

Macrobenthic Community and Ecotoxicological Status of the Nhlabane Estuary

by

Leon Vivier

Submitted to the Faculty of Science and Agriculture
in fulfillment of the requirements for the degree of

Doctor of Philosophy

in the
Department of Zoology
University of Zululand



Promoter: Prof DP Cyrus
Co-promoter: Prof V Wepener

November 2010

DECLARATION

I hereby declare that this thesis is the product of my own original work, and to the best of my knowledge, contains no material previously submitted for the award of any degree at another university. Any published information used was duly acknowledged.

DEDICATION

I dedicate this thesis to:

My lovely wife, Vicci, who supported me every step of the way and inspired me to persevere. Without her support and dedication completion of this thesis would not have been possible.

My parents, who instilled in me an enquiring mind and an appreciation of the outdoors and provided me with the opportunity to study and to pursue a career in Zoology.

ACKNOWLEDGEMENTS

This project was undertaken with the financial support of Richards Bay Minerals, with additional funding being provided by the Research Committee, University of Zululand. Richards Bay Minerals is also acknowledged for support and assistance with fieldwork. SASOL is thanked for sponsoring one of the research boats used during the study.

I am indebted to my supervisor, Prof Digby Cyrus, for his guidance, assistance and encouragement during the study. I am also grateful for all the assistance and encouragement I received from my co-supervisor, Prof Victor Wepener, and his guidance in the analysis of most of the water, sediment and tissue samples. The Department of Zoology, University of Johannesburg, are thanked for the use of their laboratory facilities and for their assistance during the preparation and analysis of the samples.

Staff from the Department of Zoology, University of Zululand, are thanked for assisting with sorting of benthic samples. I would also like to acknowledge the assistance of many people during many hours of fieldwork: Prof DP Cyrus, Prof V Wepener, Dr HL Jerling, Fiona Mackay, Steve Weerts, Louise Bakkes, Gerhard Cilliers and Alfons Vilfoen.

ABSTRACT

The Nhlabane Estuary is a small temporary open/closed estuary along the Northern KwaZulu-Natal coastline, 40 km north of Richards Bay. Historically, the Nhlabane Estuary formed the tidally influenced lower reaches of the Nhlabane coastal lake system, but in 1978, its connection with the coastal lake was permanently severed by the construction of a weir across the upper end of the estuary. The weir altered the natural flushing regime of the estuary, resulting in the estuary mouth often remaining closed for a number of years. Dune mining operations around the estuary raised concerns about trace metal concentrations in the estuary. This study was initiated following concerns about the ecological integrity of the estuary in relation to reduced freshwater input into the system and its effect on the macrobenthic community, as well as the potential trace metal contamination of the estuary water, sediment and biota due to mining activities. The study aimed to describe the macrobenthic community of the Nhlabane Estuary during the post-drought period 1996-1999, to assess the ecotoxicological status of the Nhlabane Estuary in relation to dune mining activities and to determine the suitability of two resident amphipod species, *Corophium triaenonyx* and *Grandidierella lignorum*, as potential test organisms for sediment toxicity testing in estuarine environments.

Quarterly samples of the benthos, water and sediment were collected at six sites in the estuary over the period 1996-1999. Physical water quality parameters were measured *in situ* at each sampling station. At the start of the study, the estuary showed a highly stressed benthic community characterized by low species richness and densities, caused by a combination of a naturally occurring drought event exacerbated by anthropogenically induced lack of freshwater input into the system. The number of taxa and benthic densities increased significantly after the mouth was breached. The 32 macrobenthic taxa recorded during the four year study period were dominated by estuarine crustaceans, notably the two amphipods amphipods, *C. triaenonyx* and *G. lignorum*, and the polychaetes *Ceratonereis keiskamma*, *Desdemona ornata* and *Prionospio sexoculata* and the tanaid *Apseudes digitalis*. Multivariate analysis showed a marked temporal change in the benthic community, reflected in significant changes in the number of taxa, density, species richness and species diversity during the 1996-1996 study period. This represented a recovery in the Nhlabane Estuary benthic following the extended period of mouth closure during the 1992-1995 drought.

Trace metal concentrations in the Nhlabane Estuary water, sediment and invertebrate tissues were consistently lower than in regional anthropogenically influenced estuaries. Normalization of Nhlabane

Estuary sediment metal concentrations to background concentrations for uncontaminated KwaZulu-Natal coastal areas showed the system to be uncontaminated, with dune mining not being the cause of pollution in the system. Trace metal concentrations in the two amphipod species, *G. lignorum* and *C. triaenonyx*, also compared favorably with that found in similar species, providing further evidence of the relatively uncontaminated conditions in the system.

Amphipods are commonly utilized throughout the world to test the toxicity of potentially contaminated sediments in marine and estuarine environments, because they are ecologically relevant, are sensitive to sediment contaminants and are suitable for laboratory experimentation. Laboratory studies with cadmium, zinc and copper to test the suitability of the two local amphipod species, *Grandidierella lignorum* and *Corophium triaenonyx* as test organisms in sediment toxicity tests, showed that both species accumulated Cu and Zn in their bodies in relation to increasing concentrations of the metals in the sediment. The ability to accumulate trace metals during exposure to contaminated sediments is one of the primary requirements in sediment bioassay test organisms, and results from this study have clearly shown both species to be sensitive to and accumulate bioavailable Cu and Zn in sediment.

Lack of estuarine management means that estuaries in South Africa have been subjected to increasing pressure, both indirectly from the effects of catchment utilization, which affect their water supply, and directly from the increasingly industrial and residential development along the coastal zone. As a result, many South African estuaries have become functionally degraded, which has resulted in a loss of species. The Nhlabane system is located in a forestry dominated catchment, so the system is subjected to limited industrial and residential encroachment and organic and inorganic chemical contamination. Results from this study showed that trace metal concentrations in the water and sediment were low and within environmentally acceptable levels. Construction of the Nhlabane weir, however, deprived the Nhlabane Estuary of adequate freshwater and altered the natural flushing regime. The functioning of the estuary and the state of its biotic communities are largely dependent on the maintenance of the estuarine-marine link, which in turn are directly related to freshwater input from the lake. In an Estuarine Flow Requirement (EFR) study, which forms part of the RDM based Ecological Reserve Determination process for aquatic ecosystems in South Africa, water allocations were determined to maintain the ecological integrity of the Nhlabane Estuary. The EFR study recommended that during droughts the system be permitted to close for a year, but that this was only acceptable 1 year in 3. The recommended environmental flows for the estuary have not been implemented by DWAF and no allocation of water is allowed for the Reserve for the estuary. As such, abstraction of water from the lake is still causing water levels in the lake to be consistently below overflow levels.

Results from this study showed that the Nhlabane Estuary, once regarded as one of the most pristine estuaries along our coastline, has been adversely affected by impoundment of the lake to the point where it ceases to be a functional estuary for periods of time extending over a number of years. Although this study has shown that the ecotoxicological condition of the estuary is generally good, the system is hydrologically stressed due to freshwater deprivation. There is currently no management plan for the estuary that can address these issues. Based on this, the following key issues should be addressed if the future ecological functioning of the Nhlabane Estuary is to be ensured:

It is imperative that the Ecological Reserve for the estuary be implemented, as this will ensure that freshwater allocations for the estuary be adhered to.

A management plan should be designed and implemented for the estuary by MCM in which all stakeholders are represented.

Management of the estuary should incorporate not only the estuarine environment, but also be directed at a catchment level.

A monitoring program consisting of key ecological components, which should complement the existing database and investigate long-term temporal and spatial changes in important biotic communities in the estuary, should be regarded as a priority.

The Nhlabane Estuary should be considered to be of high conservation importance as it is one of the few estuaries along the coastline that is not adversely affected by industrial development, other than dune mining, in its catchment. Motivation for the Nhlabane Estuary to be made a Estuarine Conservation Areas (ECAs) should be considered.

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List of Abbreviations

asml	above mean sea level
BCR	European Community Bureau of Reference
CCA	Canonical Correspondence Analysis
CRM	Certified Reference Material
EFR	Estuarine Flow Requirement
ERL	Effects-Range-Low
ha	Hectare
ICE	Intermittently Closed Estuary
IOC	Intermittently Open Estuary
MAR	Mean Annual Runoff
MDS	Multidimensional Scaling
NTU	Nephelometric Turbidity Units
PCA	Principal Component Analysis
TOCE	Temporary Open Closed Estuary
RBM	Richards Bay Minerals

CHAPTER 1

Introduction

1. Introduction

1.1 General Introduction

Human populations are changing the earth's ecosystems in fundamental ways, especially estuarine and inshore areas. This is evident in a number of indicators, such as changing land cover and use, reduction in natural habitats, changes in the chemistry of the Earth's atmosphere and water, fluxes of biogeochemical elements and materials and changes in diversity of life (Elliot and Quintino 2007). Consequences of rapid industrial expansion in recent decades have been an increase in demand for mineral resources, energy, housing, increased output of sewage and other waste products, and utilization of freshwater (Flemer and Champ 2006), with most of these affecting worldwide estuarine habitats. Estuaries are, by nature of their locality at the transition zone between marine and freshwater environments, traps of particulate material, including nutrients and waste material, and as such are vulnerable to nutrient over-enrichment and bioaccumulation of toxic waste.

An estuary is the meeting place of a river or lake system and the sea. Here, freshwater and seawater mix, water flow is influenced by the tides, wave action is reduced, and sediment and nutrients are deposited during normal conditions and eroded during floods. This combination of processes creates a unique and rich ecosystem known for its biodiversity, and for the important functions that it performs, such as providing nursery areas for fish and invertebrate communities, conduits for species which move between ocean and rivers and rich feeding grounds for significant populations of migratory birds (Begg 1978). Since estuaries constitute transition zones where fresh water from land drainage mixes with seawater, they create some of the most biologically productive areas on earth (Kennish 2002). Estuaries act as nursery areas for many marine and riverine organisms, support life-cycle completion of many fish and invertebrate species, are generally areas of high productivity due to the high nutrient load, provide numerous forms of recreation and are highly preferred residential areas. Financially they are important for trade and ecotourism and provide ports for commercial shipping, all which contribute significantly to the regional and national economy. However, as coastal populations increases and more people settle near estuaries and as more freshwater is abstracted from rivers, the ecological integrity of many South African estuaries is being threatened (Begg 1978, Lamberth and Turpie 2003).

South Africa is characterized by a semi-arid climate with a highly seasonal and variable rainfall pattern. Growing demand for fresh water in southern Africa has led to widespread impoundment of rivers, often without adequate consideration of the impacts on estuarine ecosystems (Whitfield 2005). When river flow patterns are altered by impoundments and by surface and subsurface water

abstraction, and the natural flow regime and the intensity and frequency of flood events are thereby modified, these disturbances change natural fluctuations within estuarine ecosystems. Changes in the frequency and magnitude of river floods can greatly alter the sedimentary processes, depth profiles and mouth configuration in estuaries, thereby affecting the frequency and duration of open mouth conditions and the size of the tidal prism within an estuary, all of which ultimately affect the ecological functioning of the system (Reddering and Rust 1990, Whitfield 2005). Estuaries are dynamic ecosystems in which resident organisms are adapted to natural fluctuations in flow and salinity. Loss of estuarine variability through alteration of the natural flow regime in rivers often leads to wide spread changes in biotic communities, with species that are well adapted to fluctuating estuarine conditions declining in abundance relative to those species that benefit from a more 'constant' environment. Freshwater deprivation also has the potential to restructure energy flow within the affected estuary, causing a shift from pelagic to benthic primary production (Allanson and Read 1995). Freshwater inflow is therefore vital to both pelagic productivity and the maintenance of a range of food chains within the estuarine environment (Schlacher and Wooldridge 1996).

What is an estuary? Due to their complex nature, estuaries vary considerably in geomorphology, hydrography, salinity, tidal characteristics, sedimentation, and ecosystem energetics. As a result, biotic communities also differ substantially in estuarine systems. Elliott and McLusky (2002) reviewed definitions for estuaries used during recent years and concluded that there are difficulties in incorporating the importance of factors such as tidal influence and geographical limits into one universally accepted definition of an estuary for all parts of the world. Although a number of definitions for estuaries have been reviewed over the years, the most widely used definition for Southern African estuaries is that proposed by Day (1980): An estuary is defined as “a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with freshwater derived from land drainage”.

1.2 Estuaries in South Africa

The southern African coastline from Maputo Bay in Mozambique in the east to Angola in the west is uniquely characterized amongst southern hemisphere coastlines by the combination of few near shore islands, strong wave action, and a very small number of sheltered bays. The estuaries along our coastline are therefore almost the only coastal habitats which are able to provide shelter from wave action and an environment in which salinities range from predominantly marine to fresh. South Africa is well endowed with estuaries, with 259 recognised functioning estuaries along its 3 100 km coastline (Whitfield 1992, 2000). Whitfield (1992) has described three biogeographic subdivisions of South African estuaries, these being the Cool Temperate zone on the west coast, the Warm Temperate zone

which extends from False Bay to the Mbashe estuary in the Eastern Cape, and the Subtropical Zone on the east coast between the Mbashe estuary and Kosi Bay. These three biogeographic regions originated from the different oceanic conditions formed off the South African east and west coasts due to the presence of the warm Agulhas current along the east coast and the cold Benguela current along the west coast. Estuaries within these zones have been shown to have relatively distinct faunal communities, and have also been found to differ significantly in their physico-chemical characteristics (Harrison 2004). Warm temperate estuaries are generally characterised by high salinities and low turbidities due to low rainfall and runoff, high seawater input and evaporative loss, while subtropical estuaries tend to have lower salinities and higher turbidity, due to relatively high runoff (Harrison 2004). The biodiversity of estuaries in the cool-temperate zone is considerably lower than in the warm-temperate zone, with the subtropical estuaries having the highest species diversity of plants and animals. Despite the increase in biodiversity from the south western to the north eastern part of the country, the proportion of endemic species is much higher in the south than along the east coast (Breen and McKenzie 2001).

South African estuaries have been classified into five types by Whitfield (1992, 2000) (Table 1.1):

- 1. Estuarine bay:** The small number of large natural and artificially formed bays are permanently linked to the sea and the salinity and water temperatures are strongly marine influenced. Marine and estuarine organisms dominate these systems and extensive wetland/mangrove swamps occur.
- 2. Permanently open estuaries:** Vertical and horizontal salinity gradients are present and are influenced by river flow, tidal range and mouth conditions. Physical conditions are marine dominated controlled during flow normal conditions and by river input during flood conditions.
- 3. River mouths:** Riverine influences dominate the physical processes in these estuaries. The mouth is generally permanently open but the tidal prism is small and strong riverine outflow prevents marine intrusion. Heavy silt loads are frequent in these estuaries often resulting in shallow mouths.
- 4. Estuarine lakes:** These are usually large (>1 200 ha) drowned river valleys separated from the sea by vegetated sand dune systems. The dune can result in complete separation of the lake from the sea resulting in a coastal lake. Estuarine lakes can be either permanently or temporarily linked to the sea and salinity within them is highly variable. The tidal prism is small, and marine and river input have little influence on water temperatures, which are directly related to solar heating and radiation. Estuarine, marine and freshwater organisms all occur depending on the salinity condition of the system.

5. Temporarily Open/Closed Estuary (TOCE): This is by far the most common type of estuary in South Africa. Sand bars often form in the mouths of these estuaries as a result of a combination of low river flow conditions and longshore sediment movement, blocking off connection with the sea. Seasonal flooding from river runoff causes breaching of the mouth, followed by rapid infilling from marine and fluvial sediment. Hypersaline conditions occur in these estuaries during times of drought. Due to their dependence on catchment runoff, these estuaries are vulnerable to poor catchment management and water abstraction. Tidal and riverine inputs control physical conditions in these systems when the mouth is open, but is independent of them when the mouth is closed. Marine, estuarine and freshwater life forms are all found in these systems, depending on the state of the mouth. Whitfield and Bate (2007) promoted the distinction between intermittently open estuaries (IOE's) and intermittently closed estuaries (ICE's), with IOE's being defined as estuaries which, on average, have no link with the sea for more than 50% of the time, while ICE's comprise estuaries which, on average, have a channel linking the lower reaches to the sea for more than 50% of the time. Due to lack of data, for most estuaries it is not possible to determine whether they are IOE's or ICE's, so for the purpose of this study, the term Temporarily Open/Closed Estuary (TOCE) is retained.

Table 1.1. Characteristics of the different types of estuaries and the number of estuaries of each type in each biogeographical zone (modified from Whitfield 2000).

Type	Tidal prism	Mixing process	Average salinity	Number per region			Total number	Total Size (ha)
				Cool Temperate	Warm Temperate	Sub-tropical		
Estuarine Bay	Large (>10 x 106 m3)	Tidal	20 – 35	0	1	3	4 (2%)	8 145 (11%)
Permanently Open	Moderate (1-10 x 106 m3)	Tidal/riverine	10 - >35	2	28	15	45 (17%)	10 961 (15%)
River Mouth	Small (<1 x 106 m3)	Riverine	<10	2	6	3	11 (4%)	1 222 (2%)
Estuarine Lake	Negligible (<0.1 x 106 m3)	Wind	1 - > 35	0	4	2	6 (2%)	47 524 (65%)
Temporarily Open/Closed	Absent	Wind	1 - > 35	7	88	98	193 (75%)	5 140 (7%)
Total				11	127	121	259	72 992

As shown in Table 1.1, the majority of South Africa's estuaries are TOCE's. This dominance is the result of the topography of our coastline which creates numerous small systems with relatively small catchments along the narrow southern and south-eastern coastal strips, with limited tidal exchange when open and a high energy surf-zone that can move large amounts of sediment into and across estuary mouths (Whitfield and Bate 2007). This numerical dominance of TOCE's is particularly evident among east coast subtropical estuaries south of the Thukela estuary, with 98 of the 121 subtropical estuaries falling into this category. North of the Thukela estuary, the coastal plain widens

considerably resulting in far fewer but larger estuaries, with the ten estuaries found north of the Thukela up to Kosi Bay comprising 95% of the estuarine area in KwaZulu-Natal, a section of coastline containing 72 estuaries. The subtropical zone contains 68% of South Africa's estuarine habitat, with Lake St Lucia alone comprising just over 50% of the country's estuarine area (350km²) (Whitfield 2000). Despite the fact that the St Lucia estuary is a World Heritage site, reductions in freshwater inputs have severely compromised its estuarine functioning as a nursery habitat during recent years (Cyrus and Vivier 1996). There is thus now a greater reliance on the remaining estuaries in the subtropical zone for maintenance of vital estuarine functions.

1.3 Environmental threats and estuarine degradation

Estuaries worldwide are subjected to a range of human impacts that increasingly compromise their ecological integrity, through rapid population growth and uncontrolled industrial development in many coastal regions (Chapman 2007a, Rainbow 2007). Ecosystem functioning and water quality in estuaries are often compromised by excessive nutrient input, the accumulation of chemical contaminants as well as direct and indirect habitat destruction (Kennish 2002). Estuarine degradation through effluent contamination and habitat alteration has far reaching ecological consequences, modifying the structure, function, and controls of estuarine ecosystems and contributing to the decline of biodiversity (Rainbow 2002).

South Africa is no exception with regard to this worldwide trend in estuarine degradation. The condition of South African estuaries has been a matter of concern since the early 1970's, particularly in KwaZulu-Natal, when it was recognised that few estuaries in this region remained in their natural state (Turpie 2004). Begg (1978) found that only 20 of the 72 KwaZulu-Natal estuaries were in a good condition. Heydorn (1986) reported that of the 66 small estuaries in KwaZulu-Natal, about half were in moderate condition, while only 24% were in good condition. Ramm (1990) found, based on the health of their fish communities, KwaZulu-Natal estuaries to be generally degraded, with more than 75% being moderately, strongly or severely degraded. In a more recent assessment on the condition of our estuaries, Whitfield (2000) also reported that only 26% of the small KwaZulu-Natal estuaries could be considered to be in a good condition (defined as no major negative anthropogenic influences on either the estuary or catchment). Turpie (2004) reviewed all available information and showed that although the overall health of South African estuaries appeared to be relatively good, in certain areas, such as the KwaZulu-Natal coastline, the majority of estuaries were in poor to fair health. In addition, 63% of subtropical TOCE were considered to be under high to very high environmental pressure with regard to freshwater deprivation and catchment management, respectively.

Estuaries are natural wastefields and repositories of contaminants generated by human activities due to their location at the transition zone between freshwater and coastal marine systems. One of the greatest concerns in estuarine areas worldwide is the increase in concentrations of trace metals in water and sediment, which tend to settle out in these areas due to the sheltered nature of estuaries (Rainbow 2007). Estuarine sediments therefore bind and accumulate trace metals to often high concentrations, which may threaten the health of biotic communities and higher trophic levels dependent on estuarine environments through bioaccumulation in animal tissues (Marsden and Rainbow 2004). Biotic communities can undergo considerable changes due to these contaminants, including the loss of rare or sensitive species, decreased species abundance, shifts in the age structure of populations, and altered trophic interactions (Rainbow 2002). Toxic substances which accumulate in estuarine food chains also pose a potential health threat to humans who consume contaminated fish and invertebrates (Kennish 2002).

Although the analysis of metal concentrations in estuarine sediment and water is usefull, it does not reflect the bioavailable and therefore potentially toxic concentration of trace metals in a system. This information can only be obtained by measuring the accumulation of trace metals in the body tissues of aquatic organisms (Rainbow 2007, Simpson and Batley 2007), thereby obtaining an accurate and essential time-integrated measure of trace metal bioavailability and the fraction of metal of direct ecotoxicological relevance within a contaminated estuary. The development of biomonitoring programmes of trace metal contamination in estuarine waters using selected resident organisms that are sensitive to pollution, has progressed rapidly over the past three decades in many parts of the world (Rainbow 2002, Chapman 2007a). Although such biomonitoring programmes are employed as standard tools in ecosystem monitoring in many coastal areas worldwide, the development of biomonitoring programmes in South Africa is almost completely lacking, even though the integrity of our estuaries has been of increasing concern during recent years. There is therefore an urgent need for the development of biomonitors for estuarine integrity in South Africa, using species that are adapted to conditions within our estuaries.

1.4 Macrobenthic invertebrates

Effective estuarine management relies not only on our understanding of the environmental and anthropogenic factors that influence the functioning of our estuaries, but also requires a basic understanding of the faunal communities characteristic of a particular system and how the biota will respond to natural and human induced changes in the estuarine environment (Chapman 2007a). Biotic communities of many of our estuaries are poorly studied and understood and as a result, decision making is often based on very limited information (Whitfield 2000).

Although all levels of biotic communities are important in estuarine functioning, benthic macroinvertebrates form a particularly important component of estuarine ecosystems as they are amongst the most sensitive biotic components of aquatic ecosystems and are thus useful for assessing ecosystem integrity (Rosenburg and Resh 1993, Chapman 2007b). Because of their life-history characteristics, benthic organisms are widely used for evaluating impacts of environmental change caused by habitat change and environmental contaminants. This results from a tubiculous or burrowing lifestyle that reflects local sediment conditions, life spans of adequate length to integrate contaminant impact over time, and a broad spectrum of tolerance to contaminants among different species within a community (Dauer, 1993). Their close association with estuarine sediment makes them intimately involved in physico-chemical processes occurring in the estuary and also renders them representative of the specific area being surveyed. Benthic macroinvertebrates should therefore form a critical component of estuarine monitoring programmes which are designed to assess temporal and spatial changes in response to natural and human induced changes in the estuarine environment (Rainbow 2002, Chapman 2007b). They also form a central element of estuarine food webs, being an important food resource for many larger crustaceans, fish and birds, and can profoundly influence the abundance and species composition of these tertiary consumers (MacKay and Cyrus 2001).

Not only do benthic organisms comprise an essential ecological component of estuarine biota, they are also commonly employed as suitable bioindicators of estuarine degradation and contamination (Marsden and Rainbow 2004, Chapman 2007b, Gale *et al.* 2006, Re *et al.* 2009). Although many different benthic organisms such as mussels, oysters and polychaetes are used in estuarine toxicity assessments, amphipods are most commonly utilized throughout the world, because they are ecologically relevant and are also known to be some of the most sensitive benthic organisms when exposed to estuarine contaminants (Rainbow 2007). Although standardized estuarine sediment toxicity tests have been developed for a many different amphipod species around the world, no estuarine toxicity tests using local benthic organisms have been developed for South African subtropical estuaries and as such there is an urgent need to assess the suitability of local species as test organisms.

1.5 The Nhlabane Estuary

The Nhlabane Estuary, the focus of this study, is a small 3.2 km long TOCE located midway between the Richards Bay Harbour/Mhlathuze Estuary complex and the St Lucia estuary. It is one of only 10 estuaries found along the 310 km section of Zululand coastline north of the Thukela estuary. Although classified as a TOCE, it was until recently regarded as part of a coastal lake (Figure 2.1). Historically, the Nhlabane Estuary formed the tidally influenced lower reaches of the Nhlabane coastal lake system, but in 1978, its connection with the coastal lake was permanently severed by the construction of a weir across the upper end of the estuary. Despite its location away from any major industrial and

residential development, the Nhlabane Estuary has a long history of being affected by surrounding coastal dune mining activities. The Nhlabane coastal lake system is situated in the dune mining lease area mined by Richards Bay Minerals (RBM), which initiated opencast mining for heavy minerals in the coastal dunes in 1977. The weir across the upper end of the Nhlabane Estuary was built by RBM in order to ensure sufficient freshwater for their opencast mining operations. The weir drastically altered the natural seasonal flushing regime of the estuary. Prior to weir construction, the estuary was exposed to regular seasonal flushing (Begg 1978), but after 1978, the weir often stopped overflowing for extended periods and as such, the estuary mouth sometimes remained closed for a number of years (Vivier and Cyrus 1999a). The mouth of the estuary remained closed for a four year period during 1992-1995 as a result of a regional drought coupled with continued water abstraction from the lake, only breaching in August 1995 after above average rainfall in the catchment caused the weir to overtop.

Mining operations raised concerns about trace metal concentrations in the estuary. On two occasions, in 1993 and 1999, RBM dredged a 6 m deep, 50 m wide channel across the estuary to allow a dredger and separation plant to float across the estuary. Dredging of estuarine sediment is known to cause resuspension of trace metals adsorbed in the sediment and can lead to elevated metal concentrations in the system (Nayar *et al.* 2004). Although the crossing area was confined by two berm walls that were constructed across the estuary to contain the slimes, repeated spills of fine sediment into the estuary in 1993-1994 caused a considerable amount of dredged slimes to flow into the estuary and settle on the bottom, smothering the benthic organisms (Hattingh and Vivier 1995).

This study was initiated after concerns were expressed by estuarine ecologists about the ecological integrity of the Nhlabane Estuary in relation to reduced freshwater input into the system caused by the weir and its effect on the benthic community, as well as the potential trace metal contamination of the estuary water, sediment and biota due to mining activities. The study thus aimed to describe the macrobenthic community of the Nhlabane Estuary during the post-drought period 1996-1999 and to assess its potential for recovery after the estuarine-marine link was re-established. The study also aimed to assess the ecotoxicological status of the Nhlabane Estuary in relation to dune mining activities and to determine the suitability of two resident amphipod species, *Corophium triaenonyx* and *Grandidierella lignorum*, as potential test organisms for sediment toxicity testing in the Nhlabane Estuary and other similar estuarine environments.

1.6 Thesis layout

This study has been divided into three main sections. Section one describes the temporal and spatial changes in the macrobenthic community of the Nhlabane Estuary (Chapter 3), Section two deals with

temporal and spatial changes in trace metal concentrations in the water, sediment and selected benthic invertebrates (Chapter 4), while Section 3 describes the suitability of two local amphipod species, *Corophium triaenonyx* and *Grandidierella lignorum*, as bioindicators of trace metal contamination in the Nhlabane Estuary (Chapter 5). These three chapters (Chapters 3-5) have each been written in the form of a separate publication and as such, there is a small degree of duplication in the introduction sections of these chapters with regards to the historical changes in the Nhlabane Estuary which can not be avoided. Chapters 1, 2 and 6 are general chapters. Chapter 1 is the general introduction to the study, Chapters 2 describes the study area, while Chapter 6 contains the general discussion and conclusion.

CHAPTER 2

Study Area

2. Study area

2.1 Nhlabane catchment

The Nhlabane coastal system (28°38'S, 32°16'E), on the southern end of the Mozambique coastal plain, consists of a bilobed freshwater lake and an estuary (Figure 2.1). Lake Nhlabane, a warm, shallow, oligotrophic lake, has a surface area of 5.1 km² at full supply level, a catchment of 107 km² and a MAR of 29 10⁶m³/a (Kemper 2000).

Surface run-off from the catchment drains into the lake through three small tributaries, the most important of which is the Nhlabane River in the northeastern corner, with two streams draining into the southwestern aspect of the northern basin. The catchment is dominated by pine and gum plantations (29%), rural settlements (43%) and indigenous forest 9% (Walmsley 1993). The establishment of extensive gum plantations has a major impact on the hydrology of the system. Due to the high rate of evapo-transpiration characteristic of gum species, run-off from the catchment and thus recharge of the lake is decreased by as much as 60% in areas of established gum plantations (Walmsley 1993). Direct atmospheric inflow of water into the lake accounts for 45% of its total water load.



Figure 2.1 Aerial photo of the Nhlabane Lake and Estuary showing the locality of the Nhlabane Weir. The sand coloured areas to the right of the lake indicate active dune mining areas. Image Google Earth.

Historical water quality data indicate generally good water quality, with low nutrient concentrations throughout the lake. Nitrogen and phosphorous concentrations compare favourably with those from other oligotrophic lakes such as Lake Mzingazi and the calculated N/P ratio for Lake Nhlabane is lower than that generally recorded in KwaZulu-Natal freshwater impoundments (Wepener *et al.* 1995,

Kemper 2000). Despite its location away from major industrial and residential development, the Nhlabane Estuary has a long history of being affected by dune mining activities. The Nhlabane coastal lake system is situated in the centre of the dune mining lease area controlled by Richards Bay Minerals (RBM). The opencast mining of heavy minerals in the coastal dunes by RBM started in 1977. Following extensive exploration since 1968, the Quebec Iron and Titanium company formed Richards Bay Minerals in 1976, and mining operations started in July 1977 (Camp 1990). After removal of the covering vegetation and topsoil, dredgers floating on a large artificial pond use heavy duty water jets to slump the dune wall in front of the pond. Dredged material pass through a floating concentrator that separates the heavy minerals from the sand. The heavy mineral concentrate is sent to the smelter, while the sand is pumped behind the pond to rebuilt the dredged dunes. RBM leaves a 300m wide strip of unmined coastal dune along the length of the Nhlabane Estuary as a condition of the mining license. This strip, known as an ecostrip, was intended to protect the estuary and serve as a source area for plant and animal recolonization (Camp 1990).

In order to supply the freshwater needs of RBM for their mining operation, a concrete weir was constructed by RBM across the upper reaches of the estuary in 1978 to increase the storage capacity of the lake and to ensure the supply of raw water to the mining operation. Originally the barrage was 3.15 m high, elevating the lake level to 3.8 m amsl. In 1984 the wall was further elevated by 0.75 m to raise the lake level to 4.55 m amsl. The barrage was finally raised to 6.0 m amsl in 2001 and a fish ladder was constructed at the same time to re-establish migration of fish. Prior to weir construction, the southern part of the lake was marine influenced and the system was characterised by a salinity gradient and an associated estuarine fauna (Begg 1978). The weir separated the estuary from the lake and halted migration of estuarine fauna into and out of the lake. Before weir construction, the estuary was exposed to regular seasonal flushing (Begg 1978). After 1978, however, the weir often stopped overflowing for extended periods and as such the estuary was only flooded when high seasonal rains caused the lake to overflow.

The region was subjected to a drought during the period 1991-1995, during which well below average rainfall was recorded. During the years 1992, 1993 and 1994, the catchment only received 45%, 65% and 66% of the mean annual rainfall (Vivier *et al.* 1998). After June 1991, the lake level dropped rapidly and by the end of 1992, was recorded at 1.9 m below the overflow level. From early 1991 to August 1995 the estuary received no direct inflow of water from the lake, although limited freshwater seepage from the lake and the adjacent mining ponds into the estuary still occurred. This, coupled with water abstraction from the estuary, caused the estuary to remain closed. This situation was only reversed during August 1995 when RBM artificially breached the estuary. The drought continued until November 1995, after which good rains again caused the lake to fill and the weir to start overflowing.

2.2 Topography and Geology

The landscape of the Nhlabane catchment is dominated by the low-lying sandy Mozambique coastal plain which is separated from the sea by a 1-2 km wide dune cordon running parallel to the coast. The coastal plain is delimited to the west by the elevated topography of the Lebombo Mountains. The coastal plain is dominated by the broad alluvial lower reaches of the Mhlathuze River and its associated coastal lakes. Four coastal lakes, Mzingazi, Cubhu, Nsezi and Mangeza occur in the catchment area. Lake Nhlabane and its associated estuary lies immediately north-east of the Mhlathuze catchment.

The coastal dune barrier, which at times reach altitudes of up to 100 m, is relatively young, possibly deposited within the last 15 000 years, and is dominated by fine grained sands. Although the dune complex is susceptible to erosion, stability of the dunes is accredited to vegetation cover. The coastal dune barrier complex sands range in colour from creamy-white and yellow to light grey, brown and red and are fine-grained, well-sorted and contain rich deposits of heavy minerals such as ilmenite, rutile and zircon, which are being extracted commercially (Camp 1990).

The coastal plain and coastal dune barrier are underlain by poorly consolidated fine grain sands, clayey silts and lignite (peat) of the Port Durnford Formation. The Port Durnford sediments overlie a Cretaceous marine siltstone deposit which is characteristic of the entire coastal plain. Cretaceous deposits have formed a mostly uniform siltstone with occasional thin clay lenses and thin bands of hardy limestone (Camp 1990). The fine siltstones are believed to be impermeable and the upper surface of this unit forms the base of the coastal aquifer. A thin deposit of calcareous Miocene sediments separate the Port Durnford sediments and siltstone beneath the dune cordon (Sudan *et al.* 2004).

The soils of the area are generally low to very low in natural fertility because of their high permeability, the rapid leaching of nutrients and because they form such a thin veneer, seldom in excess of 60 cm (Camp 1990). Despite the soils having low agricultural potential, flat topography, warm temperatures and high rainfall provides suitable conditions for sugar cane cultivation and forestry.

2.3 Marine Environment

The coastline of KwaZulu-Natal, in common with the rest of South Africa's coastline, is exposed to strong wave action. Consequently the few sheltered beaches are confined to estuary mouths and embayments (Begg 1978). The proportion of fine sand in beach sediments is low: grain sizes on the

open shore range from about 0.200-1 mm with increasing wave action. The coastline between Cape St Lucia and the Tugela River Mouth, which trends in a southwesterly direction, consists mainly of sandy beaches backed by high-vegetated dunes (Sudan *et al.* 2004). There are seven estuaries along this 137 km section of the coastline of which the Nhlabane and Siyaya are generally closed by a sandbar; the Matigulu/Nyoni is seldom closed; and the Mhlathuze, Richards Bay, Mlalazi and Tugela are permanently open.

The most important large-scale oceanographic feature affecting the coastal environment on this stretch of coast is the Agulhas Current that flows southwards bringing warm water from the tropics (Begg 1978). The continental shelf is very narrow in northern Zululand and widens to the south of Richards Bay. The Agulhas current following the edge of the narrow continental shelf flows relatively close inshore along the coast north of Richards Bay.

2.4 Climate

Coastal Zululand is typified by a subtropical climate with warm moist summers and moderate dry winters. January and July have average air temperatures of 25.5°C and 17.6°C, respectively. The mean maximum and minimum temperatures during January and June are 30.0°C and 23.4°C, and 21.1°C and 11.7°C, respectively (data from South African Weather Bureau).

The prevailing winds in the study area are predominantly north-easterly, associated with high-pressure systems and fine weather, and south-westerly winds that are associated with westerly waves and frontal weather. The annual frequency of occurrence of north-easterly winds is more than 20%, with the combined frequency of south-westerly and south-south-westerly winds also exceeding 20%. The wind speeds from both of these sectors are generally moderate with strong south-westerly winds in excess of 8 m s⁻¹ occurring at times. Other than these synoptically driven winds, a complex pattern of thermotopographically induced wind exists in the area that manifest as land-sea breezes by day and night, respectively.

The mean annual precipitation in the vicinity of the Nhlabane catchment is estimated to be 1 127 mm per annum (Kemper 2000). Most of the rain falls during the summer months (60%) from November to March with the maximum falling in the months of January and February. Winter rainfall (May to September) is usually associated with frontal weather patterns and typically coincides with cool weather. Abnormal rainfall events, which typically coincide with cyclones and cutoff low pressure systems, can produce extensive flooding at times.

2.5 Study area

The Nhlabane Estuary, a small sub-tropical temporary open/closed estuary (TOCE) only 3.2 km in length with a maximum width of 55 m in the lower reaches and a surface area of 0.17 km², assumes a meandering course through its floodplain (Whitfield 2000) (Figure 2.2). The vegetation associated with the estuary is dominated by extensive reed swamps surrounding the open water; over 50% of the estuarine plant communities are made up of the common reed, *Phragmites australis*. During periods of low salinity, proliferation of emergent macrophytes occur, notably *Potamogeton pectinatus* and *Ceratophyllum demersum*, Swamp forest species, *Barringtonia racemosa* (powder puff tree) and *Hibiscus tiliaceus* (lagoon hibiscus) occur along the estuary shoreline. By comparison with other Natal estuaries the Nhlabane has a low botanical importance rating score due to the lack of plant community diversity and small area of the system (Riddin 1999).

The mouth of the Nhlabane Estuary faces an open coastline and is directly exposed to wave action. The beaches at the mouth are very steep, which is typical for this area and is caused by the interaction of the local wave climate and the coarse sand particles on these beaches. The action of breaking waves at the beach places large quantities of sediments in suspension, which are flushed into the mouth during the incoming tide and thereby provide the main closing force at the mouth.

Prior to the construction of the barrage across the outflow of Lake Nhlabane in 1976, the mouth was normally open one to three times a year for periods of one to three weeks at a time (Huizenga and Van Niekerk 1999). It was estimated that a total cumulative inflow of 10x10⁶ m³ was required before a natural breaching would take place. After a breaching, the outflow of freshwater would have lasted for a few days, after which the intrusion of saline marine water would then have occurred at high tides until mouth closure (Huizenga and Van Niekerk 1999). Overtopping of the berm by high waves at high tides would later have caused additional inflow of marine water, especially in the period shortly after closure when the berm was still low. The influx of marine water under tidal flows would have resulted in some saline water reaching the southern basin of Lake Nhlabane as has been observed in the past (Begg 1978). Construction of the barrage disrupted the natural seasonal flushing regime of the system which normally would have ensured that the estuary breached at least once a year during periods of peak rainfall. The reduced freshwater flow into the estuary coupled with water abstraction for mining activities reduced the possibility that the estuary would breach naturally and caused the system to remain closed for more extended periods of time.



Figure 2.2 Map of the Nhlabane Estuary showing the benthic sampling stations Sites 1-6 used during the study period. The dredger crossing path, the berm walls and Nhlabane weir are also indicated.

RBM constructed two berm walls across the middle reaches of the estuary during June 1993 in order to allow the mining dredger of Mining Pond C to continue its mining operations to the north of the

Nhlabane Estuary (Vivier and Cyrus 1999a). Although operational planning called for the berm walls to be removed shortly after the dredger crossing in 1993, they remained in place for three years, only being removed in May 1996. For the 1st six months of the study period the estuary was thus divided in half by two berm walls, with limited water exchange between the upper and lower estuary. A 2nd dredger crossing occurred across the middle reaches of the estuary in March 1999, but the berm walls were removed very soon after the crossing took occurred.

2.6 Sampling sites

Six sampling sites were chosen for the study (Figure 2.2). For comparative purposes, the same site localities used during the 1992-1995 dredger crossing and drought impact study (Vivier and Cyrus 1999b) were used in this study. The six mid-channel sampling sites were:

Site 1: This site was approximately 0.4 km from the mouth opposite a group of *Casuarina* trees on the north dune (Figure 2.3). The channel, about 50 m wide and with a mean depth of 2.2 m when the system was full, ran along the southern side of the estuary, with a wide and shallow bank on the northern side. The steep southern bank was covered in natural coastal dune forest (28° 39' 34.5"S, 32° 15' 28.4"E).



Figure 2.3 Aerial photo of the lower reaches of the Nhlabane Estuary, taken shortly after the mouth was artificially breached in August 1995, showing Site 1 (white arrow) just upstream of the mouth, the dune mining operations on both sides of the estuary and Lake Nhlabane in the distance.

Site 2: This site was approximately 0.8 km from the mouth on the downstream end of the last *Phragmites* stand. The estuary at this point, about 50 m wide and with a mean depth of 1.6 m when the system was full, was fringed by *Phragmites* on both sides, while the

higher steep part of the dune was covered in natural coastal dune forest (28° 39' 25.3"S, 32° 15' 37.1"E).

Site 3: This site, about 1.2 km from the mouth, was located in the middle reaches and 50 m downstream of the 1993 dredger crossing path (see Vivier and Cyrus 1999b) (Figure 2.4). The site was initially selected as the downstream impact site for the dredger crossing study. The estuary at this point, about 40 m wide and with a mean depth of only 1.0 m when the system was full, was fringed by an extensive stand of *Phragmites* on the northern side, while the steep southern bank was covered in natural coastal dune forest (28° 39' 14.2"S, 32° 15' 45.7"E).



Figure 2.4 Aerial photo of the middle reaches of the Nhlabane Estuary, showing the locality of Site 3 (white arrow), the berm walls extending across the estuary, the dune mining operations fringing both sides of the estuary and Lake Nhlabane in the distance. This image was taken shortly before the berm walls were removed in May 1996.

Site 4: This site was also located in the middle reaches and 50 m upstream of the 1993 dredger crossing path, about 200 m from Site 3 (see Vivier and Cyrus 1999b). The site was initially selected as the upstream impact site for the dredger crossing study. The estuary at this point, about 30 m wide and with a mean depth of 2.0 m when the system was full, was fringed by *Phragmites* on both sides, although patches of *Barringtonia racemosa* trees were found along the edge. During low salinity conditions, emergent macrophytes notably *Potamogeton pectinatus* and *Ceratophyllum demersum* started to proliferate upstream of this point (28° 39' 08.1"S, 32° 15' 49.9"E).

Site 5: This site was located 0.7 km upstream of the 1993 dredger crossing path. The site, about 20 m wide and with a mean depth of 1.8 m when the system was full, was located in the meandering middle section of the system and was fringed by an extensive stand of *Phragmites* on the southern side, while the steep northern bank was covered in natural coastal dune forest (28° 38' 58.7"S, 32° 15' 56.1"E).

Site 6: This site was located in the upper reaches of the estuary, 0.45 km downstream of the Nhlabane weir (Figure 2.5). The estuary at this point was only about 15 m wide with a mean depth of 1.8 m when the system was full, but became very shallow when the system was tidal, ranging between 0.4-0.9 m in depth. The site was fringed by an extensive stand of *Phragmites* on the southern side, while the steep northern bank was covered in natural coastal dune forest. During low salinity conditions, the area became characterized by extensive stands of the emergent macrophytes *P. pectinatus* and *C. demersum* (28° 38' 42.2"S, 32° 16' 04.8"E).



Figure 2.5 The upper reaches of the Nhlabane Estuary, seen from the Nhlabane weir road bridge downstream towards Site 6 (white arrow), with fringing *Phragmites* mixed with coastal dune forest.

CHAPTER 3

Changes in the Macrobenthic Community of the Nhlabane Estuary

3. Changes in the Macrobenthic Community of the Nhlabane Estuary

3.1 Introduction

The term benthos comes from a Greek word which means "depths of the sea". Benthic organisms are generally referred to as those organisms which live in or on the bottom of an aquatic system, of which macrobenthic organisms are regarded as those retained by a 500 µm mesh sieve. They are usually the most abundant and diverse group of animals in the aquatic environment and changes in their densities and diversities are good indicators of environmental perturbation. In estuaries, benthic organisms comprise a broad assemblage of diverse forms, although the most important groups are the polychaetes, crustaceans and molluscs. Environmental factors that most affect estuarine benthos are depth, latitude, salinity gradients, shelter from wave action, fluctuations in temperature and oxygen levels and the input of detritus (Attrill and Power 2000).

Estuaries are dynamic ecosystems that are characterised by often rapid and large-scale variability in physical and chemical conditions due to changes in freshwater and marine influences. Macrobenthic organisms in estuaries have to be able to accommodate this dynamic nature of an estuary (Flint and Kalke 1986). Species-specific preferences for a distinct type of estuarine habitat or area within an estuary relate to a series of environmental variables such as salinity, grain size distribution, food availability, predator abundance, competition, and turbidity. Some estuarine benthic species are able to inhabit most parts of an estuarine environment, because of their wide-ranging environmental tolerances, but some are more specific in their habitat preference and therefore have a more limited distribution (Teske and Wooldridge 2003). The spatial distribution of macrobenthic species in relation to their varying tolerances to environmental variables is reflected in the diverse benthic communities supported by estuarine environments. Although many factors affect the abundance of benthic communities, the spatial distribution of benthic invertebrates along an estuarine gradient have been shown to be most often related to sediment composition and/or salinity (Teske and Wooldridge 2003).

Macrobenthos form an important component of estuarine ecosystems as they play a vital role in nutrient cycling. Benthic organisms are important in the ecology of estuaries both as consumers of plankton and as food for bottom feeding fish and as such provide key linkages between primary producers and higher trophic levels in estuarine food chains. Because macrobenthos is an important food resource for many large species of crustaceans, fish and birds, they can profoundly influence the abundance and species composition of these tertiary consumers (Currie and Small 2005). Humans also harvest many species of shellfish and crustaceans.

In the assessment of estuarine environmental quality through a quantitative approach, the abundance, biomass and species richness of macrobenthos have been widely utilised parameters, especially in soft-bottom communities (Giangrande *et al.* 2005). Benthic macroinvertebrates are amongst the most reliable and sensitive biotic indicators of habitat quality in aquatic ecosystems and are thus useful for assessing ecosystem integrity (Rosenburg and Resh 1993). They are largely non-mobile and their close association with estuarine sediment, where exposure to contaminants and oxygen stress is most frequent, makes them directly or indirectly involved in most physico-chemical processes in the estuarine environment. Due to their sessile nature and relatively long life cycles, benthic invertebrates are representative of the location being sampled, which allows monitoring of temporal and spatial changes in response to perturbation in the estuarine environment. Numerous studies have demonstrated that macrobenthic invertebrates respond predictably to many kinds of natural and anthropogenic stresses and they reflect environmental conditions that vary over time (Mackay and Cyrus 2001). The use of macrobenthos in estuarine and marine ecological research, and particularly in evaluating pollution impacts, is especially effective in assessing long term changes and detecting input from diffuse sources (Rosenburg and Resh 1993). Benthic macroinvertebrates include diverse species with a variety of life features and tolerances to stress, which allow their inclusion into different functional response groups. The development of biotic health indices, to evaluate the ecological integrity of estuarine ecosystems, based on benthic communities, have proved valuable tools in ecosystem management (Pinto *et al.* 2009).

The continued proper functioning of an estuary and its ability to maintain a healthy macrobenthic community is dependent firstly on the maintenance of the natural dynamism imposed on the system by both riverine and marine influences and secondly on regular contact with the marine environment. Few estuaries in South Africa are unaffected by impoundments and water abstraction schemes in their catchments, which ultimately affect their natural flushing regime and interaction with the marine environment. The over-exploitation of water resources in catchments generally have far reaching consequences for the ability of estuarine systems to perform their normal processes and functions and the effects of impoundments in rivers on the zoobenthic communities of downstream estuarine environments are well documented (Reddering and Rust 1990, Whitfield 2005).

The ecological functioning of the Nhlabane coastal lake system, which consists of a bi-lobed freshwater lake and a small estuary 3km in length (Figure. 2.1), was drastically altered in 1978 by the constructing of a weir across the upper end of the estuary, built to ensure sufficient freshwater for opencast mining operations in close proximity to the estuary. This in effect completely changed the character of the coastal lake from a tidally influenced system with regular connection to the sea, to a freshwater lake with no connection to the sea and a small estuary with an unnaturally limited flushing frequency due to impoundment and water abstraction. This resulted in a substantial loss of estuarine habitat due to the

prolonged absence of any tidal input into the lake and as a result, the benthic community of the Nhlabane Lake has become completely freshwater dominated (Vivier and Cyrus 1999a).

An understanding of the spatial distribution patterns of macrobenthos along estuarine gradients help estuarine ecologists and managers to identify the linkages between species distributions and ecological processes and therefore to gain insight into the functioning of estuarine ecosystems (Ysebaert *et al.* 2003). Limited information is available on the macrobenthic community of the Nhlabane Estuary. Despite the fact that the estuary is located within a coastal opencast dune mining area which has been actively mined since 1978, no quantitative data is available on the benthic community of the system from before 1992, when the Coastal Research Unit of Zululand (CRUZ) initiated a four year study (1992-1995) on the Nhlabane coastal lake and estuary (Vivier and Cyrus 1999a, 1999b). From 1992 to 1995 the estuary mouth was closed due to a regional drought which was aggravated by water abstraction from the adjacent Nhlabane Lake. Although the benthos of the estuary has been described as typical for a subtropical TOCE with a relatively low benthic faunal richness compared to that recorded in many open tidal estuaries (Vivier and Cyrus 1999a), the 1992-1995 study was initiated after the mouth of the estuary had been closed for about six months and hyposaline conditions had started to develop. The low species richness in the estuary during that period and the dominance of a few abundant species were seen as indications of a stressed benthic community in response to the low salinity conditions recorded in the system under closed-mouth conditions (Vivier and Cyrus 1999b).

The mouth of the estuary opened in mid 1995 and regular connection with the marine environment was re-established. In view of the complete lack of data on the macrobenthic community of the Nhlabane Estuary under “normal” open-mouth conditions, this provided an ideal opportunity to study the potential for recovery of the macrobenthic community from the drought dominated, hyposaline, closed-mouth state experienced during 1992 - mid 1995 to the marine influenced open-mouth state during the period 1996-1999. An understanding of how an estuary and its biotic communities respond under adverse conditions, natural or anthropogenic, can only be achieved by comparing the state of the system during such times to the characteristics of the system during open-mouth conditions when a tidal prism is established by regular marine and freshwater inputs. The aim of this component of the present study was therefore to describe the macrobenthic community of the Nhlabane Estuary under post-drought conditions when the mouth of the system frequently opened and to assess the recovery of the benthic community during this period in relation to that observed under drought influenced, hyposaline, closed-mouth conditions.

3.2 Material and Methods

3.2.1 Sample and data collection

Quarterly benthic samples were collected over the period 1996-1999 at six sites in the estuary (Figure 2.1). A Zabalocki-type Eckman grab, which samples 0.0236 m² to a minimum depth of 50 mm, was used to collect five replicate samples from each site. Samples were decanted five times through a 0.5 mm mesh sieve to ensure extraction of at least 95% of the animals (Cyrus and Martin 1988). Samples were preserved in a 10% formalin solution, and stained with the vital dye Phloxine B to aid sorting in the laboratory. Animals were identified to species level where possible, enumerated and densities calculated as N.m⁻².

Concurrent with the collection of benthic samples, physical water quality parameters were measured *in situ* at each sampling station. Bottom measurements were taken of temperature, salinity, electrical conductivity, dissolved oxygen, % oxygen saturation, pH and depth using a Hydrolab Datasonde 3 Water Quality Multiprobe and Logger system (Hydrolab Corporation, USA). Turbidity samples were collected and kept on ice until arrival back at the laboratory, where the turbidities were measured in Nephelometric Turbidity Units (NTUs) using a Hellige Direct Reading Turbidimeter. Sediment samples were also collected from each site and analysed for sediment particle size, sorting coefficient and organic content by the Marine Geoscience Unit, University of KwaZulu-Natal.

Rainfall data for the Nhlabane catchment (South African Weather Bureau) for the period 1961-1999 were used to determine by how much the monthly rainfall during the period 1992-1999 deviated from the long term monthly mean. Regular information on the mouth condition of the Nhlabane Estuary was difficult to obtain due to the isolated locality of the Nhlabane Estuary. The mouth status was recorded during every sampling visit, while staff from RBM were asked to provide basic information on the condition of the mouth whenever they visited the area. Water level data for the estuary during 1996-1997 was recorded during every sampling season, but in early 1998, RBM installed a telemetric water level gauge downstream of the Nhlabane weir in the estuary, with water level data being recorded and stored every 3 min.

3.2.2 Data Analysis

Diversity indices, i.e. Shannon-Wiener (H'), Margalef's species richness (D) and Species Evenness (J), were used to study changes in community structure. Single factor ANOVA was used to test for temporal and spatial differences in univariate variables. Changes in diversity between samples were further analysed using k-dominance graphs, which is a plot of the percentage cumulative abundance plotted against log species rank and in which the lower lines on the plot represent the highest diversity.

Multivariate data analysis was performed using the PRIMER Statistical Package (Clarke 1993). The log (x+1) transformed averaged benthic counts were used in determining the Bray Curtis coefficient, followed by hierarchical clustering, with group average linking, and ordination of the data through non-metric multidimensional scaling (MDS). The ANOSIM function in PRIMER was used to calculate Weighted Spearman Rank Correlation Coefficients in order to test for significant spatial and temporal differences in the benthic community. The SIMPER procedure was used to test for the influence of individual species on the similarity within community groups and the dissimilarity between community groups. The BIOENV function was used to determine the key factors that accounted for community patterns.

Principal Component analysis (PCA) and Canonical Correspondence analysis (CCA), using CANOCO (Ter Braak and Smilauer 1998), were used to test for correlation between environmental variables and between the benthic community composition and environmental variables, respectively. PCA aims to describe the variation in a set of standardized environmental variables in terms of a set of uncorrelated variables, called principal components, each of which is converted into a linear combination of the original variables. Each ordination axis is associated with an eigenvalue, which represents the variance extracted by each axis. In CCA, a direct gradient analysis, species community structure is directly related to measured environmental factors, which are displayed as arrows. The strength of the correlation of an environmental variable is reflected in the length of the arrow, and its association is reflected in the acuteness of the angle with the axis.

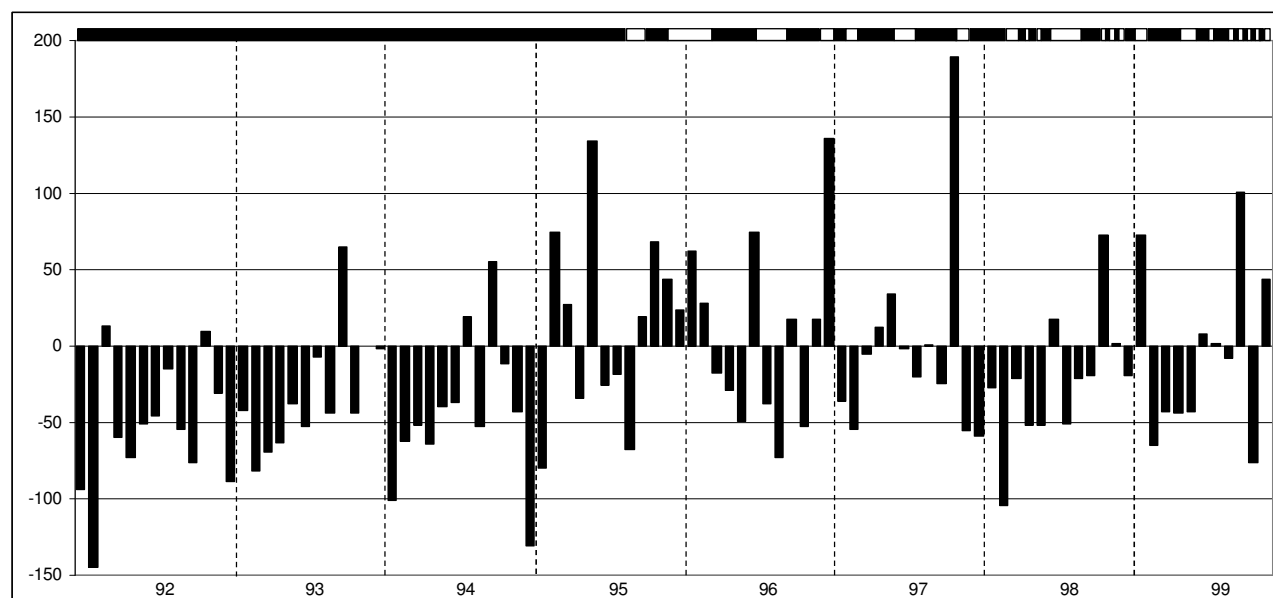


Figure 3.1 Deviation from the mean monthly rainfall for the Nhlabane catchment over the period 1992-1999. Bars indicate deviation from the 1961-1999 mean monthly rainfall. The open/closed mouth periods are indicated at the top, dark bars indicate closed conditions, clear bars indicate open mouth conditions.

3.3 Results

3.3.1 Precipitation and mouth condition

Deviation from the mean monthly rainfall in the Nhlabane catchment over the period 1992-1999 is shown in Figure 3.1. The study area was subjected to a regional drought prior to the onset of the study, over the period 1991 to mid 1995, with below average rainfall recorded during this period. This caused lower water levels in Lake Nhlabane and as a result, there was no direct flow of water from the lake into the estuary. This caused the estuary mouth to remain closed for four years until it was artificially breached in May 1995. In contrast, the study period, 1996-1999, was characterised by normal to high rainfall, which resulted in higher lake levels and frequent overtopping of the barrage. This caused the estuary mouth to open on a regular basis during the study period (Figure 3.1), particularly during 1998 and 1999. After breaching, the mouth only remained open for a few days at a time before it was closed through wave action (Figure 3.2).

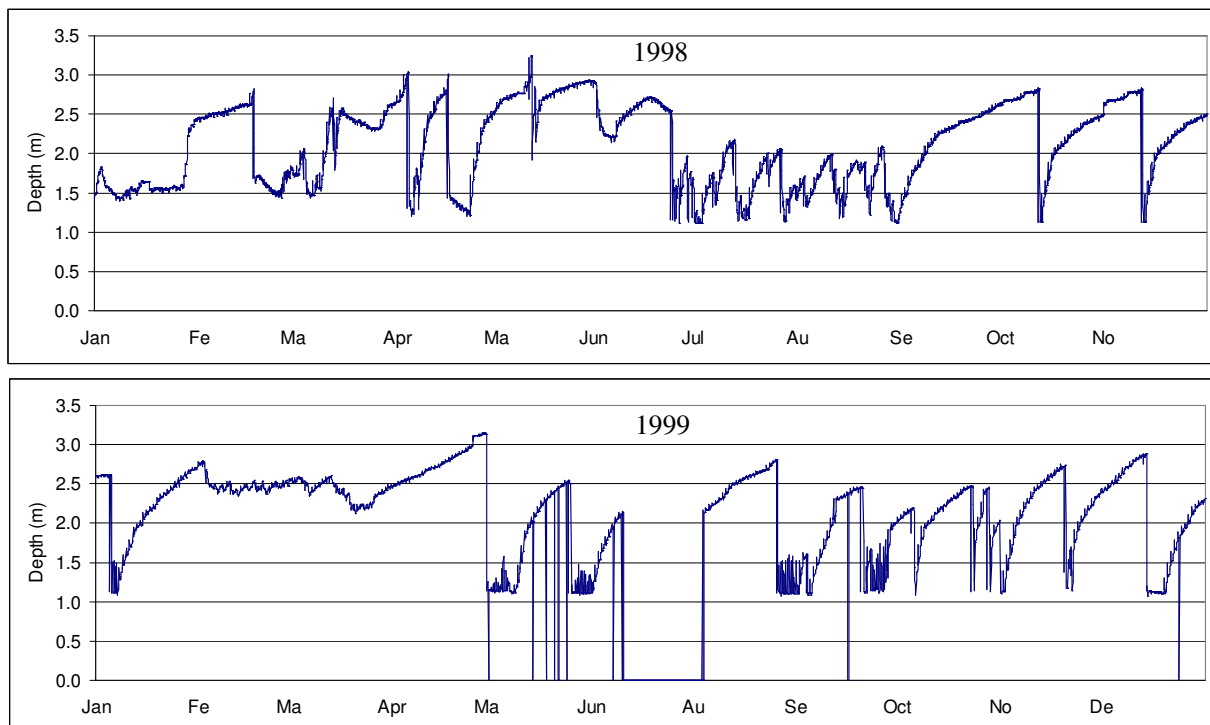


Figure 3.2 Monthly water levels in the Nhlabane Estuary during the two year period 1998-1999.

3.3.2 Physico-chemical water quality

Water temperature during the study period followed a typical seasonal pattern (Figure 3.3). Mean summer and winter temperatures were 28.0 °C (range 25.2-30.4 °C) and 20.3 °C (range 18.0-23.1 °C), respectively.

Slightly lower mean temperatures were recorded in the lower reaches at Site 1 ($\bar{x} = 23.3$, range 18.4-30.0 °C) than at Site 6 in the upper reaches ($\bar{x} = 23.9$, range 18.6-28.9 °C).

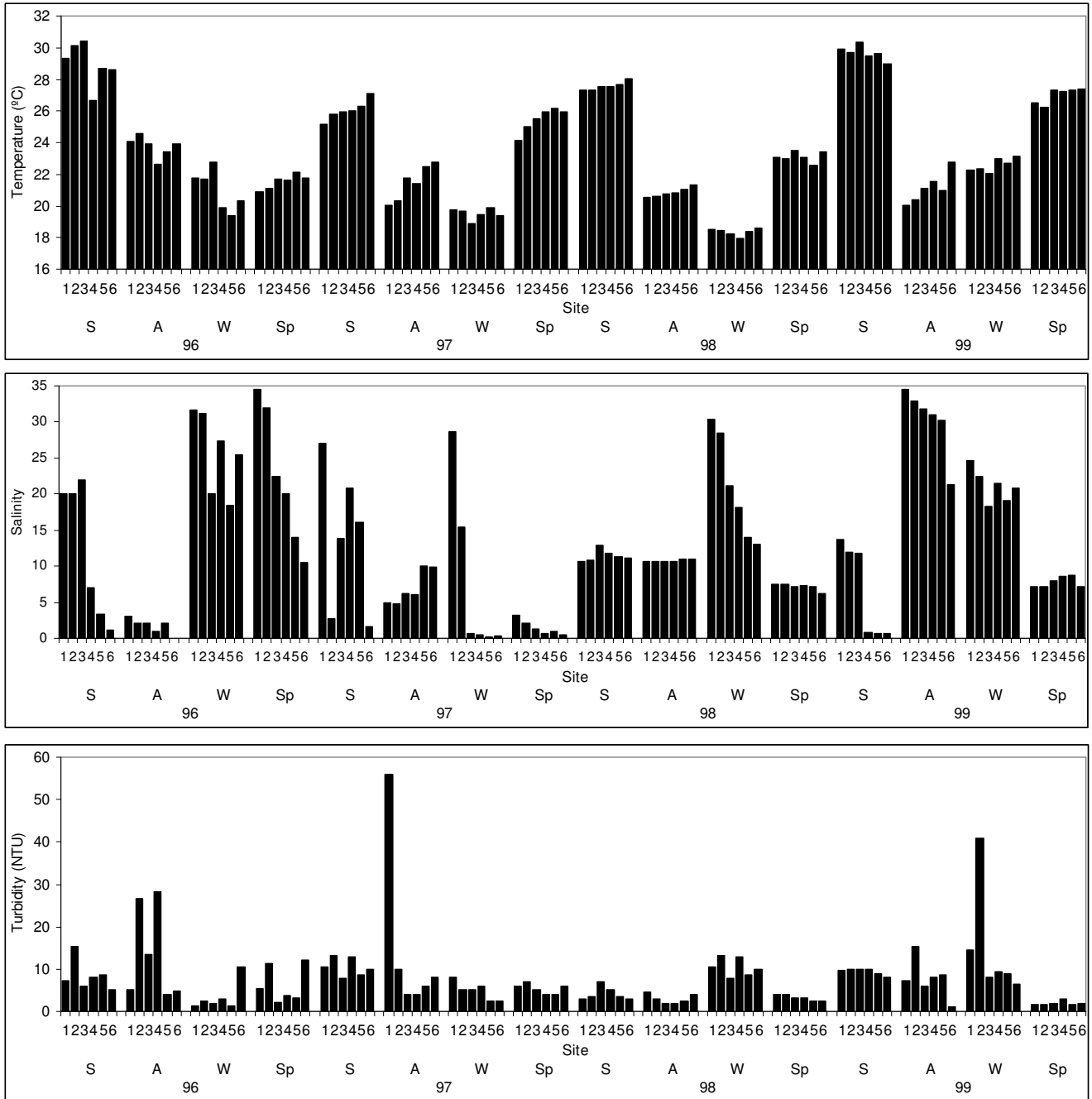


Figure 3.3 Seasonal water temperature, salinity and turbidity at Sites 1-6 in the Nhlabane Estuary over the period 1996-1999. Summer (S), Autumn (A), Winter (W), Spring (Sp).

Salinity in the estuary was largely determined by mouth conditions, with lower salinities being recorded when the mouth was closed ($\bar{x} = 6.7$, range 0.1-13.7), while higher salinities were recorded during tidal open-mouth conditions ($\bar{x} = 19.2$, range 11.0-34.5). There was a typical salinity gradient in the system during most sampling seasons with highest salinities at Site 1 close to the mouth ($\bar{x} = 18.3$, range 3-34.5) and lowest salinities in the upper reaches at Site 6 ($\bar{x} = 8.9$, range 0.1-21.3) (Figure 3.3). A strong salinity gradient existed under high flow tidal conditions when the mouth was open, with very low salinities in the upper reaches and salinities approaching marine conditions at the mouth.

Water turbidity remained low throughout the study period ($\bar{x} = 7.6$ NTU, range 3-55 NTU) (Figure 3.3). The Lake Nhlabane weir, which separated the estuary from the lake, prevented sediment laden water draining from the catchment from entering the estuary and as such, only relatively clear water flowed from the oligotrophic lake over the weir into the estuary. The reason for the uncharacteristically high turbidities recorded in the lower reaches during May 1997 and August 1999 is unclear, because elevated turbidities were not recorded elsewhere in the system.

Dissolved oxygen concentrations were generally higher in the lower reaches (Site 1: $\bar{x} = 6.8$ mg/l, range 3.5-9.3 mg/l) compared to the upper reaches (Site 6: $\bar{x} = 5.3$ mg/l, range 1.7-8.8 mg/l) (Figure 3.4). Highest oxygen concentrations were recorded during cooler winter months ($\bar{x} = 6.3$ mg/l, range 3.0-8.7 mg/l) compared to summer months ($\bar{x} = 5.0$ mg/l, range 2.2-7.5 mg/l).

Water pH during most sampling seasons ranged between 7.9-8.8 except during May 1996 when pH values as low as 6.2 were recorded at Sites 1, 2 and 4 (Figure 3.4). The reason for these low pH values is not clear, but the salinities were very low at the time (range 0.1-3.0). Concurrent with the existence of a typical estuarine salinity gradient in the system, slightly higher mean pH values were recorded at Site 1 in the lower reaches ($\bar{x} = 7.92$, range 6.6-8.5) compared to that recorded at Site 6 in the upper reaches ($\bar{x} = 7.87$, range 7.2-8.4).

The Nhlabane Estuary, when full, ranged in depth from 2.7 m at Site 1 to 1.7 m at Site 6 (Figure 3.4). The mean depth during the study period at Site 1 and Site 6 was 1.7 m and 1.1 m, respectively. The lowest depth of 0.3 m was recorded at Sites 2, 3 and 6 during 1996 at spring low tide under open-mouth conditions.

3.3.3 Sediment characteristics

The sediment in the estuary comprised medium to fine grained sand (Figure 3.5), with median phi values ranging between 1.0-2.7. No mud was recorded in the system. There was a gradual but highly significant

decrease ($F = 20.7$, $P < 0.001$) in sediment particle size from the mouth of the estuary ($\bar{x} = 1.6$, range 1.5-1.9) toward the upper reaches ($\bar{x} = 2.1$, range 1.7-2.7), with the exception of Site 5, which contained coarser sediment ($\bar{x} = 1.3$, range 1.0-1.5). The sediment ranged from well sorted to moderately sorted ($\bar{x} = 0.71$, range 0.53-0.85), with the least sorted sediment associated with the relatively coarse sediment

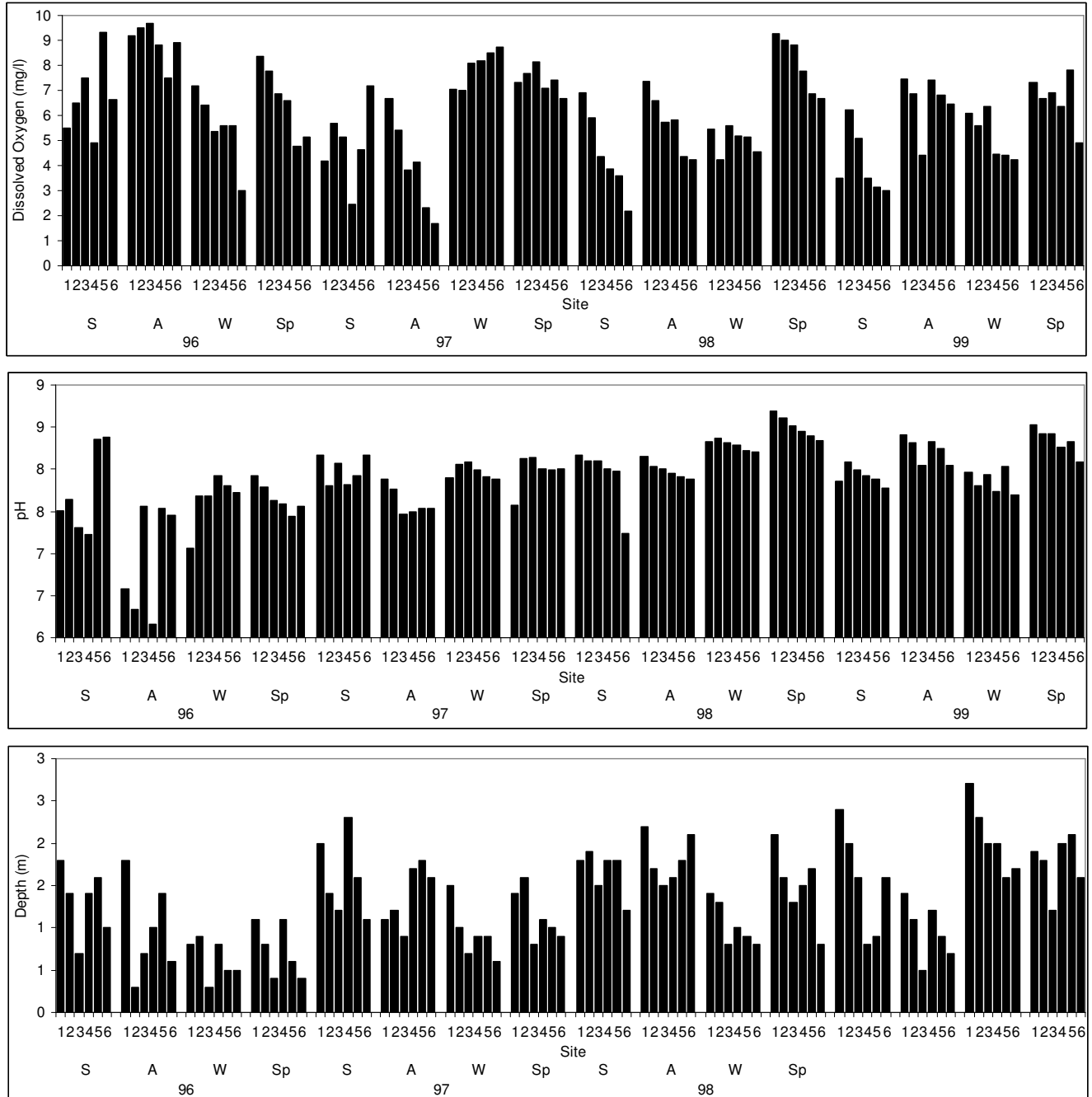


Figure 3.4 Seasonal water dissolved oxygen concentration, pH and water depth at Sites 1-6 in the Nhlabane Estuary over the period 1996-1999. Summer (S), Autumn (A), Winter (W), Spring (Sp).

recorded at Site 5 ($\bar{x} = 0.84$, range 0.84-0.85). The best sorted sediment was associated with the fine sand recorded at Site 6 ($\bar{x} = 0.65$, range 0.53-0.84). Well sorted sand indicates that the majority of the sediment sample is made up of grains of the same diameter, whilst poorly sorted sand indicates a mixture of particle sizes.

The organic content of the sediment ranged from low to moderately low (Figure 3.5). Lowest organic content was recorded at Site 3 ($\bar{x} = 0.88\%$, range 0.4-1.42%), while highest content was recorded at Site 4 ($\bar{x} = 1.33\%$, range 0.43-2.22%). There was no significant difference in organic content between sampling sites ($F = 2.1$, $P < 0.05$).

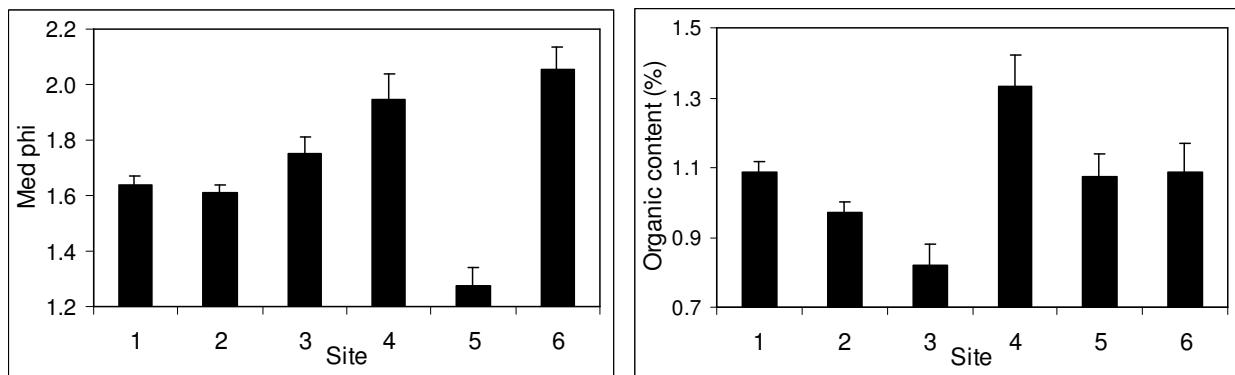


Figure 3.5 Median phi and % organic content of the sediment at Sites 1-6 in the Nhlabane Estuary over the period 1996-1999.

Table 3.1 Principal Component Analysis eigenvalues and cumulative percentage variance of the environmental variables recorded in the Nhlabane Estuary during the period 1996-1999.

Axis	1	2	3	4	Total variance
Eigenvalues	0.238	0.196	0.16	0.113	1
Cumulative percentage variance	23.8	43.4	59.4	70.8	

3.3.4 Environmental variable ordination

The results of the PCA on the environmental variables recorded in the Nhlabane Estuary are presented in Table 3.1 and Figure 3.6. The first component axis explained 23.8% of the variability in the data. The physical variables best explaining the variability in the estuarine environment were salinity and temperature, followed by pH, depth and sediment characteristics. Turbidity and to a lesser extend oxygen

concentration were not important variables. Salinity was related to open-mouth conditions, but not to organic content, oxygen levels or depth. Water temperature showed a positive correlation with closed mouth condition and negative correlation with salinity, indicating that higher temperatures during summer months coincided with low salinity closed-mouth conditions. Oxygen concentrations were positively correlated with turbidity and sediment organic content, but inversely related to depth and to a lesser degree with pH. Particle size was not related to turbidity or depth.

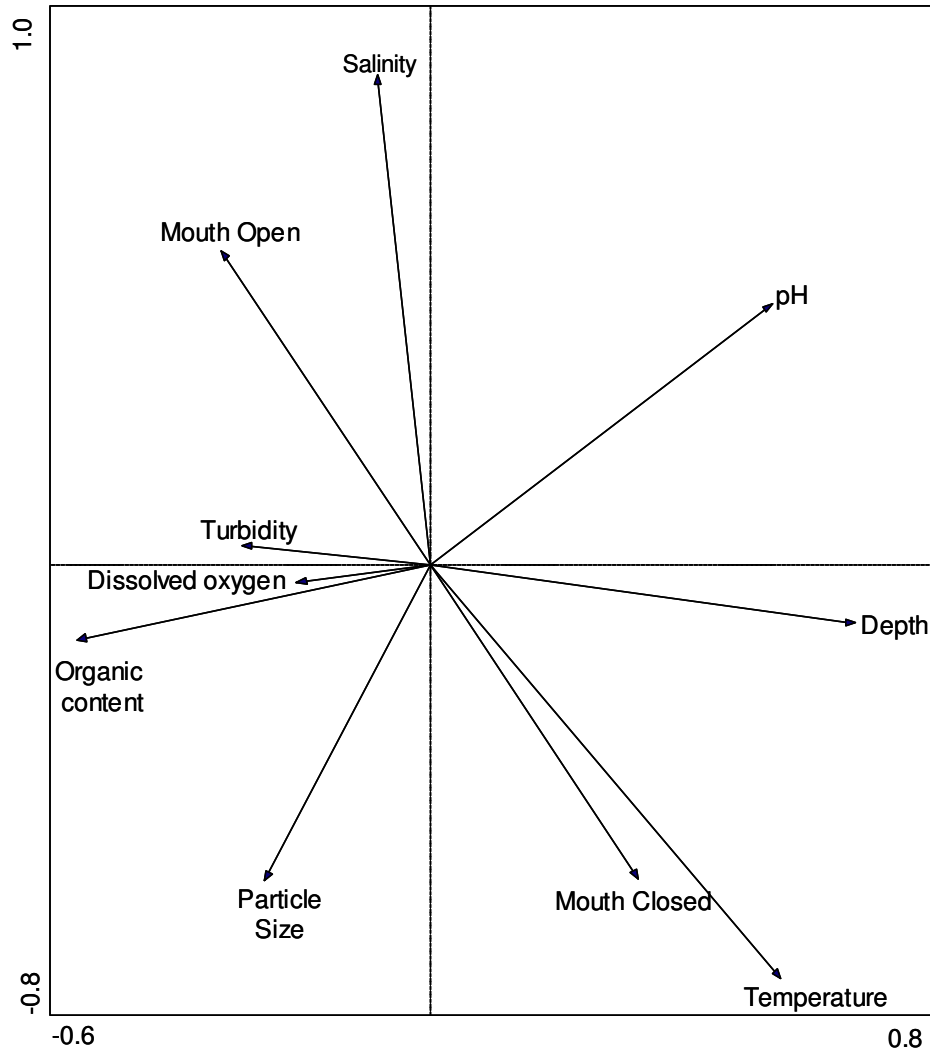


Figure 3.6 Principal component analysis (PCA) of the physical water quality parameters and sediment characteristics of the Nhlabane Estuary over the period 1996-1999.

3.3.5 Benthic species composition

A total of 32 taxa were recorded from the six sampling sites during the four year study period (Table 3.2). The total number of taxa recorded per year increased from 21 in 1996 to 29 in 1999, while there were no

notable differences in the total number of taxa between sites, which ranged between 26-29. The benthic community was dominated by amphipods, notably *Corophium triaenonyx* and *Grandidierella lignorum*, while the polychaetes *Ceratonereis keiskamma*, *Desdemona ornata* and *Prionospio sexoculata* and the tanaid *Apseudes digitalis* each contributed more than 4% to the total densities recorded. Other numerically important taxa included the bivalve *Hyathula linulata*, the cumacid *Iphinoe truncata* and oligochaetes. These nine taxa constituted 96% of the benthic organisms recorded.

Table 3.2 The benthic taxa recorded during the study period, with mean densities (N.m⁻²) per year and per site and the percentage contribution of each taxon to the total densities.

Taxa	Year				Site						Mean	% Cont
	96	97	98	99	1	2	3	4	5	6		
NEMATODA												
Nematoda spp		9	45	6	5	4	40	11	6	23	15	0.07
PLATYHELMINTHES												
Platyhelminthes spp			7	3	1	2	6	2	2	2	2	0.01
OLIGOCHAETA												
Oligochaeta spp.	52	367	456	1211	48	310	186	728	1602	253	521	2.59
POLYCHAETA												
<i>Capitella capitata</i>	2				2	1					0.4	0.00
<i>Ceratonereis keiskamma</i>	498	843	1411	2115	633	1655	1333	797	1567	1315	1217	6.05
<i>Dendronereis arborifera</i>			2	32	19	15	14	2		2	8	0.04
<i>Desdemona ornata</i>	1685	1440	903	402	544	874	1885	1124	1311	905	1107	5.50
<i>Glycera subaena</i>	24	24	7	49	49	42	50	7	9	1	26	0.13
<i>Prionospio sexoculata</i>	3185	523	1907	792	1668	1314	191	1438	2895	2105	1602	7.96
Polydora spp			1	1	2					1	0.4	0.01
HIRUDINIAE												
Hirudinae spp	1	12	95	26	23	58	54	19	31	14	33	0.16
GASTROPODA												
<i>Assiminea ovata</i>			179	210	101	34	419	7	4	19	97	0.48
<i>Melanoides tuberculatus</i>	1	0.4	74	1	1	12	71	23	5	3	19	0.09
BIVALVIA												
<i>Hyathula linulata</i>	156	902	1051	480	223	727	1569	623	259	484	648	3.22
<i>Musculus virgiliae</i>	9	4	1	0.4	3	10	1	6	1	2	4	0.02
<i>Solen cylindraceus</i>	1				2						0.4	0.01
CRUSTACEA												
<i>Iphinoe truncata</i>		12	181	1419	777	164	1309	104	38	25	403	2.00
<i>Afrochiltonia capensis</i>		6	1	14			1	7	3	21	5	0.03
<i>Boltisia minuta</i>	4		129	32	8	74	18	68	56	23	41	0.21
<i>Corophium triaenonyx</i>	5103	4512	13437	9234	10461	10870	6531	9192	6167	5208	8071	40.12
<i>Grandidierella lignorum</i>	3762	2080	6386	7214	3606	3731	3121	6759	6718	5229	4861	24.16
<i>Melita zeylanica</i>			1	2	2	1			3		1	0.01
<i>Cyathura carinata</i>	1	2	35	369	18	139	9	296	141	4	101	0.50
<i>Leptanthura laevigata</i>	7	32	77	383	386	335	17	8	3		125	0.62
<i>Munna sheltoni</i>	1	15	103	328	21	148	59	77	275	91	112	0.56
<i>Mesopodopsis africana</i>				2		3					1	0.01
<i>Apseudes digitalis</i>	956	1285	657	625	594	857	31	3024	646	133	881	4.38
<i>Hymenosoma orbiculare</i>	5	1	27	9	14	24	10	12	3	2	11	0.05
Ostracod spp	7		11	73	2	11	1	51	56	17	23	0.11
ARANEAE												
Acarina spp		1								1	1	0.00
INSECTA												
Chironomid spp	333	119	234	10	24	19	99	329	242	332	174	0.87
SIPUNCULIDAE												
Sipunculid spp		11	9	2	3	2	8	11	3	7	6	0.03
Total	15792	12200	27426	25046	19236	21435	17029	24725	22047	16220	20116	
Number of taxa	21	22	28	29	29	28	26	26	26	27	32	

The dominant taxa recorded during each sampling year are presented in Figure 3.7, while the dominant taxa recorded at each site are presented in Figure 3.8. The amphipods *C. triaenonyx* and *G. lignorum* were the numerically dominant taxa throughout the study period, contributing more than 50% of the benthic organisms recorded (Figure 3.7). These were followed by the polychaetes *P. sexoculata*, *D. ornata* and *C. keiskamma*, the tanaid *A. digitalis* and the bivalve *H. linulata*.

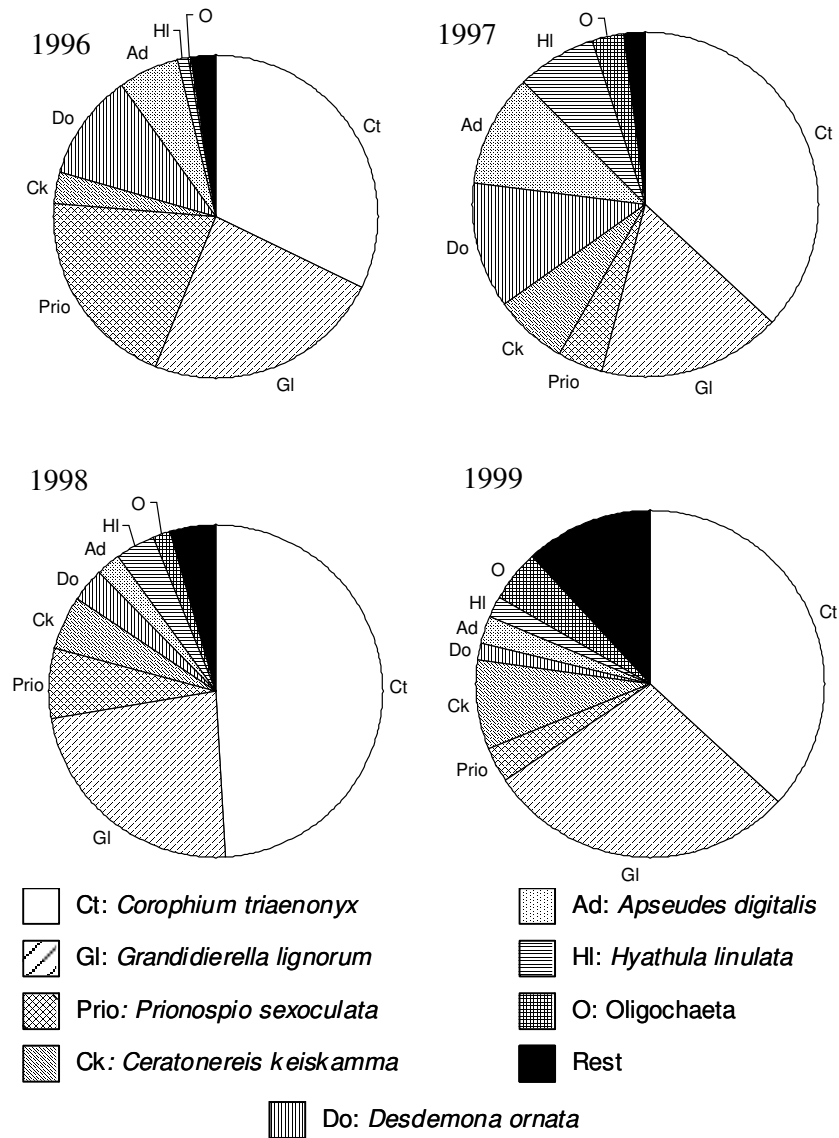


Figure 3.7 The numerically dominant taxa recorded during the sampling years 1996-1999 in the Nhlabane Estuary.

There were no marked differences in the benthic composition along the length of the system during the study period. The amphipod *C. triaenonyx* completely dominated the benthic community in the lower reaches, accounting for more than 50% of the benthos recorded (Figure 3.8). This was followed by *G. lignorum* and the three polychaetes *P. sexoculata*, *D. ornata* and *C. keiskamma*. In the upper reaches, *G. lignorum* became the dominant amphipod, although *C. triaenonyx* remained abundant.

All the dominant taxa were present during all sampling years, and at all sampling sites. Of the 32 taxa recorded, 17 were present during each of the four sampling years, while three taxa were present during a single year only. The taxa absent during 1996 or 1997, but present during 1998-1999, included the amphipods *Melita zeylanica* and *Africhiltonia capensis*, the gastropod *Assimineea ovata* and the polychaetes *Dendronereis arborifera* and *Polydora* spp. The polychaete *Capitella capitata* and the gastropod *Solen cylindraceus* were only present during 1996. The majority of taxa (72%) were recorded at all six sampling sites, while *Mesopodopsis africana*, *S. cylindraceus* and *C. capitata* were restricted to the lower reaches.

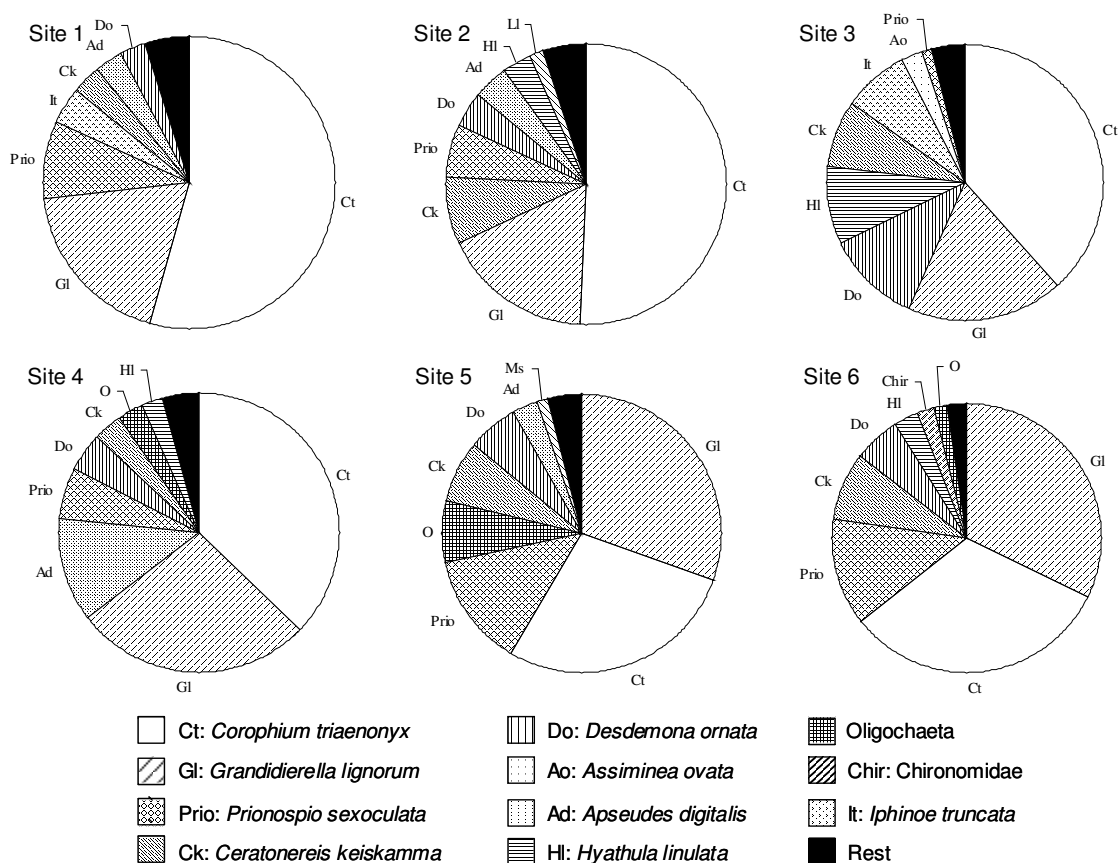


Figure 3.8 The numerically dominant benthic taxa recorded at Sites 1-6 in the Nhlabane Estuary during 1996-1999.

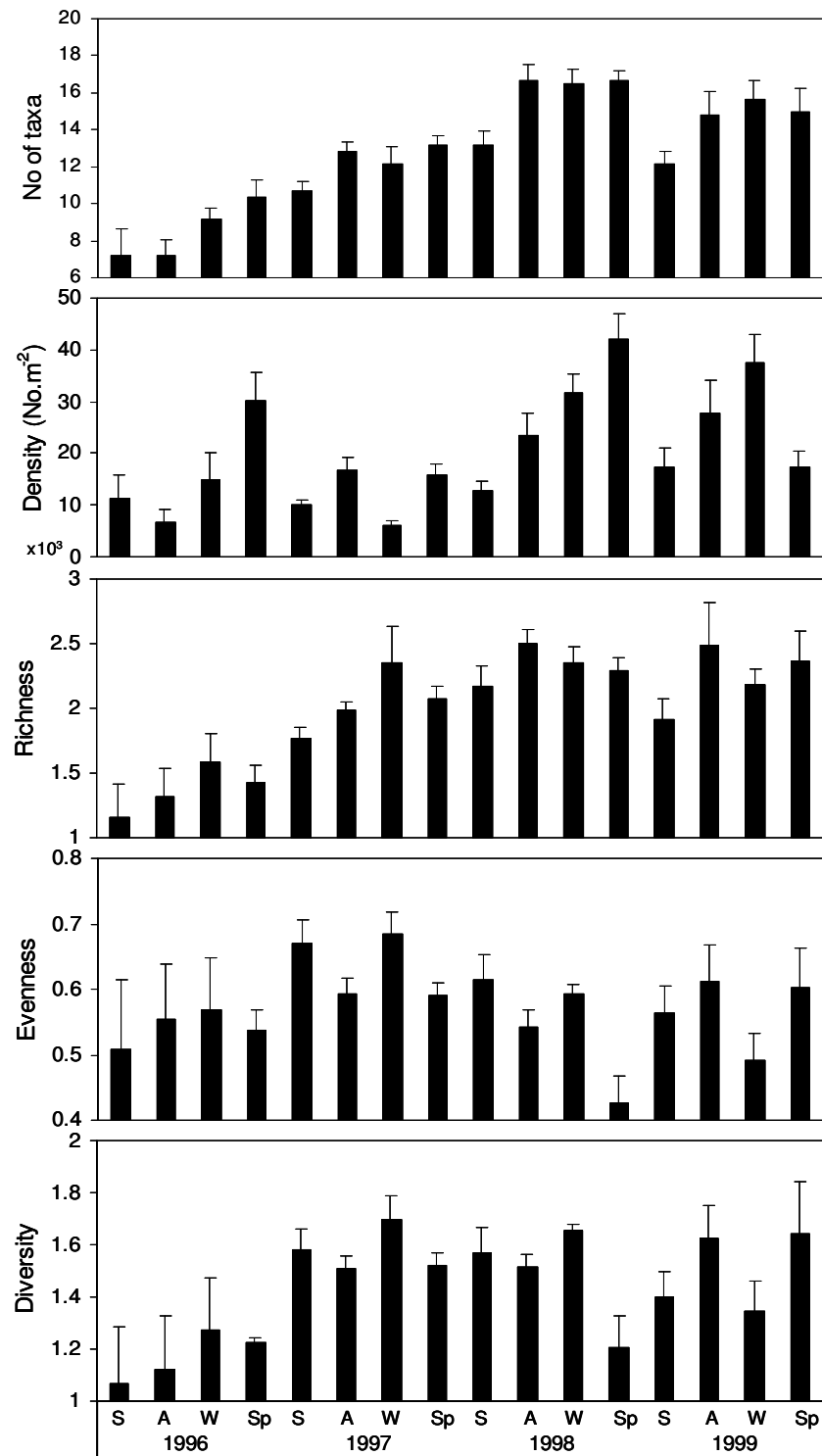


Figure 3.9 Changes in the number of taxa, densities, species richness, evenness and diversity (+1SE) in the benthic community over the 16 season sampling period 1996-1999 in the Nhlabane Estuary. Summer (S), Autumn (A), Winter (W), Spring (Sp).

The analysis of univariate indices of the benthic community are presented in Table 3.3, Figure 3.9 and Figure 3.10. There were significant changes in the number of taxa, density, species richness and species diversity over the 16 seasonal samples during the 1996-1996 study period (Figure 3.9, Table 3.3), but not between sampling sites (Figure 3.10, Table 3.3). The number of taxa, species richness and species diversity gradually increased during 1996 and 1997, reaching highs during 1998 (Figure 3.9). Benthic densities, with the exception of spring 1996, were considerably lower in 1996 and 1997 compared to 1998 and 1999. Highest densities were recorded during spring 1998 and winter 1999.

Table 3.3 ANOVA table for the univariate indices of the Nhlabane Estuary benthic community recorded during 1996-1999. ANOVA by sample refers to analysis of the 16 seasonal samples collected over the 1996-1999 period. * $P < 0.05$ ** $P < 0.005$ *** $P < 0.0005$.

Variable	ANOVA by Sample		ANOVA by site		ANOVA by year		ANOVA by season	
	Variable range	F	Variable range	F	Variable range	F	Variable range	F
No of taxa	7.2-16.7	12.43***	11.3-14.2	1.37 ns	8.5-15.8	41.07***	10.3-13.8	3.40*
Density	5974-41991	7.65***	16228-24703	0.88 ns	12161-27415	8.29***	12839-26313	4.67**
Species richness	1.2-2.5	5.44***	1.8-2.2	1.25 ns	1.4-2.3	21.29***	1.75-2.1	1.97 ns
Evenness	0.42-0.68	1.52 ns	0.45-0.58	3.05*	0.54-0.63	2.96 ns	0.53-0.58	0.69 ns
Diversity	1.16-1.53	2.59**	1.20-1.53	2.98*	1.12-1.57	7.82***	1.40-1.49	0.38 ns
d.f.	15,80		5,90		3,92		3,92	

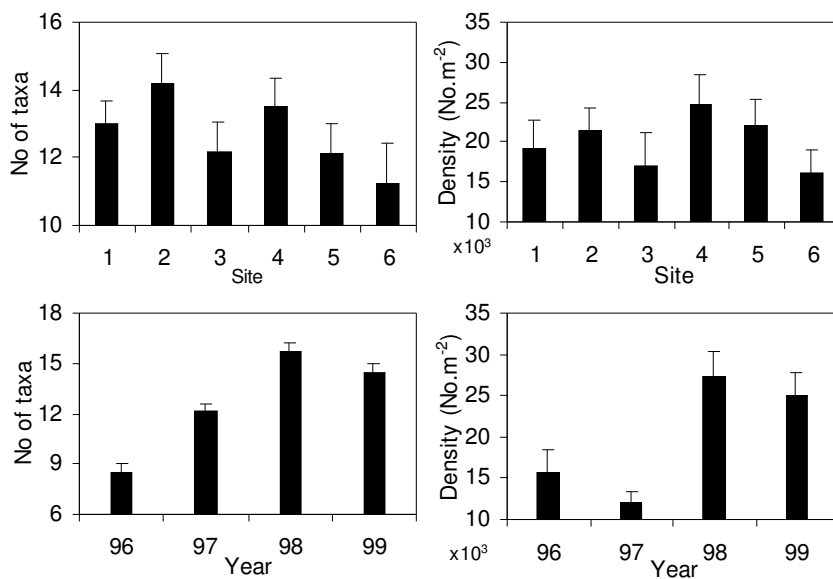


Figure 3.10 Changes in the mean number of taxa and densities (+1SE) per site and per year as recorded in the Nhlabane Estuary over the period 1996-1999.

These changes over the 16 sampling seasons were also reflected in a comparison of the benthic community between sampling years, with significant changes in all but species evenness. The mean number of taxa recorded during the four sampling years increased significantly from 7.1 in 1996 to 16.7 in 1998 (Figure 3.10, Table 3.3). Mean densities per year also changed significantly, increasing from 12 199 N.m⁻² in 1997 to 27 424 N.m⁻² in 1998. In contrast, there were no significant differences in the number of taxa, densities and species richness between sampling sites over the four year period, although there were significant differences in species evenness and diversity, with highest values at Sites 2-5 and lowest values at Sites 6 (Figure 3.10, Table 3.3). The highest densities and number of taxa were recorded at Sites 2 and 4, while lowest values were recorded in the upper reaches at Site 6. There were also significant differences in the number of taxa and density between seasons, with lowest values recorded during summer (February), increasing gradually after summer through the year to spring. There were no significant changes in species richness, evenness and diversity between seasons.

The change in species diversity of the benthic assemblage over the four sampling years is further illustrated in Figure 3.11. In a k-dominance plot, the more elevated curves represent less diverse assemblages. Diversity of the benthic assemblage increased from 1996 through to 1998, followed by a slight decrease in 1999.

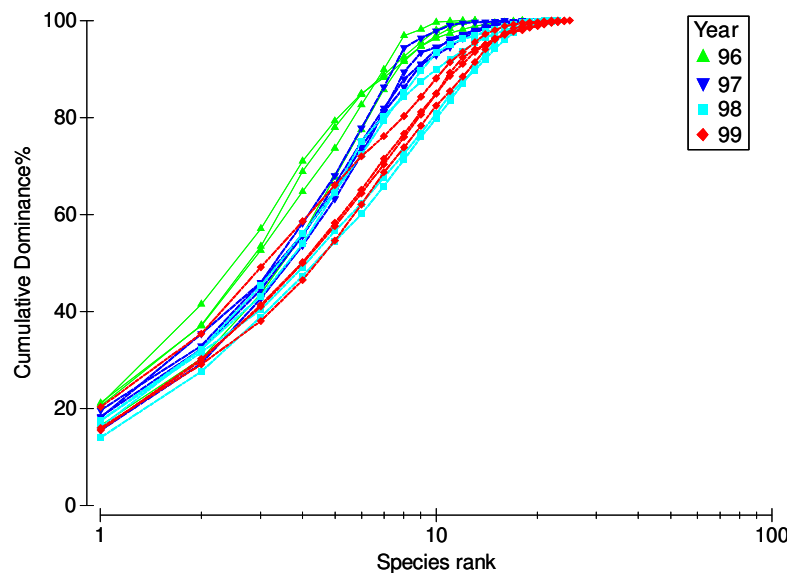


Figure 3.10 k-Dominance plot of the zoobenthic community per year as recorded in the Nhlabane Estuary over the period 1996-1999.

3.3.6 Temporal and spatial changes in the benthic community

Multivariate ordination revealed temporal and spatial shifts in the benthic community during the study period. Firstly, the benthic community recorded at Site 6 during early 1996 (summer and autumn) was so different from that recorded during the rest of the study period, that it completely masked all other temporal and spatial differences in the benthic community during the sampling period. Benthic densities from these samples were very low and only one and three taxa were recorded during summer and autumn 1996, respectively. Due to the strong influence of these samples on the benthic ordination, and in order to allow more detailed analysis of the benthic community during the four years of sampling, these two Site 6 samples from 1996 were removed from subsequent ordination analysis.

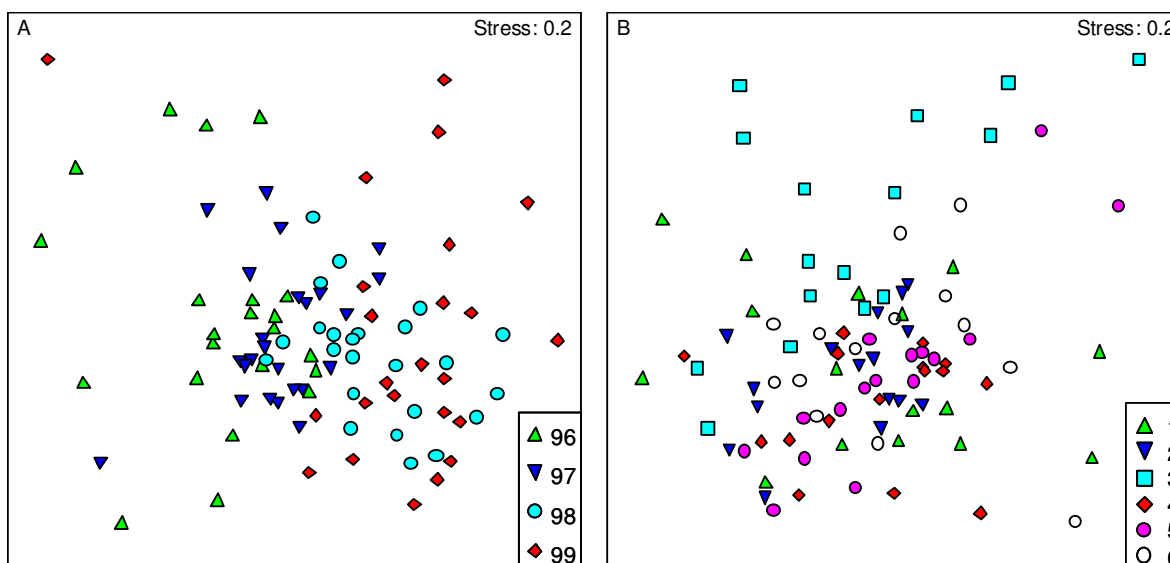


Figure 3.12 Multidimensional ordination (MDS) of the Nhlabane Estuary benthic community in relation to sampling year (A) and sampling site (B).

There was a marked temporal change in the benthic community from 1996 through to 1999 (Figure 3.12a). This represented a gradual shift in the benthic community during the four-year study period, reflecting the recovery in the Nhlabane Estuary following the extended low salinity closed mouth conditions during the 1992-1995 drought. The almost complete separation in the ordination between the 1996 and 1999 samples is indicative of the extent to which the benthic community changed over the four-year study period. These temporal changes in the benthic community were confirmed by analysis of similarity between groups of community samples (ANOSIM), which revealed highly significant differences in community structure between sampling years ($R = 0.541$, $p = 0.001$). The biggest changes were observed within the 1996 and 1999 benthic communities (Figure 3.12a), while the 1997 and 1998 benthic samples were grouped closer

together in the ordination, indicating greater uniformity in the community structure during these years.

In contrast, there were no significant differences in the benthic assemblage between sampling sites (Figure 3.12b) ($R = 0.091$, $p > 0.05$), indicating relative spatial uniformity in the benthic community of the estuary.

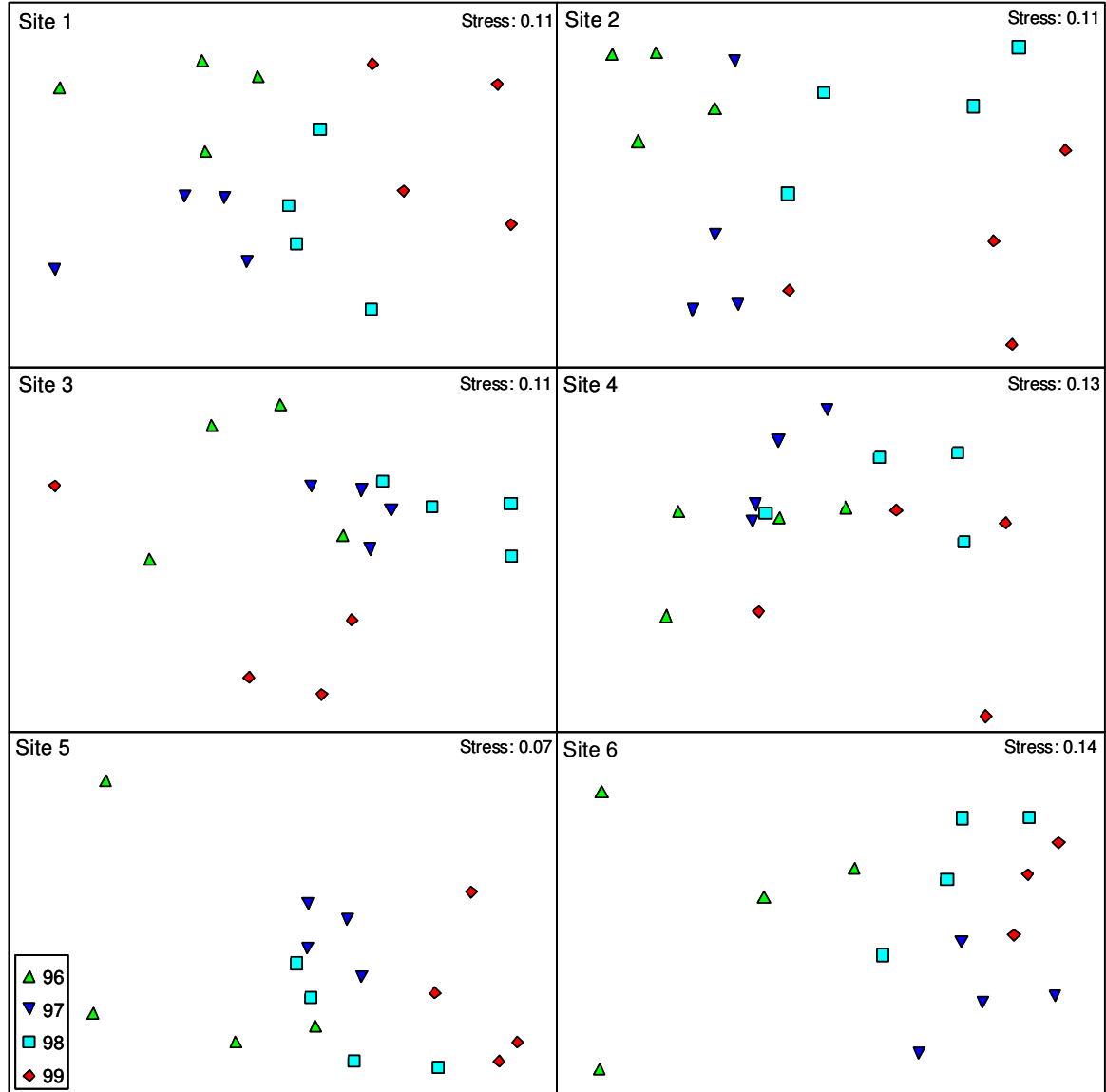


Figure 3.13 Multidimensional ordination (MDS) of the benthos recorded over the period 1996-1999 at Sites 1-6 during the period 1996-1999.

Analysis of the benthic community at each of the six sampling sites over the study period, revealed a similar gradual temporal shift in the benthic community at each site, i.e. from 1996, through 1997-1998 to 1999 (Figure 3.13). At all sites, the benthic community differed significantly between years (Site 1: $R =$

0.53, $p < 0.001$; Site 2: $R = 0.51$, $p < 0.001$; Site 3: $R = 0.43$, $p < 0.001$; Site 4: $R = 0.24$, $p = 0.01$; Site 5: $R = 0.34$, $p < 0.001$; Site 6: $R = 0.35$, $p < 0.001$). Notable changes were observed during 1996, particularly at Sites 5 and 6, and more specifically during the first two seasons of 1996. This indicates that the benthic community of the estuary underwent substantial changes throughout the study period, but mostly so during 1996, and particularly at sites 5 and 6. These changes were relatively uniform along the length of the estuary at all sampling sites, except at Site 3 (Figure 3.13). The Site 3 community showed a typical temporal change from 1996 to 1998, but the 1999 community showed a shift back towards the 1996 community, particularly during May 1999. The benthic community during May 1999 was depleted with a total density of only 556 N.m^{-2} from 11 taxa, compared to a density of 5534 N.m^{-2} at this site during February. The Site 2 and Site 4 benthic communities during February 1999 were also quite different from the rest of the 1999 samples, indicating a substantial reduction in benthic densities during this time.

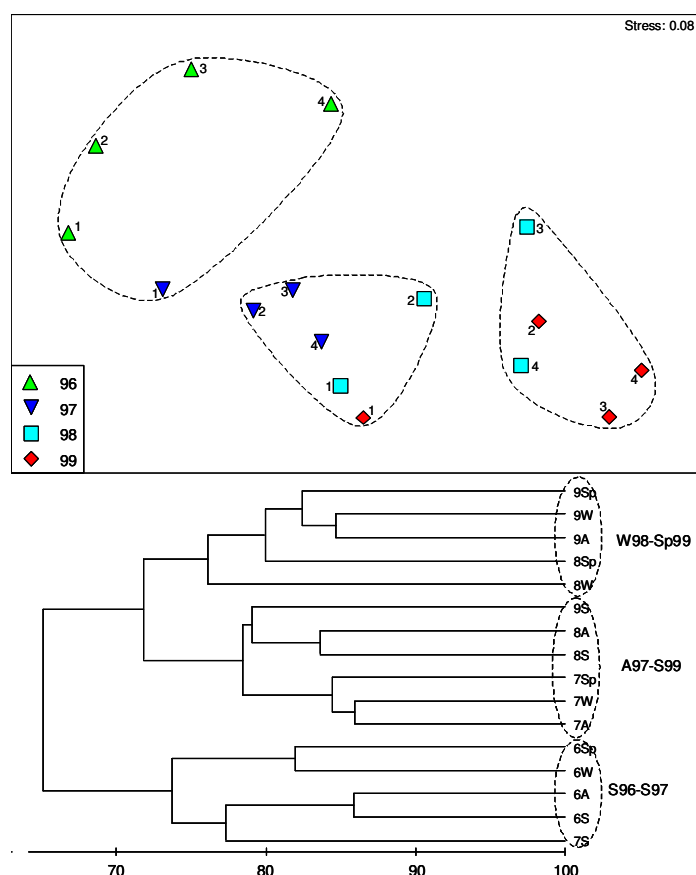


Figure 3.14 MDS plot and cluster dendrogram of the Nhlabane Estuary benthic community during the period 1996-1999. 1 = summer (S), 2 = autumn (A), 3 = winter (W), 4 = spring (Sp).

The temporal shift in the benthic community was further investigated by averaging the benthic data from the different sampling sites during each season (Figure 3.14). The analysis revealed a substantial shift in

the benthic community during the study period, particularly during 1996. Three distinct community groups were identified in the ordination, these being the 1996 and summer 1997 samples, the autumn-spring 1997 and summer-autumn 1998 samples and finally the late 1998 and 1999 samples. The 1996 and early 1997 group was separated from the remainder at a similarity level of 64%.

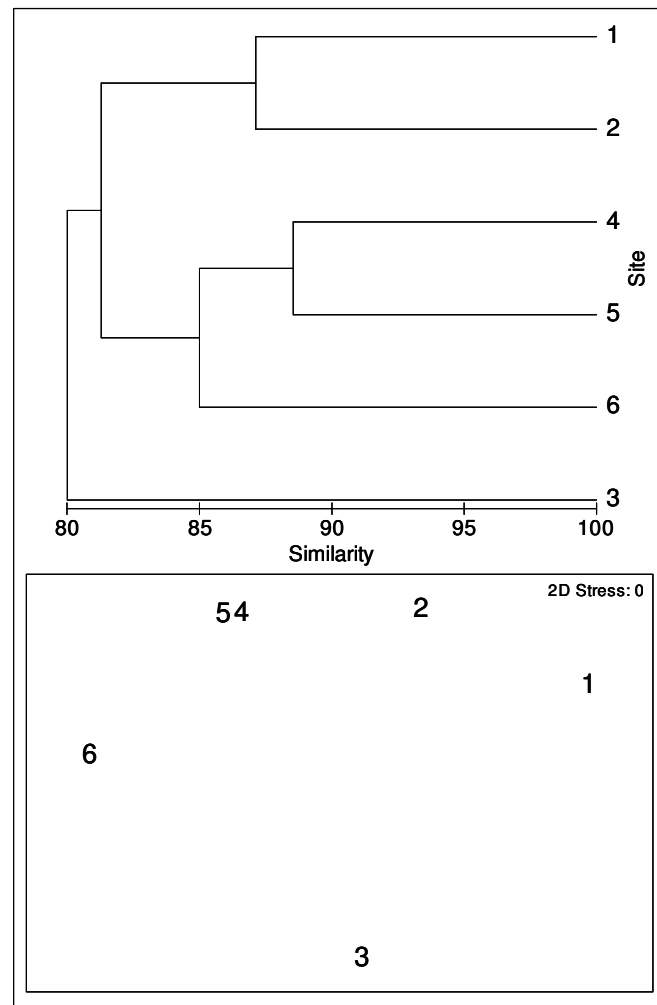


Figure 3.15 Hierarchical cluster and multidimensional ordination of the averaged benthic community recorded at Sites 1-6 during the period 1996-1999.

In order to gain better insight into the spatial distribution of the benthic community, the effect of the marked temporal variation in the benthic data was removed by averaging the data over the 16 sampling seasons. Inspection of the averaged benthic community revealed a strong linear spatial gradient from Site 1 at the mouth through the system to Site 6 in the upper reaches (Figure 3.15). The benthic community at Site 3 was exceptional as it did not follow this spatial pattern, but was found to more dissimilar to that of Sites 2 and 4 than the distant Sites 1 and 6. In contrast, the benthic communities of Sites 4 and 5 were

very similar, even though these sites were considerably further apart than Sites 3 and 4.

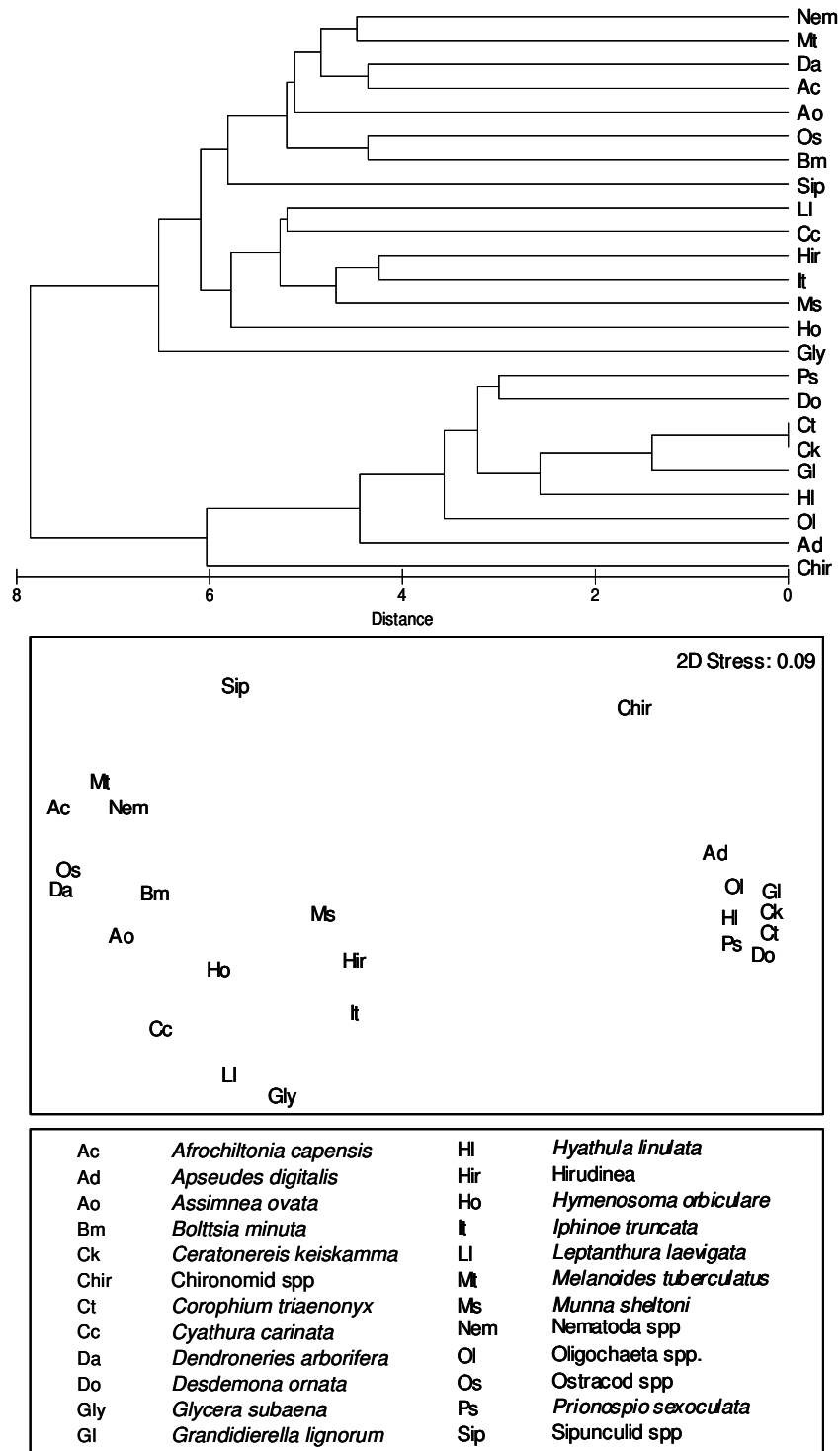


Figure 3.16 Hierarchical cluster and multidimensional ordination showing the association between benthic species within the benthic community in the Nhlabane Estuary during the period 1996-1999.

Table 3.4 Contribution of the zoobenthic taxa to the mean Bray-Curtis similarity within the three community groups identified in the community ordination during the period 1996-1999 in the Nhlabane Estuary using SIMPER analysis (See Figure 3.16). Mean % similarity defines the mean similarity within groups. Mean % dissimilarity defines the extent of dissimilarity between groups. Only higher contributing taxa, making up to 95% of total abundance, were included in the analysis.

Group 1: Average similarity: 54.9			
Species	Mean Density	Mean Similarity	% Contribution
<i>Corophium triaenonyx</i>	4942	12.8	23.2
<i>Grandidierella lignorum</i>	3196	10.3	18.7
<i>Desdemona ornata</i>	1442	7.9	14.3
<i>Ceratonereis keiskamma</i>	569	7.7	13.9
<i>Prionospio sexoculata</i>	2680	6.9	12.6
<i>Hiathula linulata</i>	437	2.8	5.0
<i>Apseudes digitalis</i>	912	2.8	5.0
Chironomidae	278	1.9	3.5
Group 2: Average similarity: 67.7			
Species	Mean Density	Mean Similarity	% Contribution
<i>Corophium triaenonyx</i>	6242	15.7	23.1
<i>Grandidierella lignorum</i>	3148	12.2	18.0
<i>Ceratonereis keiskamma</i>	1266	9.1	13.4
<i>Desdemona ornata</i>	1364	7.2	10.6
<i>Hiathula linulata</i>	755	5.5	8.1
<i>Apseudes digitalis</i>	1302	4.9	7.2
Oligochaeta	326	4.9	7.2
<i>Ceratonereis keiskamma</i>	540	4.3	6.4
Chironomidae	104	1.8	2.6
Group 3: Average similarity: 62.1			
Species	Mean Density	Mean Similarity	% Contribution
<i>Corophium triaenonyx</i>	13394	13.4	21.7
<i>Grandidierella lignorum</i>	8580	12.1	19.5
<i>Ceratonereis keiskamma</i>	1804	7.6	12.2
<i>Prionospio sexoculata</i>	1797	5.5	8.8
<i>Hiathula linulata</i>	729	4.6	7.4
<i>Ipanoe truncata</i>	1259	3.8	6.2
<i>Desdemona ornata</i>	465	2.7	4.4
<i>Munna sheltoni</i>	339	2.5	4.1
Oligochaeta	1150	2.5	4.0
<i>Apseudes digitalis</i>	344	1.7	2.7
Hirudinae	85	1.4	2.2
<i>Cyathura carinata</i>	300	1.0	1.6
<i>Bolttsia minuta</i>	125	0.9	1.5
Mean % dissimilarity between groups			
	Group 1	Group 2	Group 3
Group 1			
Group 2	41.2		
Group 3	50.7	41.1	

3.3.7 Association between benthic taxa

The association between benthic taxa within the community structure of the Nhlabane Estuary based on the presence/absence of the taxa is shown in Figure 3.16. The contribution of the benthic taxa to the mean similarity within the three main community groups and the mean dissimilarity between groups are presented in Table 3.4. Two main groups were formed on the ordination plot, the first of which comprised the dominant taxa that were present during most of the study and were recorded throughout the estuary. This included the two amphipod and four polychaete taxa that dominated the benthic community throughout the study, as well as the tanaid *A. digitalis* and the gastropod *H. linolata*. As shown in Table 3.4, these were also the taxa contributing most towards the mean similarity within the three community groups and as such were most responsible for structuring the benthic assemblage during the study period, and with the exception of oligochaetes, formed the dominant taxa in all three community groups. The second group of taxa identified in the ordination contained those taxa that were recorded infrequently. Of these, the cumacid *Ipinoe truncata*, the isopod *Munna sheltoni*, and the Hirudinae were most common, while the amphipod *Afrochiltonia capensis* and the polychaete *Dendronereis arborifera* were only recorded infrequently at higher salinities in the lower reaches.

The average similarity within a community groups is an indication of the degree of variation within the group, with lower similarities reflecting a more unstable community dominated by a few abundant taxa. The average similarity within the 1996-early 1997 community group was 54.9%, compared to the 67.7% within the 1997-1998 group (Table 3.4). In the former group, only eight taxa contributed 95% of the total density, whereas in the late 1998-1999 group, with an average similarity of 62.1%, 13 taxa contributed 95% of the total density. There was an average dissimilarity between community groups 1 and 3 of 50.7%, compared to an average dissimilarity of about 41% between groups 1 and 2 and groups 1 and 3. This is an indication of the extent of change within the benthic community during the study period.

3.3.8 Correlation between species assemblage and environmental variables

There was a significant correlation between the benthic assemblage and environmental variables as shown through the BIOENV analysis (Table 3.5). The best combination of variables responsible for structuring the benthic assemblage of the Nhlabane Estuary was pH, depth, dissolved oxygen concentration and sediment organic content ($p = 0.315$). This was followed by a combination of pH, depth, dissolved oxygen concentration, sediment organic content, and salinity. The most important single factor in structuring the benthic assemblage was pH, followed by organic content and water depth (Table 3.5). The variables usually important in structuring the benthic assemblage in estuaries, i.e. salinity and sediment particle size, were 5th ($p = 0.079$) and 7th ($p = 0.041$) on the list, respectively.

Table 3.5. Weighted Spearman Correlation coefficients between environmental variables and the benthic community structure. The best combination of variables indicated in bold.

Number of variables	Correlation ρ	Variable 1	Variable 2	Variable 3	Variable 4	Variable 5
1	0.238	pH				
1	0.174	Organic content				
1	0.096	Depth				
1	0.094	Dissolved oxygen				
1	0.079	Salinity				
1	0.069	Temperature				
2	0.286	pH	Organic content			
2	0.254	pH	Depth			
3	0.301	pH	Depth	Organic content		
3	0.287	pH	Organic content	Dissolved oxygen		
4	0.315	pH	Depth	Organic content	Dissolved oxygen	
4	0.297	pH	Depth	Organic content	Temperature	
5	0.314	pH	Depth	Organic content	Dissolved oxygen	Salinity
5	0.313	pH	Depth	Organic content	Dissolved oxygen	Temperature

Sample statistic (Rho): $\rho=0.315$, Significance level: $p=0.01$

Table 3.6 Canonical Correspondence Analysis (CCA) eigenvalues of the four principal components obtained from the correlation between the benthic assemblage and environmental variables in the Nhlabane Estuary during the period 1996-1999. Also presented is the variance accounted for by each environmental variable, as well as the significance of the contribution of each variable to the benthic assemblage.

Axis	1	2	3	4
Eigenvalues	0.238	0.196	0.16	0.113
Cumulative percentage variance	23.8	43.4	59.4	70.8
Variable	Lambda	F	P	
pH	0.06	5.82	0.002	
Organic content	0.05	4.25	0.002	
Depth	0.03	3.22	0.002	
Temperature	0.03	3.22	0.002	
Dissolved oxygen	0.02	1.8	0.012	
Salinity	0.01	1.76	0.024	
Turbidity	0.02	1.19	0.248	
Particle size	0.01	0.92	0.542	

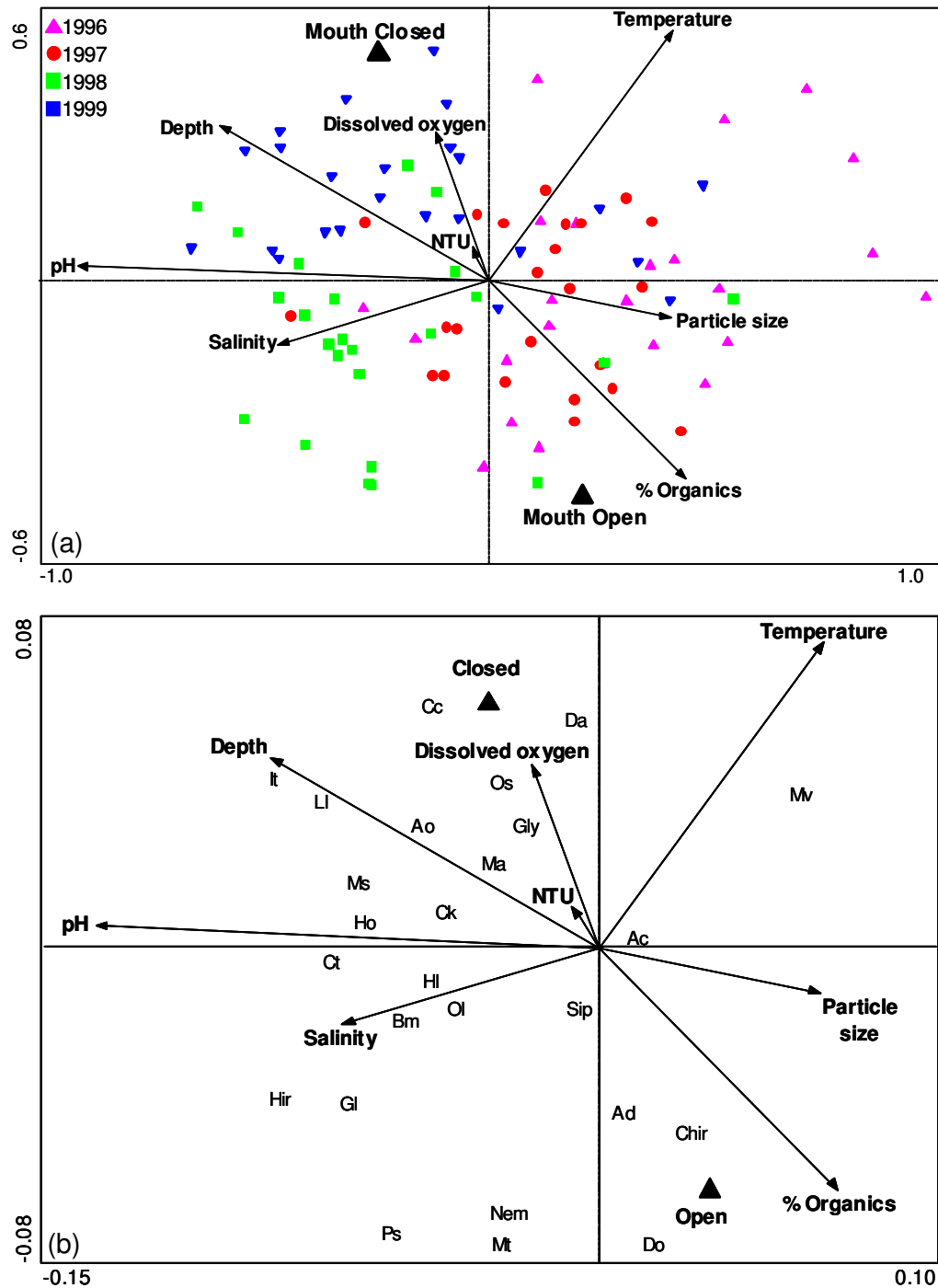


Figure 3.17 Canonical Correspondence Analysis (CCA) plots of the correlation between the benthic assemblage and environmental variables in the Nhlabane Estuary during the period 1996-1999. (a) is a biplot indicating the correlation between environmental variables and the benthic samples collected during the four sampling years. (b) is a biplot indicating the correlation between environmental variables and the benthic taxa. (see Figure 3.16 for taxa names).

These results were confirmed by the Canonical Correspondence Analysis (CCA), which showed that pH, organic content of the sediment, water depth, temperature and dissolved oxygen concentration were the environmental variables best correlated with the benthic assemblage (Table 3.6, Figure 3.17). The only variables not significantly correlated with the benthic assemblage were turbidity and sediment particle size (Table 3.6). The fact that a number of environmental variables were found to contribute significantly in structuring the benthic assemblage, suggests that the community was structured by a number of variables and not just by one or two. The first two principal components accounted for only 43.4% of the variance, thus more than 50% of the variance was accounted for by the remaining principal components. This relative equal weighting of a number of environmental variables in structuring the benthic assemblage is also clearly evident in the CCA biplots. In the environmental variables vs benthic sample biplot (Figure 3.17a), the role of pH and salinity in structuring the benthic assemblage during 1998-1999 can be seen. The effect of salinity on the benthic assemblage was strangely unrelated to mouth condition, but oxygen concentrations and water depth were closely related to closed mouth conditions. Temperature, which represents natural seasonal variability and was found to be an important variable in structuring the benthic assemblage, showed no correlation with mouth condition, water depth or sediment characteristics. More importantly, the effect of temperature on the benthic assemblage was inversely related to salinity.

Table 3.7 Correlation coefficient values of the correlation between the dominant benthic taxa and water and sediment quality variables in the Nhlabane Estuary over the period 1996-1999. Red indicates a significant correlation.

Taxa	Salinity	Turbidity	Temperature	pH	Dissolved Oxygen	Depth	Particle Size	Organic content	Mud Content
<i>Apseudes digitalis</i>	-0.201	0.192	0.064	-0.280*	-0.125	0.015	0.062	0.460*	0.137
<i>Ceratonereis keiskamma</i>	0.122	0.071	0.052	0.203*	-0.180	0.115	-0.247*	-0.238*	-0.188
Chironomid spp	-0.171	-0.027	-0.055	-0.118	0.038	-0.197	0.208*	0.325*	-0.053
<i>Corophium triaenonyx</i>	0.083	0.081	-0.122	0.334*	0.130	0.310*	-0.146	-0.135	0.028
<i>Desdemona ornata</i>	0.047	-0.153	-0.164	-0.200	-0.168	-0.209*	-0.035	0.030	-0.067
<i>Grandidierella lignorum</i>	0.264*	-0.035	-0.276*	0.177	-0.060	-0.080	-0.080	-0.193	0.031
<i>Hyathula linulata</i>	-0.038	-0.029	-0.027	0.239*	0.008	-0.008	0.083	-0.064	-0.119
<i>Iphinoe truncata</i>	0.130	0.000	-0.049	0.144	0.049	0.218*	0.026	-0.291*	-0.135
Oligochaeta spp.	0.074	-0.022	-0.047	0.149	-0.088	0.121	-0.387*	-0.198	-0.004
<i>Prionospio sexoculata</i>	0.267*	-0.026	-0.339*	0.001	-0.136	-0.197	-0.044	0.056	-0.006

The distribution of the benthic taxa in relation to the environmental variables is shown in Figure 3.17b. The distribution of the two most abundant amphipods, *C. triaenonyx* and *G. lignorum*, as well as *Bolttsia minuta*, the bivalve *Hyathula linulata* and oligochaetes, were correlated with higher salinities. The distribution of detrital feeders such as the polychaete *D. ornata*, the tanaid *A. digitalis* and Chironomidae

was related to organic content in the sediment, but not to pH and salinity. The opportunistic polychaete, *P. sexoculata*, appeared to thrive in high salinity and high organic content conditions. The cumacid *I. truncata* and the isopod *L. laevigata* were associated with deeper water, while the bivalve *Musculaus virgiliae*, was most abundant in low salinities.

With the use of multiple regression analysis, the correlation between the ten most abundant taxa and the environmental variables were investigated (Table 3.7). The correlations coefficients were generally low, even those that were significant, indicating that benthic distribution was influenced by a number of environmental variables. None of the benthic taxa showed a significant correlation with turbidity, dissolved oxygen and mud content, so these variables were of lesser importance in the distribution of the benthic taxa. Only *G. lignorum* and *P. sexoculata* showed a significant positive correlation with salinity and temperature, while *A. digitalis* and chironomid larvae showed a negative correlation with salinity. Oligochaetes and *C. keiskamma* abundance showed a significant preference for coarser sediment, while chironomid larvae showed a significant preference for finer sediment. Two taxa, *A. digitalis* and oligochaetes, showed a significant preference for sediment with high organic content, while *C. keiskamma* and *I. truncata* preferred sediment with low organic content. *Corophium triaenonyx* and *I. truncata* were most abundant in deeper areas, while *D. ornata* preferred shallower areas.

3.3.9 Recovery and recolonization of the benthic community

At the start of the study period, the benthic community was depleted, particularly in the upper reaches at Site 6, where during February and May 1996, only one and three taxa were recorded, respectively. After breaching of the estuary mouth in August 1995, the lower half of the estuary (Sites 1-3) was exposed to marine influence, but marine water was largely prevented from reaching the upper half of the estuary due to the berms walls still being in place, which were only removed after the second sampling session in May 1996. Recovery of the benthic community in the lower half of the estuary thus started shortly after breaching in August 1995, i.e. four months before the onset of this study, while recovery in the upper reaches were delayed for a further nine months until the berm walls were removed in late May 1996, i.e. after the first two sampling seasons of 1996. Changes in the dominant benthic taxa from the lower, middle and upper reaches of the estuary in relation to recovery of the benthic community following breaching of the system in August 1995 and removal of the berm walls in May 1996, are indicated in Figure 3.18.

Recovery of the benthic community of the Nhlabane Estuary after the 1992-1995 drought period was evident not only in the marked increase in the number of taxa, densities, species richness and diversity from early 1996 through to mid 1998 (Figure 3.9 and 3.10), but also in the changes in the abundance of individual taxa (Figure 3.18). Table 3.8 presents information on the taxa found to be 1st level colonizers, transition taxa and equilibrium taxa (Cardoso *et al.* 2007). Prior to removal of the berm walls, the upper

reaches were dominated by Chironomidae dipteran larvae, which are organisms known to be tolerant of disturbed and hypoxic estuarine environments. The first notable change in the benthic community of the upper reaches occurred shortly after the berm walls were removed and estuarine conditions were re-established in the upper reaches. This phase, which occurred at the time when the benthic community was severely depleted, was evident in a distinct and rapid increase in the abundance of small opportunistic polychaetes, *Prionospio sexoculata* and *Desdemonia ornata*. These species were rapidly out-competed and replaced by larger species, particularly *G. lignorum*, while another amphipod, *C. triaenonyx*, also showed a notable increase in numbers. This was followed by a gradual increase in the abundance of previously dominant taxa such as *G. lignorum*, *C. triaenonyx* and *C. keiskamma*. Taxa such as *Munna sheltoni*, *Hyathula linulata* and *Bolittsia minuta* only appeared much later, when estuarine conditions had been re-established.

Table 3.8 Taxa characteristic of the different stages of recolonization during recovery of the Nhlabane Estuary benthic community at the six sampling sites over the period 1996-1998. Stages according to Cardoso *et al.* 2007.

Site	1st level opportunists	2nd level opportunists and transition taxa	Equilibrium taxa
Site 1		<i>D. ornata</i> , <i>C. triaenonyx</i> , <i>P. sexoculata</i> , <i>A. digitalis</i> , <i>G. lignorum</i>	<i>C. keiskamma</i> , <i>C. triaenonyx</i> , <i>P. sexoculata</i> , <i>A. digitalis</i> , <i>G. lignorum</i> , <i>H. linulata</i> , <i>L. laevigata</i> , <i>M. sheltoni</i> , <i>C. aestuaria</i> , <i>I. truncata</i> , <i>A. ovata</i>
Site 2		<i>D. ornata</i> , <i>C. triaenonyx</i> , <i>Oligochaetae</i> , <i>G. lignorum</i>	<i>C. keiskamma</i> , <i>C. triaenonyx</i> , <i>G. lignorum</i> , <i>H. linulata</i> , <i>L. laevigata</i> , <i>M. sheltoni</i> , <i>C. aestuaria</i> , <i>I. truncata</i> , <i>B. minuta</i>
Site 3		<i>D. ornata</i> , <i>C. triaenonyx</i> , <i>Oligochaetae</i> , <i>G. lignorum</i>	<i>C. keiskamma</i> , <i>C. triaenonyx</i> , <i>G. lignorum</i> , <i>H. linulata</i> , <i>L. laevigata</i> , <i>M. sheltoni</i> , <i>C. aestuaria</i>
Site 4	<i>P. sexoculata</i> , <i>D. ornata</i>	<i>D. ornata</i> , <i>C. triaenonyx</i> , <i>Oligochaetae</i> , <i>G. lignorum</i> , <i>P. sexoculata</i> , <i>A. digitalis</i>	<i>C. keiskamma</i> , <i>C. triaenonyx</i> , <i>G. lignorum</i> , <i>H. linulata</i> , <i>M. sheltoni</i> , <i>C. aestuaria</i> , <i>I. truncata</i> , <i>B. minuta</i> , <i>A. digitalis</i>
Site 5	<i>P. sexoculata</i> , <i>D. ornata</i> , <i>Oligochaeta</i>	<i>C. triaenonyx</i> , <i>Oligochaeta</i> , <i>G. lignorum</i> , <i>P. sexoculata</i> , <i>A. digitalis</i>	<i>C. keiskamma</i> , <i>C. triaenonyx</i> , <i>G. lignorum</i> , <i>H. linulata</i> , <i>M. sheltoni</i> , <i>C. aestuaria</i> , <i>I. truncata</i> , <i>B. minuta</i>
Site 6	<i>P. sexoculata</i> , <i>D. ornata</i>	<i>C. triaenonyx</i> , <i>G. lignorum</i> , <i>A. digitalis</i> , <i>D. ornata</i>	<i>C. keiskamma</i> , <i>C. triaenonyx</i> , <i>G. lignorum</i> , <i>H. linulata</i> , <i>M. sheltoni</i> , <i>I. truncata</i>

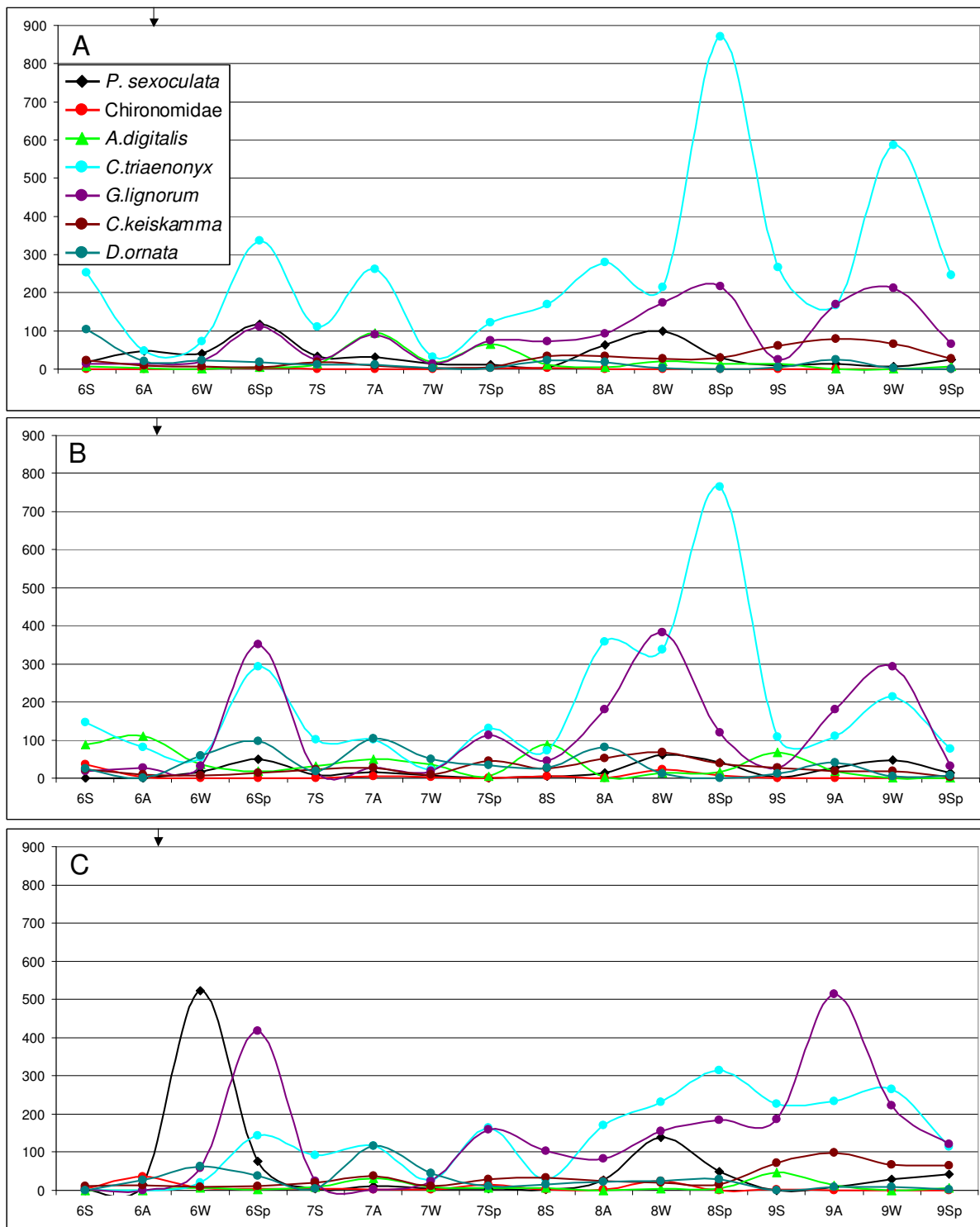


Figure 3.18. Changes in the seasonal densities (N.m⁻²) of the dominant taxa in the lower (A), middle (B) and upper (C) reaches of the Nhlabane Estuary over the period 1996-1999. The arrow indicates when the berm walls were removed. 6 = 1996, 7 = 1997, 8 = 1998, 9 = 1999

The estuarine habitat in the middle reaches was severely disturbed by the process of berm wall removal, as the area between the two berm walls had to be filled with sand to displace silt trapped between the walls and then dredged out. While the abundance of most taxa were depressed by these activities, the numbers of the opportunistic polychaetes, notably *D. ornata*, increased. This was again followed by a rapid increase in the numbers of the two dominant amphipods, *G. lignorum* and *C. triaenonyx*. This was again followed by a gradual increase in the number of taxa such as *C. keiskamma* and *A. digitalis*, while taxa such as *M. sheltoni*, *H. linulata*, *B. minuta*, *Cyathura aestuaria* and *Iphinoe truncata* only returned much later (Table 3.2). In the lower reaches, the start of the study was marked by relatively high numbers of dominant estuarine taxa such as *C. triaenonyx*, while the high numbers of *D. ornata* suggested that the initial phase of recovery had occurred in 1995 prior to the onset of the study.

3.3.10 Comparison with 1992-1995 drought benthic community

In order to gain further insight into changes in the benthic community during the study period, a multivariate analysis comparison was conducted between the benthic community recorded during the current study with that recorded during 1992-1995 drought period (Vivier and Cyrus 1999a), when the mouth of the estuary remained closed and the estuary became relatively fresh (Figure 3.19). Analysis of Similarity (ANOSIM) revealed that there was a significant difference between the benthic assemblage of the drought and post-drought periods ($R = 0.532$, $p = 0.001$). The classification and MDS ordination analysis showed a cyclical temporal shift in benthic community during the 1992-1999 period (Figure 3.19). This involved a gradual but distinct shift in the benthic community during the drought period away from the 1992 community, followed by a gradual return during the 1996-1999 post-drought period to the pre-drought community. Three groups were identified in the cluster analysis at a 65% similarity. Group I consisted of the 1992 and early 1993 community, while Group II comprised the remaining 1993 and all of the 1994-1995 samples, including the last sample of 1995 after the mouth was breached and the estuary was re-opened. Group III comprised the 1996-1999 post-drought assemblage. The 1992 community showed a greater similarity with the 1996-1999 post-drought community (62%) than with the 1993-1995 drought community (55%), indicating firstly the extend of change caused by the drought in the 1992-1995 community. This also indicates the extend of recovery in the post-drought community back towards the community that existed at the start of the drought.

3.4 Discussion

3.4.1 Benthic composition

The information in the literature on the benthic community of the Nhlabane Estuary is limited to a study conducted over the period 1992-1995 on the impact of the 1993 dredger crossing of the estuary (Vivier *et al.* 1998, Vivier and Cyrus 1999a, 1999b, MacKay and Cyrus 2001). No quantitative historical data is available on the system prior to 1992. Begg (1978) reported that “regrettably, there is very little information available on the fauna” of the system and Steffen, Robertson and Kirsten (1989), in an EIA of the 1993 Nhlabane Estuary dredger crossing, stated that “nothing definite is known about components of the aquatic fauna, such as crabs, prawns and mollusks.” The Coastal Research Unit of Zululand, University of Zululand, initiated a study on the water quality and biota of the Nhlabane system in 1992, of which the present study constitutes a component.

The 32 taxa recorded during the period 1996-1999 compares favorably with the 24 taxa reported from the Nhlabane Estuary during the 1992-1995 study (Vivier *et al.* 1998, Vivier and Cyrus 1999a, 1999b). The taxa present during the present study but not during 1992-1995 included organisms such as the polychaete *Glycera subaena* and the cumacid *Iphinoe truncata*, while all the taxa recorded during the 1992-1995 period were found during the present study. This indicates that changes in the benthic community following the 1992-1995 period of extended mouth closure and freshwater conditions, involved not only temporal and spatial changes in the assemblage of existing taxa, but also recruitment of 8 new taxa into the system.

Of the 259 estuaries along the South African coastline, 182 are classified as TOCE. Of the 73 estuaries found along the KwaZulu-Natal coastline, 59 are intermittently open (Whitfield 2000). The benthic composition of the Nhlabane Estuary, a small TOCE, was comparable to that recorded in other intermittently open systems, such as the Siyaya, Wilderness and Bot River estuaries (Davies 1984, De Decker and Bally 1985, Whitfield and Bruton 1989, Whitfield 1992, Cyrus and MacKay 1995, Mackay 1996). Being a TOCE (Whitfield 1992), the Nhlabane Estuary characteristically showed a relatively low benthic faunal richness compared to that recorded in many open tidal estuaries (Whitfield and Bruton 1989). In a study of 28 estuaries in south-eastern Australia, it was shown that entrance status of an estuary at the time of sampling was determinant in structuring the composition of benthic macrofauna, with open systems being more diverse due to open entrances being able to facilitate the intrusion of marine species (Hastie and Smith 2006). The Mhlathuze estuary, a permanently open estuarine embayment, showed a total of 86 benthic taxa, which were dominated by organisms that were primarily marine in origin (Mackay and Cyrus 1999). The 28 taxa recorded in the semi-permanently open Mlalazi estuary (Mabaso 2002) was

regarded as poor for an open subtropical estuary since such estuaries are known to be highly diverse (Whitfield 1992, Currie and Small 2005). Hill (1966) recorded 84 macrobenthic taxa in the Mlalazi estuary, which was suggested to be poor for a subtropical estuary in relation to the 378 species recorded by Day (1974) in the Morumbene estuary. Even though the Mlalazi estuary is mostly open and therefore has a more stable salinity gradient than the intermittently open Nhlabane Estuary, the dominant benthic taxa were remarkably similar in the two systems. The Mlalazi estuary was dominated by the polychaetes *Prionospio* spp and *D. arborifera*, the amphipod *C. triaenonyx*, the tanaid *A. digitalis* and the bivalve *M. virgiliae* (Mabaso 2002). With the exception of *M. virgiliae*, all these were dominant taxa in the Nhlabane Estuary.

The benthos in the Nhlabane Estuary was characterized by an abundance of estuarine crustaceans, notably the two amphipods *G. lignorum* and *C. triaenonyx*, and the polychaetes *C. keiskamma*, *P. sexoculata* and *D. ornata*, with these five estuarine species accounting for 83% of the benthic numbers. These taxa appear to be relatively common in east coast estuaries, and except for *D. ornata*, were also identified as the most abundant taxa in Eastern Cape estuaries (Teske and Wooldridge 2001). The fact that these taxa were abundant in all four types of Eastern Cape estuaries suggest that they are true estuarine endemics whose distribution in warm-temperate and subtropical estuaries is relatively independent of salinity or sediment type.

There were remarkable similarities in the dominant benthic taxa of the Nhlabane Estuary with that of a number of other intermittently open estuaries. This was particularly the case with the Wilderness estuary, even though the two systems are 1200 km apart and the Wilderness estuary is located in a warm-temperate region. Both systems were dominated by the amphipods *G. lignorum* and *C. triaenonyx*, the polychaete *C. keiskamma* and the tanaid *A. digitalis* (Davies 1984). The Nhlabane Estuary also shared a number of ecological characteristics with the Siyaya estuary, a nearby small intermittently open subtropical estuary (MacKay and Cyrus 2001). The estuarine benthic components of the two systems were very similar, and both systems were dominated by the amphipods *G. lignorum* and *C. triaenonyx* and the polychaete *C. keiskamma*. In both systems, flow dynamics had been altered by weirs in their upper reaches and both remained closed during the 1992-1995 drought. Even though a total of 88 taxa were recorded in the Siyaya estuary, compared to 32 taxa in the Nhlabane, the majority of these were freshwater taxa, which dominated the Siyaya estuary during extreme low salinity conditions.

The most notable feature of the Nhlabane Estuary benthos during the present study was the way in which the two amphipods *G. lignorum* and *C. triaenonyx* dominated the benthic community at all sampling sites throughout the study. The contribution of these two taxa at each of the six sampling sites ranged between 57-73%. This complete numerical domination by two estuarine amphipods, which was also shown in the

Siyaya and Wilderness estuaries (Davies 1984, Mackay 1996), but not in the larger Mfolozi (Ngqulana *et al.* 2010), Mlalazi (Mabaso 2002), Mhlathuze (Mackay and Cyrus 1999) and Mdloti estuaries (Blaber and Hay 1984) and the St Lucia system, appears to be characteristic of small intermittently open systems. Both *C. triaenonyx* and *G. lignorum* are euryhaline amphipods with a high tolerance to salinity and sediment variability and are common in low salinity estuaries and relict estuarine freshwater lakes in Zululand but not in larger permanently open systems (Vivier and Cyrus 1999a). Although polychaetes and amphipods are generally regarded as dominant organisms in estuaries and lagoons, estuaries worldwide are usually dominated by polychaetes (Stoner and Acevedo 1990). Polychaete worms, in particular, being generally abundant members of estuarine macrofauna communities, are represented by more genera in estuaries than any other group of organisms (Giangrande *et al.* 2005). Mackay and Cyrus (1999) reported that polychaetes formed the most abundant component of the Mhlathuze estuary benthos, while Mabaso (2002) found polychaetes to be the dominant group in terms of density and the number of taxa in the Mlalazi estuary.

3.4.2 Benthic assemblage and environmental variables

The distribution of organisms relative to their habitat is of central importance to ecology. Knowledge of the spatial distribution patterns of benthos along estuarine gradients might help to identify the linkages between species distributions and ecological processes and therefore to gain insight into the functioning of estuarine ecosystems. The nature of this distribution also provides an insight into the types of ecological processes that regulate populations and assemblages. Numerous studies have demonstrated that spatial distributions of benthic invertebrates in estuaries are affected by environmental variables (Mackay and Cyrus 2001, Teske and Wooldridge 2003, 2004, Nanami *et al.* 2005, Hastie and Smith 2006). These studies have suggested that variability in physical factors (e.g., water depth, grain size and mouth condition), chemical factors (e.g., salinity, dissolved oxygen and pH) and biological factors (e.g., macrophyte and seagrass density, predation) all contribute to provide diverse habitats in estuaries, and species specific habitat association of organisms in response to such environmental variability is common (Gonzalez-Oreja and Saiz-Salinas 1998). Snelgrove (1999) concluded that distributional patterns of individual species of shallow-water benthic fauna are reported to be largely controlled by abiotic factors at the broader scale and by biotic factors at finer scales.

Estuaries provide diverse habitats for aquatic organisms since they are located at the interface between a river and the sea, and there is often rapid and large-scale variability in physical and chemical conditions due to changes in freshwater and marine influences. Estuaries can be seen as biologically active systems structured by abiotic factors which contribute to create a complex of gradients which may determine the distribution and abundance of animal communities (Hastie and Smith 2006). To survive, benthic

organisms require a certain combination of physical and chemical conditions (Gray 1989). The effect that physico-chemical variables have on a benthic organism depends not only on the environmental preferences of the organism, but also on the tolerance range of the organism to those variables (Edgar and Barret 2002). The spatial distribution of macrobenthic species in relation to environmental variables is thus species-specific, suggesting that environmental variability provided by the estuarine environment will support diverse benthic animal communities (Flint and Kalke 1986). Many estuarine benthic species are able to inhabit most parts of an estuarine environment, because of their wide-ranging environmental tolerances, but some are more specific in their habitat preference and therefore have a more limited distribution. For example, stenohaline taxa are restricted in their distribution to lower saline ends of estuaries, while others might have strong preferences for a certain type of sediment. Teske and Wooldridge (2003) found salinity to be most important in structuring benthic communities in the upper and lower extremes of permanently open estuaries, whereas sediment type was more important at the intermediate sites, as well as throughout closed estuaries where salinity tended to be more uniform.

Results from the present study confirmed this species-specific preference for specific environmental conditions. Of the ten dominant taxa in the Nhlabane Estuary, only *G. lignorum* and *P. sexoculata* showed a significant positive correlation with salinity, while *A. digitalis* and chironomid larvae showed a negative correlation. Oligochaetes and *C. keiskamma* showed a significant preference for coarser sediment, while chironomid larvae showed a significant preference for finer sediment. Only two taxa, *A. digitalis* and oligochaetes, showed a significant positive correlation with sediment organic content.

A combination of physico-chemical factors usually affects the distribution of benthic organisms. Very seldom can a single factor be pointed out as the sole determining cause of changes in community structure, particularly on a long-term basis (Mackay and Cyrus 2001, Carvalho *et al.* 2005). Nevertheless, salinity and sediment characteristics have often been shown to be major influential environmental factors affecting the distribution of benthic organisms and numerous studies have reported that the spatial distribution of macrobenthic invertebrates along an estuarine gradient is related to sediment composition and/or salinity (Teske and Wooldridge 2003). Wolff (1983) argued that salinity is the most important variable in structuring estuarine benthic communities, whereas other studies have shown that differences in sediment type were more influential than salinity in determining the structure of benthic communities (Teske and Wooldridge 2004). In large open systems, the distribution and abundance of soft-sediment benthos have been related to sediment particle size structure and depth (Snelgrove 1999, Currie and Small 2005). In brackish Lake Hinuma, *Prionospio japonica* was most abundant in areas with larger grain size and higher salinity, while *Grandidierella japonica*, *Cyathura sp.*, *Melita sp.* and *Assimineia japonica*, were more abundant in areas with smaller grain size and lower salinity (Nanami *et al.* 2005). In Eastern Cape estuaries, the cumacean *I. truncata* was associated with sandy areas, while the tanaid *A. digitalis*, the

isopod *Cyathura aestuaria* and the polychaete *Dendronereis arborifera* were characterized as mud zone species.

The nature of the sediment was found to be more important than salinity in thirteen Eastern Cape estuaries representing all four estuarine types (see Whitfield 1992), in limiting the distribution of macrobenthos (Teske and Wooldridge 2003). These authors argued that this may set South African estuaries apart from northern hemisphere systems, in which salinity has been shown to be the major factor influencing regional distributions, while sediment characteristics strongly influenced local distributions. They further argued that this might be the result of South African estuaries being dominated by species whose distribution is relatively independent of salinity, and to which the nature of the sediment and associated variables are consequently more important variables. However, Carvalho *et al.* (2005), reported that in the permanently open Obidos lagoon in Portugal, it was generally observed that, although some taxa were associated with a given sediment-type habitat, their distribution was rarely restricted to that habitat.

Numerous studies have also reported that sediment characteristics, viz grain size and organic content, are some of the most important factors responsible for spatial distribution of macrobenthic species, particularly in terms of feeding types (Teske and Wooldridge 2003, 2004, Nanami *et al.* 2005). That is, suspension-feeders (e.g., bivalves) are usually more abundant in sandy areas in which tidal currents prevent accumulation of detritus on the bottom and brings potential food to the suspension-feeders. In contrast, deposit-feeders such as polychaetes and amphipods are usually more abundant in a muddy area in which the abundant organic matter provide an adequate source of nutrition for a large number of deposit-feeders. In organically enriched muddy sediments of estuarine systems in Portugal, deposit feeders were found to be the dominant feeding category, while suspension-feeders and carnivores were more associated with clean sandy sediments (Carvalho *et al.* 2005). In the Nhlabane Estuary, the sediment along the entire estuary consisted of medium to fine sands, so the distinction between benthic feeding types in relation to sediment characteristics was not as clear as in most other estuaries. There was no mud in the Nhlabane Estuary, and the sediment ranged from fine sand in the upper reaches to medium sand in the lower reaches, while the organic content of the sediment was low throughout. Estuaries are usually characterized by organic rich, muddy substrate in the upper to middle reaches due to settling out of fine sediment brought down from the catchment by riverine flow, and coarser marine dominated sediment in the lower reaches close to the mouth (Stoner and Acevedo 1990). Mud is the most common type of bottom in estuarine systems (Vernberg 1983). This was not the case in the Nhlabane Estuary, where the presence of the Nhlabane weir caused the fine sediment to settle out in Nhlabane Lake, with only relatively clear sediment-free water flowing into the estuary. Breaching of the estuary mouth also coincided with substantial scouring of the bottom sediments, thereby removing fine sediment and epibenthic organic material from the system (Huizenga and Van Niekerk 1998). In the Tongati and Mdloti estuaries, the lack

of mud in the upper reaches was suggested to be detrimental to the establishment of a rich infaunal benthos (Blaber and Hay 1984).

The reduction in fine sediment in estuarine environments due to impoundment of freshwater heads of estuaries have been shown in numerous studies (Whitfield and Bruton 1989, Atrill and Power 2000). Due to the relative uniformity of the sediment in the Nhlabane Estuary, sediment particle size was therefore not expected to play a major role in the distribution of benthic organisms in the estuary. In contrast, sediment organic content was shown to be the 2nd most important variable in forging the benthic community. The relatively low organic content recorded throughout the estuary suggests that organic material might be an important limiting factors in the distribution of many Nhlabane Estuary benthic taxa, and that species which have a competitive advantage in utilizing this limited food resource, will proliferate. Studies have shown that *Corophium* amphipods attain most of their nutrition not by eating detritus, but by gleaning the thin film of microflora, particularly the epipellic diatoms, and bacterial growth from the surface of particulate matter (Hughes and Gerdol 1997). In the organic poor sediments of the Nhlabane Estuary, this ability would allow these amphipods to make optimal use of a limited food source and allow them to outcompete other detritivores. This is perhaps one of the reasons why the amphipod such as *C. triaenonyx*, and *G. lignorum*, both of which are tubiculous suspension and a detritus feeders, were relatively abundant throughout the Nhlabane Estuary and occur commonly in most east coast estuaries, as they are able to optimally utilize the organic poor fine grained sand (Teske and Wooldridge 2003). The polychaetes *Desdemona ornata*, a suspension feeder, and *Prionospio sexoculata*, a detritus feeder, were also consistently recorded in the same area throughout the Nhlabane system during the study period. The low sediment organic content in the Nhlabane Estuary is related to the low nutrient levels recorded in the estuary, which could be attributed to the absence of allochthonous nutrient input through freshwater inflow, reflected in the low nitrate and phosphate concentrations at the weir (Quinn 1999). The estuary is thus oligotrophic in nature and chlorophyll-a concentrations are low. The freshwater input into the estuary is limited to overtopping of the weir, which does not allow for significant sediment and nutrient export. The emergent *Phragmites* beds appear to be the main source of nutrients in the estuary since the water that flows over the weir have reduced suspended matter and associated nutrients (Quinn 1999).

Salinity and sediment type, which usually are important in structuring benthic communities, were therefore relatively unimportant in influencing the Nhlabane Estuary benthos, to the extent that secondary limiting factors such as organic content, became important. Thus, despite the large fluctuations in salinity during the study period, salinity was not shown to be an important determining factor in the Nhlabane Estuary benthos. In thirteen Eastern Cape estuaries studied by Teske and Wooldridge (2003), a large proportion of the numerically dominant taxa were also found to be relatively independent of salinity and sediment. These included taxa such as *C. triaenonyx*, *G. lignorum* and *Prionospio* sp, which were also the

three most abundant taxa in the Nhlabane Estuary. As shown in the present study, these taxa remained abundant throughout the study period, in all sections of the estuary and under differing salinity conditions. These taxa can therefore be considered to be true estuarine endemic taxa whose distribution is not closely associated with sediment or salinity, but whose abundance and distribution might be affected by biotic constraints such as food availability, competition and predation (Flint and Kalke 1986, Teske and Wooldridge 2003, 2004). In the Blackwater River estuary in England, the distribution of *Corophium volutator*, a common inhabitant of European intertidal mudflats, was attributed to their dispersal behaviour of swimming on the flood tide, which would sweep the amphipods into the semi-enclosed creeks and bays, away from the open mudflats (Hughes and Gerdol 1997). The distribution of this amphipod was also negatively correlated with the abundance of a common polychaete, *Nereis diversicolor*, because of sediment disturbance caused by the polychaete, which deterred recruitment. High densities of microflora and a high organic matter content of the sediment have also been suggested to influence the distribution of *C. volutator* (Hughes and Gerdol 1997) and Jensen and Kristensen (1990) concluded that the local distribution of *C. volutator* was influenced by biotic rather than by abiotic factors.

3.4.3 Recovery of benthic community

The continued functioning of Southern African estuaries relies on the maintenance of their natural dynamism and of the natural range of oscillations imposed on these systems by riverine and marine influences (Whitfield and Bruton 1989). The Nhlabane Estuary is not located in an industrialized and urbanized environment and as such does not suffer directly from the pollution effluent fate found in most estuaries worldwide. Lake Nhlabane, whose catchment is dominated by forestry, is an oligotrophic lake with relatively good water quality (Vivier and Cyrus 1999a). The Nhlabane Estuary is, however, under pressure due to artificial alteration in its flow dynamics caused by the Nhlabane weir and water abstraction from the lake. The overexploitation of freshwater resources, particularly during drought conditions, often has the effect of forcing estuaries into extreme states with regard to their natural functioning and has deleterious consequences for the ability of these systems to maintain viable estuarine biotic communities. Whitfield and Bruton (1989) noted that the effects of impoundment and water abstraction on small intermittently open estuaries included a reduction in frequency and magnitude of floods, a lack of freshwater inflow during droughts, more rapid and more frequent mouth closure, a reduction in nutrient and organic material input and a decline in biotic diversity during prolonged closed phases. It should be noted that, in contrast to small Eastern Cape and most small estuaries around the world that become hypersaline during droughts and prolonged closed phases (Whitfield and Bruton 1989, Hastie and Smith 2006), small subtropical estuaries along the South African coastline, such as the Nhlabane Estuary, tend to become fresh when closed (MacKay and Cyrus 2001).

The Nhlabane Estuary was subjected to a regional drought over the period 1992-1995 during which no freshwater flowed over the Nhlabane weir, with the result that the estuary mouth remained closed from late 1991 to mid 1995. The system became fresh, with salinities throughout the system decreasing to between 1-5 and macrobenthic densities and diversity declining markedly (Vivier and Cyrus 1999b). In addition, two earthen berm walls were constructed across the middle reaches of the system in 1993 to allow passage of a RBM mining dredger across the middle of the estuary. The berm walls divided the system in half for almost three years and caused habitat changes in the estuary, preventing water and biotic movement between the upper and lower reaches and causing the estuary to develop into two separate bodies of water which attained different water qualities (Vivier *et al.* 1998). The benthos in the upper reaches became severely depleted and only one and three taxa were recorded from the head of the estuary (Site 6) during February and May 2006, respectively (Vivier and Cyrus 1999b).

During 1995, the Nhlabane Estuary was therefore characterized by a severely stressed benthic community, caused by a combination of a naturally occurring drought event exacerbated by two anthropogenically induced factors. The presence of the Nhlabane weir, coupled with continued abstraction of water from Lake Nhlabane for mining purposes, affected natural water level fluctuations in the estuary by keeping the water level artificially low, thereby ruling out any chance of the estuary breaching by itself. The resultant extended closure of the estuary mouth inhibited interaction between the estuarine and marine environments (Vivier and Cyrus 1999b). The 2nd factor was caused by the berm walls being in place across the middle reaches of the estuary for three years, causing the upper reaches to become completely freshwater dominated as well as preventing flow of marine water to the upper reaches after the system was artificially breached in August 1995.

Measures to restore the ecological functioning of the Nhlabane Estuary were initiated in August 1995 when the estuary was artificially breached and regular contact with the marine environment was restored. The berm walls were only removed in May 1996 after which the middle reaches of the estuary were rehabilitated and the channel restored (Vivier *et al.* 1998). Recovery of the estuarine habitat was therefore initiated in two phases. Breaching of the mouth in August 1995 re-established a marine influence in the lower half of the estuary, but the presence of the berm walls prevented saline water from reaching the upper half of the estuary. Recovery in this part of the estuary was only initiated after May 1996, when the berm walls were removed. The present study therefore offered the opportunity to investigate the process of recovery of the benthic community in the Nhlabane Estuary, particularly in the upper reaches, from a severely depleted freshwater condition to a marine influenced estuarine condition.

Because estuaries are such naturally variable environments, most organisms living in estuaries are able to survive within the natural range of variability shown by factors such as salinity, turbidity, temperature,

oxygen and nutrients. Organisms are eliminated when the natural range of variability in the estuarine environment is exceeded, usually as a result of added anthropogenic stress. We therefore have to distinguish between natural stress and anthropogenic stress in the estuarine environment (Elliot and Quintino 2007). Estuaries are naturally stressed environments due to their inherent variability, and organisms are adapted to this. When, however, anthropogenic stress is added to the estuarine environment, conditions are often created with which many organisms are unable to cope. This was the case in the Nhlabane Estuary during the drought conditions in 1992-1995, with anthropogenic stress caused by mining activities adding further pressure on an already stressed ecosystem, thereby creating unfavourable conditions for most benthic taxa. Ecosystems such as the Nhlabane Estuary have to deal with the effect of multiple stressors, which may act synergistically to considerably lower the resilience and resistance of the populations to disturbance.

Estuaries are, however, dynamic systems which are known to have a high tolerance to stress and an inherent resilience with the ability to return to pre-impact conditions once the cause of the impact has been removed (Gray and Pearson 1982; Whitfield and Bruton, 1989). The high natural variability in estuaries may confer an ability to withstand stress, both natural and anthropogenic, thus creating resilience to stress and the ability to recover (Elliot and Quintino 2007).

Climatic variability is known to have a significant influence on the population structures of many estuarine and marine organisms (Currie and Small 2005), as was shown in the Nhlabane Estuary during the 1992-1995 drought period (Vivier and Cyrus 1999b). Estuaries are defined by salinity change over tidal cycles, but climatic changes and associated alterations in freshwater flows (of varying intensity, frequency and duration) can alter salinity regimes dramatically to the detriment of the benthic communities. Results from a 6 year study on the Port Curtis estuary in Australia showed that drought induced changes related to a recent El Nino episode had a significant impact on the benthic fauna of the sub-tropical estuarine system, with mean species richness and abundance progressively declining by 72%. Following the end of the drought, however, the benthos subsequently recovered over the ensuing 2.5-year period to a species richness approaching that recorded at the start of the drought (Currie and Small 2005).

A similar recovery was evident in the Nhlabane Estuary during this study. The mouth of the estuary, after being breached in August 1995, remained open or breached naturally quite frequently during the study period due to increased flows of freshwater from the lake. Because of renewed marine influence, estuarine conditions returned quite rapidly, characterized by a typical salinity gradient. The mean number of taxa and benthic densities more than doubled from early 1996 to 1998. In the upper reaches (Site 6), the area most affected by prolonged freshwater conditions during the drought, the mean number of taxa increased from 2 during the pre-barrage removal period, to 12 in 1997 and 16 in 1998. The mean benthic densities in

this area increased more than tenfold during the same period. Even in the lower reaches (Sites 1-2), the area least affected by the continued drought and closed mouth conditions (Vivier and Cyrus 1999b), the mean number of taxa and benthic densities more than doubled from early 1996 to 1998. These results clearly show a remarkable recovery in the benthic community, not only in terms of benthic densities, but more importantly, in terms of species number.

Estuarine benthic communities have often been used to document biological responses to stress induced changes in environmental quality (Gray and Pearson 1982, Gray 1989). The response of anthropogenically stressed benthic infaunal communities are well known. Under severe disturbance, communities become numerically dominated by a few species with very small individuals. Such communities are characterized by small opportunistic, short-lived r-strategists and low individual biomass organisms, low diversity, with high abundances of a few species (Gray and Pearson 1982). In addition, stressed communities have a high turnover and biological productivity and a dominance by oligochaetes and oligochaetiform polychaetes (Elliot and Quintino 2007). When the recovery of such communities are initiated, only one or two opportunistic species have been found to dominate the early phases of succession (Giangrande *et al.* 2005, Cardoso *et al.* 2007). These taxa are inherently poor competitors and are usually out-competed by transition species and k-strategists if conditions improve (Cardoso *et al.* 2007). This type of response was evident in the upper reaches of the Nhlabane Estuary at the onset of the study, when the stressed benthic community became dominated mainly by *P. sexoculata* and to a lesser extent by *D. ornata*. These are small opportunistic polychaetes which rapidly colonized the disturbed area when estuarine conditions were restored. They were quickly replaced by larger transition species and/or species which are usually dominant in the system. This role was played by the estuarine amphipods, *G. lignorum* and *C. triaenonyx*, the two most abundant species in the system. In estuarine environments approaching the post-stress natural equilibrium state, diversity and the abundance of rare taxa increases, numerical dominance is of species with moderately small individuals, and biomass becomes dominated by a few species characterized by low abundance but large individual size and weight (Elliot and Quintino 2007). This was clearly evident during the study in the gradual return and increase in the abundance of relatively large organisms such as the polychaete *C. keiskamma* and the gastropod *H. linulata*.

In a recolonization study in the Mondego estuary, early colonizers were mostly small-sized species with fast growth rates, which reached high densities and with high production rates, such as *C. capitata* and *Alkmaria romijni*. As the community recovered, the larger slow growing species which are more sensitive to habitat disturbance and whose presence is indicative of a relatively stable habitat, such as nereid species *Hediste diversicolor*, replaced the opportunists (Giangrande *et al.* 2005). The two nereid species, *C. keiskamma* and *D. arborifera*, seemed to perform this role in the Nhlabane Estuary.

Prionospio sexoculata was the primary opportunistic colonizer of severely disturbed habitat in the Nhlabane Estuary. In Dokai Bay, Japan, *Prionopio pulchra* was considered an opportunistic species and a good indicator of habitat disturbance (Ueda *et al.* 1992). In India, *P. cirrobranchiata* was proposed as an indicator species of industrial pollution, while *P. sexoculata* and *P. cirrifer* were reported as species tolerant of habitat disturbance (Khan *et al.* 2004). In the organically polluted Tongati estuary, the impoverished benthic community was completely dominated by *Prionospio sp* polychaetes (Blaber and Hay 1984). The total dominance of this organism in the Tongati estuary was suggested as indicative of instability in an artificially disturbed community. These results confirm the position of *Prionospio* polychaetes as opportunistic colonizers of severely disturbed subtropical estuarine environments. The difference between the Nhlabane and Tongati estuaries lies in the fact that conditions in the Tongati estuary remained stressed as a result of ongoing industrial pollution, whereas in the Nhlabane Estuary during this study, conditions drastically improved once the system was breached and the berm walls were removed. This allowed transition and equilibrium benthic taxa to replace the opportunistic polychaetes. The Nhlabane Estuary more resembled the Mdloti estuary in terms of estuarine conditions and biotic composition, which was also dominated by taxa indicative of more stable estuarine conditions, such as *C. erythraeensis* and *Dendronereis arborifera* (Blaber and Hay 1984).

Polychaetes have been extensively used in coastal studies for monitoring purposes especially in soft-bottom habitat (Pearson and Rosenberg 1978, Gray 1989, Cardoso *et al.* 2007). They are among the most abundant and species-rich groups of estuarine benthos. Among benthic groups, polychaetes are considered to be one of the best indicators of environmental disturbance, since this group contains both sensitive and tolerant species in a gradient from pristine to heavily disturbed habitats (Giangrande *et al.* 2005, Cardoso *et al.* 2007). When referring to opportunistic species of polychaetes, which mainly belong to the Capitellidae, Cirratulidae, and Spionidae families, one immediately thinks of *Capitella capitata*, the most common taxon found in organic enriched sediments (Pearson and Rosenberg 1978, Gray and Pearson 1982). However, *C. capitata* is a complex of sibling species, differing in their environmental needs, tolerances and life-history strategies and have been found to be widely distributed in a range of natural habitats (Gray 1989). This therefore undermines its utility as an indicator taxa. In the Nhlabane Estuary, this niche was filled by *P. sexoculata* and *D. ornata*.

In a recolonization study of a previously organically polluted estuary, Sykes Creek, Florida USA, the species considered to be opportunistic and indicators of zoobenthic recovery not only included polychaetes such as *C. capitata*, but also the amphipods *Grandidierella bonnieroides* and *Corophium elleri* (Grizzle 1984). *Corophium elleri*, in particular, was very abundant in moderately disturbed sediments. Inclusion of these amphipods as opportunists was based on the fact that they also showed marked increases in densities during recovery of the benthos. Opportunistic polychaetes such as *C.*

capitata were, however, shown to be true opportunists of severely disturbed habitats as their response was marked by a rapid increase followed by a rapid decrease in numbers. The amphipods, on the other hand, did not disappear after their initial rapid colonization of the affected areas, but went on to become the dominant taxa in the estuary, in the same way that they dominated the area before habitat disturbance. In a similar way, the rapid increase in the numbers of *G. lignorum* and *C. triaenonyx* in the Nhlabane Estuary after estuarine conditions were restored, was indicative of opportunistic species that were able to re-colonise areas where they previously dominated (Vivier and Cyrus 1999b). These crustaceans are therefore not true indicators of disturbed estuarine habitats but due to some competitive advantage were able to more rapidly re-colonize moderately disturbed areas which they previously dominated, compared to most other estuarine taxa (Grizzle 1984).

CHAPTER 4

Trace metals concentrations in the water and sediment and in body tissues of the two estuarine amphipods, *Grandidierella lignorum* and *Corophium triaenonyx*

4. Trace metals concentrations in the water and sediment and in body tissues of the two estuarine amphipods, *Grandidierella lignorum* and *Corophium triaenonyx*.

4.1 Introduction

Estuaries, which are dynamic, complex, and unique systems, are among the most productive coastal ecosystems in the world (Kennish 2002). Estuaries can be seen as biologically active systems structured by abiotic factors setting up a complex of gradients which may determine the distribution and abundance of animal communities. The ability of an estuary to support healthy biotic communities is dependent on the maintenance of its water and sediment quality. Estuaries are fast becoming the wastefields and repositories of contaminants generated by human activities, which eventually affect biotic communities. Worldwide, estuaries and their biotic communities are affected by urban and industrial development and few estuaries remain unaffected by human activities (Rainbow 2007, Bat 2005).

One of the greatest ecological concerns in estuarine areas over the past few decades has been the increasing concentrations of anthropogenically derived trace metals in the water and sediment. Many studies to date have shown the extent of metal contamination in estuaries and its potential effect on biotic communities (Hennig 1985, Kennish 2002, Adamo *et al.* 2005). Marine and estuarine sediments bind and accumulate a variety of trace metals to often high concentrations, which can then directly affect resident biota or be redistributed by disturbances and anthropogenic activities such as dredging, all of which may threaten the health of the estuarine environment via bioaccumulation and biomagnification up the food chain (Marsden and Rainbow 2004, Chapman 2007a). Estuarine sediments therefore comprise an ecologically important component of the aquatic environment by mediating the exchange of accumulated contaminants between particulate, dissolved and biological phases (Bat 2005, Rainbow 2007).

What is a trace metal? Trace metals and heavy metals are generally loosely and interchangeable used terms in the field of ecotoxicology (Rainbow 1997). Nieboer and Richardson (1980) categorized metals into three categories: Class A (metals with a preference for binding with ligands with oxygen as donor atom), Class B (metals with a preference for binding with ligands with sulphur complexes as donor) and Borderline metals (metals ions exhibit intermediate behaviour in that the metal ions have a more catholic affinity for metal binding donor atoms and ligands). Generally, trace metals or heavy metals are restricted to Class B and Borderline metals (Rainbow 1985, Rainbow 1997), although aluminum, which is a Class A metal, is included in the list of metals analysed in the present study. The affinity of Class B and Borderline metals for S or N atoms in functional proteins and other molecules have allowed their evolution for active

incorporation into respiratory pigments, metalloenzymes or activators. This affinity however also means that these metals can cause toxic effects by replacing another metal in metabolically important proteins, by blocking a biofunctional site or by altering the specific conformation of a protein or related molecule (Rainbow 2002). As such, all metals with Class B or Borderline ions have the potential to be toxic, often at concentrations not much greater than that required for essential metabolic activities. Some metals are essential for metabolism, such as Co, Mn, Cu, Fe, Zn, Cr and Mg, but become toxic at elevated concentrations, while others, such as Cd, Pb, Hg, and Sn are not required for metabolism and are toxic to organisms even at low concentrations (Kennish 1997, Rainbow 2002).

Management of estuaries and coastal waters relies on our understanding of the effect of trace metal contamination in estuaries and its biological and ecological significance (Rainbow 2007, Dauvin 2008). Monitoring metal concentrations, particularly in the sediment, has therefore been the focus of attention in environmental monitoring programmes in many regions of the world during recent years (Chapman 2007a, Simpson and Batley 2007). In addition to environmental concerns there are also several pragmatic reasons for monitoring sediment metal concentrations. Concentrations of metals in the sediment are typically several orders of magnitude higher than those in the water column, and due to tidal and fluvial water movements, metals concentrations in the water column are subject to much greater temporal and spatial variability than in the sediment, which makes it difficult to obtain water samples that are representative of the contaminant status of the waterbody (Orr 2007). Most importantly, however, measurements of dissolved trace metal concentrations in estuarine water provide a measure of the total metal present, not of the bioavailable portion which is available for uptake and accumulation by estuarine organisms. It is the bioavailable fraction only that is potentially toxic and of ecotoxicological relevance (Rainbow 2002, 2007). Sediments are also long-term integrators of metals and provide a more conservative, spatially and temporally integrated measure of metal accumulation in the system and therefore a more accurate reflection of the extent of metal contamination (Simpson and Batley 2007). High total concentrations of metals in sediment, however, do not necessarily indicate anthropogenic input of metals, as elevated levels of metals may be a result of variations in sediment characteristics as well as naturally high background concentrations (Marsden and Rainbow 2004, Chapman 2007a). Sequential chemical extraction of the potentially bioavailable metal fraction in estuarine sediments is often used as a means of better understanding the potential toxicity of metals in the sediment, as it offers a more realistic estimate of the actual environmental impact of bioavailable metals (Rauret *et al.* 1999).

Analysis of sediment metal concentrations, however, still does not reveal the full ecological relevance of the bioavailable fraction of metals in a system, a component of ecotoxicology which can only be fully assessed by measuring the accumulation of trace metals in the body tissues of aquatic organisms (Simpson and Batley 2007, Dauvin 2008). Aquatic organisms accumulate trace metals from the sediment, water and

from food, often to very high body concentrations (Rainbow 2007, Casado-Martinez 2010), thereby providing an essential time-integrated measure of metal availability in the system over time. This can be easily measured and most significantly, it is a time-integrated measure of the bioavailable metal. Thus the fraction of metal of direct ecotoxicological relevance within a potentially or known contaminated estuary can be measured unambiguously in the tissues of aquatic organisms (Rainbow 1995, 2007).

Many different aquatic invertebrates are used as biotic indicators of contamination in aquatic ecosystems, including mollusks, polychaetes and amphipods (Rainbow 1995, 2007, Zauke *et al.* 2003, Re *et al.* 2009, Casado-Martinez *et al.* 2010). Such organisms are referred to as biomonitors and are widely used to establish geographical and/or temporal variations in the bioavailable concentrations of trace metals in coastal and estuarine waters (Rainbow 2007, Guerra-García *et al.* 2009). Ideal biomonitors should be sedentary, easy to identify, abundant, long lived, available for sampling throughout the year, large enough to provide sufficient tissue for analysis, resistant to handling stress caused by laboratory studies of metal kinetics and/or field transplantations, tolerant of exposure to environmental variations in physico-chemical parameters such as salinity, and net accumulators of the metal (Rainbow 1995). The organisms most commonly used for biomonitoring are bivalve mollusks, crustaceans and annelids (Rainbow 2007). Among bivalves, the most commonly used organisms are mussels and oysters of the genera *Mytilus*, *Perna* and *Crassostrea* (Rainbow and Phillips 1993). Among crustaceans, decapods regulate tissue concentrations of most trace elements, whereas barnacles and amphipods are net accumulators of trace metals and therefore have particular relevance as biomonitors (Rainbow and Moore 1986, Rainbow 2007). Amphipod crustaceans in particular have been proposed as suitable biomonitor organisms in many parts of the world, since they are generally sensitive to most contaminants, are extremely widespread, occur in high densities in most benthic communities, are often the principal food for predatory fishes and birds, and there is considerable potential for metal accumulated by amphipods to be transferred along marine food chains (Marsden and Rainbow 2004, Casado-Martinez 2007, Scarlett 2007, Re *et al.* 2009).

The Nhlabane Estuary, although largely unaffected by major industrial development in its catchment and along its shoreline, has been influenced by dune mining activities of Richards Bay Minerals (RBM) over the past 40 years (see Section 2.1 for more details). Construction of the Nhlabane Weir in 1978 across the upper end of the estuary, built to provide water for dune mining, altered the natural flow pattern in the estuary and the frequency of breaching of the mouth. On two occasions, in 1993 and 1999, a 6 m deep, 50 m wide channel was dredged across the middle reaches of the estuary to allow a dredger and separation plant to cross the estuary. Dredging of estuarine sediment causes resuspension of trace metals adsorbed in the sediment and lead to elevated metal concentrations in the system (Nayar *et al.* 2004). Two berm walls were constructed across the estuary to contain the slimes, but repeated spills of fines in 1993-1994 caused a considerable amount of dredged slimes to flow into the estuary and settle on the bottom, smothering the

benthic organisms (Hattingh and Vivier 1995). These events raised concerns about trace metal concentrations in the system. Dune mining also affects the natural mineralogical layering of sediments and minerals in the coastal dune, increasing the potential for leaching of trace metals into the estuary. No studies to date have been done on trace metal concentrations in the system. The first objective of this part of the study was therefore to assess the baseline trace metal concentrations in the water and sediment of the Nhlabane Estuary.

The development of biomonitors of trace metal contamination in estuarine waters has progressed considerably in many parts of the world over the past two decades (Re *et al.* 2009). Such developments have largely been lacking in South Africa, although the integrity of South African estuaries has been of increasing concern during recent years and attempts have been made at establishing national monitoring programs for the country (Turpie *et al.* 2002). Since biomonitoring programmes have become important standard tools for monitoring ecosystem health in estuaries worldwide, the development of such programmes in South Africa using organisms of geographical relevance should therefore be a priority. The use of amphipods that can be employed as national biomonitors is of particular relevance and as such, the second objective of this part of the study was to assess the trace metal concentrations in two estuarine amphipods species, *Grandidierella lignorum* and *Corophium triaenonyx*, with the aim to establish their potential as biomonitors of trace metal contamination in estuaries. As indicated in the previous chapter, these two amphipod species occur in high densities throughout the Nhlabane system, they tolerate varying salinities and sediment types and are easy to collect. Both species are common in estuaries along the southern and eastern seaboard of South Africa, particularly in smaller, lower salinity systems not permanently open to the sea (Griffiths 1976, Vivier and Cyrus 1999b, MacKay and Cyrus 2001). Closely related members of these two corophioid amphipods, such as *C. volutator* and *G. japonica*, have been found to be suitable biomonitors of trace metal contamination in estuaries in other parts of the world and they are regular components of estuarine biomonitoring programmes (Bat and Raffaelli 1998, Rainbow 2007, Marsden and Rainbow 2004, Han *et al.* 2005, Lee *et al.* 2005, Allan *et al.* 2007, Casado-Martinez 2007, ASTM 2008, Re *et al.* 2009).

Metals taken up by organisms from the water, sediment or food will enter in a form that is initially available to bind with metabolites in the receiving cell, with the potential to be stored or transported elsewhere in the body via the haemolymph, or have a toxic effect by binding in the wrong place at the wrong time, a feature of both essential and non-essential trace metals in excess. To avoid potential toxic effects, this newly taken up metal must either be detoxified or excreted (Rainbow 2007, Simpson and Batley 2007). Detoxification requires the metal to be bound (in soluble or insoluble form) with such a high affinity that it is unavailable to be bound to other metabolites, thereby preventing these metabolites from completing their metabolic role (Marsden and Rainbow 2004). In a crustacean that accumulates all

incoming metal in stored detoxified form, the accumulated concentration can therefore reach very high levels with no toxic effect (Rainbow 2007). If however, the metal concentrations reach toxic levels, the effect of the contaminant may be manifest in different pathways and at various levels of biological organization. The higher the accumulated metal concentration, the more energy is required by the organism to detoxify the metal, which ultimately affects its general health and reproductive potential (Marsden and Rainbow 2004, Normant and Lamprecht 2006). Toxic metals have been reported to affect the survival, growth, reproduction or fitness of individuals within a population and therefore also affect populations and communities (Hickey and Roper 1992, McGee *et al.* 1998, Nipper *et al.* 1989, Marsden 2002, Neuparth *et al.* 2005). Amphipods from clean and contaminated sites have also been found to differ in average body weight and the number of recruits (Wong 1999, in Marsden *et al.* 2003). Increasing contamination by metals in sediments has also been correlated with decreasing numbers of species and changes in the abundance and composition of the benthic communities (Conradi and Depledge 1998, Costa *et al.* 2005, Rainbow 2007).

Detection of adverse effects of metal contamination at the population or community level implies by definition that contamination has already reached a level where the survival of individual organisms has been compromised. Ideally, monitoring and measurement of toxic effects at lower levels of biological organization, such as alteration of behavioral and physiological processes within aquatic organisms, should be used as this can serve as more sensitive sub-lethal early warning tools for biological effect measurement in environmental quality assessments. The development and use of sublethal endpoints and biochemical biomarkers in toxicity assessments have therefore received much attention during recent years (Neuparth *et al.* 2005, De Lange *et al.* 2006, Kennedy *et al.* 2009, Re *et al.* 2009).

Therefore, this part of the study aimed to assess the ecotoxicological status of the Nhlabane Estuary by determining the concentrations of essential and non-essential trace metals in the water and sediment as well as in the body tissues of two ecologically relevant benthic species, the amphipods *G. lignorum* and *C. triaenonyx*, over the period 1997-1999. In order to better understand the bioavailability and therefore the potential toxicity of the metals in the sediment, sequential extraction of sediment metals was performed.

4.2 Materials and Methods

4.2.1 Collection of water, sediment and amphipods

Water and sediment samples for metal analysis were collected seasonally at six sites along the length of the Nhlabane Estuary during the period 1997-1999. See Figure 2.1 for site locations. Samples were cooled

on ice immediately after collection and stored at -20°C until analysed. Collection of benthic samples and identification and enumeration of amphipods as described in Section 2.2.

4.2.2. Preparation of water and sediment samples for analysis

Water samples collected seasonally from six sites in the estuary during the period 1997-1999 were analysed. Water samples were thawed and filtered using $0.45\ \mu\text{m}$ cellulose nitrate filter paper.

Due to the absence of mud, the consistently low organic content and the relatively uniform grain size of the sediment (See Section 3.3 for details), it was expected that there would be limited spatial and temporal variability in the sediment metal concentrations. Therefore, for the purpose of sediment analysis, the winter and summer seasons for the period July 1997 to February 1999 were considered. Sediment samples were thawed and 1g of sediment was dried at 60°C for 24 hrs, hot-plate digested using nitric acid and perchloric acid in a 2:1 ratio and then made up to 50 ml using double distilled water. Samples of the sediment were also analysed for grain size and organic content (See Section 3.2 and 3.3 for details).

In order to better understand the bioavailable fraction of each metal, sequential extraction of the sediment samples was performed using the 4-step (or 3 step and residual fraction) modified BCR method as described by Rauret *et al.* (1999). The chemical reagents and analytical conditions of the modified BCR methodology can be summarized as follows (after Sutherland and Tack 2003):

Step	Operational definition	Target	Chemical reagents and procedure
1	Exchangeable, water and acid soluble	Soluble species, carbonates, cation exchange sites	1-g aliquot, 40 ml of 0.11 M HOAc, shake for 16 h at ambient temperature; separate extract from the solid residue by centrifugation at 3000g for 20 min
2	Reducible	Iron and manganese oxyhydroxides	To Step 1 residue add 40 ml of 0.5 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ (acidify to pH 2 with HNO_3), shake for 16 h at ambient temperature; separate extract from the solid residue by centrifugation at 3000g for 20 min
3	Oxidisable	Organic matter and sulphides	To Step 2 residue add 10 ml of H_2O_2 (pH 2–3), 1h at room temp., heat to 85°C for 1 h; add a further 10 ml of H_2O_2 and heat to 85°C for 1 h; add 50 ml of 1 M NH_4OAc (pH 2) and shake for 16 h at ambient temperature; separate extract from the solid residue by centrifugation at 3000g for 20 min
4	Residual		To Step 3 residue add 3ml distilled water, 7.5 ml 6M HCL and 2.5 ml 14M HNO_3 , leave overnight, boil under reflux for 2h, cool and filter

4.2.3. Preparation of amphipod tissues for analysis

Prior to preparation for tissue analysis, the amphipods *G. lignorum* and *C. triaenonyx* were sized and only adult amphipods were included in the analysis. This was done to avoid the typical size effect observed in tissue metal concentrations in most aquatic invertebrates, with juveniles showing much higher tissue metal concentrations than adults (Moore and Rainbow 1987). Adult *C. triaenonyx* was deemed to be > 4 mm total length and *G. lignorum* > 5 mm total length. Due to low amphipod densities at certain sites during the study period, and because only adults were included in the analysis, it was decided to pool the amphipod tissue from the each of the two sites in the upper, middle and lower reaches of the system. This ensured that sufficient amount of tissue was available for analysis. The amphipods were rinsed twice in double distilled water to remove any formalin residue, dried at 60 °C to constant weight and weighed to four decimals. The amphipod tissue was then placed in capped polyethylene tubes and digested for 24 hrs in an acid solution consisting of 0.05ml H₂O₂ and 0.5 ml HNO₃. Further digestion was performed by heating samples, without boiling, in a microwave oven during four consecutive sessions of 2 minutes at 200, 300, 400 and 500W. The samples were then made up to 10ml using double distilled water, capped and stored for analysis.

4.2.4 Metal analysis

Metal concentrations in water, sediment and amphipod tissues were determined using a Varian Ultramass 700 inductive coupled mass spectrophotometer (ICP-MS). All samples were analysed for aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), titanium (Ti), vanadium (V) and zinc (Zn). Indium was used as an internal standard, while reference material (Buffalo River sediment, CRM 320) was used to test for analytical efficiency (Cu: measured 136 ± 4 µg/g, certified 138 ± 7 µg/g; Zn: measured 138 ± 5 µg/g, certified 142 ± 3 µg/g; Pb: measured 45 ± 5 µg/g, certified 42 ± 3 µg/g).

4.2.5 Geochemical normalization

High total concentrations of metals in sediment do not necessarily indicate input of metals from anthropogenic activities, as elevated levels of metals may be a result of naturally high background concentrations or due to variations in sediment characteristics (Newman and Watling 2007). Many factors affect the concentrations of metals in sediment, such as grain size, organic content and mineralogy. Determining whether aquatic sediments are metal contaminated is complex, since metal concentrations in uncontaminated sediment can vary by orders of magnitude over relatively small spatial scales due to natural mineralogy and sediment granulometry (Summers *et al.* 1996). In natural, uncontaminated sediments, strong linear relationships typically exist between Fe, Al and concentrations of most other metals, with the possible exception of Cd, As and V (Summers *et al.* 1996). The concentrations of Fe and

Al in sediments, which are naturally abundant elements in the earth's crust, are orders of magnitude higher than the concentrations of most other metals. The natural concentrations of Fe and Al are so high that it usually obscures any anthropogenic input of these metals into contaminated systems, with the result their concentrations are likely to remain relatively unchanged even in anthropogenically impacted areas (Newman and Watling 2007). Although absolute metal concentrations vary between crustal material from one region to another, the relative proportions of metals within crustal material from a particular region naturally tend to be fairly constant, a principle which is used in geochemical normalization (Newman and Watling 2007). Normalised metal/Fe ratios are used to estimate the extent of metal contamination within an estuary, and to assess whether there has been enrichment of metals from anthropogenic activities. Normalisation can provide information on unnatural alterations in the ratios of metal concentration, by comparing metal concentration ratios measured in sediment from uncontaminated locations to those in potentially metal-enriched sediment (Orr 2007). Newman *et al.* (2009) assessed baseline metal concentrations from KwaZulu-Natal estuaries and coastal waters and based on their metal/Fe ratios, the normalized metal concentrations against Fe concentrations for the Nhlabane Estuary sediment were determined and plotted. Metal concentrations on the scatter plot that fall within the 95% prediction limits of the model are considered to reflect uncontaminated sediment, while data points that fall above the upper 95% prediction limit are identified as metal enriched (Newman and Watling 2007). Since As and Cd concentrations from KwaZulu-Natal coastal sediment are poorly correlated to Fe, normalisation could not be used to determine metal/Fe ratios for these metals. Baseline concentrations above which enrichment of sediment by these metals can be inferred were therefore defined using cumulative probability and univariate concentration distribution plots, at $17.21 \mu\text{g.g}^{-1}$ for arsenic and $0.518 \mu\text{g.g}^{-1}$ for cadmium (Newman and Watling 2007).

4.2.6 Statistical Analysis

After testing for normality, single factor ANOVA was used to test for differences in the water, sediment and body metal concentrations. Pairwise comparisons were performed using Tukey's HSD test, a statistical test generally used in conjunction with an ANOVA to find which means are significantly different from one another. Pearson's Correlation coefficients were determined to test for the relationship between metal concentrations in the tissue, sediment and water. Principal Component analysis (PCA) using CANOCO (Ter Braak and Smilauer 1998), were used to further investigate the relation between metal concentrations and environmental variables.

4.3 Results

4.3.1 Trace metals in water.

Mean trace metal concentrations in the Nhlabane Estuary over the period 1997-1999 are presented in Table 4.1, while mean trace metal concentrations per season are illustrated in Figure 4.1. In comparison to other estuaries and target values for estuarine and coastal areas in South Africa, Australia and the UK, trace metal concentrations in the Nhlabane Estuary were consistently low. Concentrations of As, Cd, Ni and Pb fell far below the South African marine water quality guideline target values, whereas Cu and Zn were only slightly below the target values. Nhlabane concentrations were in most cases an order of magnitude lower than that recorded in Richards Bay Harbour and the Mhlathuze estuary, except for Mn and Zn, which were 22% and 32% of the equivalent values in the Mhlathuze estuary, respectively. It is also noteworthy that the 90th percentile concentrations in the Nhlabane Estuary were lower than the South African, Australian and the UK target values, except for Cu and Zn, indicating that for most metals, even relatively high concentrations in the Nhlabane Estuary were consistently lower than the target values.

Table 4.1 Mean water metal concentrations (+1STD) in the Nhlabane Estuary, Richards Bay Harbour uncontaminated areas (4 sites) and the Mhlathuze Estuary (8 bi-monthly samples from 1 site), as well as Target Values for South African, Australian and United Kingdom coastal regions. a - Vermeulen and Wepener 1999, b - Mzimela *et al.* 2003, c - DWAF 1995, d – ANZECC 2000, e – UK Water Research Centre 1990, – no data. (ERL - Effects-Range-Low).

Metal	Mean (±1STD)	10% Quartile ERL	90% Quartile	Richards Bay Harbour ^a	Mhlathuze estuary ^b	Target Value South Africa ^c	Target Value Australia ^d	Target Value UK ^e
	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(µg/l)
Aluminium	35 (±21)	12.4	63.1	530 (±246)	730 (±723)	-	-	-
Arsenic	3.5 (±2.6)	0.5	6.8	-	-	12.0	50	25
Cadmium	0.2 (±0.07)	0.1	0.3	-	-	4.00	2	3
Chromium	2.9 (±1.8)	1.0	5.6	59.5 (±8.6)	24.3 (±5.8)	8.0	50	15
Copper	4.5 (±2.4)	1.8	8.5	-	56.5 (±14.7)	5.0	5	5
Iron	77 (±32)	37.0	124.4	720 (±116)	1224 (±977)	-	-	1000
Manganese	29 (±30)	4.8	66.5	-	88.9 (±32.3)	-	-	-
Nickel	3.5 (±2.4)	1.3	7.3	-	-	25.0	15	30
Lead	1.8 (±0.9)	0.8	3.1	-	223 (±45)	12.0	5	25
Titanium	3.1 (±2.0)	1.1	5.6	-	-	-	-	-
Vanadium	6.8 (±7.0)	1.6	12.2	-	-	-	100	-
Zinc	17.2 (±8.3)	7.2	30.4	-	77.7 (±18.9)	25.0	50	40

Concentrations of all metals except Ti showed a significant positive correlation with Fe concentrations, whereas only Cd, Cr, Cu and Ni showed a significant correlation with Al concentrations (Table 4.2). A significant positive correlation with salinity was seen in As, Cr and Ni, while a negative but insignificant correlation with salinity was observed in Cd, Cu, Ti and V. Concentrations of all metals except Fe, Mn and V were negatively correlated with temperature. A significant negative and positive correlation with pH was observed in Cu, Pb and Mn, and in Cr, respectively. All metals but V were negatively correlated with % oxygen saturation, with As, Fe, and Mn showing a significant correlation. Most metals showed a significant temporal variation over the 12 sampling seasons during 1997-1999, while there were no significant differences in metal concentrations between sampling sites (Table 4.2).

Table 4.2 Correlation (Pearson's Correlation coefficients R) of trace metals in the water with aluminum and iron, as well as with water quality parameters and water depth. ANOVA results (F-value) of changes in trace metal concentrations in the water over the 12 quarterly sampling sessions, the three sampling years and the six sampling sites, are also presented. *denotes significant correlation/difference ($p < 0.05$), ** denotes highly significant correlation/difference ($p < 0.01$).

Metal	Correlation		Correlation					ANOVA		
	Aluminum	Iron	Salinity	Temperature	pH	Dissolved Oxygen	Depth Size	Sampling seasons	Years	Sites
Aluminum	-	0.41**	0.15	-0.11	-0.01	-0.09	0.29*	2.3 *	2.1	0.7
Arsenic	0.05	0.27*	0.49**	-0.21	0.06	-0.30*	0.09	5.1**	3.3*	0.9
Cadmium	0.45*	0.28*	-0.14	-0.20	0.07	-0.01	-0.05	2.2*	4.7	1.1
Chromium	0.35*	0.37*	0.37**	-0.21	0.29*	-0.05	-0.04	5.8**	9.3**	1.1
Copper	0.27*	0.23*	-0.02	-0.13	-0.26*	-0.16	-0.01	2.4*	2.9	1.4
Iron	0.41*	-	0.17	0.11	-0.12	-0.30*	0.13	1.0	1.3	1.0
Lead	0.06	0.24*	0.07	-0.06	-0.37**	-0.26*	0.00	4.1**	1.3	1.5
Manganese	0.16	0.34**	0.01	0.03	-0.45**	-0.63**	0.03	3.1**	6.1**	0.9
Nickel	0.24*	0.47*	0.24*	-0.12	-0.17	-0.17	0.07	3.1**	0.4	0.6
Titanium	0.21	0.19	-0.03	-0.02	-0.07	-0.08	0.04	1.2	0.5	0.2
Vanadium	0.15	0.30*	-0.04	0.09	0.13	0.05	0.10	1.7	2.0	0.7
Zinc	0.12	0.33**	0.22	-0.10	-0.18	-0.22	0.02	1.9	0.2	0.3

The association between physico-chemical variables and metal concentrations was further investigated using a PCA procedure (Figure 4.2). The majority of metals were strongly correlated with decreasing oxygen and pH levels, whereas mouth condition, temperature and to a lesser extent salinity were relatively unimportant of determining metal concentrations. The plot also shows that closed mouth conditions were closely correlated with summer temperatures

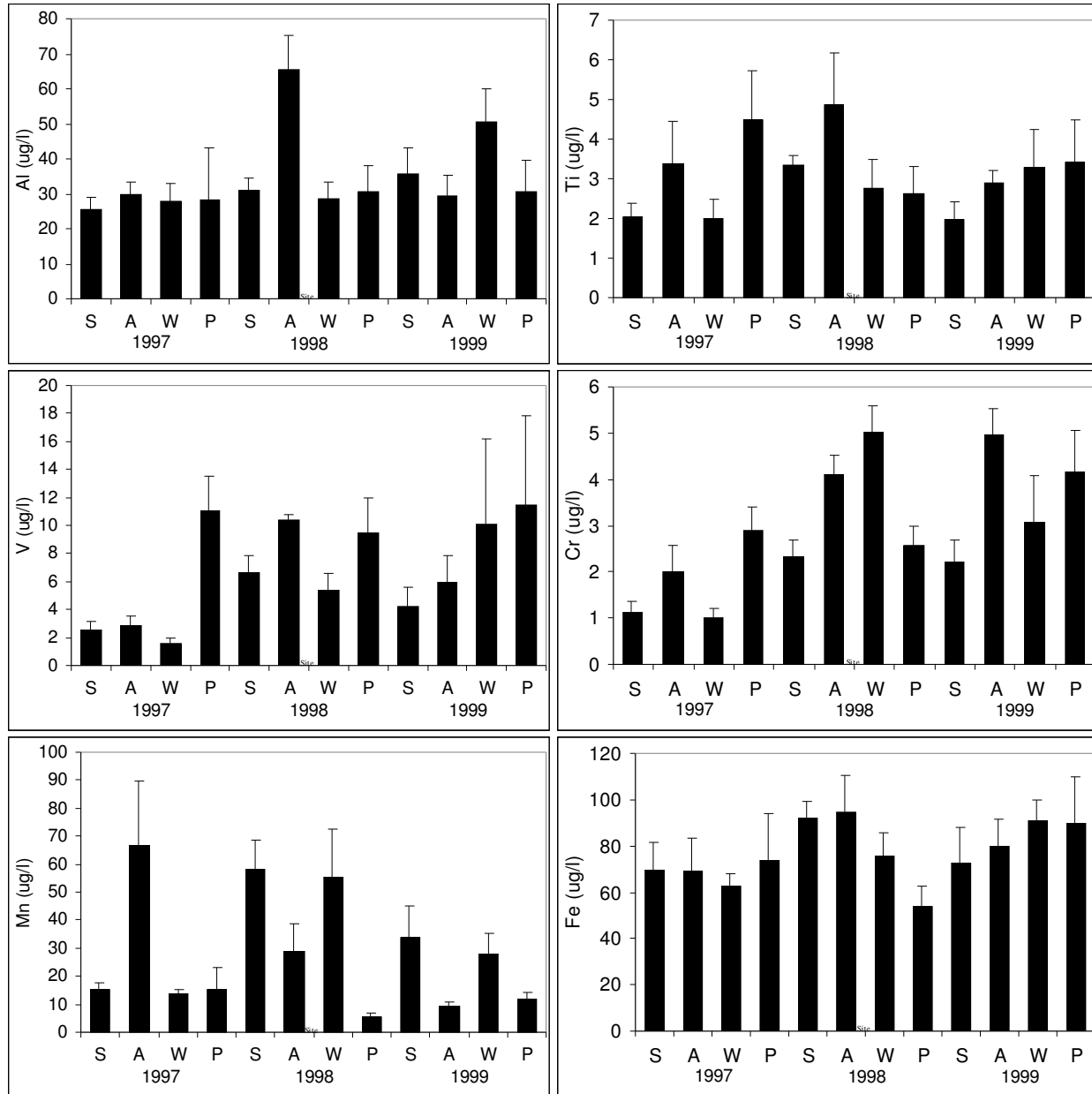


Figure 4.1a Mean (+1SE) concentrations of trace metals in the Nhlabane Estuary water during 1997-1999.

S: summer, A: autumn, W: winter, P: spring

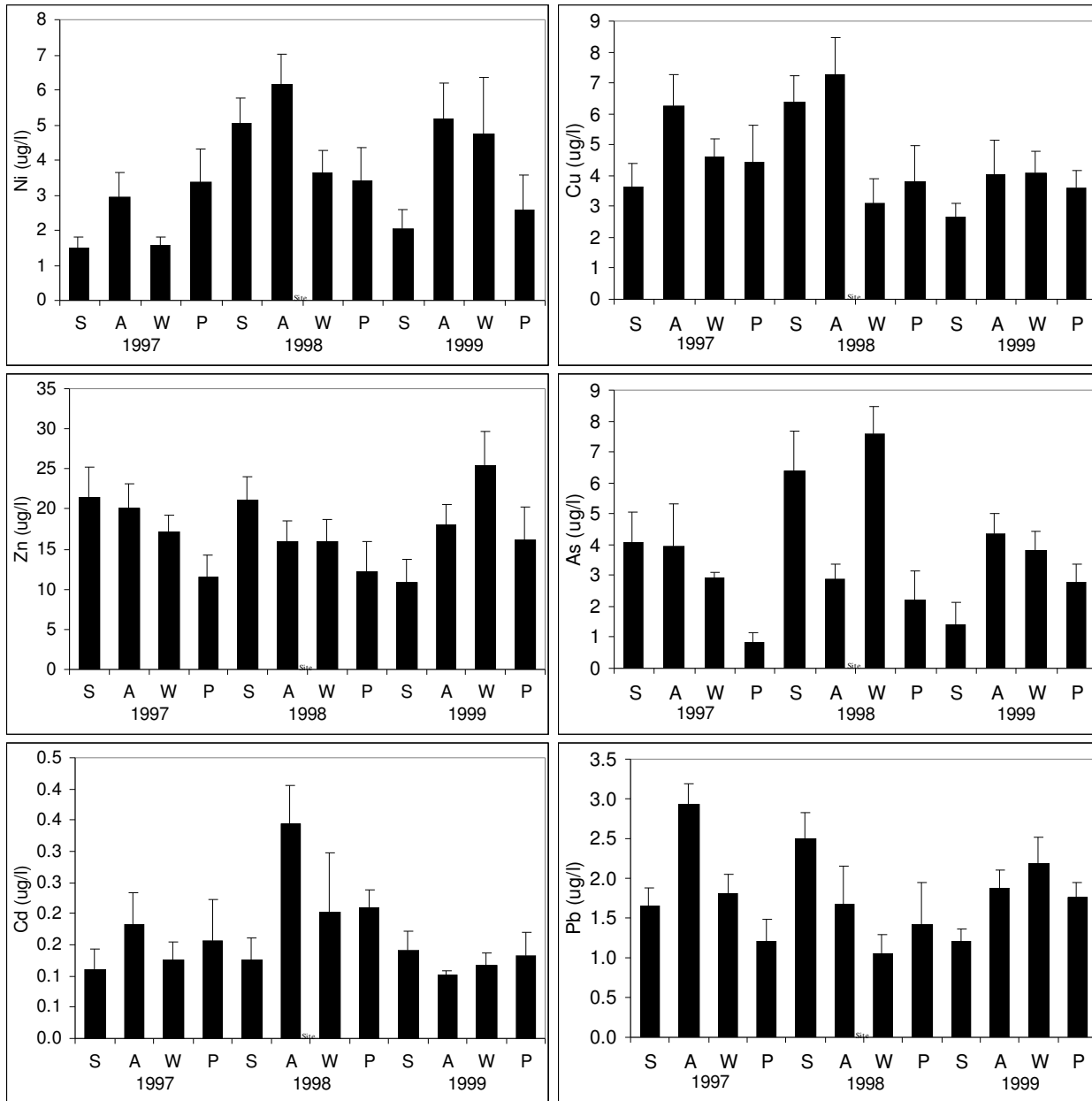


Figure 4.1b Mean (+1SE) concentrations of trace metals in the Nhlabane Estuary water during 1997-1999.

S: summer, A: autumn, W: winter, P: spring

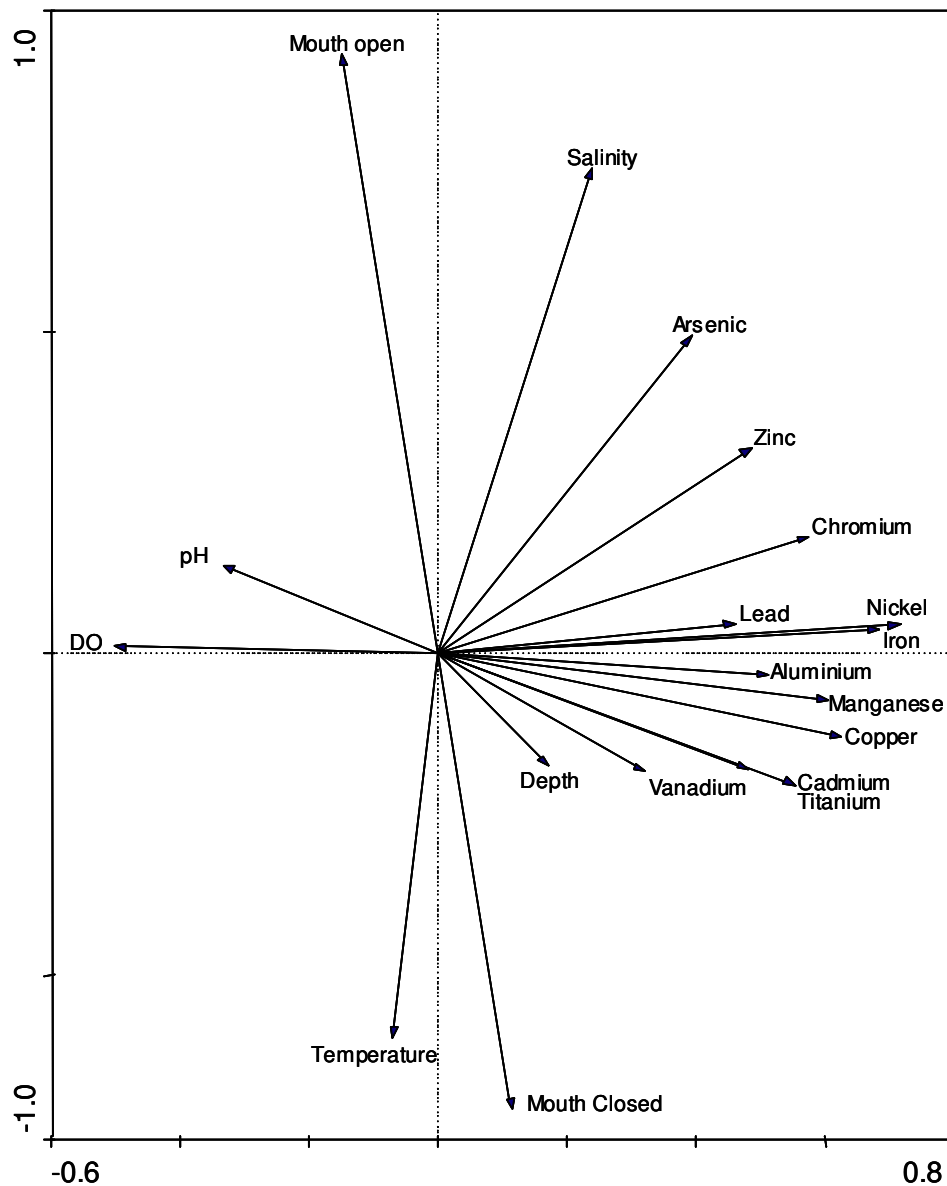


Figure 4.2 PCA biplot of the association between mouth condition, physico-chemical characteristics of the water and trace metal concentrations in the Nhlabane Estuary water during 1997-1999.

4.3.2 Trace metals in sediment.

4.3.2.1 Total sediment concentrations

Mean trace metals concentrations in Nhlabane Estuary sediment during 1997-1999 are presented in Table 4.3, together with comparative data from the Mhlathuze and Swartkops estuaries and from known contaminated and uncontaminated areas in Richards Bay Harbour. The mean concentrations of trace metals in the Nhlabane Estuary were generally low, with all metals for which sediment quality guidelines

are available being recorded at concentrations below these guidelines, while those of Al, Cu, Fe, Mn and Zn were below levels recorded in the Mhlathuze estuary and from uncontaminated sediment in Richards Bay Harbour. Although Cr concentrations were higher than in the Mhlathuze estuary and from uncontaminated sediment in Richards Bay Harbour, the mean concentration of 59.2 µg/g was still considerably lower than that found in contaminated areas in Richards Bay Harbour. No sediment quality guidelines exist for Al, Fe, Ti, Mn and V.

Table 4.3 Mean (± 1 STD) trace metals concentrations in Nhlabane Estuary sediment during 1997-1999. Metal concentrations (mean ± 1 STD) in sediment from uncontaminated (4 sites) and contaminated (5 sites) areas in Richards Bay Harbour, from the Mhlathuze Estuary (8 monthly samples from 1 site), Swartkops Estuary (7 sites) and from Australian (New South Wales) estuaries (101 sites) are also presented, as well as sediment quality guidelines (Effects-Range-Low - ERL) for Australian estuarine and coastal regions. a - Wepener and Vermeulen 2005, b - Mzimela *et al.* 2003, c – Binning and Baird 2001, d – McCready *et al.* 2006. – no data. ERL - Lower 10th percentile of the effects data and is the concentration of a contaminant below which harmful effects rarely (<10%) occurs.

	Nhlabane Estuary			Richards Bay Harbour ^a	Richards Bay Harbour ^a	Mhlathuze estuary ^b	Swartkops Estuary ^c	Australia NSW estuaries ^d	Sediment Quality Guideline Australia (ERL) ^d
	Mean (± 1 STD)	10% Quartile (ERL)	90% Quartile	uncontaminated areas	contaminated areas				
	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)
Aluminum	2198 (± 764)	1539	2718	10769 (± 2318)	47764 (± 19271)	18462 (± 15737)	-	-	-
Arsenic	4.9 (± 2.7)	2.3	7.4	-	-	-	-	21 (± 9)	8.2
Cadmium	0.24 (± 0.01)	0.23	0.26	-	-	-	-	2.8 (± 2.4)	1.2
Chromium	59.2 (± 11.1)	48.3	72.5	57.8 (± 16.9)	152 (± 50.2)	42.7 (± 32.2)	20.3 (± 12.47)	81 (± 63)	81.0
Copper	8.3 (± 3.0)	5.5	12.2	4.7 (± 2.3)	30.8 (± 15.3)	8.4 (± 6.4)	6.8 (± 4.8)	200 (± 190)	34.0
Iron	11822 (± 1892)	9685	14196	18705 (± 6696)	42148 (± 9799)	18586 (± 11977)	-	-	-
Lead	7.3 (± 1.9)	5.6	8.8	-	-	-	32.9 (± 27.6)	360 (± 310)	46.0
Manganese	150 (± 64)	86	261	201 (± 81)	580 (± 80)	308 (± 236)	114 (± 94)	-	-
Nickel	17.2 (± 3.8)	13.6	19.8	-	-	-	-	20 (± 12)	21.0
Titanium	703 (± 158)	554	935	-	-	-	99.3 (± 39.9)	-	-
Vanadium	165 (± 28)	128	196	-	-	-	-	-	-
Zinc	14.4 (± 4.3)	11.2	18.7	58.3 (± 11.4)	129 (± 43.6)	42.6 (± 18.7)	35.9 (± 26.6)	1000 (± 1500)	150

Differences in the mean trace metal concentrations between the four sampling periods during the period 1997-1999 and differences between sampling sites are illustrated in Figures 4.3 and 4.4, respectively. There were no significant differences in mean trace metal concentration between sampling sites, seasons and between the four sampling periods, the exception being a significant difference in Cu concentrations between sampling sites (Table 4.4). All trace metals except Cd, Ti and V showed higher concentrations during summer compared to winter, with Al, Cr, Cu, Fe, Ni and Zn reaching highest levels in February 1998 (Figure 4.3).

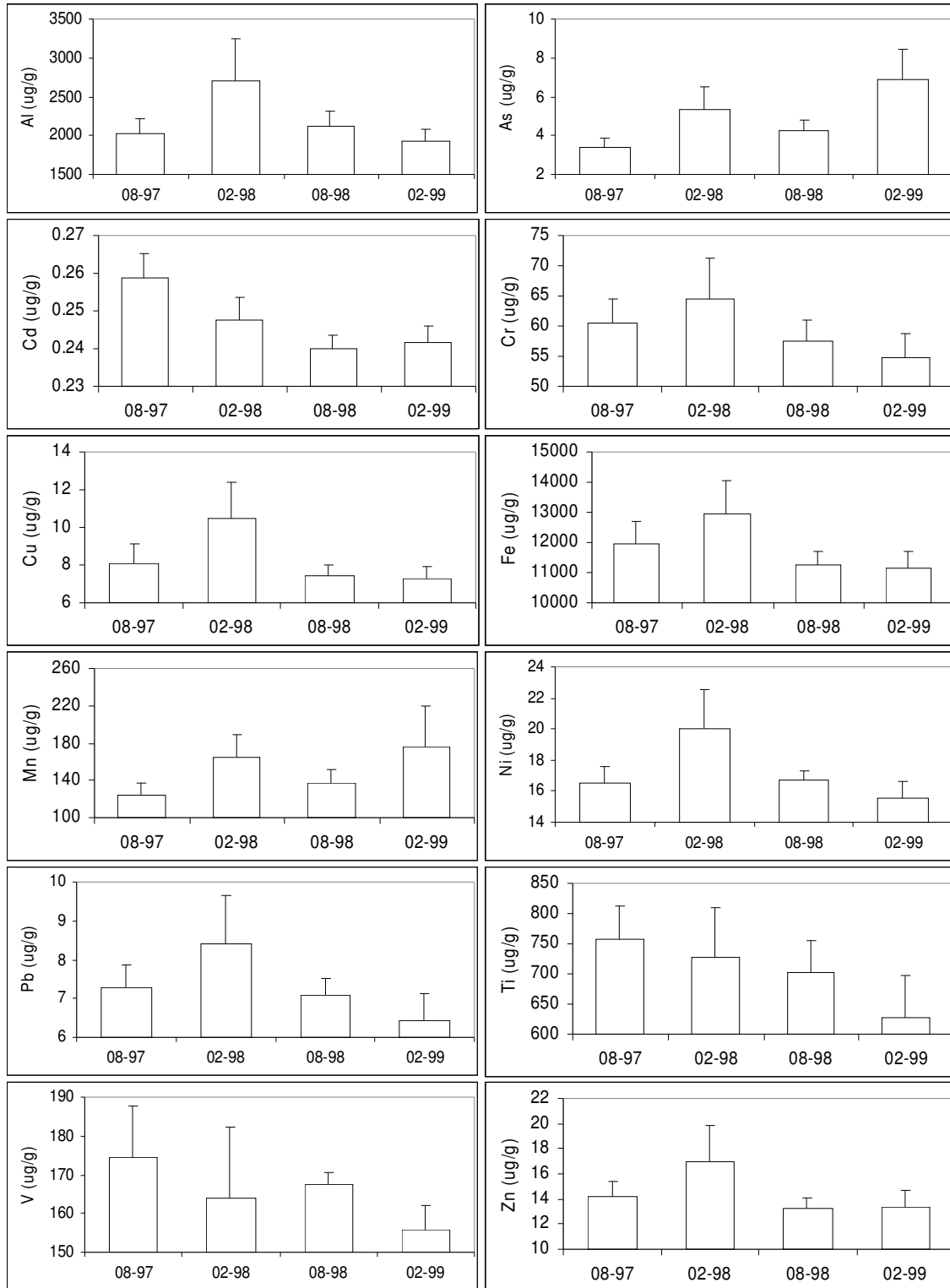


Figure 4.3 Mean (+1SE) concentrations of trace metals in the Nhlabane Estuary sediment over four sampling seasons during 1997-1999.

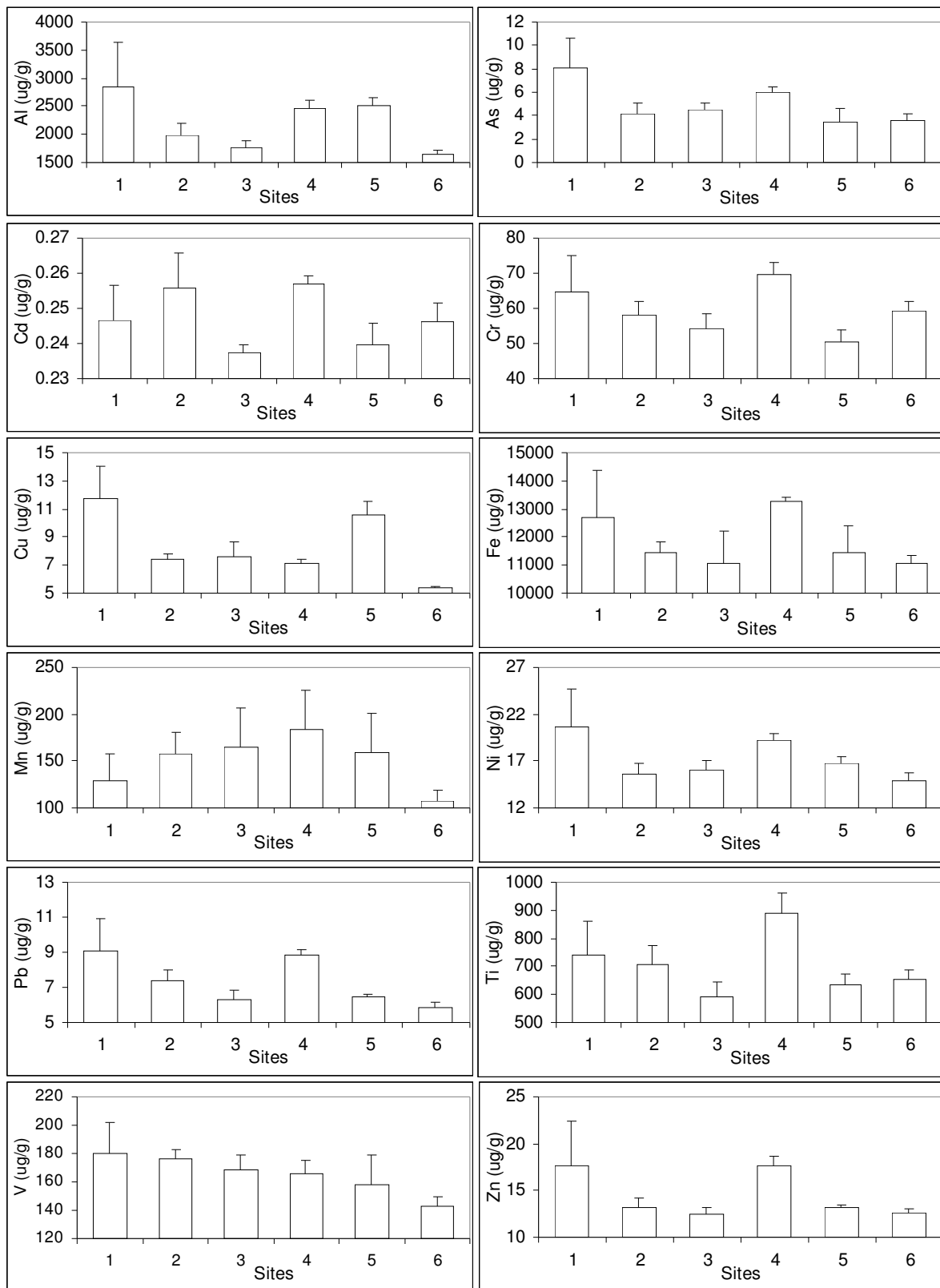


Figure 4.4 Mean (+1SE) concentrations of trace metals in the Nhlabane Estuary sediment at the six sampling stations Sites 1-6 during 1997-1999.

Table 4.4 Correlation (Pearson's Correlation coefficients R) of trace metals in the sediment with aluminum and iron, as well as with water quality parameters and sediment characteristics. ANOVA results (F-value) of changes in mean trace metal concentrations over the six sampling sites, over seasons and over the four sampling periods are also presented. * denotes significant correlation/difference ($p < 0.05$), ** denotes highly significant correlation/difference ($p < 0.01$).

Metal	Correlation		Correlation							ANOVA		
	Al	Fe	Salinity	Dissolved Oxygen	Depth	pH	Temperature	Grain Size	Organic content	Site	Season	Sampling period
Aluminum		0.64**	-0.10	0.07	-0.03	0.21	-0.01	-0.14	0.32	1.8	0.6	1.3
Arsenic	0.36	0.24	0.54*	0.14	0.51*	0.32	-0.03	-0.06	-0.21	2.0	3.0	2.1
Cadmium	0.33	0.44*	-0.21	-0.18	-0.05	-0.31	-0.23	0.22	0.15	1.4	0.7	2.6
Chromium	0.67**	0.82**	-0.12	-0.12	-0.18	-0.23	-0.08	0.56*	0.34	1.7	0.0	0.8
Copper	0.82**	0.5*	-0.28	0.35	-0.21	0.36	-0.27	-0.48*	0.10	4.2*	0.8	1.6
Iron	0.64**		-0.22	0.05	-0.24	0.08	-0.24	0.18	0.35	0.9	0.3	1.2
Lead	0.83**	0.76**	-0.17	0.11	-0.18	0.09	-0.09	0.25	0.33	2.6	0.1	1.1
Manganese	0.28	0.43*	0.16	0.04	-0.01	0.19	-0.06	-0.22	-0.12	0.7	2.4	0.8
Nickel	0.93**	0.71**	-0.26	0.12	-0.26	0.06	-0.09	0.16	0.35	1.6	0.5	1.6
Titanium	0.68**	0.68**	-0.05	-0.32	-0.03	-0.30	0.06	0.40	0.31	2.3	0.6	0.7
Vanadium	0.45*	0.75**	0.00	-0.12	-0.01	0.08	0.04	-0.04	0.02	0.9	0.9	0.4
Zinc	0.89**	0.78**	-0.07	-0.05	-0.13	-0.09	-0.15	0.30	0.28	1.4	0.6	0.9

Highest concentrations of the majority of metals (As, Cd, Cr, Fe, Ni, Pb, Ti and Zn) were recorded in the lower reaches at Site 1 and in the middle reaches at Site 4 (Figure 4.4). Copper and Al showed highest concentrations at Site 1 but low concentrations at Site 4, while the opposite was observed in Mn. Vanadium concentrations gradually decreased from the lower to the upper reaches. Low concentrations were recorded in all metals at Site 6, except in Cd and Cr. Similarly, low concentrations were recorded in all metals at Site 3, except in Mn and V.

4.3.2.2 Correlation between trace metal and environmental variables

The correlation between trace metals in sediment and environmental parameters are presented in Table 4.4. All metals showed a positive correlation with Al and Fe concentrations. Concentrations of all metals except As showed a significant positive correlation with Fe, while all metals except As, Cd and Mn showed a significant positive correlation with Al concentrations (Table 4.4). A negative correlation with salinity was seen in all metals, with the exception of V and As, with the latter showing a significant positive correlation, suggesting a marine origin of As. Similarly, all metals showed a negative correlation with temperature and depth. The exceptions were As, which showed a significant positive correlation with depth, and Ti and V, which showed a positive correlation with temperature. Cd, Cr, Ti, Zn and V were negatively correlated with dissolved oxygen concentrations, while the remainder of the metals were all

positively correlated with oxygen. Cd, Cr, Ti and Zn were negatively correlated with pH. As expected, most metals were positively correlated with sediment organic content, with the exception being As and Mn. Surprisingly, a number of metals were negatively correlated with decreasing sediment particle size (phi value), this probably being the result of the relatively uniform sediment composition in the system coupled with the lack of mud. Cr showed a significant positive correlation with decreasing sediment particle size (Table 4.4).

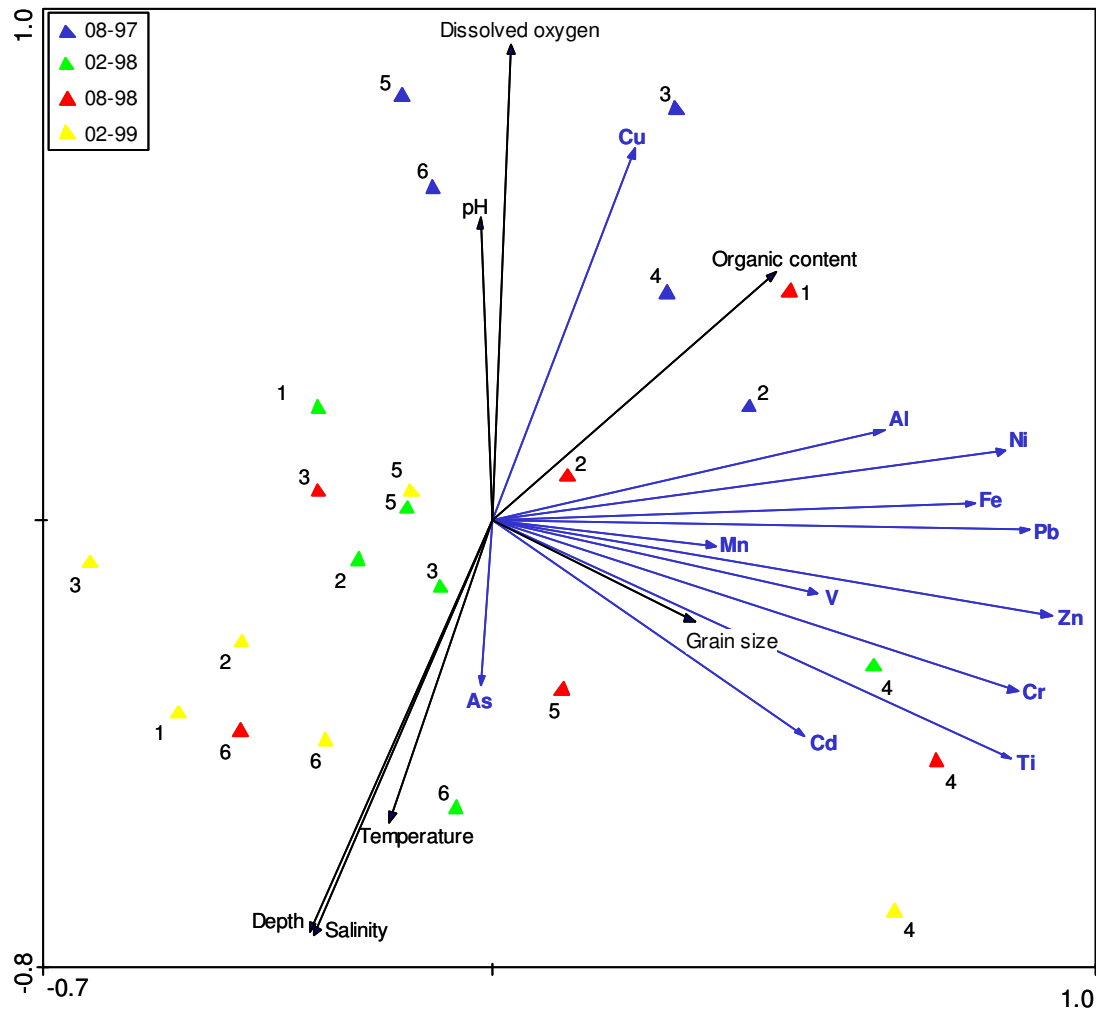


Figure 4.5 PCA triplot of association between trace metals in the sediment, environmental factors and sampling sites as recorded during four sampling seasons in the Nhlabane Estuary. Grain size represents the median phi value, with the grain size decreasing in the direction of the arrow.

The association between sediment metal concentrations and environmental factors was further investigated using a PCA triplot (Figure 4.5). The eigenvalues for the 1st and 2nd axis on the PCA plot accounted for only 49.6% of the variability within the data, while the 3rd and 4th axis only accounted for 14% and 8% of the variability, respectively. This indicates firstly that more than 50% of the variability in

the data is not accounted for by the two axis shown on the PCA plot and secondly that 28.3% of the variability in the metal data is not accounted for by the environmental factors used in the analysis, thereby suggesting that other untested parameters are also important in affecting the concentration of sediment trace metals in the Nhlabane Estuary. The PCA also confirms the strong correlation between Fe, Al and all the other metals, except for As in particular, and to a lesser extent, Cd. The behavior of As was clearly different from that of all the other metals, showing an uncharacteristic positive correlation with salinity and water depth, again suggesting a water-borne marine origin of As in the system. The same could perhaps be said of Mn, which also showed a positive correlation with salinity but an uncharacteristic negative correlation with sediment organic content and decreasing sediment particle size. The position of the majority of the metals on the right hand side of the plot (Al, Ni, Fe, Pb, Mn, Zn, V, Cr and Ti) indicates that there was little correlation between water quality parameters such as salinity, temperature and dissolved oxygen and these metals, suggesting that metal concentrations were influenced more by sediment characteristics and natural mineralogical processes. The relative importance of sediment organic content and to a lesser extent sediment grain size is illustrated on the PCA plot, with most metals increasing in concentration with an increase in organic content and a decrease in sediment particle size. The position of Cu on the PCA plot in this regard is unusual, as this metal showed a strong positive correlation with both Al and Fe, although this might be explained by the unusual significant negative correlation of this metal with decreasing sediment grain size.

4.3.2.3 Metal speciation through sequential extraction.

In order to gain further insight into the bioavailability of trace metals, sequential extraction of sediment samples was performed. The mean percentage concentration of the four metal fractions for each sediment trace metal as determined through the modified BCR method are presented in Table 4.5. The percentage contribution of the four sediment fractions to the sediment trace metal concentrations at Sites 1-6 are illustrated in Figures 4.6 and 4.7. In all metals with the exception of Cd, the highest percentage of the metal in the sediment was associated with the residual fraction, thus bound within the mineralogical structure of the sediment. This ranged from 51% in Mn to 96% in Ti. The residual fractions of Fe and Al comprised 82% and 84%, respectively. A substantial proportion (15-20%) was also associated with organic material (Fraction 3) in As, Cr and Ni, while in Pb, V and Zn, 15-20% of the metal was bound to Fe and Mn oxides in the reducible fraction (Fraction 2). In Mn, 39% of the metal was in the residual fraction. The speciation behaviour of Cd was very different from all other metals, with only 7% in the residual fraction, but 19% and 66% in the oxidisable and reducible fractions, respectively. With the exception of Cd, Mn, Ni and Zn, the proportion of trace metal that was in exchangeable or bioavailable form (Fraction 1) was mostly very low. The highest proportion of exchangeable metal was recorded in Cd (8.6%).

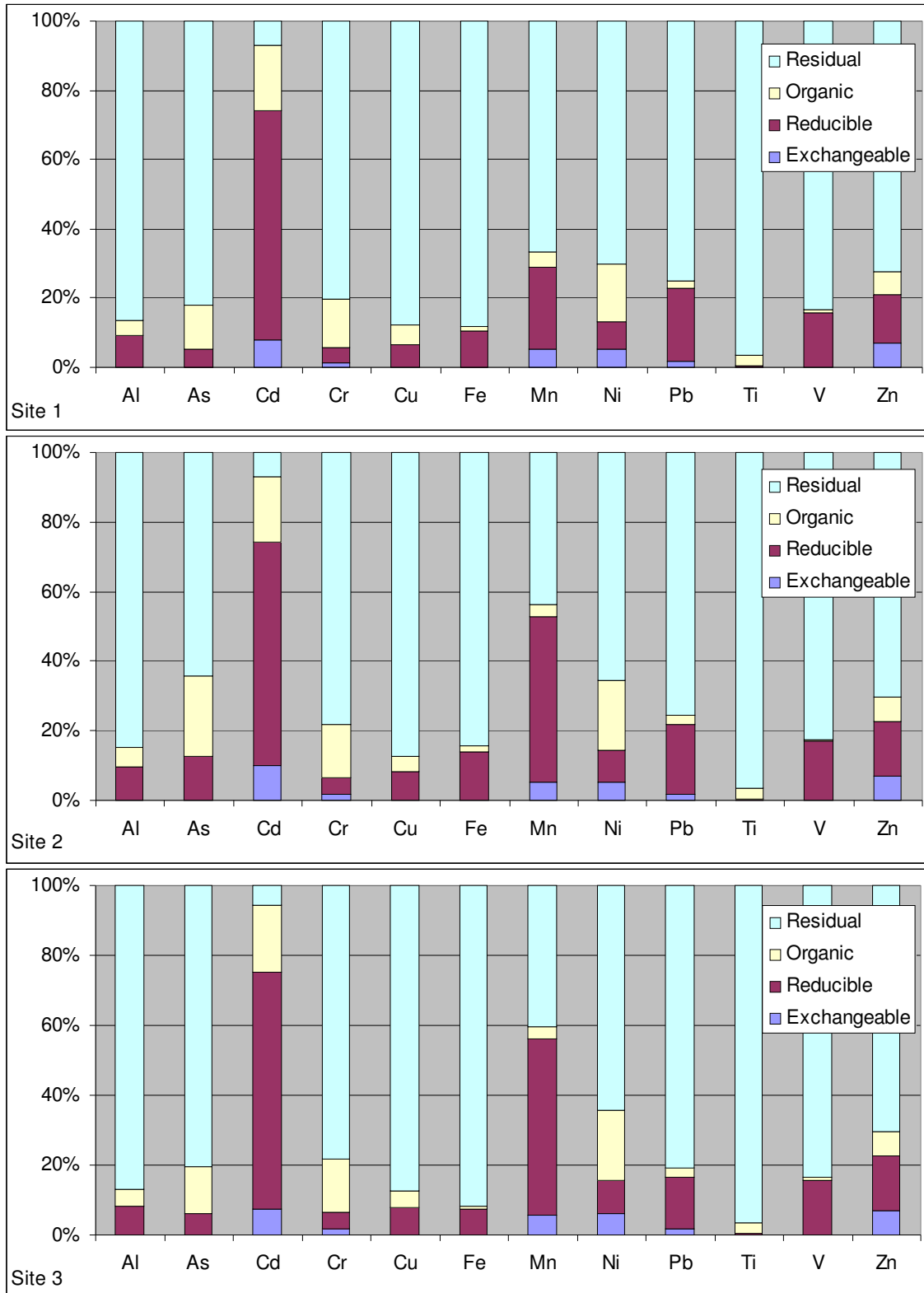


Figure 4.6 Percentage contribution of the four sediment fractions (modified BCR method) to the total trace metal concentrations in the sediment measured at Sites 1-3 in the Nhlabane Estuary over the period 1997-1999.

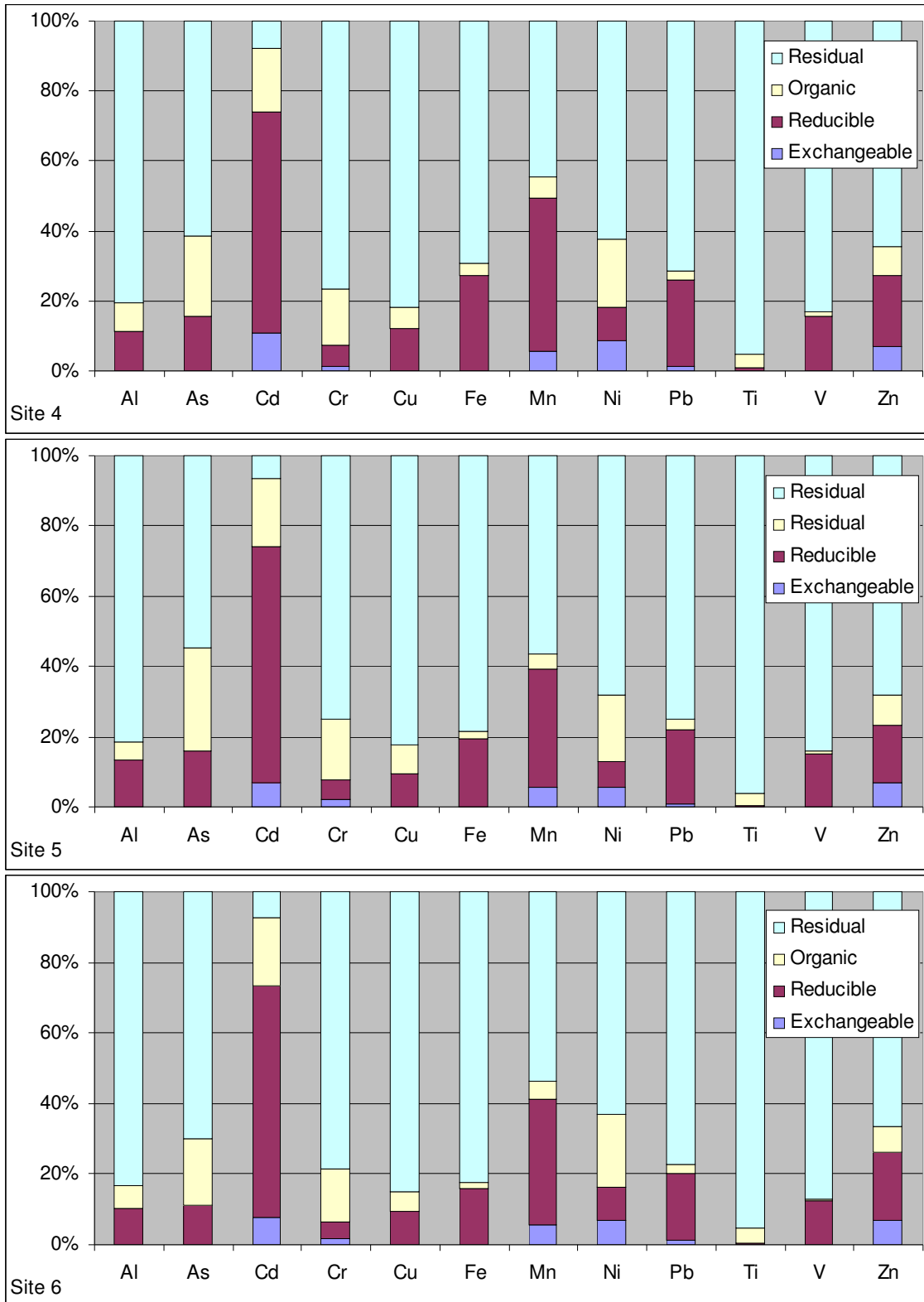


Figure 4.7 Percentage contribution of the four sediment fractions (modified BCR method) to the total trace metal concentrations in the sediment measured at Sites 4-6 in the Nhlabane Estuary over the period 1997-1999.

Table 4.5 Mean percentage (%) contribution (+1STD) of the four sediment fractions to the total metal concentration for each of the trace metals measured in the sediment in the Nhlabane Estuary over the period 1997-1999.

	Fraction 1		Fraction 2		Fraction 3		Fraction 4	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Aluminium	0.0		10.4	1.78	5.6	1.37	84.0	2.59
Arsenic	0.0		11.1	4.53	20.1	6.31	68.8	10.69
Cadmium	8.6	1.50	65.7	1.67	18.9	0.52	6.8	0.81
Chromium	1.6	0.19	5.2	0.65	15.4	1.16	77.8	1.83
Copper	0.0		9.0	1.81	5.7	1.41	85.4	2.63
Iron	0.0		15.8	7.07	1.9	0.89	82.3	7.95
Lead	1.4	0.35	20.2	3.11	2.6	0.29	75.8	3.10
Manganese	5.4	0.10	39.2	10.11	4.3	0.90	51.0	9.83
Nickel	6.3	1.27	8.9	1.03	19.3	1.46	65.6	3.05
Titanium	0.0		0.5	0.12	3.5	0.44	96.0	0.55
Vanadium	0.2	0.04	15.2	1.55	0.8	0.17	83.9	1.57
Zinc	6.8	0.10	17.0	2.43	7.5	0.77	68.7	2.91

A similar pattern was observed at all sampling sites in the Nhlabane Estuary (Figure 4.6 and 4.7). The highest proportion of residual bound metal was consistently observed in Ti, whereas the highest reducible fraction was found in Cd and Mn at all sites. The exchangeable metal proportion also remained relatively constant at all sites, being highest in Cd, followed in decreasing order by Ni, Mn, Pb and Cr.

4.3.2.4 Geochemical normalization of sediment trace metal concentrations

To gain further insight into possible metal contamination in the Nhlabane Estuary, geochemical normalization was applied to the sediment trace metals concentrations, using Fe as a normaliser. Normalisation of sediment metal concentrations in the Nhlabane Estuary to baseline sediment iron concentrations from KwaZulu-Natal coastal waters are illustrated in Figure 4.8. Metal concentrations that fall within the upper and lower 99% prediction limits (for all metals except Cd and As) on the plots are considered to fall within the expected range for granulometrically equivalent but uncontaminated sediment from the region, while concentrations that plot above the upper prediction limit are considered to be enriched. Arsenic and Cd concentrations are poorly correlated with Fe, and in these metals the 95th percentile of the metal concentration distribution was used.

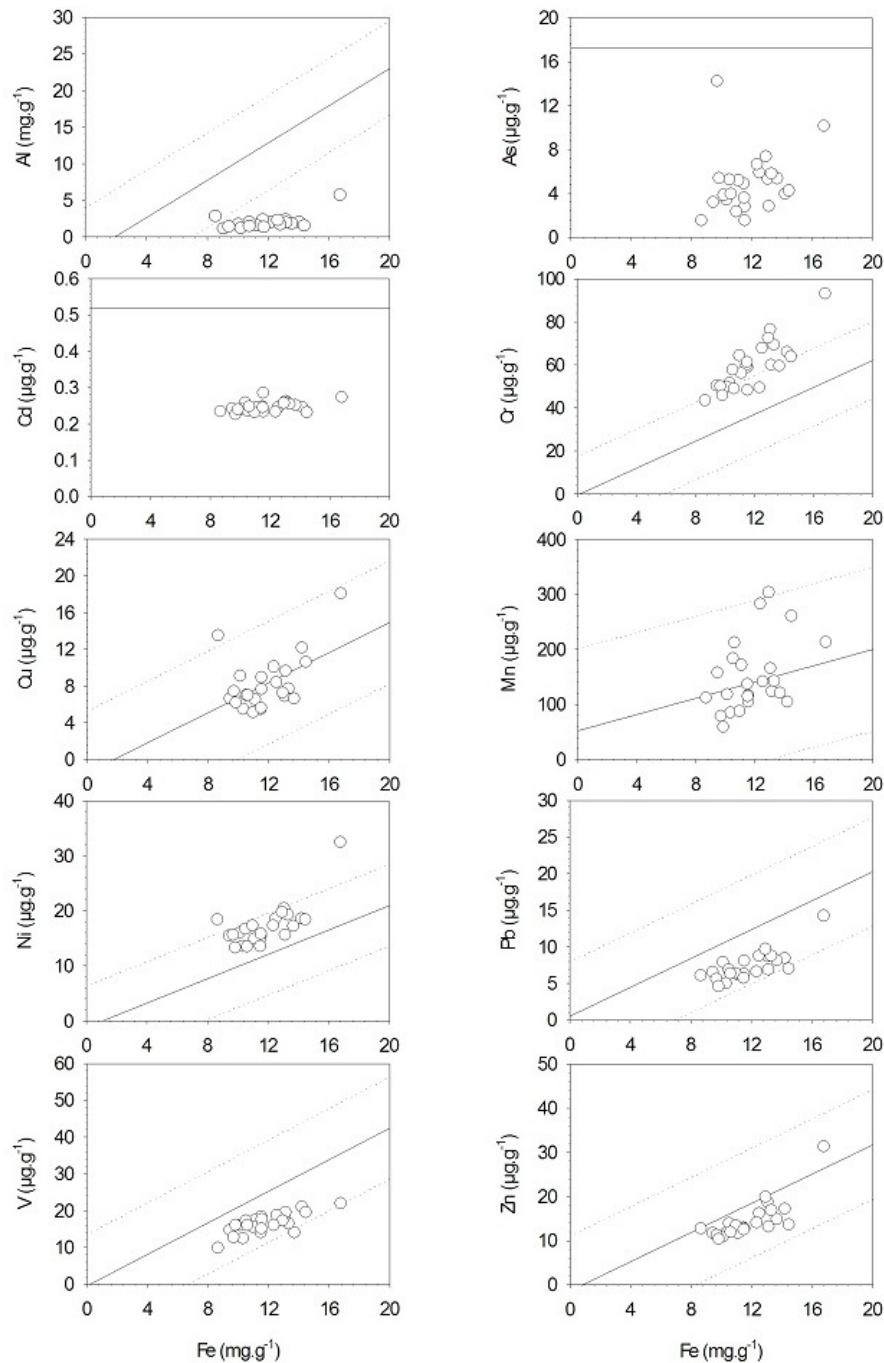


Figure 4.8 Comparison of sediment metal concentrations in the Nhlabane Estuary to baseline sediment iron concentrations from KwaZulu-Natal coastal waters. Metal concentrations that fall within the model upper and lower 99% prediction limits (----) are considered to fall within the expected range for granulometrically equivalent but uncontaminated sediment from the region, while concentrations that plot above the model upper prediction limit are considered to be enriched. For As and Cd, threshold values for uncontaminated sediment from KwaZulu-Natal coastal waters are shown.

Aluminum concentrations in Nhlabane Estuary sediment relative to normalized Fe concentrations were very low, with almost all Al concentrations falling below the lower 99% prediction limit. In all trace metals for which 99% prediction limits have been calculated, with the exception of Cr, nearly all concentrations fell below the upper prediction limit. In contrast, nearly all Cr concentrations measured fell above the 99% prediction limit, suggesting possible Cr enrichment in the sediment. The strong correlation between normalized Fe concentrations and all measured Cr concentrations, however, suggested natural Cr enrichment of the sediment in the Nhlabane Estuary. Since Cd and As concentrations are typically weakly correlated to co-occurring iron concentrations, baseline data was used to determine threshold concentrations, which are the concentrations above which enrichment of sediment by these metals can be inferred. As shown in Figure 4.8, concentrations in these two metals fell well below the threshold concentrations, notably in Cd, in which the maximum concentration of 0.3 µg/l was considerably lower than the threshold concentration.

4.3.2 Trace metals in amphipod tissue.

The mean trace metal concentrations in the two species are presented in Table 4.6. Trace metal concentrations in *G. lignorum* were generally higher than in *C. triaenonyx*, most notably in Al, As, Fe, Mn, Ti and V, while concentrations of Cd, Cu, Ni and Zn were relatively similar in the two species. There was considerable variation in the trace metal concentrations in both species during the study period, as indicated by the large standard deviations, particularly in Cd, Cr, Pb, Ni and Zn.

The mean trace metal concentrations in the amphipods *C. triaenonyx* and *G. lignorum* from the upper, middle and lower reaches of the Nhlabane Estuary over the 12 sampling seasons during 1997-1999 are illustrated in Figures 4.9 and 4.10 and in Figures 4.11 and 4.12, respectively. The amphipod tissue for each species within the different reaches of the system was pooled due to relatively low numbers of the amphipods recorded at times. Results of the ANOVA for differences in body tissue concentrations of trace metals between seasons, years and reaches in the two amphipods are presented in Table 4.6. There were significant differences in *G. lignorum* between the lower, middle and upper reaches in Al, As, Cu, Fe, Pb, Mn, Ti and V. There were also significant seasonal and annual differences in all metals in this species. In *C. triaenonyx*, there were significant differences between reaches in all metals except Cr, Cu, Ni and Zn, while there were significant differences between years except for As and Ni, and between seasons, except for Fe. Although there were some interspecies differences, for example *C. triaenonyx* showed a highly significant difference in Cd concentration between reaches but not *G. lignorum*, there were remarkably similarities between the two species in the variation in body metal concentration between reaches, seasons and years. This is illustrated by the significant positive correlation between the ANOVA

F values (differences between reaches, seasons and years) in *C. triaenonyx* and *G. lignorum* ($R = 0.71$, $p < 0.001$), indicating that the two species generally showed similar differences in metal concentrations between season, year and reach. This similarity in metal concentrations in the two species was further investigated by comparing the metal concentrations in the two species. As shown in Table 4.6, there was a positive correlation in the body concentration of all metals in the two species, this being a significant correlation in all metals except for Ni and Cr. This showed that the degree of metal uptake by the two species was comparable in all metals analysed.

Table 4.6 Mean (± 1 STD) dry mass body metal concentrations ($\mu\text{g/g}$) in *G. lignorum* and *C. triaenonyx* during 1997-1999, as well as ANOVA (F) results of differences in body metal concentration across reach (lower, middle and upper), year (1997-1999) and season (summer-spring). Correlation coefficients of body metal concentrations in the two species are also presented. *denotes significant correlation/difference ($p < 0.05$), ** denotes highly significant correlation/difference ($p < 0.01$).

	Means and STD		ANOVA						Correlation
	<i>Grandidierella</i>	<i>Corophium</i>	<i>Grandidierella lignorum</i>			<i>Corophium triaenonyx</i>			<i>C. triaenonyx</i>
	<i>lignorum</i>	<i>triaenonyx</i>	Reach	Year	Season	Reach	Year	Season	vs <i>G. lignorum</i>
Aluminum	2304 (±831)	729 (±278)	35.2**	110.7**	14.4**	7.1**	84.1**	21.8**	0.61**
Arsenic	8.3 (±4.9)	4.2 (±1.8)	17.6**	6.1**	29.1**	15.3**	1.9	18.2**	0.87**
Cadmium	0.4 (±0.3)	0.38 (±0.32)	0.7	83.6**	8.4**	42.5**	3.1*	29.5**	0.45**
Chromium	123 (±103)	80.4 (±70.4)	0.4	21.4**	38.3**	2.1	3.6*	12.8**	0.21
Copper	99.1 (±28.4)	91.7 (±54.1)	4.4*	76.8**	6.3**	0.7	65.3**	10.5**	0.65**
Iron	10760 (±3577)	4632 (±1815)	38.6**	41.4**	2.8*	39.5**	12.7**	1.1	0.70**
Lead	31.5 (±27.3)	17.6 (±14.7)	3.2*	117.9**	4.6**	9.1**	101.9**	4.9*	0.76**
Manganese	76.8 (±38.1)	27.3 (±16.8)	20.9**	89.3**	17.1**	20.5**	12.6**	4.8*	0.38*
Nickel	53.9 (±40.8)	47.0 (±39.7)	1.0	11.6**	11.8**	0.9	0.9	11.9**	0.33
Titanium	177 (±86)	48.2 (±21.1)	9.1**	123.4**	8.6**	14.6**	67.2**	28.7**	0.62**
Vanadium	13.9 (±6.8)	5.0 (±1.8)	5.5**	47.1**	18.2**	8.7**	4.5*	9.4**	0.70**
Zinc	87.7 (±80.4)	62.1 (±62.0)	1.87	29.9**	34.3**	0.2	14.6**	7.7**	0.69**

Pearson's correlation coefficients between trace metals within each amphipod species are presented in Table 4.7. In both species, all metals except As showed a significant positive correlation with Al, while all metals similarly showed a significant positive correlation with Fe, except for V in *C. triaenonyx* and As and Cd in *G. lignorum* (Table 4.7). In both species, there was a significant positive correlation between concentrations of most metals, with the exception of As, Cd and V.

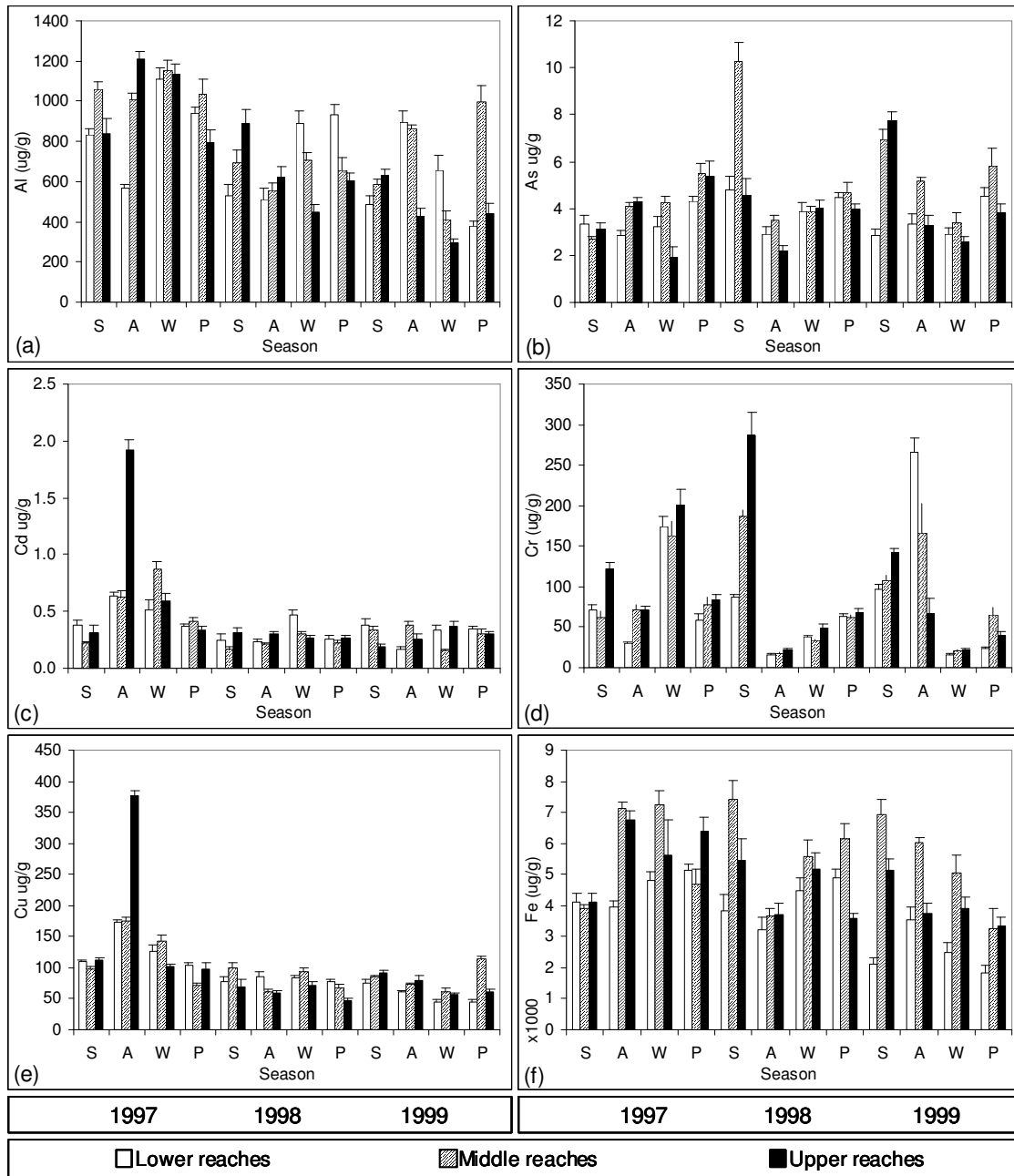


Figure 4.9 Mean seasonal concentrations (+1SE) of Al, As, Cd, Cu, Cr and Fe in *C. triaenonyx* from the lower, middle and upper reaches in the Nhlabane Estuary during the period 1997-1999. S = Summer, A = Autumn, W = Winter, P = Spring

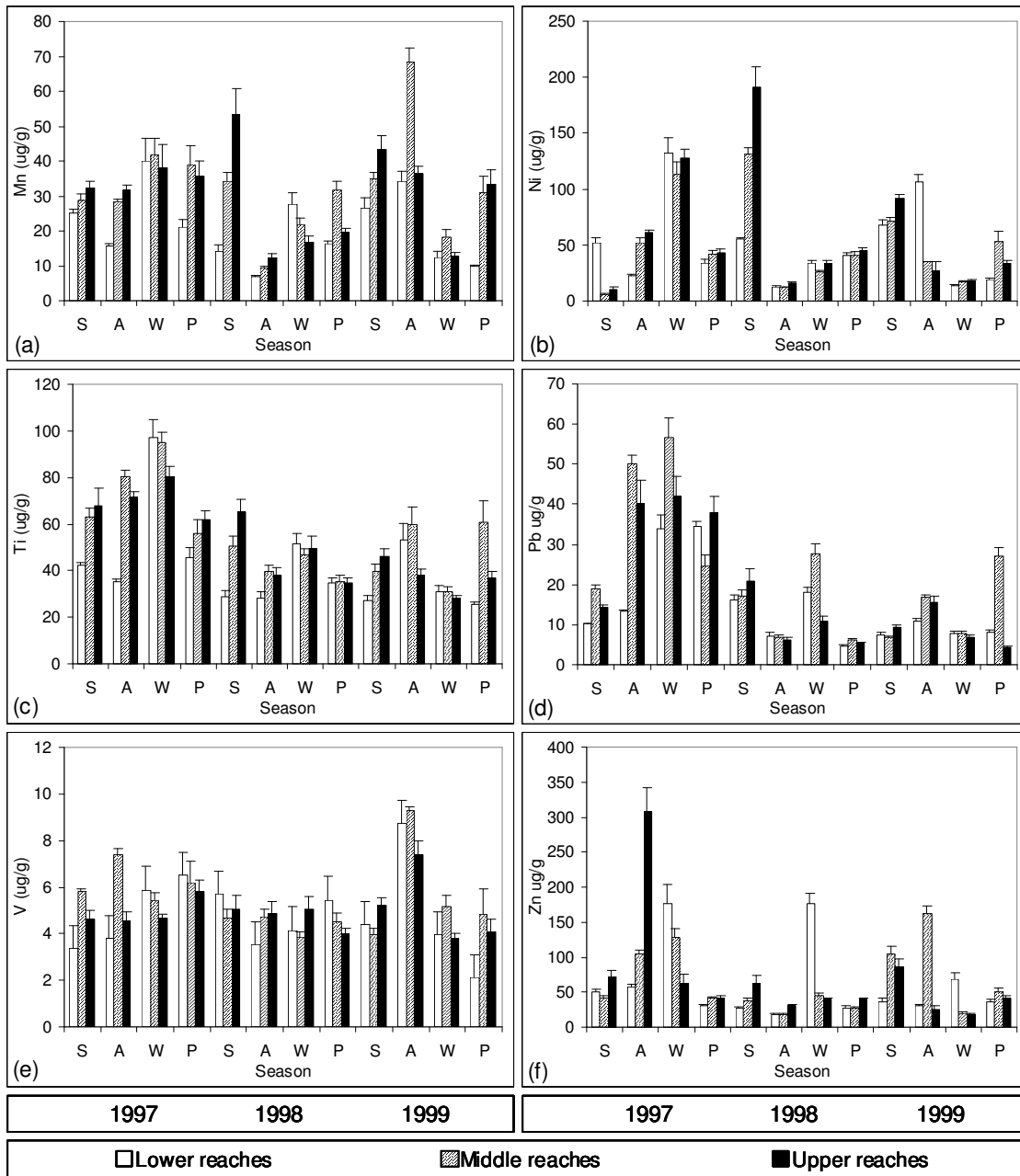


Figure 4.10 Mean seasonal concentrations (+1SE) of Mn, Ni, Pb, Ti, V and Zn in *C. triaenonyx* from the lower, middle and upper reaches in the Nhlabane Estuary during the period 1997-1999. S = Summer, A = Autumn, W = Winter, P = Spring

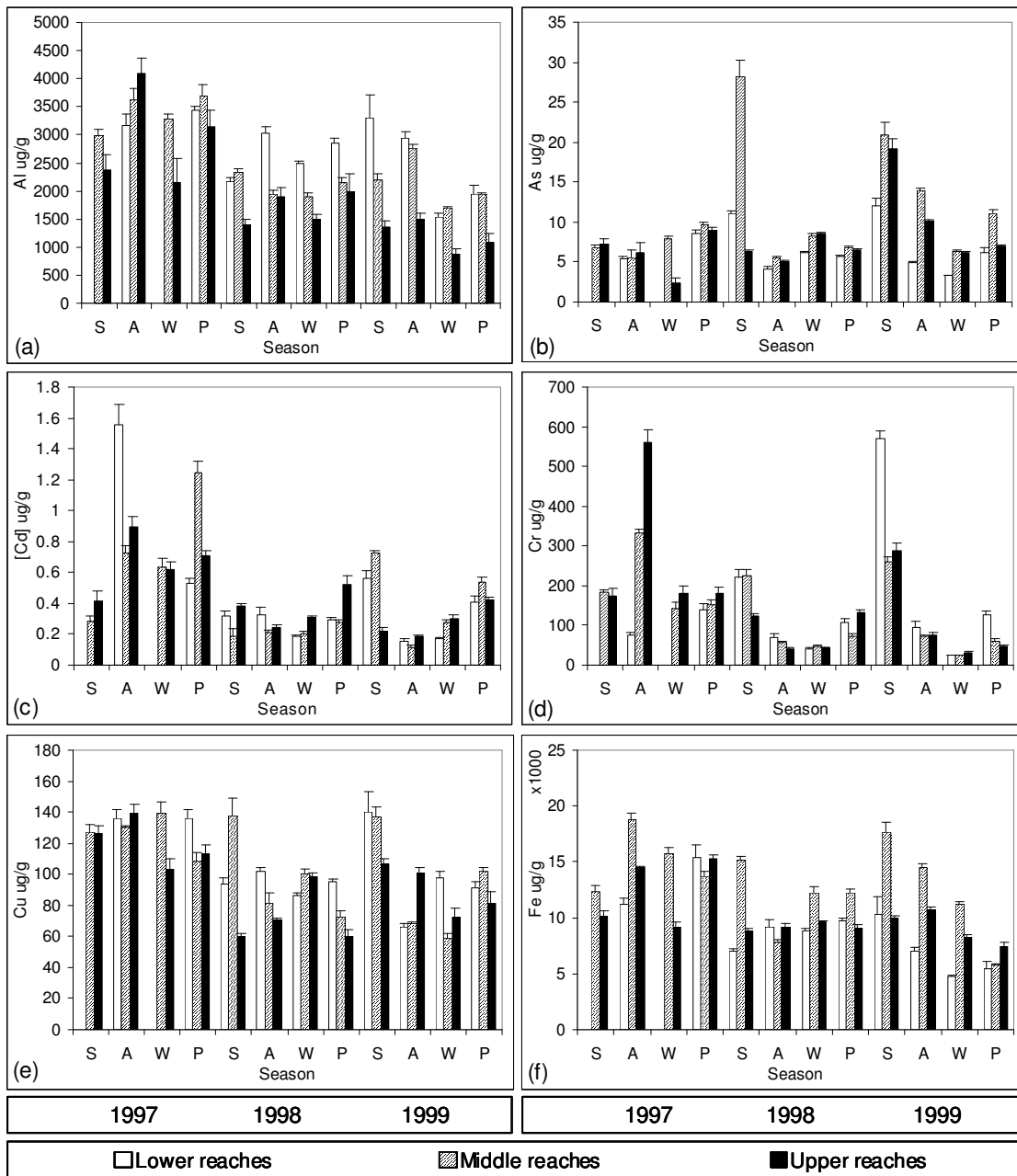


Figure 4.11 Mean seasonal concentrations (+1SE) of Al, As, Cd, Cu, Cr and Fe in *G. lignorum* from the lower, middle and upper reaches in the Nhlabane Estuary during the period 1997-1999. S = Summer, A = Autumn, W = Winter, P = Spring

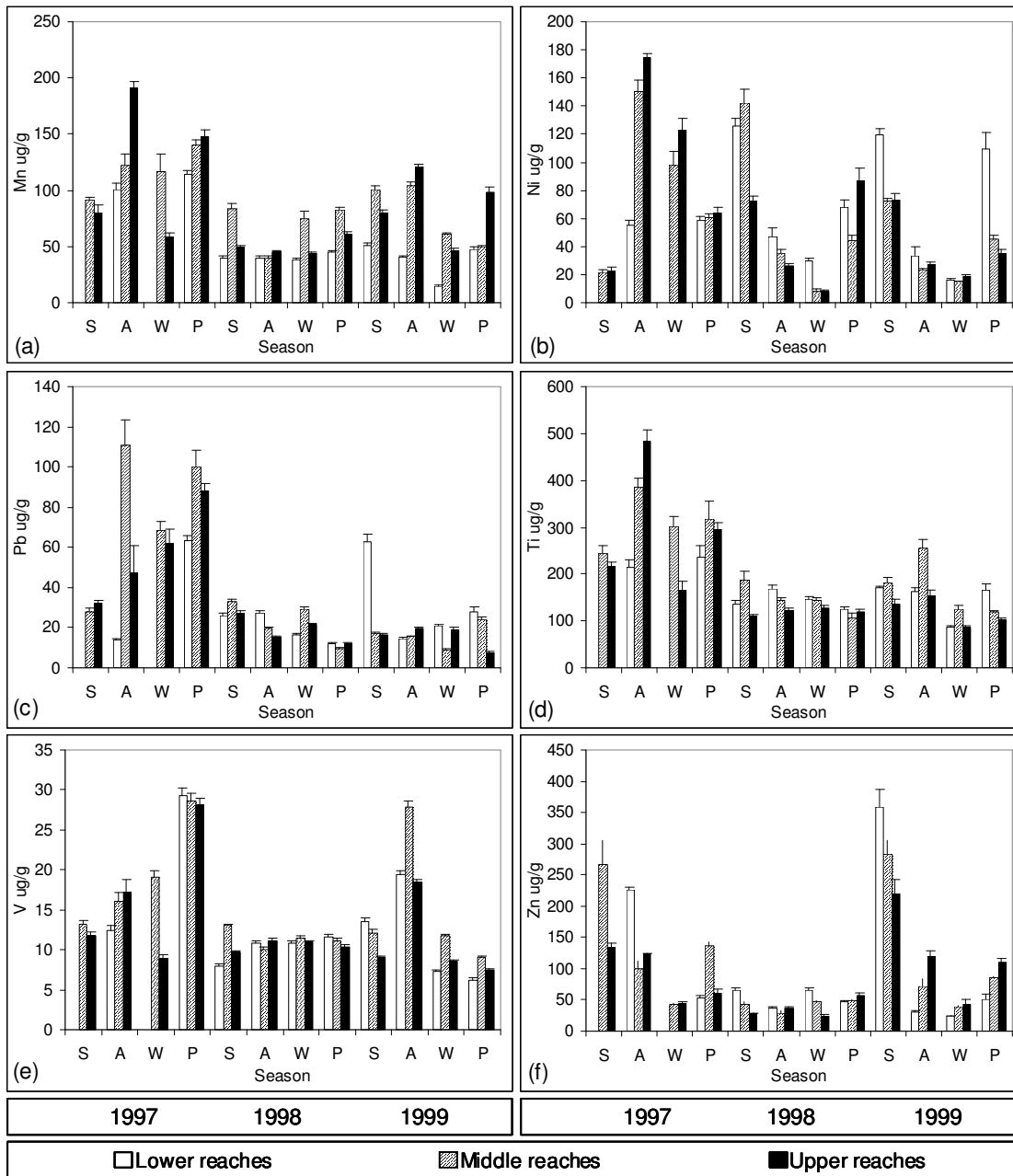


Figure 4.12 Mean seasonal concentrations (+1SE) of Mn, Ni, Pb, Ti, V and Zn in *G. lignorum* from the lower, middle and upper reaches in the Nhlabane Estuary during the period 1997-1999. S = Summer, A = Autumn, W = Winter, P = Spring

Table 4.7 Correlation (Pearson's Correlation coefficient R) of body metal concentrations in the amphipods *G. lignorum* and *C. triaenonyx* from the Nhlabane Estuary. * denotes significant correlation ($p < 0.05$), ** denotes highly significant correlation ($p < 0.01$).

	Aluminium	Arsenic	Cadmium	Chromium	Copper	Iron	Lead	Manganese	Nickel	Titanium	Vanadium
<i>Grandidierella lignorum</i>											
Aluminium											
Arsenic	-0.04										
Cadmium	0.49**	-0.14									
Chromium	0.51*	0.34*	0.29								
Copper	0.62*	0.16	0.22	0.67**							
Iron	0.71**	0.22	0.28	0.44*	0.63*						
Lead	0.63**	0.12	0.36*	0.44*	0.52**	0.88**					
Manganese	0.47**	0.28	0.31	0.96**	0.69**	0.40*	0.38*				
Nickel	0.63**	-0.05	0.13	0.46*	0.66**	0.62**	0.59**	0.43*			
Titanium	0.82**	0.07	0.44*	0.53**	0.72**	0.88**	0.82**	0.51**	0.70**		
Vanadium	0.64**	0.14	0.32	0.14	0.23	0.65**	0.62**	0.05	0.52**	0.64**	
Zinc	0.38*	0.15	0.45*	0.63**	0.45*	0.52**	0.59**	0.64**	0.13	0.60**	0.12
<i>Corophium triaenonyx</i>											
Aluminium											
Arsenic	0.03										
Cadmium	0.50*	-0.11									
Chromium	0.46*	0.28	0.02								
Copper	0.52**	0.04	0.90**	0.04							
Iron	0.45*	0.49**	0.35*	0.37*	0.44*						
Lead	0.46**	0.34*	0.13	0.70**	0.12	0.48**					
Manganese	0.42*	0.35*	0.14	0.88**	0.14	0.41*	0.52**				
Nickel	0.73**	0.04	0.57**	0.31	0.58**	0.58**	0.35*	0.38*			
Titanium	0.83**	0.04	0.45*	0.56**	0.48**	0.55**	0.57**	0.53**	0.83**		
Vanadium	0.37*	0.07	-0.05	0.45*	0.03	0.29	0.58*	0.17	0.31	0.39*	
Zinc	0.56**	0.09	0.80**	0.21	0.74**	0.43*	0.44*	0.25	0.47**	0.58*	0.12

The Pearson's Correlation coefficients between body metal concentrations in *G. lignorum* and *C. triaenonyx* and body weight, environmental factors and trace metal concentrations in water and sediment in the Nhlabane Estuary are presented in Table 4.8. In both species, there was generally a strong negative correlation between trace metal concentrations and body weight in all metals, despite the fact that only adult amphipods were analysed. This correlation was significant in all metals except As, Cd and V in *G. lignorum* and As and V in *C. triaenonyx*. In the majority of metals, there was a negative correlation with salinity, pH and depth and a positive correlation with sediment phi values and organic content. In *G. lignorum*, As, Cr and Ni were significantly correlated with increasing temperatures. In *C. triaenonyx*, only As was significantly correlated with increasing temperatures. There was a surprisingly weak correlation between body tissue and sediment metal concentrations in most metals in both species, with the exception of V, which showed a significant positive correlation. In *G. lignorum*, there was a negative correlation

between body tissue and sediment metal concentrations in Cr, Cu, Mn, Ti and Zn, while in *C. triaenonyx*, there was a negative correlation between body tissue and sediment metal concentrations in Cd, Cu, Mn and Ti. There was a weak correlation between body tissue and water metal concentrations in both species.

Table 4.8 Correlation (Pearson's Correlation coefficient R) between body metal concentrations in *Grandidierella lignorum* and *Corophium triaenonyx* and body weight, environmental factors and trace metal concentrations in water and sediment as measured in the Nhlabane Estuary during the period 1997-1999. * denotes a significant correlation ($p < 0.05$), ** denotes a highly significant correlation ($p < 0.01$).

	Body Weight	Salinity	Temp	Physical factors					Metals in water	Metals in sediment
				pH	DO	Depth	Phi	Organic content		
<i>Grandidierella lignorum</i>										
Aluminium	-0.46**	-0.18	-0.10	-0.08	0.14	-0.02	-0.01	0.21	-0.15	0.14
Arsenic	-0.20	-0.12	0.54*	-0.04	-0.33	-0.09	0.01	0.17	-0.08	0.25
Cadmium	-0.15	0.14	-0.14	-0.05	-0.03	-0.09	-0.17	0.18	-0.12	0.00
Chromium	-0.57**	-0.27	0.45*	-0.34*	-0.41*	-0.15	0.08	0.09	-0.32	-0.07
Copper	-0.62**	-0.34*	0.09	-0.42*	-0.27	0.03	0.02	0.21	0.21	-0.12
Iron	-0.45*	-0.32	-0.02	-0.37*	-0.18	-0.33	0.37*	0.32	-0.08	0.14
Lead	-0.59**	-0.43*	0.05	-0.34	0.08	-0.13	0.37*	0.17	-0.05	0.01
Manganese	-0.51**	-0.34*	0.10	-0.35*	-0.13	-0.31	0.36*	0.14	0.27	-0.18
Nickel	-0.52**	-0.27	0.34*	-0.35*	-0.39*	-0.16	0.11	0.05	-0.27	0.10
Titanium	-0.57**	-0.25	-0.05	-0.33	-0.13	-0.17	0.23	0.18	-0.29	-0.30
Vanadium	-0.16	0.02	-0.10	-0.09	0.20	-0.34**	0.21	0.04	0.04	0.59*
Zinc	-0.37*	-0.12	0.19	-0.36*	-0.50**	0.04	-0.07	0.08	-0.17	-0.37
<i>Corophium triaenonyx</i>										
Aluminium	-0.56**	-0.05	-0.18	-0.16	0.12	-0.13	0.16	0.30	-0.22	0.18
Arsenic	-0.19	-0.29	0.48**	0.05	-0.18	-0.10	0.16	0.08	-0.11	0.28
Cadmium	-0.33*	-0.14	-0.21	-0.34	-0.19	-0.12	0.00	0.11	-0.05	-0.31
Chromium	-0.55**	0.05	0.14	-0.17	0.01	-0.32	0.14	0.17	-0.24	0.22
Copper	-0.39*	-0.16	-0.13	-0.37*	-0.28	-0.08	0.01	0.21	0.14	-0.22
Iron	-0.40*	-0.25	-0.12	-0.28	-0.12	-0.46**	0.40*	0.46*	-0.11	0.27
Lead	-0.62**	-0.23	-0.25	-0.37*	0.10	-0.35*	0.38*	0.35*	0.04	0.14
Manganese	-0.58**	0.08	0.16	-0.13	-0.04	-0.40*	0.26	0.05	0.06	-0.04
Nickel	-0.52**	-0.07	0.14	-0.30	-0.11	-0.25	0.18	0.27	-0.04	0.20
Titanium	-0.74**	-0.07	-0.24	-0.29	0.04	-0.36*	0.34*	0.41*	-0.05	-0.04
Vanadium	-0.23	0.26	-0.15	-0.08	0.17	-0.20	0.08	-0.03	0.00	0.52*
Zinc	-0.41*	-0.13	-0.19	-0.22	-0.23	-0.14	-0.11	0.16	-0.12	0.02

4.4 Discussion

4.4.1 Trace metals in the water

Information in the literature on trace metal concentrations in the Nhlabane Estuary water is limited to one DWAF report on the Ecological Reserve of the Nhlabane Estuary (Quinn 2000), in which the median concentrations of Cu, Fe, Mn and Zn were reported. This study therefore provides the first detailed account of water metal concentrations in the Nhlabane Estuary.

The concentrations of Cu, Mn and Zn reported by Quinn (2000) for the Nhlabane Estuary were lower than that recorded during the present study. Quinn (2000) reported median concentrations for Cu and Zn of 1 µg/l and 1.7 µg/l, respectively, in contrast to the mean concentrations of 4.5 µg/l and 17.2 µg/l for these two metals recorded during the present study. The reasons for these differences are not clear, as no details were supplied by Quinn (2000) as to where and when the water samples were collected and analysed. Median concentrations are less influenced by highly variable data than mean concentrations and in the present study, there was considerable variation in water metal concentrations in the Nhlabane Estuary along the length of the estuary and between sampling seasons. The concentrations reported by Quinn (2000) for Cu, Mn and Zn lay at the lower end of the range of concentrations recorded during this study.

A comparison with water metal concentrations in the Mhlathuze estuary (Mzimela *et al.* 2003) and Richards Bay Harbour (Vermeulen and Wepener 1999) showed that concentrations of all metals in the Nhlabane Estuary were much lower than that from these two systems. Metal concentrations in the Nhlabane Estuary water were also much lower than South African and international target values for coastal ecosystems, indicating that the Nhlabane Estuary water was uncontaminated

A number of water quality surveys have been conducted over the past few decades on South African coastal waters and estuaries, which was summarized by Hennig (1985). Highest concentrations along the entire coastline of Cu, Zn, Cd, and Pb were reported from the northern KwaZulu-Natal coast, including Durban Bay, Richards Bay and St Lucia. These relatively high concentrations, notably at St Lucia, were regarded as indicative of geological factors leading to naturally high background concentrations of metals. Water metal concentrations in the Nhlabane Estuary compared favorably with these data, with Cd, Cu, Ni and Pb falling within the range of concentrations reported by Hennig (1985), while Zn concentrations were slightly higher in the Nhlabane Estuary. A comparison with trace metal concentrations in the Eastern Cape also showed that the mean concentrations of all metals in the Nhlabane Estuary compared favorably with that reported from these estuaries (Watling 1988).

Concentrations of Cd, Pb, Fe, Mn and Ni from the Nhlabane Estuary were at the bottom end of the range reported for Eastern Cape estuaries, while Cu and Zn concentrations approached the upper end of the range for Eastern Cape estuaries. Within these estuaries, there was considerable variation in trace metal concentrations, with the Papenkuils, Nahoon and Buffels consistently showing highest concentrations (Orr 2007).

It is noteworthy that the concentrations reported by Hennig (1985) for Richards Bay from 1974-1976 for Cu ranged between 0.3-4.8 µg/l, for Pb between 1.8-10.5 µg/l and for Zn between 1.8-12.5 µg/l. These concentrations were comparable to that reported from Richards Bay by Begg (1978), who noted that these concentrations were within normal acceptable limits. The maximum concentrations reported by Hennig (1985) were still an order of magnitude lower than that reported by Mzimela *et al.* (2003) for the Mhlathuze estuary, a permanently open estuary with limited industrial related contamination. The reason for these very large differences between historical Richards Bay (Hennig 1985) and more recent Mhlathuze estuary (Mzimela *et al.* 2003) metal concentrations is not clear, but the Mhlathuze concentrations appear to be uncharacteristically high and differences in analytical techniques should perhaps be carefully considered.

The reason for the relatively high Cu and Zn concentrations in the Nhlabane Estuary water is not clear, as its catchment is not exposed to major anthropogenic input of industrial and agricultural effluent. The relatively small catchment is largely dominated by forestry. In addition, the presence of the impounded Nhlabane Lake upstream of the estuary also means that much of the metal burden in the water would tend to settle out in the lake, with limited contamination reaching the estuary from the catchment. The concentrations of Cu and Zn in the water also showed a strong positive correlation with Fe and to a lesser extent with Al. As Fe and Al naturally occur in high concentrations, this suggests that the levels of Cu and Zn in system were influenced by natural variations in background metal concentrations. During the study period, however, the coastal dunes in close proximity to the estuary was mined by RBM, with dredgers from mining pond B and C crossing the estuary in 1993 and 1999, respectively. Leaching of water from the mined dunes at the base of the steep banks lining the sides of the estuary was observed during the study period and the colour of the water suggested ferrous oxide staining. As the metal composition of the leachate was not analysed, no conclusions can, however, be drawn about the link between dune mining activities and the metal concentrations in the estuary water.

4.4.2 Trace metals in sediment

There is no historical data in the literature on sediment metal concentrations in the Nhlabane Estuary. Sediment metal concentrations in the estuary were generally low and were comparable to those from the Mhlathuze estuary (Mzimela *et al.* 2003) and from uncontaminated areas in Richards Bay harbour. Concentrations were, however, considerably lower than that from contaminated areas in the harbour (Wepener and Vermeulen 2005). As indicated in Table 4.1, there are no sediment quality guidelines for South African coastal waters, but concentrations were considerably lower than the Effects Range Low (ERL) values for Australian estuaries, which is the lower 10th percentile of the metal concentration data and is the concentration of a contaminant below which harmful effects rarely (<10%) occurs (Long *et al.* 1995). The relatively low metal concentrations in the Nhlabane Estuary, based on these ERL values, is therefore suggested to be indicative of an uncontaminated estuary with metal concentrations not expected to adversely affect benthic organisms.

Hennig (1985) provided a comparative summary of sediment trace metal concentrations in estuaries along the northern KwaZulu-Natal coastline based on data recorded over the period 1975-1979. The mean concentrations of all eight metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the Nhlabane Estuary for which comparative data is provided by Hennig (1985), were at the lower end of the range of metal concentrations reported for the St Lucia estuary and Richards Bay Harbour, this also being indicative of the relative uncontaminated nature of the Nhlabane Estuary sediment. No data on Al, As, Ti and V concentrations were supplied by Hennig (1985) for the northern KwaZulu-Natal coastline. A comparison with sediment metal concentrations from Eastern Cape estuaries (Allanson and Baird 1999) showed that mean concentrations in the Nhlabane Estuary for Cu, Pb, Zn, and Fe were at the lower end of the range for eastern Cape estuaries, Mn and Cd were in the middle of the range, while only Ni and Cr were at the upper end of the range. In uncontaminated control areas in the Firth of Clyde in Scotland and the Avon estuary in England, concentrations of all the metals tested (Cu, Pb, Zn, Fe, Cd and Ni) were higher than in the Nhlabane Estuary, providing further evidence that the Nhlabane Estuary sediment during the study period was not contaminated (Bryan and Langston 1992).

4.4.3 Sequential extraction

Sedimentary particulate matter consists of many different components and phases, including crystalline minerals, carbonates, hydrous metal oxides and organic substances. Metals may be bound to the particulate matter by a variety of mechanisms including surface adsorption, ion exchange, co-precipitation and complexation with organic substances. The distribution on trace metals in the

different phases determines their mobility, bioavailability and toxicity (Usero *et al.* 1998, Rauret *et al.* 1999). Total metal concentrations in aquatic sediments therefore do not necessarily reflect bioavailability and high metal concentrations do not imply high toxicity. In order to better understand the bioavailability and potential toxicity of trace metals to aquatic organisms, it is useful to study the different speciation forms in which metals occur rather than the total metal concentration (Tessier *et al.* 1979). Although most studies dealing with particulate metals in sediment in aquatic systems have been concerned mainly with total metal concentrations, sequential extraction techniques have been developed since the 1980's in which selective reagents are used to consecutively extract the different chemical forms of the metal (Rauret *et al.* 1999). In this study, the optimized BCR (European Community Bureau of Reference) method was used, as developed by the European Commission in order to create a standard extraction methodology using standardized reference materials (Rauret *et al.* 1999, Sutherland and Tack 2003). The optimized BCR procedure is currently the only scheme that is standardized, and reference samples covering a range of levels of sediment contamination is available. This is a significant advantage and provides for a degree of comparability between research laboratories generally not obtainable for most of the other procedures reported in the literature (Sutherland and Tack 2003). Adamo *et al.* (2005) also showed a close agreement between the fractional total concentrations using the BCR methodology and the total concentration as determined through standard single step analysis procedures, indicating that enhanced recovery of metal through the sequential extraction process is not an issue of concern. Sequential extraction procedures such as the BCR method should however always be applied with full consideration of their limitations in terms of the ability of the various reagents used to extract metal mixtures (Rauret *et al.* 1999).

Of most relevance to toxicity assessments of estuarine sediments is the proportion of a metal in the sediment that is bioavailable in the exchangeable and water and acid soluble fraction (Rainbow 2007, Re *et al.* 2009). This is the fraction that has the greatest potential to be toxic to aquatic organisms (Adamo *et al.* 2005). Simpson and Batley (2007) pointed out that the bioavailability of metals in sediments is influenced by a complex set of factors which include metal speciation, sediment–water partitioning relationships, organism physiology and the feeding dynamics of organisms. As reflected in Figures 4.6 and 4.7 and Table 4.5, the proportion of metal in the exchangeable fraction for the majority of metals in the Nhlabane Estuary was below 0.2%, while for Cr and Pb, it was below 2%, suggesting that in these metals, the amount of bioavailable metal that could potentially be toxic was very low. The exchangeable proportion in only four metals (Zn, Ni, Mn and Cd) was above 5%. Such relatively high exchangeable fractions was however not unusual in Zn and Cd in uncontaminated sediment (Adamo *et al.* 2005). In the majority of metals, the largest proportion (>80%) was found in the residual fraction and thus tightly bound within the mineralogical content of the sediment. These results parallel those reported from Richards Bay Harbour, where the exchangeable portion of Al, Cr,

Cu and Fe was also found to be negligible in some areas (results were based on the 5 step Tessier extraction method) (Wepener and Vermeulen 2005). The conservative metals Fe and Al were also found in very similar proportions in the two systems, but in the harbour, some contamination by a number of metals was observed, as the reducible fraction of Cr, Cu, Mn and Zn in the harbour sediment was much higher than in the Nhlabane Estuary.

In a comparative assessment of reference sediments from European coastal waters using the modified BCR method, metal concentrations in reference sediment known as CRM601 was reported by Sutherland *et al.* (2002). Despite being classified as only mildly contaminated by Sutherland *et al.* (2002), concentrations of Cu, Zn, Pb and Mn in this sediment were at least an order of magnitude higher than in the Nhlabane Estuary sediment. The most notable difference between the CRM601 and Nhlabane Estuary sediment was found in the relatively high proportions of the exchangeable and reducible fractions, the two fractions most likely to be toxic to aquatic organisms. For example, the exchangeable Cu fraction in the CRM601 and Nhlabane sediment was 34% and 7 % respectively, while the reducible Pb fraction in the CRM601 and Nhlabane sediment was 83% and 20 % respectively. The relatively low exchangeable and reducible fractions in the Nhlabane Estuary sediment for all metals, with the possible exception of Cd, therefore suggest that the sediment in the system was not contaminated. In a partitioning study of the Port of Naples sediment, Adamo *et al.* (2005) also found a considerable increase in the exchangeable and reducible fractions in contaminated sediments. These authors reported a 25%, 3% and 24% increase in the exchangeable fraction in Cd, Pb and Zn from control sediment to contaminated port sediment, respectively, while in Cr, Cu, Pb and Zn, there was a corresponding 40%, 45%, 15% and 40% decrease in the contribution of the residual fraction to the total metal concentration, respectively. What is notable in the present study, however, is that the exchangeable fraction in the Nhlabane Estuary sediment was as low as or lower than that in the uncontaminated control sediment from Naples. The exchangeable fraction in Cd, Cr, Cu, Pb and Zn in the Naples sediment comprised 26%, 1%, 14%, 1% and 18%, respectively, compared to 9%, 2%, 0%, 1% and 7% for Nhlabane sediment. This again suggests that metal concentrations in the Nhlabane Estuary closely resembled natural background levels with no apparent indication of contamination.

The distribution of Cd between the extractable fractions from Nhlabane sediment was very different from that of all other metals tested, with 9% in the exchangeable fraction, 66% in the reducible fraction and only 7% in the residual fraction. These values correspond to those from other studies, as Rauret *et al.* (1999) reported 69 % of Cd in the reducible fraction and only 1 % in the residual fraction in reference sediment CRM483, while Adamo *et al.* (2005) found 22% Cd in the reducible fraction

and 25% in the residual fraction in control sediment in the Port of Naples. No comparative data for Cd was available from Richards Bay Harbour.

The value of sequential metal extraction as an evaluation tool in toxicity assessments of contaminated and dredged sediments has been realized more in more in recent years as it provides an indication of the potential for mobilization, bioavailability and ultimately toxicity of metals in aquatic sediments (Wepener and Vermeulen 2005). To this end, the European Commission has made a concerted effort to develop and standardize the BCR methodology with the ultimate goal to develop a method that can be successfully utilised and cross-referenced by researchers across the globe. In this study, sequential extraction was used to provide additional evidence that concentrations of sediment fractions that could potentially be toxic were low for all metals tested.

4.4.4. Correlation and Geochemical Normalisation of sediment

Estuarine sediments are known to effectively sequester chemical pollutants and high concentrations of trace metals and organic pollutants in sediment are often associated industrialized and urbanized estuarine and coastal environments (Rainbow 2002, McCready *et al.* 2006). As a consequence of the sheltered nature of estuaries, these materials tend to settle out in estuaries, and thereby increase the propensity for further contamination and accumulation in these environments (Rainbow 2002). Metals, which typically bind to fine grained sediments and organic matter, are however not indefinitely bound to sediments and may be re-mobilized and returned to solution via physical, chemical and biological processes (De Groot *et al.* 1976). Metals may thus occur in various chemical forms whilst being transported to the sea, such as ions in solution, organic or inorganic complexes in solution, adsorbed onto the surface of suspended matter or sediment particles, bound to sulfides or incorporated into solid organic particles (Kennish 2002).

There are many factors that influence the concentration and therefore the toxicity of metals in sediment, the most important of which are natural background concentrations, salinity, pH, speciation, granulometry and organic content of the sediment (Rainbow 2007). It is inappropriate to consider metal concentrations in the sediment of a given area without taking these factors into account.

In estuaries, salinity is one of the key controlling factors for the partitioning of contaminants between sediments and overlying waters (Chapman and Wang 2001). The free ionic component of metals and therefore the bioavailable fraction generally increase with a decrease in salinity, thus a decrease in salinity of exposure waters increases the toxicity of metals such Ni, Zn, Cu and Cr to aquatic organisms. Similarly, in freshwater and low salinity systems, pH controls the transition of metals

adsorbed in the sediment into the free ionic and bioavailable form and thus the toxicity of metals to aquatic organisms (Chapman and Wang 2001).

Different sediments can have different capabilities of adsorbing contaminants. In uncontaminated sediments, metal concentrations tend to increase as grain size decreases due to the fact that the surface area of sediments is grain size dependant, and thus smaller particles provide a larger surface area for the adsorption of metals (Summers *et al.* 1996). Fine grained sediment such as silt and mud naturally have a higher metal content compared to coarse-grained sand, and as a consequence, there is typically a strong inverse correlation between sediment grain size and the metal content of sediment. In contrast, coarse grained sand particles are composed primarily of quartz and potassium and are less reactive. A similar trend exists for organic content, as it is widely acknowledged that metallic cations have strong adsorptive tendencies and a capacity for complex formation with organic matter (Rainbow 1995). Organic carbon thus acts as a matrix to which metals can readily complex, and several studies have shown strong linear relationships between total organic carbon and metal concentrations. Generally, muddy sediments have a higher organic content than sandy sediment (Chapman and Wang 2001).

Although all sediment metal concentrations during this study, except for As, showed a negative correlation with salinity, temperature and depth, ordination analysis (PCA) revealed that water quality parameters (salinity, temperature, depth, pH, and dissolved oxygen) were only weakly correlated with metal concentrations and were therefore relatively unimportant in determining sediment metal concentrations. It would appear that sediment metal concentrations were mostly influenced by the natural geology of the estuarine environment as well as sedimentary characteristics. Of all the parameters tested, the best correlation between metal concentrations and environmental conditions was observed with sediment grain size and organic content. As particle size and organic content are such influential factors in controlling metal concentrations, it would also explain the generally low metal concentrations in the Nhlabane Estuary sediment. The sediment in the system was characterized by fine to medium fine grained sand with no mud and consistently low organic content (See Section 3.3). The absence of mud and low organic content in the system is related to the Nhlabane weir, which prevents fine sediment and detritus entering the lake via surface runoff from the catchment to reach the estuary. This lack of mud in the sediment and the unusually low organic content is therefore expected to result in naturally low sediment metal concentrations (Chapman and Wang 2001, Newman and Watling 2007). The relatively uniform sediment type in the system would also explain the absence of notable spatial and temporal differences in sediment metal concentrations observed in the system during the study period.

As indicated in Table 4.4, the most obvious anomaly with regard to the influence of physico-chemical water quality parameters on metal concentrations was observed in As. This metal showed a significant positive correlation with salinity and depth, in stark contrast to all the other metals. As expected, highest salinities were recorded at the mouth when the estuary was open. The deepest sampling sites were also at the mouth. This strongly suggests that the As concentration in the system was marine driven, with the metal entering the system dissolved in seawater when the mouth was open and settling out onto the sediment.

Metals in estuaries often show naturally high concentrations in some areas, regardless of the factors mentioned above (Rainbow 2002). Determining whether estuarine sediment is metal contaminated is complicated because metal concentrations in uncontaminated sediment can vary by orders of magnitude over relatively small spatial scales due to the natural mineralogy and sediment granulometry (Newman and Watling 2007). High metal concentrations therefore do not automatically imply that contamination has occurred, but may simply reflect natural concentrations in the sediment (Orr *et al.* 2008).

As indicated in Table 4.4, the concentrations of all metals, except for As, showed a very strong positive correlation with Fe, while all but As, Cd and Mn also showed a similar strong correlation with Al concentrations. The natural concentrations of Fe and Al in sediments, respectively the third and fourth most abundant elements in the earth's crust, are orders of magnitude higher than the concentrations of most other metals from a toxicological perspective and reflect the natural geochemical composition in the immediate environment (Rainbow 1995). The high natural concentrations of Al and Fe are considered to mask the usually low inputs of these metals to the environment from anthropogenic sources, and their concentrations are therefore likely to remain relatively unchanged even in anthropogenically impacted areas (Summers *et al.* 1996, Orr *et al.* 2008). Because Fe and Al are so abundant in the earth's crust; given the naturally occurring high levels of these elements, they are poorly influenced by anthropogenic input (Dauvin 2008). The low natural concentrations of trace metals are in contrast far more sensitive to anthropogenic inputs, with the result that the concentration ratio between the trace metals and the Al or Fe is changed when contamination occurs. In uncontaminated sediment, the ratio between Fe and Al concentrations with that of the other metals therefore remains relatively constant within the same geographical area. This principle is used in geochemical normalization, which is based on the fact that while absolute metal concentrations vary between crustal material from one region to another, the relative proportions of metals within crustal material from a particular region naturally tend to be fairly constant (Newman and Watling 2007). Normalisation thus serves to detect unnatural alterations in the ratios of metal concentration, by comparing metal concentration ratios measured in sediment from uncontaminated

locations to those in potentially metal-enriched sediment. As noted by Chapman and Wang (2001), geochemical normalization can compensate for both the granulometric and mineralogical variability of metal concentrations in sediments, with the result that Fe is often used successfully for normalization of trace metals in order to assess possible anthropogenic input of contaminants into aquatic ecosystems.

Normalization of Nhlabane Estuary sediment metal concentrations to background concentrations for uncontaminated KwaZulu-Natal coastal areas showed that with the exception of Cr, concentrations of all metals tested fell within the prediction limits and as such are indicative of uncontaminated sediments. The fact that most Nhlabane Estuary Cr concentrations plotted above the prediction limits was unexpected as these Cr levels cannot be directly linked to any known source of Cr contamination. As mentioned earlier, sources of possible industrial contamination in the Nhlabane catchment is limited as the catchment is dominated by pine and gum plantations (29%), rural informal settlements (43%) and indigenous forest (9%). Furthermore, mean Cr concentrations in the system (59.2 µg/g) were only slightly higher than that recorded in known uncontaminated areas in Richards Bay Harbour (57.8 µg/g) and in the Mhlathuze Estuary, but considerably lower than that recorded in known contaminated areas in the harbour (152.2 µg/g) as well as the sediment quality guideline (ERL 81.0 µg/g) for Australian estuaries. Hennig (1985) also reported Cr concentrations in South African coastal sediments ranging from 2.6-388 µg/g, which compared favorably with the range of concentrations of 35-307 found in other parts of the world. A high level of contamination in a trace metal is also usually associated with a high bioavailable fraction. This was not the case with Cr in the Nhlabane Estuary, as the bioavailable fraction of this metal remained very low throughout the system, as indicated in Figure 4.8. The strong positive correlation between Cr and Fe concentrations (0.82) also suggested that Cr concentrations closely followed that of the conservative, naturally abundant Fe, this usually being indicative of trace metal concentrations of uncontaminated sediment.

4.4.5 Amphipods as biomonitors

All aquatic invertebrates accumulate trace metals in their tissues, whether or not these metals are essential to metabolism. Different invertebrates accumulate trace metals to different concentrations in their body tissues (Rainbow and White 1989). Whether an accumulated trace metal concentration is high or low, therefore, cannot be assessed on an absolute scale, the significance of an accumulated concentration depending on the specific tissue or the specific invertebrate involved (Rainbow 2007).

A trace metal has the potential to bind to any molecule with an affinity for that metal. Since trace metals typically have an affinity for sulphur and nitrogen, and proteins are made up of amino acids,

many of which contain sulphur and/or nitrogen, there is no shortage of potential binding sites for trace metals within cells. Such affinities make all trace metals potentially toxic, binding to proteins or other molecules and preventing them from functioning in their normal metabolic role (Rainbow 2007). To avoid potential toxic effects, newly taken up metal must either be detoxified or excreted. As long as the combined rates of detoxification and excretion exceed the rate of metal uptake, the incoming metal will not have a toxic effect on the crustacean. If the rate of uptake exceeds the maximum combined rate of detoxification plus excretion, then the metabolically active metal may reach a threshold at which first sublethal and finally lethal toxic effects are manifested (Marsden and Rainbow 2004, Simpson and Batley 2007, Pastorinho *et al.* 2009).

The choice of a biomonitor needs to consider the potential sources of metals to the biota. Suspension feeders such as mussels, oysters and barnacles take up metals directly from seawater and from the suspended particles collected during feeding (Weeks and Rainbow 1993, Rainbow 1995, 2007). Deposit feeding amphipods and bivalves, in contrast, will reflect the bioavailability of metals in the surrounding water via respiratory currents and from detritus in and on the sediment. In a sediment burrowing organism such as a burrowing polychaete, the soft epidermis may be bathed directly by interstitial water within the sediment with a redox potential probably very different from that of the overlying water (Bat and Raffaelli 1998, Simpson and Batley 2007). A key feature to the choice of biomonitors is therefore an understanding of their biology, for example method of feeding, extent of preferred habitat, life history and breeding season, length of life and age structure of population (Rainbow 2007). Ideally, biomonitoring studies should also employ species with a wide or even cosmopolitan geographical distribution, as there is an inherent danger in comparing bioaccumulation strategies in different species, due to the fact that even closely related species from the same location may have significantly different accumulated concentrations (Marsden and Rainbow 2004). Due to the variable accumulation of trace metals in crustaceans, any meaningful comparison of relative concentrations in aquatic invertebrates should therefore be intraspecific, rather than between groups or families. Zinc concentrations that might be low for a barnacle would perhaps be extraordinarily high for a caridean decapod (Marsden and Rainbow 2004, Rainbow 2007). However, within the crustacea there appears to be consistent differences between major taxonomic groups. Decapod crustaceans have evolved mechanisms to regulate the body concentrations of essential metals such as Zn and Cu. Other crustaceans such as barnacles and amphipods accumulate both essential and non-essential metals without excretion (Rainbow 2002).

What makes a good bioindicator? Ideally, a good bioindicator of metal contamination in estuarine sediment should not only be a net accumulator of the metal, it should also be a sediment dweller or feeder and should be exposed to contaminants in sediment and/or in porewater. A biomonitor must be

a net accumulator of the metal in question in order to increase the power of resolution between potentially contaminated sites (Rainbow 2007). Amphipods have been widely used as trace metal biomonitors not only because they are net accumulators of metals, but they also conform to other criteria including being relatively sedentary, abundant, easy to identify and resistant to handling stress (Rainbow 2002, Marsden and Rainbow 2004, Re *et al.* 2009). The amphipods *G. lignorum* and *C. triaenonyx* tolerate a wide range of salinity and sediment conditions, they are geographically widespread, abundant within the benthic community and relatively easy to collect. There are however, certain disadvantages to using these amphipods as biomonitors. Corophioid amphipods such as *G. lignorum* and *C. triaenonyx* are not true sediment burrowers and because they construct tubes within which they remain partially partitioned from the sediment, they are not directly exposed to the contaminants in the sediment (Wang *et al.* 2004). These amphipods are also not true sediment feeders, as they feed selectively on epibenthic periphyton, epiphytes and associated bacteria that they glean from particulate matter (Wang *et al.* 2004). In addition, *C. triaenonyx*, in particular, is predominantly a filter feeder and as such is more exposed to contaminants dissolved in the water column than contained within the sediment. Contaminant uptake by this amphipod in its natural habitat would therefore be expected to be primarily from overlying water or from food that is in equilibrium with contaminants in the overlying water (Wang *et al.* 2004). In this regard, sediment dwelling or burrowing amphipods have been promoted as more suitable biomonitors rather than pelagic or tube-dwelling amphipods (Rainbow 2007, ASTM 2008). There are however no burrowing amphipods that occur in sufficient densities in local estuaries such as the Nhlabane Estuary.

There are no historical data in the literature on metal concentrations in Nhlabane Estuary benthic amphipods, or for any KwaZulu-Natal estuary. In fact, not a single reference could be found on metal concentrations in estuarine amphipods in South African estuaries in the well known text, “*Estuaries of South Africa*” (Allanson and Baird 1999), which perhaps reflects the scarcity of information on this topic in South Africa. O’Donoghue (2005) conducted a detailed survey of marine and estuarine pollution research in South African and reported that of the 284 publications found from 1960, only a very small number dealt with estuarine benthic organisms as bioindicators of estuarine contamination. The only toxicity work done specifically on estuarine amphipods in South Africa was published by Connell and Airey (1979, 1982). In contrast, much work has been done on amphipod toxicity in many other parts of the world, notably Europe, Australia and the USA, although a lot of the research focused on laboratory based experimental studies. Marsden and Rainbow (2004) provided a review of field-derived metal concentrations in amphipods, which showed that the majority of available data concerned littoral and supralittoral talitrid amphipods with relatively little information on metal concentrations in benthic amphipods from inter and subtidal estuarine areas. A summary of the available information on metal concentrations in amphipods from different aquatic habitat types are

presented in Table 4.9. In the table, amphipods were divided into ecologically relevant groups in terms of habitat preference as it is believed that the biology of an organism will influence the uptake of metals from the environment. Due to the marked differences in the aquatic habitat and feeding habits of littoral and benthic amphipods, a direct comparison between metal concentrations in local benthic corophoid amphipods and littoral talitrid species is therefore of limited relevance Rainbow *et al.* 1998)

Table 4.9 Metal concentrations ($\mu\text{g/g}$) in field sampled amphipods from various habitat types. U = unpolluted, P = polluted.

Species		Cu	Cd	Zn	As	Fe	Mn	Pb	Ni	Habitat	Reference
Benthic estuarine/marine											
<i>Grandidierella lignorum</i>		99.1	0.44	87.8	8.3	1076	76.8	31.6	54.0	sand/mud estuarine	This study
<i>Corophium triaenonyx</i>		91.7	0.39	62.1	4.2	463	27.3	17.6	47.0	sand/mud estuarine	This study
<i>Corophium volutator</i>		86–115								sand/mud estuarine	Eriksson and Weeks 1994
<i>Paracorophium excavatum</i>		158–207								sand/mud estuarine	Marsden <i>et al.</i> 2003
<i>Gammarus locusta</i>		93–134		81–562		71–1290				sand/mud marine	Rainbow and Moore 1986
<i>G. oceanicus</i>		14–28	0.7–1.0	61–68				0.5	1.3–2.4	Intertidal rocky shore	Zauke <i>et al.</i> 2003
<i>G. salinus</i>		75–135	0.1–0.5	62–91				1.6–2.8		sand/mud estuarine	Zauke <i>et al.</i> 2003
<i>G. locustra</i>		64	0.12	51				2		sand/mud estuarine	Zauke <i>et al.</i> 2003
<i>G. setosus</i>			0.8							sand/mud estuarine	Zauke <i>et al.</i> 2003
Intertidal seaweed											
<i>Caprella penantis</i>	U	7		81		117	21		<0.1	intertidal seaweed	Guerra-García <i>et al.</i> 2009
	P	173		544		1886	122		21		
<i>Hyale schmidt</i>	U	<0.1		21	<0.1	28	101		<0.1	intertidal seaweed	Guerra-García <i>et al.</i> 2009
	P	86		42–96	6–9	83–340	607		4.2		
Pelagic											
<i>Themisto abyssorum</i>		25								pelagic	Ritterhoff and Zauke 1997
<i>Themisto compressa</i>		39	70	76						pelagic	Rainbow 1989
Littoral/Supralittoral											
<i>Gammarus duebeni</i>		158–193								supralittoral marine	Rainbow and Moore 1986
<i>Echinogammarus pirloti</i>		91–245								eulittoral	Rainbow and Moore 1986
<i>Talorchestia saltator</i>	U	36–74		145–181							Rainbow and Moore 1990
	P	112		306–332							
		37–75	2.4–4.6	165–241		163–329	23–44	14–19	3.8–6.5		Rainbow <i>et al.</i> 1998
<i>T. quoyana</i>		16–48	6.4–18	97–481						littoral strandline	Marsden <i>et al.</i> 2003
<i>Transorchestia chiliensis</i>		42–108	2.1–6.5	112–171						supralittoral	Marsden <i>et al.</i> 2003
<i>Orchestia gammarellus</i>	U		1.5							supralittoral	Rainbow and Moore 1990
	P		7.5								
		55–123		132–361		69–1800					Rainbow and Moore 1986
<i>O. tenuis</i>		31–49								littoral strandline	Rainbow 1993

A comparison with ecologically relevant intertidal and subtidal benthic amphipods shows that metal concentrations in *G. lignorum* and *C. triaenonyx* compare favorably with that found in most of these species (Table 4.9). Although Cu concentrations in the two amphipods were relatively high, it fell within the range for species such as *C. volutator* and *Gammarus locusta*, but was considerably lower than the range shown for *Paracorophium excavatum*. In terms of metabolic requirements, Cu levels in both local amphipods were sufficient to adequately cover metabolic needs. Copper and Zn are essential trace metals for crustaceans, and theoretical calculations suggest that Cu concentrations

below 26.3 µg/g are insufficient to satisfy both enzyme requirements and haemocyanin production (White and Rainbow 1985).

Cadmium concentrations in the Nhlabane amphipods were much lower than that found in any other species, and as Cd is a toxic metal of concern in many industrialized estuaries and coastal areas throughout the world (Luoma 1983), this perhaps also reflects the low contamination potential in the Nhlabane system. Similarly, Zn, As, Fe and Mn concentrations in the Nhlabane amphipods were within the concentration range for other intertidal and benthic species, although As, Fe and Mn concentrations in *G. lignorum* and *C. triaenonyx* were closer to the upper end of the range and approached the values characteristic of polluted areas in *Caprella penantis* and *Hyale schmidt* (Table 4.9). Copper concentrations in *G. lignorum* and *C. triaenonyx* were also similar to that found in littoral talitrid amphipods, while that of Ni, Fe, Pb and Mn were considerably higher in Nhlabane Estuary amphipods.

When comparing the metal concentrations in the two amphipods analysed in this study, it was clear that for the majority of metals, concentrations in *G. lignorum* were much higher than in *C. triaenonyx*. This can not be size related, as both species showed a clear size effect in concentration, and *G. lignorum* is a considerably larger amphipod than *C. triaenonyx*. Differences in metal concentrations can either be related to differences in uptake of metals via dissolved and particulate fractions, or to differences in storage, detoxification and excretion. The latter possibility was not investigated during this study and needs to be considered during future studies. The former possibility deals mainly with differential uptake of metals from the dissolved fraction and from food.

It has become increasingly appreciated in recent years that uptake of trace metals from the diet may be the major pathway of metals accumulation in aquatic invertebrates (Rainbow 2007). Benthic amphipods employ a variety of feeding mechanisms, which include filter feeding, herbivory, predatory behaviour, scavenging and deposit feeding (Wang *et al.* 2004). Trace metal accumulation occurs from food (including sediment) as well as from solution (Rainbow 2007). Tubiculous corophioid amphipods such as *G. lignorum* and *C. triaenonyx* are both filter feeders inside their tubes using pleopod induced tube-currents, and deposit feeders, which gather detritus from the sediment outside their tubes. In *Hyalella azteca*, a tubiculous freshwater amphipod, a metal partitioning study showed that most of the Cd in the amphipod was taken up from the overlying water compartment and little from the sediment compartment. In contrast, a sediment-burrowing tubificid oligochaete was found to take up nearly all its Cd from the sediment. In *H. azteca*, similar findings were reported for Pb, Cu and Ni, which showed that in this tubiculous amphipod, most of its metal is derived from the water column and not from the sediment (Wang *et al.* 2004). Since concentrations of metals in the

sediment is usually much higher than in the water, it is therefore expected that true burrowing organisms or those that are more directly in contact with sediment and ingest sediment directly would tend to accumulate higher concentrations of metals than their tubiculous counterparts (Chapman and Wang 2001, Rainbow 2007). In the Nhlabane Estuary, the higher body metal concentrations recorded in *G. lignorum* compared to *C. triaenonyx* is suggested to reflect the difference in feeding strategy between the two species. *Corophium triaenonyx* spends more time filter-feeding in its tube than *G. lignorum* and as such is more exposed to dissolved metals in the water column than *G. lignorum*, which spends more time outside its burrow and is more in direct contact with the sediment. Based on the results from this study, *G. lignorum* would therefore appear to be better a biomonitor of trace metal contamination in estuarine sediments than *C. triaenonyx* and a more suitable candidate as a biomonitor in estuarine monitoring programmes (Rainbow 2007).

In species living in estuaries, trace metal toxicity generally increases as salinity decreases and as temperature increases (McLusky *et al.* 1986, Atkinson *et al.* 2007). In estuaries, salinity can be regarded as one the major factors controlling the distribution of contaminants in sediment and in overlying or interstitial waters (Dauvin 2008). Many studies have investigated the separate and combined influence of temperature and salinity on the response of amphipods to toxicants (McLusky *et al.* 1986, McGee *et al.* 1998, Marsden and Rainbow 2004, Perez-Landa *et al.* 2008). These studies have confirmed that in most metals, toxicity decreases when salinity increases and temperature decreases due to increased desorption resulting from increasing complexation with seawater anions and/or increasing competition for particle sorption sites with seawater cations, as well as coagulation, flocculation and precipitation (Chapman and Wang 2001). Salinity changes thus have the potential to affect the bioavailability and hence the toxicity of different trace metals to estuarine organisms (Atkinson *et al.* 2007). In their review on the effects of temperature and salinity on the toxicity of trace metals for marine and estuarine invertebrates, McLusky *et al.* (1986) showed that temperature and salinity generally has a clear effect on Cu toxicity, which tended to increase as salinity decreased and temperature increased. However, McLusky *et al.* (1986) also found that body tissue metal concentrations in some amphipods appeared to be unaffected by salinity. Weeks and Rainbow (1993) investigated the effects of short-term exposure in both hyper and hypo-saline conditions on Cu and Zn accumulation in the body tissues of talitrid amphipods and found that the adults of all three species tested were tolerant of a wide range of salinities and the metal concentrations in the blood and various tissues were unaffected by the salinity. These authors concluded that changes in salinity did not significantly affect the Cu and Zn concentrations within body tissues in these littoral talitrid amphipods. The biology of the different species therefore seem to be influential in determining the effect of salinity on metal accumulation, which in intertidal and subtidal true benthic amphipods appear to be affected differently compared to littoral and supralittoral species. During the present

study, body metal concentrations in both species were negatively correlated with salinity and temperature. Although the expected correlation with salinity was thus observed, the same was not true in the case of temperature. The anomalous negative correlation with temperature was perhaps related to the fact that the mouth of the system was open more frequently during winter and as such higher salinities were recorded during the cooler winter months.

Eriksson and Weeks (1994) found that low dissolved oxygen increased mortality and caused altered behaviour in *C. volutator* exposed to Cu concentrations in varying oxygen conditions, although the accumulation of Cu was not affected by the low oxygen conditions. This would explain the consistent negative correlation between body metal concentrations and dissolved oxygen in the present study.

In the Nhlabane Estuary, there was a strong positive correlation between metal concentrations in both amphipod species, with the notable exception of As, Cd and V. This suggests that both species are net accumulators of metals from the environment, as essential metals were not preferentially stored or accumulated relative to non-essential metals such as Pb and Cr. As discussed in more detail later, the anomalous accumulation of As and Cd is thought to be related to the marine origin of these metals, with increased accumulation coinciding with high salinities and open mouth conditions. Marsden and Rainbow (2004) reviewed the influence that different metals have on the accumulation of other metals by amphipods and concluded that the literature on the interaction of one trace metal on the uptake or accumulation of another metal by aquatic invertebrates is confusing without any clear patterns. In a study of interactions between the uptake rates of Zn and Cd in different populations of *Orchestia gammarellus*, the presence or absence of one metal had an inconsistent effect on the rate of uptake of the other (Rainbow *et al.* 2000). Although Zn and Cd might share common routes of uptake from solution by crustaceans, these metals do not consistently interact competitively or synergistically at the exposure concentrations investigated. In *Transorchestia chileensis*, body Cu and Zn concentrations did not reflect the sediment metal concentrations or any salinity variations in the system (Marsden *et al.* 2003).

In the Nhlabane Estuary, not all metals displayed a strong correlation between metal concentrations in body tissues and in the sediment and water. There was no consistent pattern or trend in body metal concentrations against sediment and water metal concentrations during this study, for example the negative correlation between Zn concentrations in the tissue, sediment and water in *G. lignorum*. The data do however suggest differential uptake of metals from water and sediment, as body Fe concentrations in both species showed a negative correlation with Fe in the water, but a positive correlation with Fe in the sediment. This suggests that body concentrations were closely linked to Fe concentrations in the sediment. In contrast, body Cu concentrations in both species showed a positive

correlation with Cu in the water, but a negative correlation with Cu in the sediment, suggesting that body Cu concentrations were closely linked to dissolved Cu in the water.

Information in the literature on seasonal changes in metal concentrations and rate of metal accumulation in amphipods is confusing and there is not always a feasible explanation for many seasonal variations in metal content (Marsden and Rainbow 2004). In *Leptocheirus plumosus*, seasonal differences in sensitivity to sediment contaminants was suggested to be at the time when population densities were lowest and that they may be due to limited food resources (McGee *et al.* 1998). In a subtropical estuary such as Nhlabane Estuary, seasonal changes in population densities of amphipods occur and the observed differences in metal concentrations between seasons could perhaps be explained by decreased general conditions in the estuary in cooler winter months when food resources are lower. Seasonal moulting and reproduction have also been suggested to affect the accumulation of metals in crustaceans. The frequency of moulting, which is related to growth rate, increases during summer and as result, crustaceans accumulate more Cd in summer than in winter, as Cd uptake increases during moulting. This was found to be the case in *Corophium multisetosum*, which showed a higher sensitivity to Cd in summer (Perez-Landa *et al.* 2008). In contrast, the moult cycle was found not to have any effect on body concentrations of Cu and Zn in the littoral strandline dweller *Orchestia gammarellus* (Rainbow 1995). Sensitivity changes of *Corophium urdaibaiense* to ammonium was also found to be related to its reproduction pattern, with increased sensitivity during summer months of peak reproductive activity (Perez-Landa *et al.* 2008).

The strong correlation between amphipod body weight and body metal concentrations during this study was unexpected, since only adult amphipods were used in the analysis. Juveniles are generally more sensitive to contaminants than adults due to increased metal accumulation caused by their relatively larger surface area, and as a result, size effects on metal concentrations are regularly compensated for in toxicity studies (Moore and Rainbow 1987, McGee *et al.* 1998). During earlier work on the two amphipods used in this study, a typical non-linear negative relationship between body weight and Cu concentration was recorded in these two species, with juvenile amphipods showing much higher metal concentrations than adults (Xulu 2003). Based on the results from this earlier study, and in order to avoid this well known effect of body size on metal concentrations, only adult *C. triaenonyx* of >4mm total length and adult *G. lignorum* of > 5mm total length were used for metal analysis during this study. In a closely related species, *C. urdaibaiense*, the minimum size of an adult was found to be 3.7 mm (Perez-Landa *et al.* 2008). The decision to only use adult amphipods during this study was based on work done by Moore and Rainbow (1987) on talitrid amphipods, in which only amphipods >2 mg were used for analysis of trace metal concentrations. Results from their work showed that size effects were largely eliminated by using only adult amphipods.

Future work on *C. triaenonyx* and *G. lignorum* should therefore compensate for size effects by standardizing body size to a pre-determined weight which must reflect the most appropriate body size for the species investigated. Perez-Landa *et al.* (2008) also emphasized that size effect differences in sensitivity must be taken into account in toxicity assessments and that in order to avoid extreme values of sensitivity, toxicity tests should be performed outside the breeding period. Zauke *et al.* (2003) questioned whether significant differences in metal concentrations in biota are due to a geographical heterogeneity or due to a size dependency. These authors found significant differences in metal concentrations in *Gammarus oceanicus* due to a geographical heterogeneity only when no significant linear correlation existed between metal concentration and body size.

CHAPTER 5

Acute sediment toxicity test using two
estuarine amphipods, *Grandidierella*
lignorum and *Corophium triaenonyx*,
exposed to zinc and copper

5. Acute sediment toxicity test using two estuarine amphipods, *Grandidierella lignorum* and *Corophium triaenonyx*, exposed to zinc and copper.

5.1 Introduction

Estuarine ecosystems include numerous interlinked and interdependent species, habitats and processes, all of which are dependent on water and sediment quality in the estuarine environment for successful ecosystem functioning. Increasing industrial and recreational demands on coastal and estuarine environments lead to habitat deterioration and pollution with deleterious consequences for benthic communities. This occurs through direct contact of organisms with contaminated water and sediment or by resuspension of contaminated sediment particles into the overlying water (Bat 2005 Rainbow 2007, Casado-Martinez 2007). Estuarine sediments support a diverse community of benthic organisms which by virtue of their close association with the sediment, are highly susceptible to contamination of the sediment (Simpson and Batley 2007, Re *et al.* 2009).

Trace metal contamination of estuarine water and sediment has become one of the greatest concerns worldwide, as metals have the potential to be toxic to aquatic organisms if present at high concentrations (Rainbow 1995, Chapman and Wang 2001, Simpson and Batley 2007). Metals naturally occur in varying concentrations in the environment and processes that contribute to the input of metals into aquatic ecosystems systems include natural weathering of rocks, windblown dust, volcanic and hydrothermal vent activity, forest fires and the deposition of organic matter (Kennish 1997). The concentration of metals in pristine sediments thus reflects the natural abundance of metals in that area, however, anthropogenic input in many industrialized estuarine areas exceed the natural input of metals into the water (Rainbow 2007).

Many factors affect the concentrations of metals in sediment, such as grain size, total organic carbon content, pH and mineralogy (Chapman and Wang 2001, Rainbow 2002). In uncontaminated sediments, metal concentrations usually increase concomitant with a decrease in sediment grain size, due to the relatively larger surface area associated with smaller sediment particles which provide a larger surface area for the adsorption of metals. Organic carbon also acts as a matrix to which metals can complex, and there is usually a shown strong linear relationship between total organic carbon and metal concentrations (Kennish 1997, Simpson and Batley 2007).

Anthropogenically derived metal contamination has been studied in many estuarine systems during recent years, largely due to the persistent and toxic nature of many metals (Matthiessen and Law 2002, Chapman 2007a). Some metals are essential for metabolism (Co, Cu, Fe, Zn and Mg), but become toxic at elevated concentrations, while others such as Cd, Pb, Hg, and Sn are not required for metabolism and are toxic to organisms even at low concentrations (Kennish 1997, Rainbow 2002, Simpson and Batley 2007). When introduced into the estuarine and marine environment, organic and inorganic contaminants, particularly heavy metals, eventually accumulate in sediment, where they pose considerable risk to the health of benthic communities and eventually to higher trophic levels through bioaccumulation in animal tissues (Chapman and Wang 2001, Bat 2005). Marine and estuarine sediments bind and accumulate a wide variety of trace metals to often high concentrations, which can then be redistributed by disturbance and anthropogenic activities such as dredging and may threaten the health of the estuarine environment via bioaccumulation (Marsden and Rainbow 2004). Estuarine sediments comprise an ecologically important component of the aquatic environment by mediating the exchange of accumulated contaminants between particulate, dissolved and biological phases (Simpson and Batley 2007). The protection of estuarine faunal communities from being adversely affected by contaminants requires an understanding of both the sensitivity of benthic organisms to contaminants and their ecological requirements.

The words “contaminant” and “pollutant” are often used interchangeably with little understanding for their actual meaning (Chapman 2007a, Orr *et al.* 2008). It is thus useful to define them to avoid any confusion. Pollution is the indirect or direct introduction of a substance into the environment by man. Contamination is the presence of elevated concentrations of a substance above that of its background concentration in the environment or biota concerned. A contaminant may become a pollutant if it is introduced by man, and has some damaging effect on the environment. Elevated levels of contaminants may be insignificant unless the contaminant causes direct or indirect changes in biological populations (Rainbow 2007).

All aquatic invertebrates accumulate trace metals in their tissues, whether or not these metals are essential to metabolism (Rainbow 2007). Heavy metals are often accumulated by many marine and estuarine organisms to very high body concentrations (Rainbow 1995). These accumulated concentrations in body tissues are easily measured, not liable to contamination, and provide a time-integrated measure of metal supply over weeks, months, or even years, according to the species analysed. Toxicity tests, particularly sediment toxicity tests, have been shown worldwide to be an appropriate way of increasing our understanding of the sensitivity of organisms to contaminants and also have the advantage of being a time-integrated measure of the supply of the bioavailable fraction of a contaminant, which can be very different from the total amount present in the environment (Bat

2005, Chapman 2007a, Rainbow 2007, Re *et al.* 2009, Casado-Martinez 2010). It is the bioavailable fraction only that is potentially toxic and of ecotoxicological relevance (Rainbow 2007). Thus the fraction of metal of direct ecotoxicological relevance can be accurately measured through sediment toxicity tests. Such tests are now widely used to establish geographical and/or temporal variations in the bioavailable concentrations of heavy metals in coastal and estuarine waters (Rainbow 2007).

A variety of sediment toxicity tests have been developed for assessing the toxicity of chemicals in coastal sediments using amphipods, midges, polychaetes, mussels, oysters, barnacles, or cladocerans (Rainbow 1995, Chapman and Wang 2001, Bat 2005, ASTM 2008). Although a variety of endpoints have been studied to measure potential effects of contaminants in sediment including survival, growth, behavior, or reproduction; however, survival of test organisms in 10-day exposures is the endpoint most commonly reported (ASTM 2008, Kennedy *et al.* 2009). These short-term exposures that only measure effects on survival can be used to identify high levels of contamination in sediments, but may not necessarily be able to identify moderate levels of contamination in sediments, conditions which are more commonly tested with sublethal endpoints.

The choice of a test organism in sediment toxicity testing has a major influence on the relevance, success, and interpretation of a test, as different organisms respond differently to toxicant concentrations under similar conditions (Chapman and Wang 2001, Rainbow 2007). Ideally, a number of test organisms should be used to develop a better understanding of the effects of toxicants and contaminated sediments on aquatic communities in estuaries (Rainbow 2002, ASTM 2008). A suitable sediment toxicity test organism should ideally demonstrate relative sensitivity to a range of contaminants in the sediment appropriate to the objectives of the study, be in direct contact with the sediment by living below the sediment–water interface thus ensuring maximum and consistent exposure to sediment contaminants, be easily cultured or maintained in the laboratory or be easily accessible in sufficient numbers by field collection, be easily identified and ecologically important, have a high survival rate under control conditions, have a broad geographical distribution, have environmental requirements appropriate to the conditions prevalent at the site being evaluated and most importantly, be tolerant of a broad range of sediment physico-chemical characteristics such as grain size and distribution (Swartz *et al.* 1982; DeWitt *et al.* 1988, Bat *et al.* 1998). In addition, test organisms should be net accumulators of the metal in question with a simple correlation between metal concentration in tissues (body) and average ambient bioavailable metal concentration (Rainbow 1995).

Amphipods are commonly utilized throughout the world to test the toxicity of potentially contaminated sediments in marine and estuarine environments, because they are ecologically relevant,

have a short life cycle and are suitable for laboratory experimentation (Swartz *et al.* 1982; DeWitt *et al.* 1996, Rainbow and White 1989, Ciarelli *et al.* 1997, Rainbow 1997, Chapman and Wang 2001, Marsden 2002, Rainbow 2007, Marsden and Rainbow 2004, Bat 2005, Gale *et al.* 2006, ASTM 2008, Re *et al.* 2009). Chapman (1998) produced a detailed review of the suitability and use of amphipods in sediment toxicity testing. Benthic amphipods often occur in such high densities that they dominate benthic communities and often comprise the principal food for many predatory fish and birds, making them ecologically important organisms with considerable potential for metals accumulated within their body tissues to be transferred along estuarine food chains. Amphipods are also useful sediment test organisms because they are geographically widely distributed, occurring in habitats from the abyssal depths, throughout the ocean and extending into estuaries, freshwater and groundwater, are easy to identify and most are in close contact with the contaminated sediment due to their burrowing or tubiculous habits (Marsden and Rainbow 2004, King *et al.* 2006). Most importantly, amphipods are also known to be among the most sensitive benthic organisms when exposed to sediment contaminants such as PAHs and heavy metals (Ciarelli *et al.* 1997, Gale *et al.* 2006, ASTM 2008, Re *et al.* 2009). To date, the majority of estuarine sediment test organisms are in fact amphipods (Chapman and Wang 2001).

Standardized sediment toxicity tests have been developed for a number of amphipod species such as the burrowing species *Rhepoxynius abronius* and *Leptocheirus plumulosus* (Swartz *et al.* 1982, King *et al.* 2006, ASTM 2008) and the tubiculous species *Corophium volutator* and *Grandidierella japonica* (Ciarelli *et al.* 1997, Bat 2005, ASTM 2008, Re *et al.* 2009). Considerable attention has been paid to the development of sediment toxicity tests using species of the genus *Corophium* (Bat 2005, Re *et al.* 2009). Environment Canada (1992) has recommended the use of *C. volutator* for sediment toxicity testing. *Corophium volutator* (Ciarelli *et al.* 1997, Chapman and Wang 2001, Rainbow 2002, Peters and Ahlf 2005, Stronkhorst *et al.* 2003), *C. multisetosum* (Castro *et al.* 2006, Ré *et al.* 2007, Re *et al.* 2009) and *C. orientale* (Onorati *et al.* 1999), have been shown to be suitable indicators of metal contamination in European estuarine areas and are regularly employed as test organisms in estuarine biomonitoring programs. *Corophium spinicorne* and *C. arenarium* (Chapman and Wang 2001) have also been shown to be suitable test organisms for marine and estuarine sediment toxicity testing in the USA and Canada. *Paracorophium excavatum* has been shown to be a potential test organism for sub-lethal sediment toxicity tests in New Zealand (Marsden 2002). *Corophium* amphipods are cosmopolitan burrowing or tubiculous benthic organisms inhabiting temperate and tropical shallow marine and estuarine environments, feeding preferentially on detritus and epipellic microalgae and often establishing populations of extremely high densities. They are thus considered a key food source in intertidal marine and estuarine areas for fish and shorebirds (Cunha *et al.* 2000). Due to their abundance and high productivity in tidal areas, *Corophium* spp. are considered as “keystone

species'' because they reflect the ecological integrity (structure and productivity) of the surrounding community (Re *et al.* 2007). Similarly, a number of studies have been conducted on the suitability of *Grandidierella* amphipods, notably *G. japonica* in New World and Asian estuaries (Nipper *et al.* 1989, Greenstein and Tiefenthaler 1997, Han *et al.* 2005, ASTM 2008) and *G. lignorum* and *G. lutosa* in South African estuaries (Connell and Airey 1982) as bioindicators of contaminated sediments.

The purpose of this study was to determine the accumulation of copper and zinc in two South African estuarine amphipod species, *C. triaenonyx* and *G. lignorum*, and to assess the suitability of these amphipods as potential test organisms for sediment toxicity testing in estuarine environments. These two amphipods were the most abundant taxa in the benthic community in the Nhlabane Estuary throughout the study period and have been shown to be tolerant of variable salinity and sediment conditions (Vivier and Cyrus 1999a). *Corophium triaenonyx* has a western Indian Ocean distribution extending from the Southern Cape to the eastern shore of India (Griffiths 1973, Shyamasundari 1976). *Grandidierella lignorum* is a Southern African endemic, ranging in distribution from False Bay, Western Cape, to southern Mozambique (Griffiths 1973). In southern Africa, these two species form a dominant component of the benthic community in Southern Cape and Zululand relict estuarine coastal lakes (Hart 1995, Bolt 1969) and are abundant in low to medium salinity reaches of estuaries (Whitfield 1989, Vivier and Cyrus 1999a, Vivier and Cyrus 1999b, Teske and Wooldridge 2001). *Grandidierella lignorum*, in particular, has been shown to be more abundant under low salinity conditions (Vivier and Cyrus 1999b), while *C. triaenonyx* appears to be less restricted by variation in salinity and has been recorded in all types of estuaries along the South African east coast under all salinity conditions (Blaber *et al.* 1983, Davies 1984; De Decker and Bally 1985; Whitfield 1989, Whitfield and Bruton 1989; Whitfield 1992; Cyrus and Mackay 1995, MacKay and Cyrus 1999, Teske and Wooldridge 2001, Mabaso 2002).

Copper and Zn were chosen for this study as both are essential metals for crustaceans, there is extensive literature on the toxicity effects of these metals on aquatic biota (Rainbow 1993, Weeks and Rainbow 1993, Chapman *et al.* 1997, Conradi and Depledge 1998, Chapman and Wang 2001, Marsden 2002, Marsden and Rainbow 2004, Simpson and Batley 2007) and they have been identified as potential contaminants in the Port of Richards Bay (Wepener and Vermeulen 2005). Being essential metals, there is a certain quantity of the metal required in the body to meet essential metabolic needs, which have been estimated for amphipods (White and Rainbow 1985). Any further accumulation of these essential metals in metabolically available form has the potential to be toxic, with deleterious consequences for aquatic organisms (Rainbow 2007).

5.2 Materials and Methods

The methodology applied during this study was based on the techniques specified in the methodology developed by the US-EPA for 10-day static sediment toxicity tests for estuarine amphipods (ASTM 2008). Although the EPA methods were originally developed for use in whole sediment toxicity tests from suspected contaminated areas, they have also been adapted to experiments with spiked sediments. These methods have been applied since their development by numerous research agencies throughout the world using a variety of estuarine and marine amphipods (Chapman and Wang 2001, ASTM 2008). Although the methods described by the EPA was designed specifically for use with New World amphipods, such as the marine amphipods *Ampelisca abdita* and *R. abronius* and the estuarine amphipods *Eohaustorius estuarius* and *L. plumulosus*, they were adapted during this study for use with the two South African estuarine amphipod species, *G. lignorum* and *C. triaenonyx*. Exposure is static, the organisms were not fed over the 10 day exposure period and the endpoint measured was survival.

5.2.1 Collection and handling of amphipods

Grandidierella lignorum and *C. triaenonyx*, were collected from the Nhlabane Estuary using a benthic grab, after which they were placed into clean, plastic containers with water and a thin layer of sediment collected *in situ*. Containers with amphipods were immediately transported to the laboratory where the amphipods were sorted by species. Cultures of the two species were maintained under controlled conditions in climate chambers (25 °C and 12 hrs daylight) in aerated culture trays in sediment collected from the Nhlabane Estuary at a salinity of 20. Water renewal was undertaken once a week with diluted filtered seawater and the amphipods were fed once a day with TetraminTM fish flakes.

5.2.2. Collection and preparation of test sediment

Sediment used in toxicity testing was obtained from a known uncontaminated area in Richards Bay Harbour (Wepener and Vermeulen 2005) of similar sediment characteristics to those in the Nhlabane Estuary where the amphipods were collected. After collection, the sediment was sieved with a 500 µm mesh stainless steel sieve to remove macrofauna and debris, after which it was stored in acid washed plastic containers at 4 °C for a minimum of two weeks prior to use. Sediment was acclimated at room temperature for a minimum of 24 hours prior to use.

The suitability of the sediment as test sediment was tested prior to the metal spiked exposure experiments by conducting a 10 day static test with *G. lignorum* at a salinity of 20 using only clean

sediment from the Nhlabane Estuary and from the chosen uncontaminated site and a known contaminated site in the harbour. After 10 days, the Nhlabane Estuary and uncontaminated sediment showed survival rates of >90%, while the survival rate in the contaminated sediment (60%) was significantly lower ($F = 8.75$, $p = 0.004$, d.f. = 12).

The spiking procedure to create the test sediment was based on the method described by Swartz *et al.* (1985), with minor modifications. The metals used in the exposure experiments, Cu and Zn, as CuCl and ZnCl₂, were directly added from the stock solution to the control sediment to achieve the required nominal concentrations (expressed on a dry weight basis). This was followed by 30 min of mechanical mixing. The sediments were then allowed to equilibrate overnight at 4 °C.

5.2.3 Toxicity test procedures

Toxicity tests were conducted using standard protocols with some modifications for the use of local species (EPA 1994, ASTM 2008). The acute toxicity of Cu and Zn spiked sediments to the two species of amphipods were determined by conducting 10-day static, non-renewal exposure tests without feeding under controlled conditions in an environmental chamber at a temperature of 25 ± 0.2 °C. Amphipods were exposed to five metal concentrations and control sediment with five replicates per concentration in 250 ml beakers containing 200 ml of test water and 2cm of sediment. Each beaker was filled with 2 cm (about 40 g) of homogenized test sediment, after which the water was added, without disturbing the surface of the sediment. Plastic petri dish lids, each with a small hole drilled through the center to allow aeration of the test beaker with a glass pipette, were placed over the beakers to minimise evaporation. Beakers were supplied with a gentle flow of air (3-5 air bubbles per second) throughout the tests to maintain dissolved oxygen concentration in the overlying water at >80% saturation. Sediments and diluted filtered sea water were added to the beakers at least 12 hours prior to the start of a test and placed in an environmental chamber at 25 °C to allow the sediment to settle overnight.

Immediately prior to the start of the toxicity test, the amphipods were isolated from the stock cultures by gentle sieving using a 500 µm sieve. Only adult amphipods were selected for the toxicity tests (*C. triaenonyx* > 4mm, *G. lignorum* > 5 mm), as amphipods have a known and marked body size effect on metal accumulation with juvenile amphipods displaying a relatively high body metal concentrations due to their higher surface area to volume ratio (Moore and Rainbow 1987, Pérez-Landa *et al.* 2008). The amphipods were gently rinsed with filtered test water to ensure removal of any remaining culture sediment and then kept in aerated 1 l beakers filled with test water at salinity of 20 until the start of the experiment. The amphipods were carefully monitored and any inactive amphipods were removed.

At the start of each test, 20 randomly selected amphipods were gently released in each beaker using an inverted glass Pasteur pipette, to avoid injury to the amphipods. After release, the amphipods were carefully monitored for 1hr to check for burrowing activity and any inactive amphipods or individuals showing erratic behavior were replaced. Test beakers were monitored daily to ensure that there was adequate aeration and to record and remove any dead amphipods. The number of amphipods on the surface of the sediment was also noted. For quality assurance purposes, physico-chemical parameters including dissolved oxygen concentration (>80%), ammonium (<0.8 mg/l), pH (7.5–8.2), salinity (20 ± 1) and temperature (25 ± 0.2 °C) were monitored periodically throughout the test and at test termination to ensure that all variables remained within acceptable limits throughout the test.

At the end of the 10-day period, water and sediment samples were randomly collected from three beakers per exposure for metal analysis, after which the surviving amphipods were gently sieved from the sediment and counted. The amphipods from each beaker were then blotted dry, rinsed twice in double distilled water to remove any residual sediment and debris and stored for metal analyses. An average amphipod survival rate of 90% or greater in the control sediment was regarded as acceptable.

All glass and plastic ware used during the toxicity testing were cleaned by soaking for 24 hrs each in phosphate-free detergent and 5% HNO₃ acid diluted with double distilled water, followed by a rinse in double distilled water.

5.2.4 Laboratory procedure for test metal concentrations.

The amphipods from each beaker were oven-dried at 60 °C for 24 hrs, weighed to four decimals and then digested for 24 hrs using 0.05 ml H₂O₂ for and 0.5 ml HNO₃. Further digestion was performed by heating samples in a microwave oven during four consecutive steps of 2 minutes at 200, 300, 400 and 500 W (Blust *et al.* 1988). The samples were then filtered and the volume made up to 10ml using double distilled water. Sediment samples were dried at 60 °C for 24 hrs, hot-plate digested using nitric acid and perchloric acid in a 2:1 ratio and then made up to 50 ml using double distilled water. Water samples from the toxicity test were filtered and stored until analysed. Copper and Zn concentrations in water, amphipod tissues and sediment samples were measured by ICP-MS by the Accredited Analytical Laboratory, Mhlathuze Water, Richards Bay. For quality control purposes, each batch of samples analysed included one blank and a certified reference material. The percent recovery of the reference material (CRM 320) from the analysis of the samples remained > 90%.

Due to the much smaller body size of *C. triaenonyx* compared to *G. lignorum*, the amount of *C. triaenonyx* body tissue available at the end of the toxicity tests was very small and as a result, the Cu and Zn concentrations in *C. triaenonyx* were below detection limits for the ICP-MS analyses, except for the highest exposure concentrations. This meant that Cu and Zn concentrations in *C. triaenonyx* after the spiking experiment could not be determined. This problem was not observed in the *G. lignorum* experiments.

5.2.5 Reference toxicity test

Cadmium chloride is commonly used as a reference toxicant to test the relative sensitivity of organisms to toxicants. The sensitivity of the two test species, *C. triaenonyx* and *G. lignorum*, to CdCl_2 was determined by 96hrs static water only tests. Amphipods were added to aerated beakers containing 200 ml of filtered and diluted seawater at a salinity of 20 containing CdCl_2 at nominal concentrations ranging between 0.4-6.4 mg/l under similar conditions as described earlier.

5.2.6 Statistical Analysis

The LC_{50} for metal concentrations in *C. triaenonyx* and *G. lignorum* was determined using the EPA Probit Analyses software. After the data were tested for normality, one way ANOVA was used to test for significant differences in the body Cu concentrations. Pairwise post-hoc comparisons were performed using Tukey's HSD test, a statistical test used in combination with ANOVA to find which means are significantly different from one another. Pearson's Correlations coefficients (R) were determined to test for the relationship between metal concentrations in the tissue, sediment and water.

5.3 Results

5.3.1 Environmental conditions

The environmental parameters measured at the start and end of the *C. triaenonyx* and *G. lignorum* sediment toxicity tests are presented in Table 5.1. All the parameters remained within or close to the allowed range and as such, did not affect the outcome of the experiments. Although the allowed application limit of ammonia was 0.8 mg/l, this was the recorded concentration at the start of the experiment in the control water and the levels did not change appreciably during the duration of the toxicity tests, attaining a maximum of 0.9 mg/l.

Table 5.1 Environmental variables measured at the start of the sediment toxicity tests and at the end of the 10-day period. Application limits are based on ASTM (2008), modified for local conditions. Values given are the median concentrations after 10 days for all four tests.

Variable	Unit	Application Limits	Start	After 10 days		
				Median	Min	Max
Oxygen saturation	%	>80%	>80%	80.9	77.3	81.1
pH	-	7-8	7.43	7.56	7.43	7.87
Ammonia	mg/l	0.8	0.8	0.85	0.7	0.9
Temperature	°C	25±1	24.8	24.7	24.5	24.9
Salinity	-	20±1	20.1	20	19.8	20.1

5.3.2 Reference toxicity test

The 96 h water-only LC₅₀ of *C. triaenonyx* and *G. lignorum* for CdCl₂ at 25 °C and at a salinity of 20 was 1.6 mg/l and 1.1 mg/l, respectively. These values are comparable to results obtained for similar species elsewhere under similar experimental conditions (DeWitt *et al.* 1996).

5.3.3 10-day sediment toxicity test

Mean survival in all the controls was >90%, demonstrating that the test procedures, amphipod handling techniques, water and control sediment were acceptable for conducting the 10-day sediment toxicity test, as required in the standard protocol (Swartz *et al.* 1985, ASTM 2008).

Copper Bioassays

The LC₅₀ value for *G. lignorum* exposed to sediment Cu with concentrations ranging from 4-64 µg/g Cu during the 10-day sediment toxicity test, was found to be 26 µg/g Cu (Table 5.2). The LC₅₀ value for *C. triaenonyx* during similar exposure concentrations was found to be 20 µg/g Cu. The LC₁₀

values for *G. lignorum* and *C. triaenonyx* were 17.8 µg/g and 3.3 µg/g Cu, respectively, indicating that *C. triaenonyx* was slightly more sensitive to Cu than *G. lignorum*.

Table 5.2 LC₅₀ concentrations for Cu and Zn in *G. lignorum* and *C. triaenonyx* (µg/g) as measured through 10-day sediment toxicity tests. LC₁₀ and LC₉₀ concentrations are also shown.

	LC10	Copper LC50	LC90	LC10	Zinc LC50	LC90
<i>Grandidierella lignorum</i>	17.8	26.7	40	7.4	44.6	267
<i>Corophium triaenonyx</i>	3.3	20.1	120.1	3.6	53.1	1932

Body Cu concentrations in *G. lignorum* were found to increase with increasing sediment Cu concentrations (Figure 5.1). Body Cu concentrations changed significantly during exposure to increasing sediment Cu concentrations ($F = 28.2$, $p < 0.001$). Body Cu concentrations were not significantly different between the control exposure and sediment Cu concentrations of 4 and 8 µg/g, but were significantly different from the control exposure at sediment concentrations of 16 µg/g and above (Figure 5.2). The body Cu concentrations at a sediment concentration of 64 µg/g were significantly different from that recorded at all other concentrations (Figure 5.1). There was a significant positive correlation between sediment and body Cu concentrations ($R = 0.95$, $t = 6.43$, $p = 0.003$) (Figure 5.2).

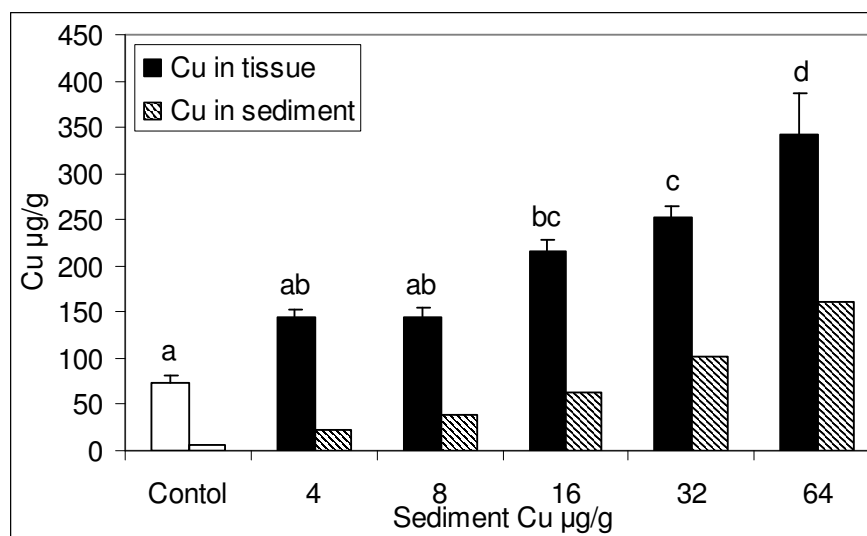


Figure 5.1 Mean tissue Cu concentration (+1SE) in *G. lignorum* and sediment Cu concentrations measured (µg/g dry weight) after a 10-day static sediment toxicity test with exposure concentrations ranging from 4-64 µg/g Cu. Bars with common superscript do not differ significantly.

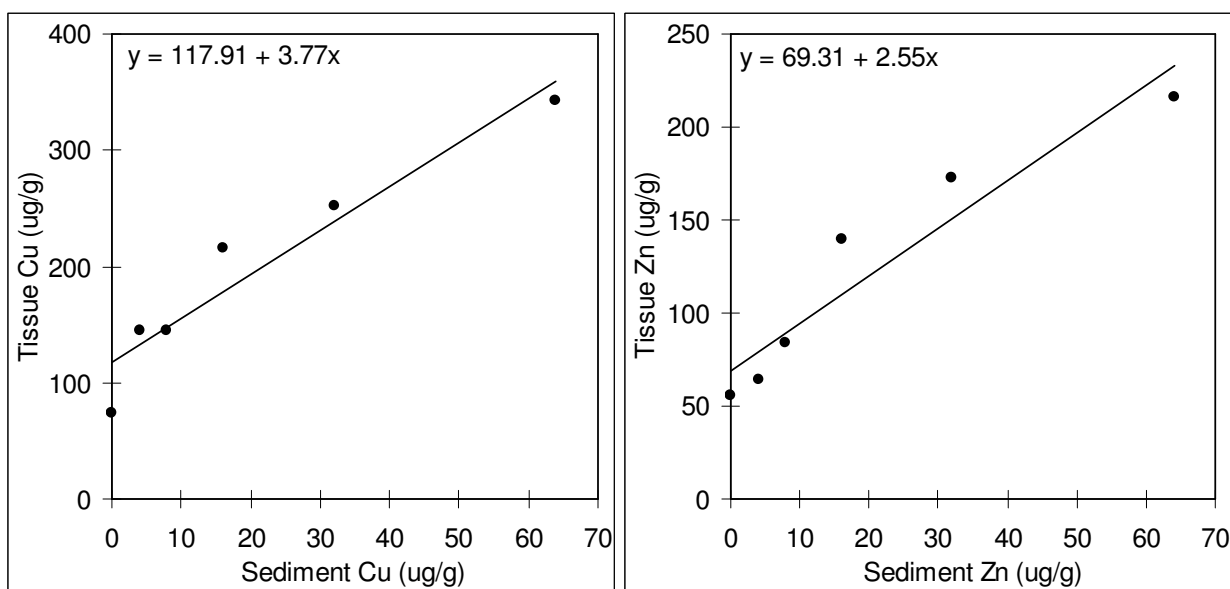


Figure 5.2 Correlation between body tissue Cu and Zn against spiked sediment Cu and Zn concentrations in *G. lignorum* after a 10-day static sediment toxicity test with exposure concentrations ranging from 4-64 µg/g.

Zinc bioassays

The LC₅₀ value for *G. lignorum* exposed to sediment with Zn with concentrations ranging from 4-64 µg/g Zn, was found to be 44.6 µg/g Zn (Table 5.2). Due to the relatively high survival (60%) of *G. lignorum* at 32 µg/g Zn, the Zn concentrations of the spiked sediment for the subsequent *C. triaenonyx* sediment toxicity test was increased to 6-96 µg/g Zn. The Zn LC₅₀ value for *C. triaenonyx* was 53.1 µg/g Zn. The LC₁₀ values for *G. lignorum* and *C. triaenonyx* for Zn was 7.4 and 3.6 µg/g Zn., respectively.

Body Zn concentrations in *G. lignorum* were found to increase concurrent with the increase in spiked sediment Zn concentrations (Figure 5.3). Body Zn concentrations changed significantly during exposure to increasing sediment Zn concentrations ($F = 8.9$, $p < 0.001$). Pairwise post-hoc comparison revealed that body Zn concentrations were not significantly different between the control exposure and sediment Zn concentrations of 4 and 8 µg/g, but were significantly different from the control exposure at sediment concentrations of 16 µg/g and above (Figure 5.3). The body Zn concentration at a sediment concentration of 64 µg/g Zn were significantly different from those recorded at all other sediment concentrations. Similarly, the body Zn concentrations at sediment concentrations of 8 and 16 µg/g Zn were also significantly different from that recorded at all other

sediment concentrations (Figure 5.3). There was a significant positive correlation between sediment and body Cu concentrations ($R = 0.95$, $t = 6.43$, $p = 0.003$) (Figure 5.2).

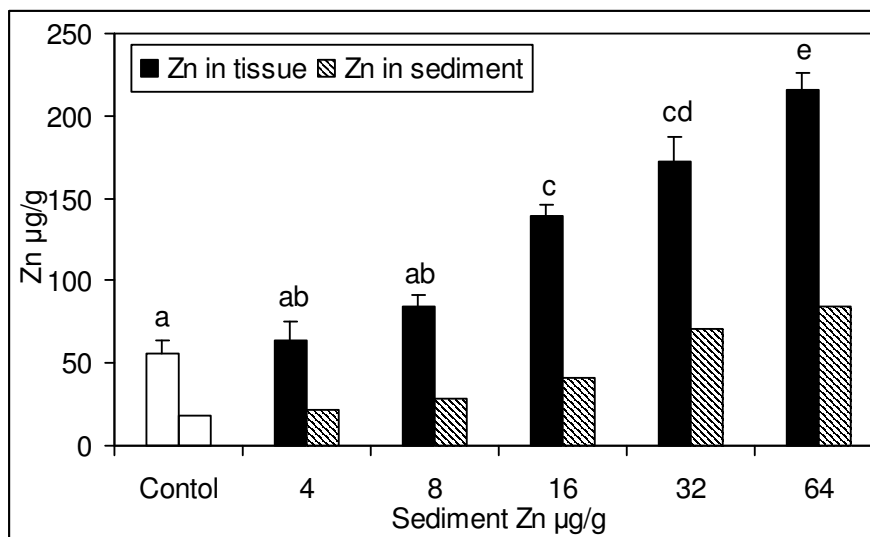


Figure 5.3 Mean body tissue Zn concentration (+1SE) in *G. lignorum* and sediment Zn concentration measured ($\mu\text{g/g}$ dry weight) after the 10-day static sediment toxicity test with exposure concentrations ranging from 4-64 $\mu\text{g/g}$ Zn. Bars with common superscript do not differ significantly

5.3.4. Emergence in *G. lignorum* during 10 day sediment bioassay

Following the 10 day sediment bioassay, the mean percentage emergence of *G. lignorum* from the sediment increased significantly with an increase in sediment Cu ($F = 37.1$, $p < 0.001$) and with sediment Zn ($F = 31.9$, $p < 0.001$) (Figure 5.4). With regard to Zn, emergence increased significantly from zero in the control sediment, to 17.6% in 4 $\mu\text{g/g}$ and 72.6 in 8 $\mu\text{g/g}$ sediment (Figure 5.4). Highest percentage emergence was observed in the highest Zn concentration of 64 $\mu\text{g/g}$. Similarly, emergence in Cu spiked sediment increased significantly from the control, to 24.4% in 4 $\mu\text{g/g}$ and 68.5% in 8 $\mu\text{g/g}$ sediment. Highest percentage emergence was observed in the 32 $\mu\text{g/g}$ sediment, while there was no survival of amphipods in 64 $\mu\text{g/g}$. There was a strong but not significant correlation between spiked sediment metal concentrations and percentage emergence of *G. lignorum* from both the Zn and Cu spiked sediment experiments. There was no emergence from the sediment recorded in *C. triaenonyx* during the 10 day bioassays.

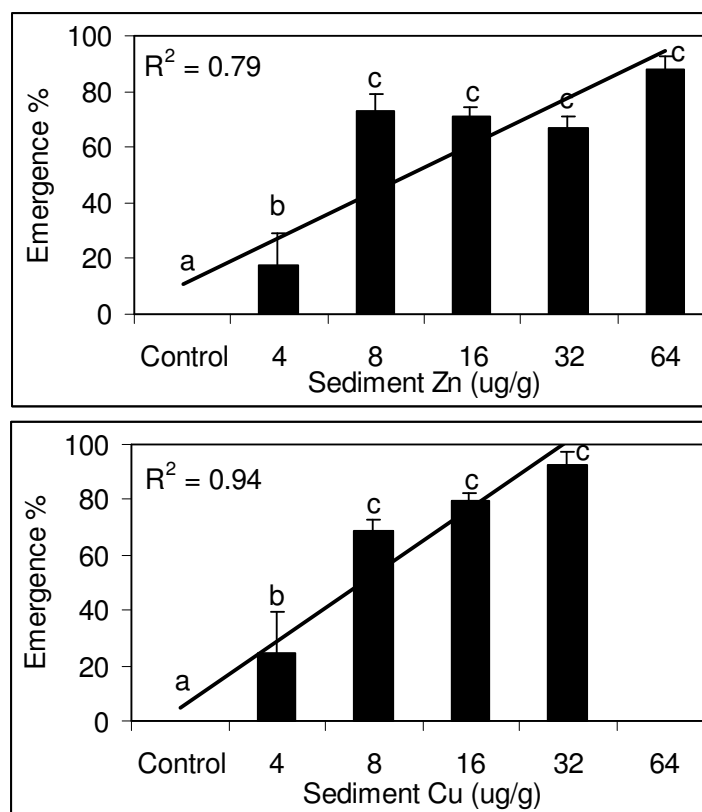


Figure 5.4 Mean emergence (%) in *G. lignorum* after a 10-day sediment toxicity test with Zn and Cu spiked sediment ranging from 4-64 µg/g. Bars with common superscript do not differ significantly

5.4 Discussion

5.4.1 Experimental procedure

Although the standardized methods prescribed for sediment bioassays (ASTM 2008) require 1 l beakers and about 200 g sediment per beaker, these parameters were miniaturized during this study, using 250 ml beakers and about 40 g of sediment per beaker. Ferreti *et al.* (2002) evaluated the effects of reducing the water and water volume during sediment bioassays and found that survival was consistently the same between standard volume tests and reduced volume tests. Due to the advantage offered by the reduced volume tests in terms of space required to run the tests and the volume of sediment needed to be spiked, it was decided to follow the methodology specified by Ferreti (*et al.* 2002) during this study. Although one of the concerns related to reduced volume tests was the possibility of overcrowding the organisms, tests by these authors showed the amphipod density in the 250 ml beakers was still much lower than nominal densities of test organisms in the field. The two amphipods used during this study consistently occurred at higher densities in the field than that measured in the test beakers (8400 N.m⁻²; see chapter 3).

5.4.2 10 day LC₅₀

This study showed that *G. lignorum* and *C. triaenonyx* are sensitive to metal contaminated sediment, with Cu being more toxic to these species than Zn. A similar response was not observed in *C. volutator*, which was less sensitive to Cu than to either Zn or Cd. The Cu, Zn and Cd 96 h water LC₅₀ values for this species was reported to be 20.74 (16.77–25.65), 9.79 (7.41–12.92) and 9.03 (6.15–13.26) mg/l, respectively (Bat *et al.* 1998). Copper is generally more toxic to aquatic organisms than Zn, and this has been reported for a number of amphipod species other than *C. volutator* (ASTM 2008).

The 10-day LC₅₀ values determined for *G. lignorum* and *C. triaenonyx* during this study for Cu and Zn (see Table 5.2) corresponded favorably to that found in benthic amphipods from elsewhere, such as in *C. volutator* for which values of 36.9 µg/g and 31.9 µg/g were recorded for Cu and Zn, respectively (Bat and Raffaelli 1998). A 10-day LC₅₀ value for a closely related species *G. japonica* of 31 µg/g was reported in a Cu-spiked sediment (Black 2003, in King *et al.* 2006), while the LC₅₀ for Cu in *Paracorophium excavatum* in New-Zeeland was reported to be 55 µg/g (Marsden and Wong 2001). The slightly higher sensitivity of *G. lignorum* and *C. triaenonyx* during this study compared to *C. volutator* and *G. japonica* could perhaps be attributed to the lower salinity (salinity of 20) at which these tests were conducted compared to the salinity of 32 for the *C. volutator* (Bat and Raffaelli 1998) and 35 for the *G. japonica* (Black 2003, in King *et al.* 2006) bioassays. Salinity has been reported to affect the toxicity of trace metals to aquatic organisms, with a decrease in salinity closely related to an increase in toxicity (Chapman and Wang 2001). The relatively high LC₅₀ value for *P. excavatum* was surprising, given that the test was run at a salinity of 20. In *G. locusta*, the 10-day LC₅₀ value for Cu of 6.8 µg/g was much lower than that for other amphipods for which data could be obtained, although Costa *et al.* (1998) cautioned that toxicity of Cu in the sediment is closely related to the organic content and that the LC₅₀ for Cu in this species has been shown to increase markedly with higher organic content. Definitive conclusions about the comparative sensitivity of different test organisms to trace metals in sediment should therefore ideally only be drawn by doing simultaneous experiments at the same salinity with the same sediment. As an alternative, 96h water only reference toxicity tests were introduced in an attempt to provide a standardized means of comparing the sensitivity of different test organisms to trace metals, and information in the literature show that these tests are a useful and robust means of directly comparing the sensitivity of amphipods to trace metals (ASTM 2008).

Corophium triaenonyx was more sensitive to Cu than *G. lignorum*, but not for Zn. This relative sensitivity of *C. triaenonyx* to Cu was unexpected, as the literature indicates that *Corophium* amphipods are generally less sensitive to metal concentrations than most other groups of amphipods (Bat and Raffaelli 1998), including the genus *Grandidierella*, although the sensitivity of the latter group is based on one species only, namely *G. japonica*. In 96 h water only Cu toxicity tests, the LC₅₀ values of *G. japonica* was 1.5 mg/l, compared to 4.5 mg/l for *C. colo* and a very high 20.7 mg/l for *C. volutator*, indicating that these two *Corophium* species were markedly less sensitive to Cu than *G. japonica*. The sensitivity of *G. japonica* compared favorably with that of another known sensitive species *Melita awa* (0.7 mg/l). However, in a comparative study exposing several North American and European species to contaminated North Sea sediment, *C. volutator* was found to be the most sensitive species. This again highlights the difficulty of making indirect comparisons between species used in sediment bioassays that were not tested under exactly the same conditions.

Results from this study showed that *G. lignorum* amphipods accumulated Cu and Zn in their bodies during the bioassay and the concentrations of the metals in the tissues increased with increasing concentrations of the metals in the sediment. The Cu (range 74-342 µg/g) and Zn (range 55-216 µg/g) concentrations in *G. lignorum* body tissues at the end of the 10 day test compared favorably with that reported for other species. Marsden and Wong (2001) reviewed the body Cu concentrations in amphipods and showed that in estuarine benthic species such as *P. excavatum* and *C. volutator*, Cu concentrations were 90-207 µg/g and 84-550 µg/g, respectively. Body Zn concentrations in *C. volutator* were reported to range between 79-303 µg/g (Bat and Raffaelli 1998). In a species regarded as sensitive to trace metals, *M. awa*, Cu and Zn tissue concentrations increased from 110 to 330 µg/g Cu and from 110 to 370 µg/g Zn over the 10-d exposure period (King *et al.* 2006), which in the case of Cu was remarkably similar to that recorded in *G. lignorum* during this study. In sediment toxicity tests exposing a series of amphipods to Cu and Zn spiked sediment, it was reported that a closely related species, *G. japonica*, was the species to consistently accumulate the highest concentrations of Cu (390 ± 60 µg/g) and Zn (330 ± 34 µg/g) relative to the control sediment of the eight species tested (King *et al.* 2006). In these experiments, *G. japonica* was also shown to be substantially, but not significantly, more sensitive to sediment Cu and Zn than two *Corophium* species, *Chaetocorophium lucasi* and *C. colo*. Bryan (1976) also reported *C. volutator* to be a relatively Cu-tolerant species, both in laboratory experiments and in contaminated field conditions and questioned the suitability of this species as a test organism for Cu contaminated sediments. The accumulation of Zn in *G. lignorum* during this study was considerably lower than that reported for *M. awa* and for *G. japonica*. In a comparative study of Cu and Zn accumulation in a decapod, an amphipod and a barnacle, it was found that the amphipod, *Echinogammarus pirloti*, accumulated Cu and Zn in a similar fashion but at a lower rate to that observed in the barnacle, which is generally believed to be the organism with the highest

rate of metal accumulation without any excretion (Rainbow and White 1989). The ability to accumulate trace metals during exposure to contaminated sediments is one of the primary requirements in sediment bioassay test organisms, and results from this study have clearly shown *G. lignorum* to be sensitive to and accumulate bioavailable Cu and Zn in sediment.

5.4.3 Reference toxicity test

In the reference toxicity test (96 h Cd LC₅₀ water only), *G. lignorum* (1.1 mg/l) was more sensitive than *C. triaenonyx* (1.6 mg/l) to Cd. Comparative data show that the sensitivity of *G. lignorum* and *C. triaenonyx* to the reference toxicant compared favorably to that reported for closely related species, this being indicative of the relative sensitivity of the two species to contaminants. The LC₅₀ for *G. japonica* was reported to be 1.17 (0.94–1.46 mg/l) (ASTM 2008). Lee *et al.* (2005) reported the LC₅₀ for *G. japonica* to range from 1.2–3.1 mg/l, while for *Monocorophium acherusicum*, is was slightly less, ranging from 0.7–1.4 mg/l. For *C. orientale*, from two distinct clean areas in France, the LC₅₀ was reported to be considerably higher at 4.28 (2.96–5.63 mg/l) and 2.91 (2.09–3.73) mg/l, respectively (Onorati *et al.* 1999), while for *C. volutator* from the Netherlands, the LC₅₀ ranged from 1.85 (1.27–2.69) to 5.30 (3.72–7.54) mg/l (Ciarelli 1994). In *Gammarus locusta*, a relatively low LC₅₀ of 0.85 mg/l (0.43–1.64) was argued to suggest that the species is a possible suitable alternative to commonly used less sensitive test species such as *C. volutator* (Costa *et al.* 1998). For *R. abronius* and *Leptocheirus plumulosus*, two species very commonly used in sediment toxicity testing in the USA and regarded as very sensitive to sediment contaminants, the LC₅₀ was reported to be 1.92 (1.47–2.51) mg/l and 1.06 (0.85–1.33) mg/l, respectively (ASTM 2008). In *C. lucasi*, the LC₅₀ value was 1.2 mg/l, which is very close to that reported for *G. lignorum* in this study. Of particular interest is that the 96h LC₅₀ value in this amphipod was found to be very similar to that measured in the 10 day *in situ* toxicity test, this being indicative of the ecological relevance of the sensitivity of the amphipod to the reference toxicant (DeWitt *et al.* 1996). Other species such as *Eohaustorius estuarius* were reported to be less sensitive, with a LC₅₀ value of 9.33 (7.20–12.09) mg/l (Lee *et al.* 2005). The sensitivity of *G. lignorum* and *C. triaenonyx* to the reference toxicant thus lies within in the range displayed by the more sensitive species mentioned above, notably that of *G. lignorum*, which closely paralleled that of *G. japonica*. As seen in *C. triaenonyx*, *C. orientale* and *C. volutator*, amphipods of the genus *Corophium* generally seem to be less sensitive to trace metals when compared to other species used in sediment bioassays. As the purpose of the reference toxicity test is to measure the relative sensitivity of a test species to a universally used toxicant under standardized conditions, it can be concluded that *G. lignorum* in particular, but also *C. triaenonyx*, are suitable candidates as test organisms for sediment bioassays, based on the relative sensitivity of these two species to Cd.

5.4.4 Emergence

In the 10 day toxicity tests, *G. lignorum* displayed avoidance behavior to contaminated sediment by emerging from the sediment. The percentage emergence in this species increased significantly with an increase in trace metal concentration, whereas all amphipods remained in their burrows in the control sediment. This sub-lethal behaviour has been reported in a number of other amphipods used in sediment bioassays (Mearns *et al.* 1986) and is regarded as a behavioural means of reducing exposure to metal in the sediment (Bat and Raffaelli 1998). *Rhepoxynius hudsoni* has been shown to emerge throughout 10 days of exposure to sediment containing 8.09 and 9.34 µg/g Cd, with a 68% emergence in Cd concentrations of 12 µg/g (Swartz *et al.* 1985). In *C. volutator*, a 25% emergence was recorded in sediment containing 30 µg/g Cu (Mearns *et al.* 1986). Bat and Raffaelli (1998) reported that in this species, emergence increased with increasing metal concentrations indicating that the contaminants had a sub-lethal effect. Few *C. volutator* amphipods emerged from clean (control) sediment, whereas many amphipods emerged from contaminated sediment. This behavior is similar that found during the present study, with *G. lignorum* emerging from all but the control sediments. Emergence behavior is not reported for all species, as *P. excavatum* was found to remain mostly buried in the sediment during a 10 day exposure to Cu, with a maximum emergence of 2.1% in 85 µg/g Cu (Marsden and Wong 2001). Compared to other amphipods, *G. lignorum* during the present study was found to emerge in large numbers at relatively low trace metal concentrations, with emergence of 68% and 72% being recorded at a Cu and Zn concentration of 8 µg/g, respectively. In addition, emergence in this species increased very rapidly in Cu and Zn exposure concentrations between 4 to 8 µg/g, at which point survival after 10 days was still more than 70%. This implies that emergence from contaminated sediment in this species is a sensitive sub-lethal measure of sediment toxicity of trace metals at relatively low contaminant concentrations. Future studies should focus on emergence as an ecologically relevant endpoint in risk assessments of potentially contaminated sediment.

In this regard, survival is typically the chosen endpoint in 10-day sediment toxicity tests. In recent years, however, the development and application of bioassays has also shifted from short-term assays focusing merely on mortality towards long-term exposures in which sublethal effects such as reproduction and growth at lower contaminant concentrations are the main endpoints (Scarlet *et al.* 2007, Kennedy *et al.* 2009). While short-term bioassays are still a valuable tool, chronic bioassays are considered to be more sensitive to pollution (Gale *et al.* 2006). It can thus be used to predict more subtle effects on a population level and can therefore provide a greater degree of environmental protection (Rainbow 2007). As a result, chronic bioassays are often considered to be an ecologically more relevant tool for risk assessment (Marsden 2002, Gale *et al.* 2006, van den Heuvel-Greve *et al.* 2007).

5.4.5 Ecological relevance

In *C. volutator*, a species commonly used in European sediment bioassays (Re *et al.* 2009), most surviving amphipods were capable of burrowing normally at the end of a 10-day exposure period, once removed from the source of contamination. Bat and Raffaelli (1998) considered this as clear indication that *C. volutator* is a robust species capable of surviving quite high levels of sediment contaminants and remarked that the relative low sensitivity of the species to contaminants may be related to their tubiculous habits. In contrast to the burrowing *R. abronius*, which is notably more sensitive to contaminants, the tube dwelling *C. volutator* is mostly exposed to overlying water which is pumped through the burrow so that regular contact between the amphipod and the sediment is thought to be limited, thereby reducing direct exposure to particle-bound contaminants in the sediment. Burrowing amphipods such as *R. abronius* are thought to experience more direct exposure to sediment bound contaminants (Bat and Raffaelli 1998, Simpson and Batley 2007). Both *G. lignorum* and *C. triaenonyx* are tubiculous amphipods and are therefore less exposed to sediment contaminants than burrowers, but at the same time, they feed on microalgae, particularly diatoms, that are gleaned from the surface of sediment and detritus particles and in that way they are continually in close contact with the sediment and detritus. They also ingest organic and inorganic particles <60 µm either by deposit feeding or suspension feeding (Icely and Nott 1985). Observations in the laboratory have shown that *G. lignorum* in particular regularly extend the upper parts of their bodies out of the tubes to grab sediment particles which they drag into their tubes and glean with their gnathopods and mouthparts to remove the microalgae (Vivier *pers obs.*). *Corophium triaenonyx*, using the well adapted row of long setae on their first gnathopods, spent much more time suspension feeding than *G. lignorum*, by creating a strong water current through their tubes with their pleopods and as such are probably less frequently in direct contact with the contaminated sediment than *G. lignorum*. King *et al.* (2006) reported that the relative insensitivity of *C. colo* to metal-spiked sediments was related to it being predominantly a suspension-feeder, whereas deposit feeders and macroalgae grazers were more sensitive to and also accumulated the higher amounts of Cu and Zn in their body tissues. The relative insensitivity of *C. orientale* to contaminated harbour sediment was also linked to its suspension feeding lifestyle with limited direct contact with contaminated sediment. In this species, only those chemical fractions released into solution in the water column were shown to cause mortality under laboratory conditions and therefore the species may be suitable to assess sediment toxicity of samples containing soluble contaminants rather than sediment bound contaminants (Onorati *et al.* 1999). Simpson and Batley (2007) recently reviewed the approaches available for predicting metal toxicity in sediments. They highlighted the difficulties to produce a ‘one-size-fits-all’ approach for benthic species toxicity assessment according to the diversity of modes of life of animals living in contact

with sediments. Simpson and Batley (2007) urged that a better understanding of the toxicity of sediment to benthic organisms can only be achieved by distinguishing between different exposure pathways and taking into account the role played by metal accumulation dynamics and the process of detoxification. Using a multipathway bioaccumulation model that accounts for uptake from solution and food, the organism's exposure to metals or net uptake can be determined (Simpson and Batley 2007, Casado-Martinez *et al.* 2010).

Tubicolous amphipods have been shown to cause extensive bioturbation of the sediment where they occur in large numbers through their tubicolous nature. This only brings them into regular contact with contaminated sediment, but also increases the flux of trace metals from the water into the sediment (Peterson *et al.* 1998, De Deckere *et al.* 2000). *Corophium volutator* has been found to increase Cd concentrations 3-6 times in the upper 2 cm of the sediment due to their activities, while the polychaete *Arenicola marina* actively caused Cd transport down to at least 10-13 cm in the sediment (Peterson *et al.* 1998). In addition, the sediment immediately around the tubes of the polychaete *Nereis diversicolor* have been found to be high in trace metals which are thought to readily adsorb onto the mucus linings of tubes. Peterson and Kristensen (1998) also mentions that in some nematodes, the mucus lining of their tubes have been found to have a high ability to bind and adsorb heavy metals. *Nereis diversicolor* increase the flux of arsenic and Zn from contaminated sediments into the overlying water through their burrowing and tube-building activities in the sediment (Riedel *et al.* 1989). This information suggests that a tubicolous nature does not necessarily limit exposure of the organisms to sediment bound metals, although a higher frequency of suspension feeding, as seen in *C. triaenonyx*, will expose the organism more frequently to contaminants in the water. Knowledge of the burrowing and feeding habits of test organisms is therefore important in assessing their suitability as bioindicators of sediment contamination, which in the case of *G. lignorum* in particular, has shown that it is a valuable addition to the array of potential organisms that can be used in sediment bioassays.

Rainbow (2007) provided a detailed review of trace metal accumulation patterns in aquatic invertebrates, notably crustaceans. As suggested by Rainbow (2007), knowledge of accumulation patterns is a prerequisite to understanding why aquatic invertebrates accumulate trace metals to such different body concentrations. All aquatic invertebrates accumulate trace metals in their tissues, but to varying degrees. Thus, aquatic invertebrates living in the same habitat under similar conditions may well have very different body concentrations of trace metals (Luoma and Rainbow 2005, Simpson and Batley 2007). Decapod crustaceans for example are known to regulate body concentrations of metals to a large extent, whereas some other crustaceans, most notably the barnacles, accumulate most trace metals without any significant excretion (Rainbow and White 1989, Rainbow 1997, 2007). This

implies that crustaceans such as barnacles will suffer from the toxic effects of the accumulated trace metals when the bioavailability of the metal is such that the rate of uptake exceeds the rates of detoxification (Rainbow 2007). At this point, the concentration of bioavailable metal will pass the critical threshold, with lethal consequences, even though it represents only a small portion of the total concentration of the metal in the body (Simpson and Batley 2007, Re *et al.* 2009). The lack of any form of excretion of accumulated trace metals makes barnacles the ultimate bioindicator of contamination, as their body tissues reflect the full fraction of bioavailable metal in the environment. Barnacles are unfortunately, by nature, not closely associated with bottom sediments, whereas benthic amphipods are known to have a close association with sediment due to their burrowing and feeding habits, making them more suitable indicators of contaminated sediments (Chapman 1998, Rainbow 2007). Amphipods have been reported to be net accumulators of trace metals, but some excretion of accumulated metal, such as Cu, Zn and Co, takes place through the excretion of metal rich granules from the detoxified store in the body (Rainbow 2007). These metal rich granules are formed and stored in the ventral caeca and eventually passed out through the alimentary canal. The total body concentration of metal therefore still reflects the amount of bioavailable metal associated with the sediment, making amphipods suitable bioindicators of trace metal bioavailability (Rainbow and White 1989). This explains the close correlation between body and sediment metal concentrations observed in *G. lignorum* in the present study and confirms the suitability of the species as a bioindicator of trace metal bioavailability. In order to predict toxic effects, the dynamics of metal exposure and assimilation and the site and mode of toxicity within the organism need to be understood. Recent advances in our understanding of accumulation dynamics of metals in aquatic invertebrates were offered by the application of biodynamic modeling, which takes into account the importance of different exposure pathways in metal accumulation (Luoma and Rainbow 2005, Casado-Martinez *et al.* 2010). Biodynamic-based models show great promise for interpreting metal accumulation by organisms with multiple exposure pathways (Simpson and Batley 2007). This is supported by Pastorinho *et al.* (2009), who concluded that there is a significant uptake and accumulation of metals such as Cu and Zn within the exoskeleton of amphipods, which though adding to the full body burden would add little to toxicity through lack of bioavailability.

There are a number of non-contaminant factors that may influence amphipod survival in sediment toxicity tests, of which the most important are sediment particle size, salinity, oxygen saturation and ammonia concentrations (ASTM 2008). These have to be taken into account in the interpretation of the test results. Ammonia concentrations during this study (0.7-0.9 mg/l) remained at or just above the applications limit of 0.8 mg/l specified for estuarine amphipods in acute sediment toxicity tests and could potentially have influenced the test results (ASTM 2008). Ammonia, particularly the un-ionized form (NH₃), is acutely toxic to most aquatic organisms (Kohn *et al.* 1994), with the tolerance limit

ranging from 0.07 mg/l in some freshwater fish to 19.1 mg/l in the oyster *Crassostrea virginica*. Although no information is available on the ammonia tolerance range of the two test organisms, the LC₅₀ for un-ionized ammonia in *G. japonica* and *E. estuarius* (salinity of 30) was reported to be 3.35 and 2.49 mg/l, respectively, which is considerably higher than the maximum of 0.9 mg/l recorded during this study. The ammonia concentrations of 0.7-0.9 mg/l recorded during this study are therefore not believed to have influenced the outcome of the sediment toxicity tests, although this will have to be verified in further studies. In fact, the relatively high tolerance of *G. japonica* to ammonia was thought to be advantageous to the species when used as test organisms in sediment bioassay. Since ammonia is an important pollutant in benthic environments, it is often attributed to being a confounding factor in sediment bioassays and as such, a low sensitivity to ammonia, as seen in *G. japonica*, will increase the interpretive relevance of the test result.

Conditions under which the toxicity tests were conducted during this study, viz. at 25 °C and a salinity of 20, are considered to be suitable for acute toxicity tests using these estuarine amphipods. Although *G. lignorum* has been shown to prefer the low salinity upper reaches of estuaries (Vivier and Cyrus 1999a), Connell and Airey (1979) conducted life-cycle bioassays with *G. lignorum* at salinities of 25 to determine the sub-lethal effects of marine pollutants. These authors found that reproduction and survival in *G. lignorum* were not affected at the relatively high salinity of 25 and that healthy cultures of the amphipod were maintained for more than a year. *Grandidierella lignorum* and *C. triaenonyx* have both been found to have a wide tolerance of sediment grain size and salinity, with both species being recorded in estuarine as well as freshwater environments (Bolt 1969, Cyrus and Martin 1988, Vivier and Cyrus 1999a, Vivier and Cyrus 1999b). Connell and Airey (1979) concluded that *G. lignorum* has significant potential as indicator species in sediment toxicity tests, notably for sediment contaminated with fluoride, due to its relative sensitivity to contaminated sediments, its abundance and wide distribution in many estuaries along the east coast and its wide tolerance with regard to salinity. These results were confirmed during the present study.

In this regard, further studies should also focus on *G. bonnieriodes*, a common amphipod in larger, more marine dominated east coast estuaries (Mabaso 2002), which has a circum-tropical distribution and has potential as a sediment toxicity test species. The species occurs commonly in the contaminated Port of Richards Bay and therefore the development of sediment toxicity tests using this species will be directly relevant to determining the potential ecological risk associated with contaminated sediment in the port. The species does not occur in the Nhlabane Estuary (Vivier and Cyrus 1999a) and was therefore not selected as a test organism during this study.

5.4.6 Amenability

Results from the 10 day toxicity test indicate that *G. lignorum* is a useful test organism for the evaluation of contaminated sediments. Acute sediment toxicity tests are feasible with the species and are sensitive to sediment contaminant levels. *Grandidierella japonica*, a common species in tropical brackish lagoons in different parts of the world, which appear to have very similar habitat requirements as *G. lignorum*, and can also tolerate a variety of sediment types (sands, silts, or clays) and other environmental conditions such as temperature and salinity, has also been indicated as being a suitable test organism for sediment toxicity testing (Nipper *et al.* 1989, Han *et al.* 2005, Lee *et al.* 2005). An interlaboratory comparison indicated that the 10-day survival response of *G. japonica* was similar to that of *R. abronius*, an amphipod regarded as highly sensitive to contaminated sediments and widely used in sediment toxicity tests (Nipper *et al.* 1989). The ASTM (2008) concluded that *G. japonica* is a suitable test species as its tolerance to a variety of sediment types gives broad application for the use of the species in regulatory applications. Nipper *et al.* (1989) remarked that the sediment bioassay with *G. japonica* has definite ecological relevance as the results from this species complement the bioassays done for other species such as *R. abronius* due to differences in life history characteristics and the relative tolerance of *Grandidierella* species to sediment type and salinity. These authors recommended that the development of sediment bioassays with congeners of *G. japonica* should be encouraged as it would facilitate the application of amphipod test methods for sediment toxicity testing, as the genus occurs throughout the world. In the only other bioassay to date using *G. lignorum*, Connel and Airey (1982) tested the chronic effect of fluoride on *G. lignorum* and a closely related species, *G. lutosa*. They concluded that these two species are suitable test organisms for fluoride toxicity. In a comparative test using these amphipods, turbellarians and copepods, the amphipods were shown to be most sensitive to ecologically relevant fluoride concentrations. These authors also commented on the suitability of these amphipods as test organisms due to the ease of maintaining healthy cultures, their apparent tolerance to a range of salinities and sediment types as well as the ease of handling the amphipods. The same was observed during the present study as it was found that *G. lignorum* was easy to breed, they required little maintenance and they were generally easy to handle during the experimental setup. In contrast, it was found that the second species used in the bioassays, *C. triaenonyx*, presented a bigger challenge as they were less amenable to laboratory testing. They were considerably more difficult to breed and maintain in the laboratory, they appeared more sensitive to water quality and it was considerably more difficult to extract them from their tubes. It was found that often the amphipods would only leave their tubes when forced to do so by exposure to air, the effect of which must be assessed during further studies. In addition to more research being required to further develop the sediment bioassays with *G. lignorum* and *C. triaenonyx*, it is advised that a species closely related to the former, *G. bonnieroides*, also be evaluated as a potential sediment

test organism. This species has a circum-tropical distribution and occurs widely in marine dominated estuarine systems in southern Africa, where it often forms a dominant component of the benthic community. Since it occurs more frequently in larger, higher salinity systems, it has potential as a test organism in industrial influenced systems such as Richards Bay and Durban Bay Harbours.

CHAPTER 6

General Discussion

6. General Discussion

6.1 Freshwater input and estuarine integrity

Since the ecological importance and value of estuaries are often not appreciated, the management of estuaries in South Africa during the past decades has been inadequate and never a priority (Allanson and Baird 1999). Lack of management and priority research generally meant that estuaries have been subject to increasing adverse pressures, both indirectly from the effects of catchment utilization, which affect their water supply, and directly from the increasingly industrial and residential development along the coastal zone (Breen and McKenzie 2001). As a result of all of these pressures, many South African estuaries have become functionally degraded, and this has frequently been accompanied by a loss of species (Allanson and Baird 1999).

The locality of the Nhlabane system in a forestry dominated catchment means that the system does not yet suffer from the same fate as do most other estuaries around the world, i.e. industrial and residential encroachment, organic and inorganic chemical contamination and excessive nutrient loading. There is relatively little input of industrial chemical and solid waste, agricultural fertilizer, and sewage related nutrients into the system. Huizenga and Van Niekerk (1998) considered the low nutrient levels in the estuary to be due to the absence of allochthonous nutrient input through freshwater inflow, demonstrated in the low nitrate and phosphate concentrations at the weir. Results from this study also showed that trace metal concentrations in the water and sediment were low and within environmentally acceptable levels.

The future health of South Africa's estuaries is dependent on two main interrelated factors: their direct management and the quantity and quality of freshwater inputs (Hay and McKenzie 2005). Very little consideration has been given to either in the past, but both of these aspects are currently under review in South Africa. The management of estuaries now falls under Marine and Coastal Management (MCM), Department of Environment Affairs, and is regulated by the Marine Living Resources Act (Act 18 of 1998), while their water allocation is controlled under the new National Water Act (Act 36 of 1998). Through the new Water Act, the Resource Directed Measures (RDM) was created as a legal framework to ensure a freshwater supply or 'Reserve' for estuaries to maintain their ecological functioning. Since the initial RDM methods were published, the process has been revised and an updated version of the RDM procedures for estuaries has been published (DWAF 2004), detailing the step by step process of setting the Reserve for estuaries. A detailed conceptual framework stipulating the monitoring procedures to be followed after the Reserve for an estuary has been implemented was also designed by Taljaard *et al.* (2003). Further recent advances in estuarine management in South

Africa include a detailed assessment of the conservation importance of our estuaries (Turpie *et al.* 2002, Turpie 2004), a water quality index for estuarine water quality management (Wepener *et al.* 2006) and a procedure guide for managing estuaries in South Africa (Hay and McKenzie 2005). Considerable advances have thus been made at the policy making level for estuarine management in South Africa, but the implementation of these policies is sadly lacking far behind.

Various studies have explored the importance of freshwater for estuaries and have shown that the timing, frequency, and magnitude of freshwater flows are critical features of estuarine biotic productivity (Currie and Small 2005, Whitfield 2005, Flemer and Champ 2006, Whitfield and Bate 2007). Since much of the high biological variability in estuaries may be attributed to variations in freshwater flow, it is increasingly recognised that the natural dynamics of estuaries are vulnerable to upstream water extraction and diversion processes and that large-scale anthropogenic modifications of freshwater inflow to estuaries are threatening the existence of estuarine habitats. In some catchments in South Africa, the storage capacity of reservoirs exceeds the annual runoff from rivers feeding them (Schlacher and Wooldridge 1996, Whitfield and Bate 2007). As the ecological consequences of severely reduced river inflow on estuarine systems are predictably negative, it is imperative that water management plans incorporate a comprehensive assessment of down-stream effects. In particular, regulatory processes should consider the economic, social and environmental costs associated with reduced estuarine biodiversity and fisheries productivity (Currie and Small 2005).

Construction of the Nhlabane weir deprived the Nhlabane Estuary of adequate freshwater. In an assessment of the effect of the weir on the hydrodynamics of the estuary, Huizenga and Van Niekerk (1998) reported that, prior to weir construction in 1978, flow from the catchment would have ensured that the system breached naturally at least once per year. During most years one or two breachings would have occurred and the mouth would rarely have been open four times or more per year, although during drought periods the mouth probably stayed closed for over a year. During post-construction years, contrastingly, the weir overtopped only infrequently and as a result, the estuary was deprived of direct freshwater inflow for extended periods of time. The Nhlabane weir therefore changed the natural flow regime of the system, with no breachings over the four year period 1991-1995, while during 1998 and 1999, the mouth breached at least eight times. As seen during this study, the estuary will not breach by itself unless it receives inflow from the lake, but during below average rainfall periods, rainfall from the catchment is first absorbed by the lake. The lake needs to fill up before it will overtop into the estuary. Abstraction of water from the lake for mining purposes aggravates the situation, inhibiting any chances that the estuary would breach during any period of below average rainfall. In 1999, RBM raised the level of the weir to 6m amsl, which as shown during this study, resulted in even more water deprivation for the estuary.

Seepage around the weir and from the surrounding dunes is insufficient to allow the system to breach under these conditions and the estuary mouth remained closed from July 1991 to August 1995. The mouth was artificially breached in August 1995, after which above average rainfall allowed the estuary mouth to open more frequently. This assisted in re-establishing a salinity gradient in the system and contributed largely towards improving estuarine habitat quality. The functioning of the estuary and the state of its biotic communities are therefore largely dependent on the maintenance of the estuarine-marine link, which in turn are directly related to freshwater input from the lake. As shown in this study, the benthic community gradually recovered after the mouth was breached in 1995. Recovery is, however, a long process and the benthic recovered to pre-drought conditions in 1998 and 1999.

6.2 Ecotoxicological status

Ecotoxicological assessments of coastal regions have increasingly integrated data on measures of sediment chemistry, toxicity and benthic invertebrate assemblage to best explain sediment quality in estuaries (Chapman *et al.* 1997, Chapman 2007a). This resulted in the development of the Sediment Quality Triad (SQT), a conceptual framework for collecting synoptic measurements of sediment chemistry, toxicity and benthos, and the use of these measurements collectively to assess relative sediment quality (Chapman 1990). The SQT consists of three components: sediment chemistry, which measures contamination; sediment bioassay, which measures toxicity; and in-situ determination of the benthic community structure to measure alteration due to contamination. The information provided by each component is complementary as all three measures are essential for determining sediment quality, in terms of the integrated accumulation of contaminants in sediments and of biological responses. The basic principle of the SQT was applied during this study in that sediment chemistry, bioassays and benthic assemblages were used to measure estuarine habitat integrity in terms of water and sediment quality in the Nhlabane Estuary. Results from this study showed that trace metal concentrations in the water, sediment and amphipod body tissues in the Nhlabane Estuary were lower than South African and international target values for coastal ecosystems, indicating that the Nhlabane Estuary was uncontaminated. The study also showed that local amphipods, notably *Grandidierella lignorum*, were suitable test organisms that could be used in sediment toxicity testing from contaminated estuarine areas. The relatively low trace metal concentrations in the water, sediment and body tissues recorded during this study also suggest that the Nhlabane Estuary would be a suitable reference site in future toxicity assessment of regional estuarine areas. The use of a reference area of suitable habitat quality is considered as a critical component in toxicity studies on aquatic ecosystems (Chapman 2007a).

6.3 Sediment toxicity test development in South Africa

Because of increasing industrial and recreational demands on coastal areas, especially estuarine environments, there is an increase in habitat deterioration and pollution. This affects faunal communities, through direct contact of organisms with contaminated sediment or by resuspension of contaminated particles into the overlying water (Rainbow 2002, Bat 2005). When introduced into the estuarine and marine environment, organic and inorganic contaminants, particularly heavy metals, eventually accumulate in sediment. Toxicity tests, particularly sediment toxicity tests, have been shown worldwide to be an appropriate way of increasing our understanding of the sensitivity of organisms to contaminated sediments and also have the advantage of being a time-integrated measure of the supply of the bioavailable fraction of a contaminant, which can be very different from the total amount present in the environment (Chapman and Wang 2001, Bat 2005). It is the bioavailable fraction only that is potentially toxic and of ecotoxicological relevance in biomonitoring studies of contaminated sediments (Rainbow 1995). This ecotoxicologically relevant fraction can be measured unambiguously through sediment toxicity tests. Such tests are now used widely to establish geographical and/or temporal variations in the bioavailable concentrations of heavy metals in coastal and estuarine waters (Rainbow 1995).

South Africa has experienced an accelerated growth in its economy over the past decade which has resulted in an increase in the demand for bulk cargo and container handling capacity throughout South African ports. As an example, the Port of Richards Bay, the largest deep-water port in South Africa built largely for the export of coal, is set to enter a long-term expansion phase that will see a three-fold increase in surface area and five-fold extension of existing quay length over the next 40 years. Despite being an important shipping facility for the import and export of bulk cargo, the port still serves as a fully functional estuary and contains ecologically highly important habitats for aquatic fauna. Richards Bay and the adjacent Mhlathuze Estuary have been shown to be of particular regional importance because between them they offer almost the complete range of habitat types found in tidal reaches of subtropical South African estuaries (Forbes *et al.* 1996, Weerts 2002). The port typically plays an important role in the life histories of many marine species that associate with estuaries at some stage in their life, by acting as nursery area for juveniles of many marine species (Weerts *et al.* 2003). Port environments, such as Richards Bay, are amongst the most susceptible to contamination as many port and industrial associated operational activities have the potential to introduce contaminants to the estuarine environment. Storage, handling and spillage of unprocessed materials, especially metal ores, are particularly problematic. A variety of approaches can be used to evaluate the hazard posed by contaminated sediments to ecological receptors. These approaches include water and sediment chemistry measurements, sediment toxicity tests, benthic invertebrate community

surveys, and active bioaccumulation assessments (Rainbow 2002). Water and sediment chemistry data by itself are of limited value, unless such data can be linked to biotic responses or observed adverse biological effects, thereby providing an efficient means for evaluating the ecological risk of sediment contamination (EPA 2005). Throughout the world, sediment toxicity tests are increasingly being used as important and relevant monitoring tools to assess the ecological risk associated with the potential toxicity of contaminated sediment. South Africa, unfortunately, is far behind the rest of the world in developing and incorporating sensitive sediment bioassays into biomonitoring programs. This study has shown that the amphipods *G. lignorum* and *C. triaenonyx* have potential to be used in assessments of sediment toxicity in estuarine environments, although further research is needed to address some issues related to laboratory procedures. In addition, much needed research must be done on other potentially suitable amphipod species, such as *G. bonnieroides*, in order to increase the array of suitable test organisms that can be used under different environmental conditions to assess the ecological relevance of sediment toxicity in our ever increasingly contaminated estuarine areas.

6.4 Long-term Monitoring

Because of their dynamic nature, the faunal composition of an estuary may vary considerably at spatial scales ranging from a few meters to a few kilometers and temporal scales of hours to years. Long-term monitoring programmes are essential for defining long-term trends of environmental degradation. Unfortunately, few monitoring programmes worldwide have studied long-term (>3 years) changes in soft-sediment estuarine benthic communities related to human induced pressures, mainly due to the expense associated with the collection, processing and analyses of benthic infauna and toxicological samples. Truly long-term data sets with high spatial and temporal replication are therefore rare but invaluable in determining long-term trends in estuarine biotic integrity. Understanding long-term temporal and spatial change in invertebrate community structure, and the abiotic and biotic factors underpinning them, is essential to the better understanding and management of estuarine ecosystems. In an assessment of the state of scientific knowledge of South African estuaries, Whitfield (2000) concluded that, of the 250 systems assessed, the state of information on 68% of the estuaries was “nil” to “poor”. Of the remaining estuaries, the state of information of 22% was classified as “moderate” while only 10% were regarded as having “good” or “excellent” information. Thus the necessary baseline information for most estuaries does not exist that would permit effective management of coastal resources. There is therefore an urgent need for information to be collected on the ecological functioning of these estuaries through monitoring of key elements. This study formed part of a long-term eight year investigation into the water quality, biota and ecotoxicology of the Nhlabane Estuary, which in the future will prove invaluable in our understanding of the ecological functioning of the system and in assisting estuarine managers to make decisions

based on sound scientific data if it can be continued. There is however a need for such long-term monitoring of the estuary to continue, given the ecological importance of the Nhlabane Estuary on the one hand and the adverse effect of continued freshwater deprivation due to the weir on the other hand.

6.5 Ecological Integrity and Management options

In an Estuarine Flow Requirement (EFR) study, which forms part of the Ecological Reserve Determination process for aquatic ecosystems in South Africa (Quinn 1999), water allocations were determined for the Nhlabane Estuary for drought flows during dry spells and maintenance flows during normal rainfall periods. The reference state for the Nhlabane Estuary was considered to be the pristine condition of the estuary, in other words, the state of the estuary prior to the construction of the weir and any alteration in freshwater flow patterns or volumes. Under these conditions the estuary was considered to be part of an estuarine lake (Whitfield 1992). As a clear estuarine lake, the Nhlabane was considered an important estuary due to its rarity of type, and because of its geographical location (35 km from the nearest adjacent estuary), it was considered to have high importance locally (Quinn 1999). The macrobenthos component of the system was considered to be of high importance, mainly due to the high densities of certain benthic organisms, while five endemic species at a national level were recorded in the estuary. These included *Ceratoneries keiskamma*, *Grandidierella lignorum*, *Bolttsia minuta*, *Lepthanthura laevigata* and *Hymenosoma orbiculare*.

Table 6.1 Assessment of present state for the Nhlabane Estuary, as determined through the Estuarine Flow Requirement (EFR) study in 1999 (from Quinn 1999).

COMPONENT	RATING & CONFIDENCE (%)		RATIONALE
Physical	D	60	• considerable changes have taken place
Water quality	B	80	• clearwater sytem, small number of localised impacts
Vegetation	C	60	• only lower basin significant
Zooplankton	E	80	• extensive loss of habitat (80%)
Zoobenthos	E	80	
Macrocrustacea	E	80	• extensive loss of habitat (80%)
Fish	E	80	• extensive loss of habitat (80%)
Birds	D	80	• extensive loss of habitat (80%)
OVERALL SCORE	D/E without fishway C with fishway		Despite being an important system, the Nhlabane has been significantly impacted by development. However should the fishway work, the link with the lake will be established and most of the above components would be increased to a C category

Based on the present status of the estuary relative to the pristine condition of the reference state, the estuary was classified as a D/E category estuary, corresponding to a seriously to largely modified system (Table 6.1). The EFR study recommended the establishment of base flows throughout the year and a permanent connection with the sea through the construction and management of a fish ladder across the weir, that the provision of flood events and freshets should emulate natural conditions and that during droughts the system be permitted to close for a year, but that this was only acceptable 1 year in 3. It was also recommended that mechanical breaching was required if the system remained closed for more than a year, and openings should coincide with spring and autumn equinoxes (Quinn 1999).

Since the EFR study was completed and the environmental flows determined for the Nhlabane estuary, little has, however, been done to improve the ecological integrity of the system. The recommended environmental flows for the estuary have not been implemented by DWAF and no allocation of water is allowed for the Reserve for the estuary as abstraction of water from the lake is still causing water levels in the lake to be consistently below overflow levels. Primarily due to this reason, the estuary has been closed from 2004 to present as there has been no outflow over the weir. A fish ladder was built across the Nhlabane weir, but operation of the weir is entirely dependent on water levels in the lake (Mastenbroek 2002) and as such, the fish ladder has not been in operation since 2004.

Results from this and previous studies show that the Nhlabane Estuary, once regarded as one of the most pristine estuaries along our coastline (Begg 1978), has been adversely affected by human related actions to the point where it ceases to be a functional estuary for periods of time extending over a number of years (Vivier and Cyrus 1999b). Although this study has shown that the ecotoxicological condition of the estuary is generally good, the system is hydrologically stressed due to freshwater deprivation. There is currently no management plan for the estuary that can address these issues and that can ensure that the Ecological Reserve for the estuary, which was determined in 1999 at great cost in terms of financial resources and scientific input, can be implemented. It is a huge tragedy that the New Water Act of 1998, which was promulgated to ensure the ecological integrity of aquatic systems, has produced all the necessary tools in the form of the Ecological Reserve with which to legally and effectively manage the Nhlabane Estuary, and yet it still has not been implemented a decade later. All the policies and conceptual monitoring plans are in place and all the hard work has been done in setting the Ecological Reserve, but the implementation of the Reserve by DWAF is still required.

As stated earlier, the future health of the Nhlabane Estuary is dependent on effective management and

the quantity and quality of freshwater inputs. Based on this, the following are regarded as key issues that should be addressed if the future ecological functioning of the Nhlabane Estuary is to be ensured:

Based on the legal framework created by the New Water Act, it is imperative that the Ecological Reserve for the estuary be implemented, as this will ensure that freshwater allocations for the estuary be adhered to. This will increase the frequency with which the estuary mouth breaches, thereby re-establishing the vital estuarine-marine link.

A management plan should be designed for the estuary by the responsible management agency, in this case MCM, in which all stakeholders are represented. As noted by Hay and McKenzie (2005), who compiled a detailed recent framework and manual on estuarine management in South Africa, stakeholders include other state and provincial departments, local and district municipalities and traditional leadership, who, together with industry, should all be represented in a Nhlabane Estuary management forum.

Management of the estuary should take place at both an estuary level, and at a catchment, or national, level. Regulation at the national level, regarding water supply and catchment management is a priority, mainly due to the fact that the system is hydrologically stressed.

A monitoring program of key ecological components in the Nhlabane Estuary, which should complement the existing database and investigate long-term temporal and spatial changes in important biotic communities, should be regarded as a priority. This will allow management decisions to be made on relevant, up to date scientific information. Long-term monitoring programmes in estuaries are important in that they highlight long-term trends, help document ecosystem variability that is important for the interpretation of trends, generate hypotheses on fundamental ecological relationships and allow measurement of compliance to legal limits for fish and bait quotas (Hay and McKenzie 2005). The monitoring framework designed for the implementation of the Reserve should be followed as this will enable comparison of the biotic integrity of the system once the Reserve has been implemented (Taljaard *et al.* 2003).

Input of contaminants, particularly trace metals, into the estuary is limited. In this regard, the Nhlabane Estuary is considered to be of high conservation importance, as it is one of the few estuaries along the coastline that is not adversely affected by industrial development, other than dune mining, in its catchment. It is also the only estuary along a 70 km section of coastline between Richards Bay Harbour, a heavily industrialized port earmarked for considerable expansion in the near future, and St Lucia, an ecologically severely stressed and degraded coastal lake during recent years. The ecological importance of the Nhlabane Estuary as an alternative sheltered estuarine area for marine migrant fauna in view of the substantial regional loss of estuarine habitat should be realised and motivation for the Nhlabane Estuary to be made a Estuarine Conservation Areas (ECAs) should be considered (Hay and McKenzie 2005).

CHAPTER 7

References

7. References

- American Society for Testing and Materials (ASTM). 2008. *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates*. Method E1367-08, West Conshohocken, PA.
- ANZECC. 2000. *Australian and New Zealand guidelines for fresh and marine water quality*. National Water Quality Management Strategy No 4. Canberra, Australia.
- Adamo, P., Arienzo, M., Imperato, M., Naimo, D., Nardi, G. and Stanzione, D. 2005. Distribution and partition of heavy metals in surface and sub-surface sediments of Naples city port. *Chemosphere* **61**: 800-809.
- Allan, Y. T., Thain, J. E., Haworth, S. and Barry, J. 2007. Development and application of long-term sublethal whole sediment tests with *Arenicola marina* and *Corophium volutator* using Ivermectin as the test compound. *Environmental Pollution* **146**: 92-99.
- Allanson, B. R. and Baird, D. 1999. *Estuaries of South Africa*. Cambridge University Press, Cambridge.
- Allanson, B. R. and Read, G. H. L. 1995. Further comment on the response of Eastern Cape Province estuaries to variable freshwater flows. *African Journal of Aquatic Science* **21**: 56-70.
- Atkinson, C.A., Jolley, D. F. and Simpson, S. L. 2007. Effect of overlying water pH, dissolved oxygen, salinity and sediment disturbances on metal release and sequestration from metal contaminated marine sediments. *Chemosphere* **69**: 1428–1437.
- Attrill, M. J. and Power, M. 2000. Effects on invertebrate populations of drought- induced changes in estuarine water quality. *Marine Ecology Progress Series* **203**: 133-143.
- Bat, L. and Raffaelli, D. 1998. Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. *Journal of Experimental Marine Biology and Ecology* **226**: 217-239.
- Bat, L., Raffaelli, D. and Marr, I. L. 1998. The accumulation of copper, zinc and cadmium by the amphipod *Corophium volutator* (Pallas). *Journal of Experimental Marine Biology and Ecology* **223**: 67-184.
- Bat, L. 2005. A review of sediment toxicity bioassays using amphipods and polychaetes. *Turkish Journal of Fisheries and Aquatic Sciences* **5**: 119-139.
- Begg, G. W. 1978. The Estuaries of Natal. *Natal Town and Regional Planning Report* **41**: 1-657.
- Binning, K. and Baird, D. 2001. Survey of heavy metals in the sediments of the Swartkops River Estuary, Port Elizabeth South Africa. *Water SA* **27**: 461-466.
- Blaber, S. J. M., Kure, N. F., Jackson, S. and Cyrus, D. P. 1983. The benthos of South Lake, St. Lucia following a period of stable salinities. *South African Journal of Zoology* **18**: 311-319.
- Blaber, S. J. M. and Hay, D. G. 1984. The ecology of two degraded estuaries on the north coast of

- Natal South Africa. *South African Journal of Zoology* **19**: 224-240.
- Blust, R., Van Der Linde, A., Verheyen, E. and Decler, W. 1988. Evaluation of microwave heating digestion and graphite furnace atomic absorption spectrometry with continuum source background correction for the determination of Fe, Cu and Cd in brine shrimp. *Journal of Analytical Atomic Spectrometry* **3**: 387–393.
- Boltt, R. E. 1969. The benthos of some South African Lakes. Part III. The epifauna and infauna of the benthos of Lake Sibaya. *Transactions of the Royal Society of South Africa* **38**: 249-269.
- Breen, C. M. and McKenzie, M. 2001. *Managing Estuaries in South Africa: An Introduction*. Pietermaritzburg, Institute of Natural Resources. 66 pp.
- Bryan, G. W. 1976. *Heavy metal contamination in the sea*. In: R. Johnston (Ed.), Marine Pollution. London Academic Press. London. 185-302
- Bryan, G. W. and Langston, W. J. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental Pollution* **76**: 89–131.
- Camp, P. D. 1990. Rehabilitation after dune mining at Richards bay Minerals. *South African Mining World* **1990**: 34-37
- Cardoso, P. G., Bankovic, M., Raffaelli, D. and Pardal, M. A. 2007. Polychaete assemblages as indicators of habitat recovery in a temperate estuary under eutrophication. *Estuarine, Coastal and Shelf Science* **71**: 301-308.
- Carvalho, S., Moura, A., Gaspar, M. B., Pereira, P. and Drago, T. 2005. Spatial and inter-annual variability of the macrobenthic communities within a coastal lagoon (Óbidos lagoon) and its relationship with environmental parameters. *Acta Oecologica* **27**: 143-159.
- Casado-Martinez, M.C., Forja, J.M. and DelValls, T.A., 2007. Direct comparison of amphipod sensitivities to dredged sediments from Spanish ports. *Chemosphere* **68**: 677–685.
- Casado-Martinez, M.C., Smith, B.D., Luoma, S.M., Rainbow, P.S. 2010. Metal toxicity in a sediment-dwelling polychaete: Threshold body concentrations or overwhelming accumulation rates? *Environmental Pollution* **158**: 3071-3076.
- Castro, H., Ramalheira, F., Quintino, V. and Rodrigues, A. M. 2006. Amphipod acute and chronic sediment toxicity assessment in estuarine environmental monitoring: An example from Ria de Aveiro, NW Portugal. *Marine Pollution Bulletin* **53**: 91-99.
- Chapman, P. M. 1990. The sediment quality triad approach to determining pollution-induced degradation. *The Science of the Total Environment* **98**: 815-825.
- Chapman, P. M., Anderson, B., Carr, S., Engle, V., Green, R., Hameedi, J., Harmoni, M., Haverland, P., Hyland, J., Ingersoll, C., Long, E., Rodgers, J., Salazar, M., Sibley, P. K. and Smith, P. J. 1997. General Guidelines for using the Sediment Quality Triad. *Marine Pollution Bulletin* **34**: 368-372.

- Chapman, P. M. 1998. *Death by Mud: Amphipod Sediment Toxicity Tests*. In: Microscale testing in aquatic toxicology: advances, techniques and practices. (Eds) P.G Wells, CRC Press, Florida, 451 - 463pp.
- Chapman, P. M. 2007a. Determining when contamination is pollution — Weight of evidence determinations for sediments and effluents. *Environment International* **33**: 492 - 501.
- Chapman, P. M. 2007b. Do not disregard the benthos in sediment quality assessments. *Marine Pollution Bulletin* **54**: 633-635.
- Chapman, P. M. and Wang, F. 2001. Assessing sediment contamination in estuaries. *Environmental Toxicology and Chemistry* **20**: 3–22.
- Ciarelli, S. 1994. *Guideline for conducting 10-day static sediment toxicity tests using marine or estuarine amphipods*. Report from the Tidal Water Division, Middelburg, The Netherlands.
- Ciarelli, S., Vonck, W. and Van Straalen, N. 1997. Reproducibility of spiked sediment bioassays using the marine benthic amphipod, *Corophium volutator*. *Marine Environmental Research* **43**: 329-343.
- Clarke, K. R. 1993. “Non-parametric multivariate analyses of changes in community structure.” *Australian Journal of Ecology* **18**: 117-143.
- Connell, A. D. and Airey, D. D. 1979. Life-cycle bioassays using 2 estuarine amphipods, *Grandidierella lutosa* and *G. lignorum*, to determine detrimental sublethal levels of marine pollutants. *South African Journal of Science* **75**: 313-314.
- Connell, A. D. and Airey, D. D. 1982. The chronic effects of fluoride on the estuarine amphipods *Grandidierella lutosa* and *Grandidierella lignorum*. *Water Research* **16**: 1313-1317.
- Conradi, M. and Depledge, M. H. 1998. Population responses of the marine amphipod *Corophium volutator* (Pallas, 1766) to copper. *Aquatic Toxicology* **44**: 31-45.
- Costa, F. O., Correia, A. D. and Costa, M. H. 1998. Acute marine sediment toxicity: a potential new test with the amphipod *Gammarus locusta*. *Ecotoxicology and Environmental Safety* **40**: 81-87.
- Costa, F. O., Neuparth, T., Correia, A. D. and Costa, M. H. 2005. Multi-level assessment of chronic toxicity of estuarine sediments with the amphipod *Gammarus locusta*: II. Organism and population-level endpoints. *Marine Environmental Research* **60**: 93-110.
- Cunha, M. R., Moreira, M. H. and Sorbe, J. C. 2000. The amphipod *Corophium multisetosum* (Corophiidae) in Ria de Aveiro (NW Portugal). II. Abundance, biomass and production. *Marine Biology* **137**: 651-660.
- Currie, D. R. and Small, K. J. 2005. Macrobenthic community responses to long-term environmental change in an east Australian sub-tropical estuary. *Estuarine, Coastal and Shelf Science* **63**: 315-331.

- Cyrus, D. P. and Mackay, C. F. 1995. Siyaya Estuary: A survey of the fish and benthic fauna. Unpublished CRUZ Report.
- Cyrus, D. P. and Martin, T. J. 1988. Distribution and abundance of the benthos in the sediments of Lake Cubhu: a freshwater coastal lake in Zululand, South Africa. *Journal of the Limnological Society of Southern Africa* **14**: 93-101.
- Cyrus, D.P. and Vivier, L. 2006. Status of the estuarine fish fauna in the Lake St Lucia Estuarine System, South Africa, after 30 months of mouth closure. *African Journal of Aquatic Science* **31**: 71–81.
- Dauer, D. M. 1993. Biological criteria, environmental health and estuarine macrobenthic community structure. *Marine Pollution Bulletin* **26**: 249–257.
- Dauvin, J. 2008. Effects of heavy metal contamination on the macrobenthic fauna in estuaries: The case of the Seine estuary. *Marine Pollution Bulletin* **57**: 160-169.
- Davies, B. R. 1984. The zoobenthos of the Touw River floodplain. *Journal of the Limnological Society of Southern Africa* **10**: 62-73.
- Day, J. H. 1974. The ecology of Morrumbene estuary, Mocambique. *Transactions of the Royal Society of South Africa* **41**: 43-97.
- Day, J. H. 1980. What is an estuary? *South African Journal of Science* **76**: 198.
- De Decker, H. P. and Bally, R. 1985. The benthic macrofauna of the Bot River estuary, South Africa, with a note on its meiofauna. *Transactions of the Royal Society of South Africa* **45**: 379-397.
- De Deckere, E. M., Koppel, J. V. and Heip, C. H. 2000. The influence of *Corophium volutator* abundance on resuspension. *Hydrobiologia* **426**: 37-42.
- De Groot, A. J., Salomons, W. A. and Allersma, E. 1976. Processes affecting heavy metals in estuarine sediments. In: Burton LJW and Liss PS (Eds.) *Estuarine Chemistry*. Academic Press, London. 131-157.
- De Lange, H. J., Noordoven, W., Murk, A.J., Lurling, M. and Peeters, E. T. H. M. 2006. Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. *Aquatic Toxicology* **78**: 209–216.
- DeWitt, T. H., Ditsworth, G. R. and Swartz, R. C. 1988. Effects of Natural Sediment Features on Survival of the Phoxocephalid Amphipod, *Rhepoxynius abronius*. *Marine Environmental Research* **25**: 99-124.
- DeWitt, T. H., Swartz, R. C., Hansen, D. J., McGovern, D. and Berry, W. J. 1996. Bioavailability and chronic toxicity of cadmium in sediment to the estuarine amphipod *Leptocheirus plumulosus*. *Environmental Toxicology and Chemistry* **15**: 2095-2101.
- DWAF. 1995. *South African Water Quality Guidelines For Coastal Marine Waters*. Volume 1: 1-160.

- DWAF. 2004. *Water Resource Protection and Assessment Policy Implementation Process*. Resource Directed Measures for protection of water resources: Methodology for the Determination of the Ecological Water Requirements for Estuaries. Version 2. Pretoria.
- Edgar, G. J. and Barrett, N. S. 2002. Benthic macrofauna in Tasmanian estuaries: scales of distribution and relationships with environmental variables. *Journal of Experimental Marine Biology and Ecology* **270**: 1 - 24.
- Elliott, M. and McLusky, D. S. 2002. The Need for Definitions in Understanding Estuaries. *Estuarine, Coastal and Shelf Science* **55**: 815-827.
- Elliott, M. and Quintino, V. M. 2007. The Estuarine Quality Paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin* **54**: 640-645.
- Environment Canada. 1992. *Biological Test Method: Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods*. Conservation and Protection, Ottawa, Ontario Report EPS1/RM/26.
- EPA. 1994. *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*. EPA 600R94025.
- Eriksson, S. P. and Weeks, J. M. 1994. Effects of copper on hypoxia on two populations of the benthic amphipod *Corophium volutator* (Pallas). *Aquatic Toxicology* **29**: 73–81.
- Ferretti, J. A., Calesso, D. F., Lazorchak, J. M. and Durham, C. O. 2002. Evaluation of reduced sediment volume toxicity test procedures using the marine amphipod *Ampelisca abdita*. *Environmental Toxicology and Chemistry* **21**: 2372–2377.
- Flemer, D. A. and Champ, M. A. 2006. What is the future fate of estuaries given nutrient over-enrichment, freshwater diversion and low flows? *Marine Pollution Bulletin* **52**: 247-258.
- Flint, R. W. and Kalke, R. D. 1986. Niche characterization of dominant estuarine benthic species. *Estuarine, Coastal and Shelf Science* **22**: 657-674.
- Forbes, A. T., Demetriades, N. T. and Cyrus, D. P. 1996. Biological significance of harbours as coastal environments in KwaZulu-Natal, South Africa. *Aquatic Conservation: Marine and Freshwater Ecosystems* **6**: 331-341.
- Gale, S.A., King, C.K., Hyne, R.V., 2006. Chronic sublethal sediment toxicity testing using the estuarine amphipod, *Melita plumulosa* (Zeidler): evaluation using metal-spiked and field-contaminated sediments. *Environ. Toxicol. Chem* **25**: 1887–1898.
- Giangrande, A., Licciano, M. and Musco, L. 2005. Polychaetes as environmental indicators revisited. *Marine Pollution Bulletin* **50**: 1153-1162.
- González-Oreja, J. A. and Saiz-Salinas, J. I. 1998. Exploring the relationships between abiotic variables and benthic community structure in a polluted estuarine system. *Water Research* **32**: 3799-3807

- Gray, S. J. 1989. Effects of environmental stress on species rich assemblages. *Biological Journal of Linnean Society* **37**: 19–32.
- Gray, S. J. and Pearson, T. H. 1982. Objective selection of sensitive species indicative of pollution-induced change in benthic communities I. Comparative methodology. *Marine Ecology Progress Series* **9**: 111–119.
- Greenstein, D. and Tiefenthaler, L. 1997. Reproduction and population dynamics of a population of *Grandidierella japonica* (Stephensen) (Crustacea: Amphipoda) in Upper Newport Bay, California. *Bulletin of the Southern California Academy of Sciences* **96**:34-42.
- Griffiths, C. L. 1973. The amphipoda of Southern Africa. I. The Gammaridea and Caprellidea of southern Moçambique. *Annals of the South African Museum* **60**: 265-306.
- Griffiths, C. L. 1976. *Guide to the benthic marine amphipods of Southern Africa* South African Museum, Cape Town, 106 pp.
- Grizzle, R. E. 1984. Pollution indicator species of macrobenthos in a coastal lagoon. *Marine Ecology Progress Series* **18**: 191-200.
- Guerra-García, J., Baeza-Rojano, E., Cabezas, M., Díaz-Pavón, J., Pacios, I. and García-Gómez, J. 2009. The amphipods *Caprella penantis* and *Hyale schmidtii* as biomonitors of trace metal contamination in intertidal ecosystems of Algeciras Bay, Southern Spain. *Marine Pollution Bulletin* **58**: 765-786.
- Han, J., Quan, X., Wang, J. and Yan, Q. 2005. Bioavailability of zinc in the sediment to the estuarine amphipod *Grandidierella japonica*. *Hydrobiologia* **541**: 149-154.
- Harrison, T. 2004. Physico-chemical characteristics of South African estuaries in relation to the zoogeography of the region. *Estuarine, Coastal and Shelf Science* **61**: 73-87.
- Hastie, B. F. and Smith, S. D. 2006. Benthic macrofaunal communities in intermittent estuaries during a drought: Comparisons with permanently open estuaries. *Journal of Experimental Marine Biology and Ecology* **330**: 356 - 367.
- Hattingh, R. P. and Vivier, L. 1995. *Summary report on the fine sediment intrusion into the Nhlabane Estuary, including recommended rehabilitation measures*. Report EHS/W/30/94 for RBM.
- Hart, R. C. 1995. South African coastal lakes. In: *Wetlands of South Africa*. pp. 103-130. Cowan, G.I (Eds). Department of Environmental Affairs and Tourism, Pretoria.
- Hay, D. and McKenzie, M. 2005. *Managing Estuaries in South Africa: A step by step guide*. WRC Report No. TT 243/04.
- Hennig, H. 1985. *Review of metal concentration in Southern African coastal waters, sediment and organisms*. Foundation for Research Development, Council for Scientific and Industrial Research Report No 108. pp140.
- Heydorn, A. E. 1986. *Assessment of the state of the estuaries of the Cape and Natal in 1985/86*. SANSP Report 130. pp 44.

- Hickey, C. and Roper, D. 1992. Acute toxicity of cadmium to two species of infaunal marine amphipods from New Zealand. *Bulletin of Environmental Contamination and Toxicology* **49**: 165-170.
- Hill, B. J. 1966. A contribution to the ecology of the Umlalazi estuary. *Zoologica Africana* **2**: 1-24.
- Hughes, R. G. and Gerdol, V. 1997. Factors affecting the distribution of the amphipod *Corophium volutator* in two estuaries in South-east England. *Estuarine, Coastal and Shelf Science* **44**: 621-627.
- Huizenga, P. and Van Niekerk, L. 1998. *Nhlabane Estuary - Physical Aspects*. Report prepared for IWR Environmental. CSIR Report ENV/S-C 98107.
- Huizenga, P. and Van Niekerk, L. 1999. Nhlabane Estuary. In Quinn, N. (Eds): *Nhlabane estuarine flow requirements: an initial assessment*. Mhlathuze Ecological Reserve Study, IWR Environmental.
- Icely, J. D. and Nott, J. A. 1985. Feeding and digestion in *Corophium volutator* (Crustacea: Amphipoda). *Marine Biology* **89**: 183–195.
- Jensen, K. T. and Kristensen, L. D. 1990. A field experiment on competition between *Corophium volutator* (Pallas) and *Corophium arenarium* Crawford (Crustacea: Amphipoda) effects on survival, reproduction and recruitment. *Journal of Experimental Marine Biology and Ecology* **137**: 1–24.
- Kemper, N. 2000. Nhlabane Lake water level requirements: an initial assessment. *Mhlathuze Ecological Reserve Study*, IWR Environmental.
- Kennedy, A.J., Jeffery, A., Steevens, J. A., Lotufo, G. R., Farrar, J. D., Reiss, M. R., Kropp, R. K., Doi, J. and Bridges, T. S. 2009. A comparison of acute and chronic toxicity methods for marine sediments. *Marine Environmental Research* **68**: 118–127
- Kennish, M. J. 1997. *Practical handbook of estuarine and marine pollution*. CRC Press: New York.
- Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* **29**: 78-107.
- Khan, S. A., Murugesan, P., Lyla, P. S. and Jaganathan, S. 2004. A new indicator macro invertebrate of pollution and utility of graphical tools and diversity indices in pollution monitoring studies. *Current Science* **87**: 1508-1510.
- King, C. K., Gale, S. A., Hyne, R. V., Stauber, J. L., Simpson, S. L. and Hickey, C. W. 2006. Sensitivities of Australian and New Zealand amphipods to copper and zinc in waters and metal-spiked sediments. *Chemosphere* **63**: 1466–76.
- Kohn, N. P., Word, J. Q. and Niyogi, D. K. 1994. Acute toxicity of ammonia to four species of marine amphipods. *Marine Environmental Research* **38**: 1–15.
- Lamberth, S. J. and Turpie, J. K. 2003. The role of estuaries in south African fisheries: economic importance and management implications. *African Journal of Marine Science* **25**: 131-157.

- Lee, J. S., Lee, K. T., Kim, D. H., Kim, C. K., Lee, J. H., Park, K. H. and Park, G. S. 2005. Application of indigenous benthic amphipods as sediment toxicity testing organisms. *Ocean Science Journal* **40**: 17-24.
- Long, E. R., MacDonald, D. D., Smith, S. L. and Calder, F. D. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management* **19**: 81-97.
- Luoma, S. N. 1983. Bioavailability of trace metals to aquatic organisms - a review. *Science of the Total Environment* **28**: 1-22.
- Mabaso, S. H. P. 2002. The macrobenthos of the Mlalazi estuary, KwaZulu-Natal. Unpublished MSc Thesis, University of Zululand. 148pp.
- Mackay, C. F. 1996. The benthos of the Siyaya estuary: Species composition, density and distribution. Unpublished MSc Thesis, University of Zululand.
- Mackay, C. F. and Cyrus, D. P. 1999. A review of the macrobenthic fauna of the Mhlathuze estuary: setting the ecological reserve. *African Journal of Aquatic Science* **24**: 111-129.
- Mackay, C. F. and Cyrus, D. P. 2001. Is freshwater quality adequately defined by physico-chemical components? Results from two drought-affected estuaries on the east coast of South Africa. *Marine and Freshwater Research* **52**: 267-281.
- Marsden, I. D. 2002. Life-history traits of a tube-dwelling corophioid amphipod, *Paracorophium excavatum*, exposed to sediment copper. *Journal of Experimental Marine Biology and Ecology* **270**: 57-72.
- Marsden, I. D. and Rainbow, P. S. 2004. Does the accumulation of trace metals in crustaceans affect their ecology - the amphipod example. *Journal of Experimental Marine Biology and Ecology* **300**: 373-408.
- Marsden, I. D., Rainbow, P. S. and Smith, B. D. 2003. Trace metal concentrations in two New Zealand talitrid amphipods: effects of gender and reproductive state and implications for biomonitoring. *Journal of Experimental Marine Biology and Ecology* **290**: 93 - 113.
- Marsden, I. D. and Wong, C. H. T. 2001. Effects of sediment copper on a tube-dwelling estuarine amphipod, *Paracorophium excavatum*. *Marine and Freshwater Research* **52**: 1007-1014.
- Mastenbroek, W. K. 2002. Fish and macro-invertebrate migration through a fishway on the Nhlabane Estuary in KwaZulu-Natal, South Africa. Unpublished MSc Thesis, University of Natal Durban. 83 pp.
- Matthiessen, P. and Law, R. J. 2002. Contaminants and their effects on estuarine and coastal organisms in the United Kingdom in the late twentieth century. *Environmental Pollution* **120**: 739-757.

- McCready, S., Birch, G. and Long, E. 2006. Metallic and organic contaminants in sediments of Sydney Harbour, Australia and vicinity – A chemical dataset for evaluating sediment quality guidelines. *Environment International* **32**: 455-465.
- McGee, B. L., Wright, D. A. and Fisher, D. J. 1998. Biotic factors modifying acute toxicity of aqueous cadmium to estuarine amphipod *Leptocheirus plumulosus*. *Archives of Environmental Contamination and Toxicology* **34**: 34–40.
- McLusky, D. S., Bryant, V. and Campbell, R. 1986. The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. *Oceanography and Marine Biology Annual Review* **24**: 481–520.
- Mearns, A. J., Swartz, R. C., Cummins, J. M., Dinnel, P. A., Plesha, P. and Chapman, P. M. 1986. Inter-laboratory comparison of a sediment toxicity test using the marine amphipod, *Rhepoxynius abronius*. *Marine Environmental Research* **19**: 13–37.
- Moore, P. G. and Rainbow, P. S. 1987. Copper and zinc in an ecological series of talitroidean Amphipoda (Crustacea). *Oecologia* **73**: 120– 126.
- Mzimela, H. M., Wepener, V. and Cyrus, D. P. 2003. Seasonal variation of selected metals in sediments, water and tissues of the groovy mullet, *Liza dumerelii* (Mugilidae) from the Mhlathuze Estuary, South Africa. *Marine Pollution Bulletin* **46**: 659-676.
- Nanami, A., Saito, H., Akita, T., Motomatsu, K. and Kuwahara, H. 2005. Spatial distribution and assemblage structure of macrobenthic invertebrates in a brackish lake in relation to environmental variables. *Estuarine, Coastal and Shelf Science* **63**: 167-176.
- Nayar, S., Goh, B. and Chou, L. 2004. Environmental impact of heavy metals from dredged and resuspended sediments on phytoplankton and bacteria assessed in in-situ mesocosms. *Ecotoxicology and Environmental Safety* **59**: 349-369.
- Neuparth, T., Correia A. D., Costa F.O. and Costa M. H. 2005. Multi-level assessment of chronic toxicity of estuarine sediments with the amphipod *Gammarus locusta*: I. Biochemical endpoints. *Marine Environmental Research* **60**: 69-91.
- Newman, B. K. and Watling, R. J. 2007. Definition of baseline metal concentrations for assessing metal enrichment of sediment from the south-eastern Cape coastline of South Africa. *Water SA* **33**: 675-681.
- Ngqulana, S. G., Owen, R. K., Vivier, L. and Cyrus, D. P. 2010. Benthic faunal distribution and abundance in the Mfolozi – Msunduzi estuarine system, KwaZulu-Natal, South Africa. *African Journal of Aquatic Science* **35**: 123-133.
- Nieboer, E. and Richardson, D. H. S. 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environmental Pollution* **1**: 3-26.

- Nipper, M. G., Greenstein, D. J. and Bay, S. M. 1989. Short- and long-term sediment toxicity test methods with the amphipod *Grandidierella japonica*. *Environmental Toxicology and Chemistry* **8**: 1191-1200.
- Normant, M. and Lamprecht, I. 2006. Does scope for growth change as a result of salinity stress in the amphipod *Gammarus oceanicus*? *Journal of Experimental Marine Biology and Ecology* **334**: 158 - 163.
- O'Donoghue, S. 2005. An investigation into marine pollution biomonitoring in KwaZulu-Natal using physiological and cytological biomarkers in the common sand prawn, *Callinassa kraussi* *stebbing*. Unpublished MSc dissertation, University of KwaZulu-Natal, Durban
- Onorati, F., Bigongiari, N., Pellegrini, D. and Giuliani, S. 1999. The suitability of *Corophium orientale* (Crustacea, Amphipoda) in harbour sediment toxicity bioassessment. *Aquatic Ecosystem Health and Management* **2**: 465-473.
- Orr, K. K., Burgess, J. and Froneman, P. 2008. The effects of increased freshwater inflow on metal enrichment in selected Eastern Cape estuaries, South Africa. *Water SA* **34**: 39-52.
- Orr, K. K. 2007. Spatial and temporal variations in metals in the sediment and water of selected eastern Cape estuaries, South Africa. Unpublished MSc Thesis, Rhodes University, South Africa.
- Pastorinho, R.M., Telfer, T.C. and Soares, A. M. V. M. 2009. Amphipod susceptibility to metals: Cautionary tales. *Chemosphere* **75**: 1423–1428
- Pearson, T. R. and Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual review* **16**: 229–311.
- Perez-Landa, V., Belzunce, M. J. and Franco, J. 2008. The Effect of Seasonality and Body Size on the Sensitivity of Marine Amphipods to Toxicants. *Bulletin of Environmental Contamination and Toxicology* **81**: 548-552.
- Peters, C. and Ahlf, W. 2005. Reproduction of the estuarine and marine amphipod *Corophium volutator* (Pallas) in laboratory for toxicity testing. *Chemosphere* **59**: 525-536.
- Peterson, K. and Kristensen, E. 1998. Influence of bioturbating animals of flux of cadmium into estuarine sediment. *Marine Environmental Research* **45**: 403-415.
- Pinto, R., Patricio, J., Baeta, A., Fath, B. D., Neto, J. M. and Marques, J. C. 2009. Review and evaluation of estuarine biotic indices to assess benthic condition. *Ecological Indicators* **9**: 1-25.
- Quinn, N. 1999. *Nhlabane Estuarine Freshwater Requirements: An initial assessment*. Report Prepared for IWR Environmental.
- Quinn, N. 2000. *Mhlatuze System Ecological Reserve Study: Report on Estuary Freshwater Requirements Nhlabane Estuary Supporting Report No 4*. DWAF Report W120-00-1599.

- Rainbow, P. S. 1985. The Biology of Heavy Metals in the Sea. Intern. *Journal of Environmental Studies* **25**: 195-211.
- Rainbow, P.S. 1989. Copper, cadmium and zinc concentrations in oceanic amphipod and euphausiid crustaceans, as a source of heavy metals to pelagic seabirds. *Marine Biology* **103**: 513-518.
- Rainbow, P. S. 1993. The significance of trace metal concentrations in marine invertebrates. In: Dallinger, R., Rainbow, P.S. (Eds.), *Ecotoxicology of Metals in Invertebrates*. Lewis Publishers, Chelsea, USA, 3–23.
- Rainbow, P. S. 1995. Biomonitoring of Heavy Metal Availability in the Marine Environment. *Marine Pollution Bulletin* **31**: 183-192.
- Rainbow, P. S. 1997. Trace metal accumulation in marine invertebrates: marine biology or marine chemistry? *Journal of the Marine Biological Association UK* **77**: 195–210.
- Rainbow, P. S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what. *Environmental Pollution* **120**: 497-507.
- Rainbow, P. S. 2007. Trace metal bioaccumulation: Models, metabolic availability and toxicity. *Environment International* **33**: 576–582.
- Rainbow, P. S., Amiard, J. C., Smith, B. D. and Langston, W. J. 2000. Observations on the interaction of zinc and cadmium uptake rates in crustaceans (amphipods and crabs) from coastal sites in UK and France differentially enriched with trace metals. *Aquatic Toxicology* **50**: 189 - 204.
- Rainbow, P. S., Fialkowski, W. and Smith, B. D. 1998. The sandhopper *Talitrus saltator* as a trace metal biomonitor in the Gulf of Gdansk, Poland. *Marine Pollution Bulletin* **36**: 193–200.
- Rainbow, P. S. and Moore, P. G. 1986. Comparative metal analyses in amphipod crustaceans. *Hydrobiologia* **141**: 273–289.
- Rainbow, P. S. and Moore, P. G. 1990. Seasonal variation in copper and zinc concentration in three talitrid amphipods (Crustacea). *Hydrobiologia* **196**: 65-72.
- Rainbow, P. S. and Phillips, D. J. H. 1993. Cosmopolitan biomonitors of trace metals. *Marine Pollution Bulletin* **26**: 593-601.
- Rainbow, P. S. and White, S. L. 1989. Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in a decapod, an amphipod and a barnacle. *Hydrobiologia* **174**: 245–262.
- Ramm, A. E. L. 1990. Application of the community degradation index to South African estuaries. *Water Research* **24**: 383–389.
- Rauret, G., Lopez-Sanchez, J., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A. and Quevauviller, P. 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *Journal of Environmental Monitoring* **1**: 57-61.

- Re, A., Rodrigues, A. M. and Quintino, V. 2007. Acute toxicity testing with the European estuarine amphipod *Corophium multisetosum*. *Hydrobiologia* **587**: 89-99.
- Re, A., Freitas, R., Sampaio, L., Rodrigues, A. M. and Quintino, V. 2009. Estuarine sediment acute toxicity testing with the European amphipod *Corophium multisetosum* Stock, 1952. *Chemosphere* **76**: 1323–1333.
- Reddering, J. S. V. and Rust, I. C. 1990. Historical changes and sedimentary characteristics of southern African estuaries. *South African Journal of Science* **86**: 425-428.
- Ridden, T. 1999. The botanical importance of the Nhlabane Estuary. Unpublished MSc Thesis, University of Port Elizabeth, Port Elizabeth
- Riedel, G., Sanders, J. G. and Osman, R. W. 1989. The role of three species of benthic invertebrates in the transport of arsenic from contaminated estuarine sediment. *Journal of Experimental Marine Biology and Ecology* **134**: 143-155.
- Ritterhoff, J. and Zauke, G. 1997. Influence of body length, life-history status and sex on trace metal concentrations in selected zooplankton collectives from the Greenland Sea. *Marine Pollution Bulletin* **34**: 614-621
- Rosenberg, D. M. and Resh, V. H. 1993. *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman and Hall, New York, United States of America.
- Schlacher, A. and Wooldridge, H. 1996. Patterns of selective predation by juvenile, benthivorous fish on estuarine macrofauna. *Marine Biology* **125**: 241-247.
- Scarlett, A., Rowland, S.J., Canty, M., Smith, E.L. and Galloway, T.S. 2007. Method for assessing the chronic toxicity of marine and estuarine sediment-associated contaminants using the amphipod *Corophium volutator*. *Marine Environmental Research* **63**: 457–470.
- Shyamasundari, K. 1976. Effects of salinity and temperature on the development of eggs in the tube building amphipod *Corophium triaenonyx*. *Biological Bulletin* **150**: 286-293.
- Simpson, S.L., Batley, G.E., 2007. Predicting metal toxicity in sediments: a critique of current approaches. *Integrated Environmental Assessment and Management* **3**: 18-31.
- Snelgrove, P. V. R. 1999. Getting to the bottom of marine biodiversity: sedimentary habitats. *Bioscience* **49**: 129-138.
- Stronkhorst, J., Schipper, C. A., Brils, J., Dubbeldam, M., Postma, J. F. and van de Hoeven, N. 2003. Using marine bioassays to classify the toxicity of Dutch harbour sediments. *Environmental Toxicology and Chemistry* **22**: 1525–1547.
- Sudan, P., Whitmork, G., Uken, R. and Woodhourn, S. 2004 Quaternary evolution of the coastal dunes between Lake Hlabane and Cape St Lucia, KwaZulu-Natal. *South African Journal of Geology* **107**: 355-376.
- Summers, J. K., Wade, T. L., Engle, V. D. and Maleb, Z. A. 1996. Normalization of metal concentrations in estuarine sediments from the Gulf of Mexico. *Estuaries* **19**: 581-594.

- Steffen, Robertson and Kirsten. 1989. *EIA for the raising of the Nhlabane Weir*. Unpublished Report for RBM.
- Stoner, A. W. and Acevedo, C. 1990. The macroinfaunal community of a tropical estuarine lagoon. *Estuaries* **13**: 174-181.
- Sutherland, R. A. and Tack, F. M. 2002. Determination of Al, Cu, Fe, Mn, Pb and Zn in certified reference materials using the optimized BCR sequential extraction procedure. *Analytica Chimica Acta* **454**: 249-257.
- Sutherland, R. A. and Tack, F. M. 2003. Fractionation of Cu, Pb and Zn in certified reference soils SRM 2710 and SRM 2711 using the optimized BCR sequential extraction procedure. *Advances in Environmental Research* **8**: 37-50.
- Swartz, R. C., De Ben, W. A., Sercu, K. A. and Lamberson, J. O. 1982. Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, USA. *Marine Pollution Bulletin* **13**: 359–364.
- Swartz, R. C., Ditsworth, G. R., Schults, D. W. and Lamberson, J. O. 1985. Sediment toxicity to a marine infaunal amphipod: cadmium and its interaction with sewage sludge. *Marine Environmental Research* **18**: 133–153.
- Taljaard, S., Van Niekerk, L., Huizinga, P. and Joubert, W. 2003. *Resource monitoring procedures for estuaries for application in the ecological Reserve determination and implementation process*. WRC Report No. 1308/1/03.
- Ter Braak, C. F. J. and Šmilauer, P. 1998. *CANOCO Reference Manual and User's Guide to CANOCO for Windows: Software for Canonical Community Ordination* (Version 4). Microcomputer Power (Ithaca, NY, USA), 352pp.
- Teske, P. R. and Wooldridge, T. H. 2001. A comparison of the macrobenthic faunas of permanently open and temporarily open/closed South African estuaries. *Hydrobiologia* **464**: 227–243.
- Teske, P. R. and Wooldridge, T. H. 2003. What limits the distribution of subtidal macrobenthos in permanently open and temporarily open/closed South African estuaries? Salinity vs. sediment particle size. *Estuarine, Coastal and Shelf Science* **57**: 225-238.
- Teske, P. R. and Wooldridge, T. H. 2004. Affinities of some common macroinvertebrates to salinity and sediment type: empirical data from Eastern Cape estuaries, South Africa. *African Zoology* **39**:183–192.
- Tessier, A., Campbell, P. G. C. and Bisson, M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* **51**: 844-851.
- Turpie, J. K., Adams, J. B., Joubert, A., Harrison, T. D., Colloty, B. M., Maree, R. C., Whitfield, A. K., Wooldridge, T. H., Lamberth, S. J., Taljaard, S. and Van Niekerk, L. 2002. Assessment of the conservation priority status of South African estuaries for use in management and water allocation. *Water SA* **28**: 191 - 206.

- Turpie, K. 2004. South African National Spatial Biodiversity Assessment 2004 Technical Report Volume 3: Estuary Component. Pretoria: *South African National Biodiversity Institute* **3**: 1-31.
- Ueda, N., Tsutsumi, H., Tokusaki, K., Takeuchi, R. and Kido, K. 1992. Sediment condition and macrobenthic community in the nearshore area of Dokai Bay. *Benthos Research* **42**: 55-62.
- Usero, J., Gamero, M., Morillo, J. and Gracia, I. 1998. Comparative study of three sequential extraction procedures for metals in marine sediments. *Environment International* **24**: 487-496.
- Van den Heuvel-Greve, M., Postma, J., Jol, J., Kooman, H., Dubbeldam, M., Schipper, C. and Kater, B. 2007. A chronic bioassay with the estuarine amphipod *Corophium volutator*: test method description and confounding factors. *Chemosphere* **66**: 1301–1309.
- Vermeulen, L. and Wepener, V. 1999. Spatial and Temporal Variations of Metals in Richards Bay Harbour, South Africa. *Marine Pollution Bulletin* **39**: 304-307.
- Vernberg, W. B. 1983. Responses to estuarine stress. In: *Estuaries And Enclosed Seas. Ecosystems of the World* 26. Ed BH Ketchum. Elsevier Scientific Pub. Co. New York.
- Vivier, L., Cyrus, D. P., Jerling, H. L. and Cilliers, G. J. 1998. *Effect of the dredger crossing on the Nhlabane Estuary*. CRUZ Investigational Report No. 57.
- Vivier, L. and Cyrus, D. P. 1999a. The zoobenthic fauna of the Nhlabane coastal lake system, KwaZulu-Natal, South Africa, 20 years after construction of a barrage. *Water SA* **25**: 533-542.
- Vivier, L. and Cyrus, D. P. 1999b. Dune Mining and the Nhlabane Estuary, South Africa: the Effect of a Dredger Crossing on the Zoobenthic Community. *Marine Pollution Bulletin* **39**: 308-314.
- Walmsley Environmental Consultants. 1993. *Environmental Impact Assessment for raising the weir at Lake Nhlabane*. Unpublished Report for RBM WO24.
- Wang, F., Goulet, R. R. and Chapman, P. M. 2004. Testing sediment biological effects with the freshwater amphipod *Hyaella azteca*: the gap between laboratory and nature. *Chemosphere* **57**: 1713-1724.
- Water Research Centre 1990. Proposed Water Quality Criteria for the Protection of Aquatic Life from Intermittent Pollution, Report PRS 2498-NM, Water Research Centre, Medmenham, UK.
- Watling, R. J. 1988. Metal studies in the coastal environment of the Eastern Cape: a review. In: *'Towards an environmental plan for the Eastern Cape'*. Grocott and Sherry: Grahamstown, South Africa. pp 367-377.
- Weeks, J. M. and Rainbow, P. S. 1993. The relative importance of food and seawater as sources of copper and zinc to talitrid amphipods (Crustacea; Amphipoda; Talitridae). *Journal of Applied Ecology* **30**: 722–735.

- Weerts, S. P. 2002. Habitat utilisation by juvenile fishes in Mhlathuze Estuary and Richards Bay Harbour. Unpublished MSc thesis, University of Zululand, KwaDlangezwa.
- Weerts, S. P., Cilliers, G. and Cyrus, D. P. 2003. Estuarine macrocrustacea of Richards Bay Harbour, South Africa, with particular reference to the penaeid prawns. *African Zoology* **38**: 285-296.
- Wepener, V., Fleischer, L. and Vivier, L. 1995. *Biomonitoring: a literature survey*. Unpublished CRUZ Report for Richards Bay Minerals.
- Wepener, V. and Vermeulen, L.A. 2005. A note on the concentrations and bioavailability of selected metals in sediments of Richards Bay Harbour, South Africa. *Water SA* **31**: 589-596.
- Wepener, V., Cyrus, D. P., Vermeulen, L. A., Obrien, G. C. and Wade, P. 2006. *Development of a water quality index for estuarine water quality management in South Africa*. WRC Report : 1163/1/06: 1-118.
- White, S. L. and Rainbow, P. S. 1985. On the metabolic requirements for copper and zinc in molluscs and crustaceans. *Marine Environmental Research* **16**: 215–229.
- Whitfield, A. K. 1989. The benthic invertebrate community of a Southern Cape estuary: structure and possible food sources. *Transactions of the Royal Society of South Africa* **47**: 159-179.
- Whitfield, A. K. 1992. A characterization of southern African estuarine systems. *African Journal of Aquatic Sciences* **10**: 89-103.
- Whitfield, A.K. 2000. *Available Scientific Information on Individual South African Estuarine Systems*. Water Research Commission Report No. 577/3/00. Pretoria.
- Whitfield, A. K. 2005. Fishes and freshwater in southern African estuaries – A review. *Aquatic Living Resources* **18**: 275-289.
- Whitfield, A. K. and Bruton, M. N. 1989. Some implications of reduced fresh water inflow into eastern Cape estuaries, a preliminary assessment. *South African Journal of Science* **85**: 691-694.
- Whitfield, A. and Bate, G. 2007. *A Review of Information on Temporarily Open/Closed Estuaries in the Warm and Cool Temperate Biogeographic Regions of South Africa, with Particular Emphasis on the Influence of River Flow on These Systems*. Interim report to the Water Research Commission on the Project: The freshwater requirements of intermittently open Cape estuaries. WRC Report No 1581/1/07: 1-232.
- Wolff, W. J. 1983. Estuarine benthos. In: *Estuaries And Enclosed Seas. Ecosystems of the World* 26. BH Ketchum (Eds). Elsevier Scientific Pub. Co. New York
- Xulu, C. I. 2003. Effects of body size and sex on accumulation of copper in amphipod, *Corophium triaenynox*. Unpublished BSc Honours Thesis, Zoology Department, University of Zululand, South Africa.
- Water Research Centre. 1990. *Design guide for marine treatment schemes. Volume II. Environmental design and data collection*. Report No UM 1009. Water Research Centre, Swindon, UK

- Ysebaert, T., Herman, P. M., Meire, P., Craeymeersch, J., Verbeek, H. and Heip, C. H. 2003. Large-scale spatial patterns in estuaries: estuarine macrobenthic communities in the Schelde estuary. *Estuarine, Coastal and Shelf Science* **57**: 335-355.
- Zauke, G., Clason, B., Savinov, V. and Savinova, T. 2003. Heavy metals of inshore benthic invertebrates from the Barents Sea. *Science of the Total Environment* **306**: 99 - 110.