*	*			
Blood				*	*		*	
Gill				*	*	*	*	*
Gonads (F)	*							
Gonads (M)	*							
Gut		*	*	*	*			
Kidney		*		*	*			
Liver		*		*	*	*	*	*
Muscle	*	*	*	*	*	*	*	*
Opercular bone	*					*	*	*
Scales	*					*	*	*
Skin	*		*					
Vertebrae	*			*	*	*	*	*

* Fish organs to sample for metal analysis

Organs of *B. marequensis* with highest metal concentrations

Histopathological studies should be done in addition to metal analysis

10. APPENDICES

Nussey, G. 1994. The effect of copper on the blood coagulation and general haemotology of *Oreochromis mossambicus* (Cichlidae). M.Sc. RAU.

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