RELATIONSHIP BETWEEN SEDIMENT STRUCTURE AND INFAUNAL AMPHIPOD COMMUNITIES ALONG THE DURBAN OUTFALLS REGION ON THE EAST COAST OF SOUTH AFRICA

BY

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Abstract

Increased human habitation brings associated pressures with it, such as the introduction of contaminants to coastal waters. The major sources of these occur along the KwaZulu-Natal coast via Sappi Saiccor discharge points, Tioxide, AECI, the Mlaas canal, Central Works Outfall and Southern Works Outfall. This study investigated the effects of sediment structure on benthic amphipod communities exposed to sewage and industrial waste from the Central Works and Southern Works Outfalls along the Durban coastline, and used a 4-year dataset of sediment grain size analysis, metal concentrations, Total Kjeldahl Nitrogen (TKN) and Chemical Oxygen Demand (COD) at impacted and reference sites. Results exhibited that the levels of effluent being discharged onto the Durban coast from the Southern Works and Central Works Outfalls do not accumulate in the fine grained sediments in sites where it would be expected. The Mdloti reference site which was dominated by coarse sediment showed the highest concentrations of metals. In addition, the outfalls do not have significant effects on the amphipod communities in the vicinity. Community structure between sites with similar grain sizes tends to be very similar thereby highlighting the possible influence of grain sizes on determining community patterns. Overall, there seemed to be no effect of pollutants on the biology or accumulation in the receiving environment.

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1. Introduction

1.1. General overview of pollution

Coasts around the world have long been of economic importance, contributing to countries' economies via agricultural and industrial production, fisheries and residential developments, amongst others (Taljaard *et al.*, 2006). Due to the increasing number of people that live by and rely on the coast in Africa, and in particular South Africa, pressure on coastal resources in this region is increasing significantly (Calamari *et al.,* 1987; Meyer-Reil & Koster, 2000; O'Donoghue & Marshall, 2003). Increased human habitation brings associated pressures with it, such as the introduction of contaminants to coastal waters. With these pressures arises the concept of ecosystem health. Ecosystem health has been defined as a system free from "distress syndrome", is stable, sustainable and resilient to stress. It is active and maintains its organisation and autonomy over time. Distress syndrome is the irreversible breakdown of the system leading to system collapse (Constanza, 1992; Rapport *et al.,* 1998).

Contaminants enter coastal waters via sewage and industrial effluent discharges, storm water run-off, contaminated ground water seepage, mining and agricultural return flows, dredging and deep-sea outfalls (Zauke *et al.,* 1999; O'Donoghue & Marshall, 2003; Taljaard *et al.*, 2006). These pollutants are introduced into the marine environment from either point or non-point sources. Point sources include discharge points of industrial and municipal effluent and urban runoff, while non-point sources are those from urban and agricultural runoff, groundwater and atmospheric inputs (Meyer-Reil & Koster, 2000). The major sources of effluent discharges occur along the KwaZulu-Natal coast via Sappi Saiccor discharge points, Tioxide, AECI, the Mlaas canal and the Durban outfalls. The focus of this study is on the two Durban outfalls, *viz*. the Southern Works Outfall and Central Works Outfall. The Central Works Outfall deals with sanitary waste while Southern Works deals with both sanitary and industrial wastes. The reason for investigating these pipelines is to look at influence of these on the benthic community structure in those areas in comparison with reference sites. This study provides insight into the possible effects of these pipelines on the physico-chemical conditions and the sedimentary invertebrate community structure by looking at data representing a four year snapshot of the benthos.

Chemical contaminants may have negative effects on ecosystems and can change the well-being of organisms and the consequent biogeochemical properties of the biota (Gadzala-Kopciuch *et al.*, 2004). The pollutants may be inorganic chemicals as well as natural (i.e. organic material of biological origin) and synthetic organic compounds. Such pollutants can attach to particulates in the water and sink to the bottom of the ocean (Reish, 1993). Although dilution of pollutants occurs, some settlement of particulates takes place, resulting in their accumulation in the sediment (Fleeger *et al.,* 2003). Many contaminants have low water solubility and are particle reactive through their ability to sequester high levels of certain metals. Contaminants tend to be scavenged to bottom sediments via flocculation, coagulation and sedimentation. The concentrations of contaminants in sediment have been found to be significantly higher than that in the water column (Huh *et al.,* 1992; Mwanuzi & De Smedt 1999; Hatje *et al.,* 2003; Newman & Watling, 2007). Sediments act as final acceptors of pollutants and therefore are used in marine contaminant studies worldwide (Sprovieri *et al.,* 2007). Chemical analyses of sediments help determine the extent and the nature of contamination in an area (Chapman *et al.,* 1987). Sediment grain size analyses, together with measurements of metal concentrations, total Kjeldahl nitrogen and chemical oxygen demand provide a comprehensive understanding of how the system responds to to contamination. These can then be applied in order to determine the possible effects of the anthropogenic sources on the biological system.

1.2. Sediments and contaminants

Sediments are comprised of diverse components of a variety of sources, which are classified into the following categories: detrital material primarily from terrigenous origin, products of volcanism, skeletal remains and organic matter from dead organisms, inorganic precipitates from seawater, and products from chemical transformations taking place in the sea (Sverdrup *et al.,* 1962). Sediment is transported to the sea, settling through the water while being carried by ocean currents (Sverdrup *et al.,* 1962). The Agulhas Current, one of the most important currents along the east coast of South Africa, together with wind action and fluvial fluxes play a role in sediment transport to and along the coast (McClurg, 1988; Schumann, 1988; de Ruijter *et al.,* 1999). Many rivers flow into the sea off KwaZulu-Natal thereby affecting the shelf benthos. In addition, high rainfall as well as urbanisation and increased agricultural activities result in erosion and large amounts of sediment, particularly silt and clay, being discharged to the sea (Mclurg, 1988). This sediment determines the distribution and survival of benthic fauna and resulting ecosystem processes along the KwaZulu-Natal coast (Schumann, 1988).

Sediment characteristics, such as the organic carbon content, grain size composition, mineral constituents (e.g. oxides of iron or manganese), and acid volatile sulfides (Di Toro *et al.*, 1990) provide supporting information regarding the distribution and composition of pollutants in a particular area (de Mora *et al.*, 2004). These characteristics are important factors to consider when studying macrobenthic infauna due to the fact that these organisms are exposed to sediment-bound contaminants through their life spans. This study focuses primarily on sediment particle size composition and other measurements of selected physico-chemical properties of the anthropogenic inputs, such as the concentrations of metals, Total Kjeldahl Nitrogen (TKN) and Chemical Oxygen Demand (COD) on the benthic communities.

1.2.1. Sediment grain size

The particle size grade composition of sediment is determined by sieving and determining the settling velocity of the smaller particles. Consequently, sediment is classified into gravels, sands, silts, mud and clays according to size grades. Although fine-grained material may be formed in the sea, most of it is of terrigenous origin, having previously undergone mechanical and chemical weathering *en route* to the coast (Sverdrup *et al.,* 1962). Fine material may be introduced via rivers, resuspension by wave action in shallow water, and from the remains of planktonic animals (Sverdrup *et al.,* 1962). The small size of these particles causes them to be transported far from their point of entry to the nearshore. Coarse material is mainly transported to the coast from land, and being larger, settles quickly to the seafloor in the vicinity of point of entry.

Sediment grain size is important in influencing the concentration of metals in sediment (Lin *et al.,* 2002; Zhang *et al.,* 2002). According to de Mora *et al*. (2004), fine silt and clay retain more contaminants than coarse sandy sediment. There tends to be a preferential adsorption of compounds onto suspended fine-grained particles, and these particulates are ultimately introduced into the benthic sediment through sedimentation (Lowrey, 1993). Fine grained sediments have a greater surface area to volume ratio and a higher surface electric charge than coarse sediments, thereby making them more chemically reactive (Plumb 1981; Power & Chapman, 1995; Newman & Watling, 2007). Sites with fine grained sediments therefore exhibit higher metal contamination compared to coarse grained sediments in areas where anthropogenic inputs of metals are important.

The introduction of man-made structures like pipelines may alter soft-bottom habitats in a number of ways. They affect wave fields and change current patterns resulting in changes of sand ripple patterns and sediment grain size. In addition they trap organic material which can cause organic build-up in the sediment, and shells from fouling organisms modify sediments (Davis *et al.,* 1982). Braga *et al.* (2000) have shown that water around the diffusers was high in organic nutrients and they concluded that the high organic content in sediments was due to the sewage discharge. This however, would not apply to pipelines disposing of industrial pollutants.

A study by Abessa *et al.* (2005) showed evidence for the potential for sewage to influence sediment grain size distribution and increase organic content. Sediments close to the outfall diffusers were finer and organically enriched (Abessa *et al.,* 2005). In order to predict the effects of these man-made structures, Davis *et al.* (1982) concluded that the impacts are dependant on the size and complexity of the structure, the time passed since it was constructed and the behaviour of mobile predators to the structure in terms of foraging. In addition, the flora and fauna attached to the structure and the susceptibility and resilience of bottom dwelling communities to physical and biological changes can contribute to the impacts caused.

1.2.2. Metals

Metals that tend to pose an ecological or biological risk include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) and iron (Fe) (Duruibe *et al.*, 2007). These are found naturally in sediment, however their levels can be increased through their introduction via anthropogenic sources; they are considered to be persistent environmental contaminants as they cannot be degraded or destroyed (Duruibe *et al.*, 2007; Newman & Watling, 2007; Marín-Guirao *et al.,* 2008). Anthropogenically introduced metals can enter the sea via many sources, such as through ground water, domestic and industrial developments, urban and riverine runoff, sewage treatment plants, mining activities and effluent disposal (Stoffers *et al.,* 1986; Wong *et al.,* 2000; Duruibe *et al.*, 2007).

Metal accumulation in sediments is affected by sediment characteristics such as grain size and organic content (Rainbow, 1995a). Organic enrichment is often found in sewage impacted areas. It has been found that most sewage sludge has toxic metals that are bound to small particles which are often organic matter. This organic matter has been found to be involved in the complexing and incorporation of metals within sediment. Significant correlations have been found between the metals zinc, copper, lead and cadmium and organic matter (Cheggour *et al.,* 2000). The release of metals from the sediment into the water column can result through disturbances such as bioturbation, storms and dredging (Newman & Watling, 2007). Some metals, such as lead, do not have a physiological role (Marín-Guirao, *et al.*, 2008), while other metals such as copper and zinc are essential and needed for normal metabolic functions. However, if intracellular metal concentrations exceed that required by the organism they can exert their toxic effect (Correia *et al.,* 2002; Marín-Guirao, *et al.*, 2008). Those metals that do not have physiological roles act on organisms in a number of ways. Some may cause colour or shape changes of an organism, histological changes in tissue material, decreased development of larvae, abnormal larvae structures, inhibition of growth, prevent the settlement of sessile organisms, delay or prevent sexual maturity or spawning and behavioural changes (Bryan, 1971). Table 1 lists the sources of essential and non-essential metals encountered in this study, and their biological roles. Non-essential substances are defined as those substances that may not always be present in biologically significant amounts in the local environment of a species-population while essential substances are those substances that co-occur in the environment with biota in biologically significant amounts (Barata *et al*., 1998).

Table 1: Essential and non-essential metals considered in this study, their natural and anthropogenic sources and biological roles.

METAL	NATURAL AND ANTHROPOGENIC SOURCE	BIOLOGICAL ROLE/EFFECT	REFERENCE
ESSENTIAL METALS			
Iron	Sediment; atmospheric aerosols; rain; mining.	Component of haemoglobin and respiratory cytochromes; biochemical effects of photosynthesis: reduced pigment content, decrease in light absorption, decrease in storage carbohydrates, increased photoinhibition susceptibility; participates in two geochemical reactions that influence primary production in marine systems; decreased iron can limit phytoplankton productivity; needed by some enzymes for energy production and protein metabolism; the cytochrome system requires iron to produce energy.	Soria-Dengg & Horstmann (1995); Zhuang *et al.* (1995); Schiff & Weisberg (1999); Chambers *et al.* (2001); Haas & Levin (2006); Pankowski & McMinn (2009).
Copper	Anti-fouling coatings on hulls of ships; treated wood; natural erosion; mining; smelting; municipal and industrial wastewater effluents; pesticides.	Required for the respiratory pigment haemocyanin in invertebrates; oxygen carrier in blood of molluscs; micronutrient for aquatic organisms; found in many enzymes; copper enzymes play a role in oxygen-free radical metabolism thereby having an anti-inflammatory effect.	Weis *et al.* (1992); Hall & Anderson (1999); Thomas *et al.* (2000); Lamontagne & Foerster (2002); Haas & Levin (2006).
Chromium	Chromated Copper Arsenate (CCA treated wood.	Taken up by phytoplankton; in its +6 oxidation state is carcinogenic and mutagenic; production of insulin; plays a role in lipid metabolism.	Nabrzyski (1991); Weis et al. (1992).

Table 1 continued: Essential and non-essential metals considered in this study, their natural and anthropogenic sources and biological roles.

Zinc	Sewage treatment works; mining.	Essential part of the enzyme carbonic anhydrase; needed for lactate and malate dehydrogenases which are used in energy production; synthesis of nucleic acids; aids in chemical detoxification thereby increasing the ability to withstand environmental chemicals and toxins; required for essential catalytic functions in enzymes.	Coleman (1992); Stevenson & Betty (1999); Johansen *et al.* (2000); Haas & Levin (2006).
Nickel	Sewage treatment works; electroplating; pigment in oils and paints; wind blown soil and dust; coal and oil combustion.	Serves as a chemical defense in plants against to protect against being eaten	Martens & Boyd (1994); Stevenson & Betty (1999).
Cadmium	By-product of zinc mining; electroplating; nickel-cadmium batteries.	Essential nutrient for marine phytoplankton; can denature enzymes making them inactive; kidney damage	Morel & Malcolm (2005).
NON-ESSENTIAL METALS			
Mercury	Combustion of fossil fuels; weathering of mercury-containing rocks; emissions from deep-sea hydrothermal vents.	Decreases enzyme activity; accumulates in kidneys causing kidney failure.	Mackey *et al.* (1996); Goyer (1997); Kennish (2002).
Lead	Lead-acid batteries; manufacture of solder and alloys; atmospheric pollution.	Toxic to the nervous system; causes behavioural changes in organisms; alters the metabolism of brain neurotransmitters.	Bryan (1971); Caurant *et al.* (2006); Shukla & Singhal (1984).

There are two major pathways, discussed by Luoma (1989), in which metals are taken up by deposit feeding and detritus feeding marine organisms. The first is the ingestion of sediment or suspended particles that are enriched during feeding; the second is uptake from solution. Benthic fauna living in the sediment can therefore be exposed to anthropogenic metal inputs and uptake of these pollutants may occur.

Metal toxicity to organisms occurs by the reaction of free metal ions with physiologically active binding sites. This process is represented as the development of a metal-biotic ligand complex (Di Toro *et al.,* 2001). A ligand is a specific receptor in an organism where the complexation of metals leads to acute toxicity (Arnold *et al.,* 2005). At the organisms-water interface, free metal ions react with binding sites. The metals form ligands with organic compounds, resulting in altered protein structure (Rao *et al*., 2006). It is this reaction that is identified as the metalbiotic ligand complex (De Schamphelaere & Janssen, 2002). The behaviour of metal ions in seawater is related to many different types of binding sites or ligands, which possess differing complexation strengths and concentrations (Town & Filella, 2000).

Free metal ion concentrations can be changed without altering the total dissolved metal concentrations by changing the availability of these metal-binding ligands (Rainbow, 1995b). Ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) are synthetic chelating agents and may be present in coastal systems. The presence of these chelators tends to reduce metal accumulation (Ray, 1984). Zinc uptake by organisms can be reduced by adding the chelating agent EDTA to the bioavailable form in solution. This decreases the absolute equilibrium concentration of free zinc ions (Rainbow, 1995b). It must be noted that metal toxicity in aquatic environments can also be influenced by factors such as pH and dissolved organic carbon (DOC) (Barata *et al.*, 1998).

Sewage tends to have a typical composition, usually comprised of high solid and nutrient contents and low concentrations of metals, hydrocarbons and pesticides. However, this is not always observed. High metal concentrations have been found in effluent discharges from three different outfalls in Sydney (Abessa *et al.,* 2005). High lead and zinc concentrations in Turkey were found to be confined to those sites that receive effluent. This enrichment of metals has been attributed to steel and petroleum manufacturing and domestic sewage (Ergin *et al.,* 1998). A study by Gonzalez *et al.* (1999) of a submarine sewage outfall showed that the hydrodynamics of the area played a role in concentrating pollutants in the some regions due to surface and bottom currents; however the highest levels of metals were still found at the discharge points. The main pollutants at the sampling sites were copper, lead and zinc of which the same dominated the wastewater discharge.

1.2.3. Total Kjeldahl Nitrogen (TKN) and Chemical Oxygen Demand (COD)

Globally, the input of nitrogen into marine ecosystems has increased by a factor of 20 since 1860 to the current production of nitrogen being ~150 Tg N yr^{-1} (Rabalais, 2002). Urban and rural wastewater, fertilisers, animal waste, atmospheric deposition of fossil fuel, other combustion products, agricultural emissions and nitrogen enriched groundwater all contribute to the availability of nitrogen to the marine environment (Paerl, 1997; Cornell *et al.,* 2003). Rivers play an important role in nutrient delivery to the ocean. Nitrogen enrichment occurs extensively in estuaries or in the nearshore coastal ocean and it has been found in parts of the world that anthropogenic nitrogen inputs via rivers exceed any other source of nitrogen input. Nitrogen is the primary limiting nutrient in marine waters (Rabalais, 2002). Aquatic systems are influenced by inorganic dissolved forms (nitrate, nitrite, ammonium), various dissolved organic compounds (amino acids, urea, composite dissolved organic nitrogen) that are collectively called DON, and particulate nitrogen (PON) (detritus, phytoplankton) (Frankovich & Jones, 1998; Rabalais, 2002). Total nitrogen in a system is the sum of the inorganic and fixed organic nitrogen. One of the methods used to determine total nitrogen is by the use of acid Kjeldahl digestion of organic nitrogenous compounds. The result of this method is a value that includes organic N and NH_4^+-N (not NO_2^- -N and NO_3^--N) (D'Elia *et al.,* 1977).

As aerobic bacteria decompose the organic matter that settles on the sea floor, oxygen levels in the sediment decrease until eventually anoxia develops. This results in biological and geochemical changes in the benthic environment (Pearson & Rosenburg, 1978; Diego-McGlone *et al.,* 2000; Rabalais, 2005). Organic carbon and nitrogen, microbial biomass, microbial decomposition potential for substrata and oxygen consumption in communities increase with increasing eutrophication (Rabalais, 2002). Eutrophication can result in the increase of inorganic nutrients into the system, a decrease in the turbidity of water or a decrease in grazing pressure. The most common factor increasing the supply of organic matter to ecosystems is nutrient enrichment (Nixon, 1995). Oxygen depletion results from eutrophication, and can affect detrital matter decomposition, bioturbation of sediments, nutrient regeneration and the exchange of material across the water-sediment interface (Heip, 1995; Kennish, 2002).

Chemical oxygen demand is the measure of the amount of organic and inorganic matter that can be broken down by chemical oxidation. It results from the oxidation of reduced compounds through anaerobic metabolism and provides information on anaerobic activity (Kristensen, 1985; Lee *et al.,* 1999). COD is usually higher than Biological Oxygen Demand (BOD) due to the fact that chemical oxidation breaks down biological materials as well as solvents, hydrocarbons and pesticides, while BOD breaks down decaying animal and plant matter (Newman *et al*., 2007). BOD is defined as a measure of the amount of biodegradable organic compounds in terms of the oxygen that is needed for their decomposition. COD and BOD are generally specific to a body of water due to the amount and nature of pollutants differing between sources (Lee *et al.,* 1999). Hydrogen sulphide (H_2S) is considered to be a key contributor to chemical oxygen demand in marine sediments (Boucher *et al.,* 1994; Findlay & Watling, 1997). COD concentrations decrease in the vicinity of outfalls while further from the pipeline these concentrations are higher (Cheggour *et al.,* 1999). High COD levels are associated with maximum concentrations of organic matter, which can be detrimental to marine organisms. However, Shanmugam *et al.* (2007), found contradictory patterns with high concentrations of COD, particularly in areas where there was sewage deposition resulting in a high organic content.

1.3. Ecological and biological consequences of pollution on amphipods

Pollution impacts on benthic organisms are highly probable due to the accumulation of pollutants in the sediment which these organisms inhabit. The focus here is on the consequences of pollution on amphipods due to amphipods being possible indicators of pollution in the areas studied as well as the availability of long-term datasets of amphipod communities at the selected test sites.

1.3.1 Sediment grain size

Sediment particle size composition affects marine invertebrates in various ways. Larval settlement is affected by particle grain size (Morgans, 1956), and it plays a role in the feeding behaviour and burrowing of amphipods. Some amphipods require clay and silt particles as they are only able to feed on bacteria absorbed to particles between 3 and 4 µm in diameter (Fenchel *et al.,* 1975). Sanders (1958) found that different amphipods tend to inhabit different types of sediment, with some flourishing in sediments of fine particle size and others in coarser particle sizes. Sandy sediments have been found to be abundant in some amphipods such as ampeliscids. Ampeliscids are an important constituent of the marine benthos along the KwaZulu-Natal (McClurg, 2004). This dominance by *Ampelisca* spp. was also observed in other studies (Miyadi, 1940; Stickney & Stringer, 1957; Le Bris & Glemarec, 1996). However, contradicting studies have shown *Ampelisca* spp. associated with mud or muddy-sand (Stickney & Stringer, 1957). In contrast to these finding, amphipods have also been found to inhabit coarse sediment (Bergen *et al.,* 2001).

This preference of certain sediment grain sizes by different groups of amphipods has been linked largely to their feeding strategies. Deposit-feeders that survive on organic matter dominate finer grained sediments while suspension feeders, which also rely on organic matter as a food source, tend to occupy coarser grained sediments (Sanders, 1958). Sediment grain size preferences also play a role in amphipod burrowing and their choice of habitats. The presence of certain grain sizes would determine the species of amphipods that inhabit that area. Grain size affects the burrowing capabilities of amphipod groups, as mechanically it is difficult to burrow in certain size grades (Oakden, 1984). The same study by Oakden (1984) also showed this preference in Phoxocephalid amphipods. These amphipods have been found to react to changes in grain size. They will either not burrow in unacceptable grain sizes, exhibit avoidance behaviour if the grain size is not ideal, or they will burrow through the unsuitable sediment to find more desirable sediments.

1.3.2. Metals

Metal accumulation affects the reproduction and development of marine crustaceans. Increased metal concentrations may have the following effects on marine organisms: histological or morphological changes in tissues; physiological changes such as the suppression of growth and development or impaired swimming abilities); biochemical changes such as enzyme activity; behavioural changes; and reproductive changes (Shanmugam *et al.,* 2007).

Amphipods store excess metals from their diets in detoxified granules in the ventral caeca. As metal exposure increases, these granules increase in number. As the cells pass through a cell cycle they are passed long the caecum and released in the gut as mature cells are expelled from the caecum epithelium. Increased metal bioavailabilities and increased numbers of granules per cell increases the accumulated metal concentration in the ventral caeca and therefore in the body of the amphipod (Fialkowski, 2003).

The variability in regulatory abilities of different amphipods to copper and zinc is evident. Some species are able to regulate copper at all concentrations but not zinc; some have the ability to regulate zinc and not copper; other amphipods cannot regulate copper or zinc while another group has the ability to regulate both copper and zinc. At low metal concentrations and long term exposure, metal uptake may be gradual. This could result in the deposition of metal in non-critical tissues such as the exoskeleton, thereby increasing the overall body metal concentrations (Rainbow & Moore, 1986; Borgmann *et al.,* 1993). Copper has been found to be responsible for the elimination of sensitive or intolerant species of macrofauna from the community (Cheggour *et al.*, 1999). Amphipods exhibit a significant correlation between the uptake of zinc and cadmium of individuals which could be related to the shared uptake routes of these two metals (Rainbow *et al.,* 2000).

Metal accumulation in amphipods has been linked to the size of the animal and season. Some studies have shown seasonal patterns in some metals but not others (Thompson, 1999; Fialkowski, 2003). Animals of the same size may not be of the same age and therefore would exhibit differences in their metal concentrations. This is due to older amphipods having been exposed for longer time periods and therefore having higher accumulation (Fialkowski, 2003). Metal contents of marine invertebrates can be divided into two components: i) metal absorbed into the body, which is subject to physiological control; and ii) passive adsorption of metal onto the body surface, which is beyond metabolic control. Small amphipods have been found to have high metal concentrations, particularly iron, copper, zinc and lead. A decrease in size means increased surface area:volume ratio. This results in the latter becoming an increasingly larger proportion of the total body metal burden. The effect of size is strongest on dissolved iron compared with copper, zinc and lead due to its strong ability to be absorbed onto body surfaces (Rainbow & Moore, 1986; Rainbow, 1989).

1.3.3. Total Kjeldahl Nitrogen (TKN) and Chemical Oxygen Demand (COD)

Nitrogen is an essential element in the growth and productivity of primary producers. The different forms mentioned above are utilised by phytoplankton in differing proportions, resulting in effects on growth, size structure and community composition (Rabalais, 2002; Zehr & Ward, 2002). Significant increases in the concentration of organic nitrogen can result in eutrophication, which causes increased biomass of nuisance or harmful algae, and an accompanying reduction of species diversity (Carpenter *et al.,* 1998; Meyer-Reil & Koster, 2000; Rabalais, 2002). Ultimately, the effects are on biodiversity and oxygen availability, which are both reduced (Shanmugam *et al.,* 2007).

Particulate organic nitrogen (PON) additions can have direct and indirect effects on the associated environment. Indirect consequence of particulate organic nitrogen (PON) result in a reduction in grazing, increased organic matter flux which leads to hypoxia and changes in trophic dynamics (Rabalais, 2002). Algal blooms can additionally lead to decreased oxygen concentrations and hypoxia. This affects the displacement of pelagic organisms and the selective loss of demersal and benthic organisms. The breakdown of organic matter also results in reduced levels of oxygen and a decrease in organic matter in sediment. A decrease in oxygen concentration also causes less mobile organisms to become stressed and to move out of the sediments and seabed, resulting in death. An increase in organic matter on the seabed provides a rich food source for deposit feeding organisms; however, decomposition of organic matter in organic-rich sediment uses dissolved oxygen and causes physiological stress to benthic infaunal invertebrates. In severe cases this may lead to the death of benthic organisms and this reduces their density, biomass and alters the community composition (Segar & Berberian, 1976; Thomas *et al.,* 1976; Gross, 1978; Heip, 1995). It has been found that as oxygen levels decrease from extremely hypoxic to totally anoxic ($0 - 0.5$ mg L^{-1}), decrease in benthic infaunal diversity, abundance, and biomass is observed. Short-lived, smaller benthic groups replace larger long-lived ones with certain invertebrates being completely absent (Rabalais, 2002). This thereby influences benthic communities by changing their species abundances, distribution and diversity (Pearson & Rosenburg, 1978; Diego-McGlone *et al.,* 2000; Meyer-Reil & Koster, 2000; Borja, 2003). Oxygen depletion results in the reduction of some groups of macroinvertebrates (crustaceans, polychaetes), while others (oligochaetes, molluscs) may thrive in these conditions (Myslinski & Ginsburg, 1977; Hooda *et al.,* 2000; Rabalais *et al.,* 2000). It has a significant impact on benthic communities by altering their distribution and species abundance and diversity (Shanmugam *et al.,* 2007).

Although nitrogen concentrations do fluctuate naturally, drastic changes can occur due to anthropogenic influences, thereby resulting in a change in the system functioning (Carpenter *et al.*, 1998; Newman *et al.,* 2007). A direct consequence of the addition of PON, caused by increases in organic matter content, can result in a richer food source for sediment-dwelling deposit feeders. This can, however, cause physiological stress in other organisms, thereby changing the density, biomass and composition of benthic communities (Heip, 1995; Kennish, 2002). Studies have shown that macrofauna are almost completely absent from sediments that have a high oxygen demand. Decomposition of organic matter causes increased COD, thereby resulting in sediments lacking macrofauna (Findlay & Watling, 1997; Hedmark & Scholz, 2008). The COD of organic matter accounts for >90% of oxygen flux in the benthos. Oxygen uptake in marine sediments is due to the combined effect of aerobic metabolism of benthic organisms in conjunction with the reaction of oxygen that diffuses into sediment and decreased organic compounds that are diffusing out, (Kristensen, 1985).

In order to survive hypoxic or anoxic conditions, benthic organisms have developed specialised adaptations that allow them to overcome these stresses. Some species can access oxygen above the sediment-water interface. Tube building amphipods build tubes that protrude 2 – 3 mm above the sediment surface thereby allowing the amphipod to overcome the anoxic conditions in the sediment (Gallagher & Keay, 1998). Colonising amphipods like *Ampelisca* spp. are found in large groups, using their numbers as a mechanism by which they aerate and detoxify organically rich sediments. Ampeliscids bioturbate sediment that was previously anoxic, and a small oxidised cylinder is created within the sediment in the process. An oxic-anoxic gradient around the tube occurs leading to increased protozoan and aerobic bacterial (nitrifying bacteria) activity. Nitrifying bacteria oxidise ammonia to nitrite and nitrite to nitrate. Some of this nitrate and nitrite will be used by anaerobic denitrifying bacteria in the sediment for respiration, thereby producing nitrous oxide and nitrogen gas (Gallagher & Keay, 1998).

1.4. Biomonitoring and bioindicators

The rate of pollutant accumulation in organisms varies depending on the intensity and duration of exposure (Fleeger *et al.,* 2003). In order to determine the extent of this exposure to pollutants, various methodologies and techniques have been developed that incorporate analysis of both biological and physico-chemical aspects of water and sediment from contaminated and control areas. Biological and physico-chemical variables such as abundance, diversity, salinity, depth, pH, sediment type, total nitrogen, COD and pollutant concentrations are interlinked, and analysing them provides an indication of the health of the ecosystem with reference to bench mark data and control areas. Governments in many parts of the world actively manage these problems, with biomonitoring playing an important role in assessing the health of the marine system (Carballo & Naranjo, 2002). South Africa has in the past 13 years improved on its policies and legislation in terms of marine pollution through environmental quality objectives, scientific assessment studies, implementing specifications of critical limits and mitigating actions, and long-term monitoring programmes (Taljaard *et al*., 2006).

Biomonitoring of outfall discharges in the marine environment usually involves analysis of sediment and associated benthic organisms. The distribution of these organisms is often structured by pollution gradients. However, marine biological assemblages experience fluctuations in their pattern of distribution and abundance because of natural physical and biological processes (Roberts, 1996). Natural physical processes include disturbances such as storms, whilst biological processes include settlement and recruitment, competition and predation (Dayton, 1984). Anthropogenic disturbance has the potential to alter marine assemblages at various scales of organisation within the community and the effects of industrial and sewage pollution have received considerable attention (Lindegarth & Hoskin, 2001; Ellingsen, 2002; Mucha *et al*., 2004; Cruz-Motta & Collins, 2004; Van Hoey *et al*., 2004; Currie and Isaacs, 2005; Stark *et al*., 2005). The structure of local benthic communities is widely used as a measure of the impact that an effluent discharge may have in the marine environment (Warwick *et al*., 1990). Benthic organisms are well suited for this purpose as they are generally sedentary or have limited mobility (Boening, 1997) and cannot simply move away from adverse conditions (Warwick, 1993). They are almost entirely dependent on local conditions for survival and reproduction. In harsh conditions some sensitive species might be eliminated and open the way for more opportunistic species to proliferate. The net effect would be a skewing of the community structure that will tend to reflect the general state of the environment. Pollution impact would be manifested by shifts in the abundance of component species, a reduction in species diversity and/or a relative proliferation of opportunistic species (Rao *et al*., 2006).

At the end of the 1970s, bioindicators were introduced to represent spatio-temporal changes in the marine benthic environment due to pollution. These were aimed at the macrobenthic group of organisms due to their sensitivity to pollution gradients (Gómez Gesteira & Dauvin, 2000). Biological indicators have been classified as environmental indicators, ecological indicators and biodiversity indicators. These categories tend to overlap with some falling between categories and others belonging to more than one category (Niemela, 2000). Bioindicators in particular have been defined as organisms that provide information on the environmental conditions of their habitat by its presence, absence or changes in their behaviour (Wilson, 1994; van der Oost *et al.,* 2003). Bioindicators have been used in marine pollution monitoring programmes for three reasons: i) to assess those pollutants that are bioavailable and most important; ii) to establish the effects of contaminants on biological organisms at levels lower than chemical analytical detection limits; and iii) to assess synergistic, additive or antagonistic relationships among pollutants (Maher & Norris, 1990). Bioindicators have been classified as opportunistic species or sensitive species. Opportunistic species increase under pollutant exposure and are considered positive indicators while sensitive, less tolerant species are negative indicators (Belan, 2003). According to Carballo & Naranjo (2002), bioindicators can be organisms, species or communities, and may serve as subjects of biological and chemical monitoring.

When used efficiently, bioindicators should be able to provide information which could aid development planning and decision-making (Salas *et al.*, 2006). Formal, quantitative risk assessments have become the focus of environmental policy-making due to the escalating awareness of the prevention of damage from toxic chemicals (Russell & Gruber, 1987). van der Oost *et al.* (2003) define environmental risk assessment (ERA) as the procedure by which the likely or actual adverse effects of pollutants and other anthropogenic activities on ecosystems and their components are estimated with a known degree of certainty using scientific methodologies. Risk assessments have multiple advantages in decision-making in that they establish quantitative platforms against which comparing and prioritising risks can occur (van der Oost *et al.,* 2003). Risk assessments also allow a systematic way of improving the understanding of risks and estimating clear, consistent endpoints. Bioindicators are biological responses and can be used to assess organisms' health status and used as early warning signals of environmental risks (van der Oost *et al.,* 2003). Bioindicators provide information based on their responses to contaminants that contribute to environmental monitoring programs which are designed for surveillance, hazard assessment, compliance or the documentation of remediation. They allow information to be obtained that is not possible from chemical residue measurements environmentally and biologically (van der Oost *et al.,* 2003). Bioindicators focus on the important molecular events that occur after exposure and result from metabolism. Their use overcomes the problems that arise with intrinsic and extrinsic barriers. When multiple sources of degradation are present in water, bioindicators prove to be useful in assessing that ecological risk. Bioindicators are able to identify a specific pollutant causing environmental damage in a multiple pollutant scenario (Keeler & McLemore, 1996).

Bioindicators are useful in the assessment of possible hazards and therefore in the decision-making process (Keeler & McLemore, 1996; van der Oost *et al.,* 2003). An ecological risk assessment is a decision-making process, promoting sound environmental decisions (Finizio & Villa, 2002). If properly chosen, bioindicators represent objective systems of evaluation and information, serve as key tools for policy objectives, may facilitate the communication of countries' data priorities and allow reporting complex situations in simple ways that policy makers and the public can understand (Casazza *et al.,* 2002). Casazza *et al.* (2002) divided the use of marine benthic bioindicators into three categories: i) indicators at the level of species (the presence of a particular species or group of species can act as indicators of environmental stress or to identify the community); ii) those at the level of community structure (variation in number of species, abundance and biomass during specific period of time have been related to different indices such as Shannon-Weiner diversity indices and Simpson's dominance index); and iii) integrated indices that combine faunal data with chemical and/or ecotoxicological components in order to identify distinct areas that are affected by different pollution types.

Depending on the rationale behind the relevant study, organisms used in marine monitoring programmes should be selected based on specific criteria. These organisms should have a limited mobility. Sedentary benthic organisms are particularly useful for bioaccumulation estimates thereby revealing the status of the environment. These organisms are in close contact with sediments which are considered to be sinks of many contaminants (Gadzala-Kopciuch *et al*., 2004; Gaspar & Carvalho, 2005; Rao *et al.,* 2006). The organisms should be abundant and have a widespread distribution, be simple to identify and sample, have a high tolerance for the pollutants being analysed, have population stability and high pollutant accumulating capacities (Gadzala-Kopciuch *et al*., 2004).

1.4.1 Amphipods as bioindicators of pollution

Amphipods contain many of the specific characteristics of bioindicators. It is for this reason that they are common bioindicators of marine pollution worldwide. Amphipods make up a significant portion of the macrobenthic communities sampled along the KZN coast. Past data, based on the same sites highlighted in this study, have revealed annual differences in amphipod abundance between impacted and reference sites. This study uses these organismal and historical data series, available from 2003 to 2006, to evaluate the outcome of long term exposure of amphipods to pollutants originating from the selected effluent pipelines along the KZN coastline. In this study annual differences are not analysed. Instead, four years of data are combined in order to determine the outcome of the long term exposure. Amphipods occur in a variety of habitats, including deep ocean environments, freshwater and groundwater (Marsden & Rainbow, 2004). They are usually small with a limited mobility, and lack a planktonic larval stage, thereby reducing dispersal effects. Their limited mobility, which together with their sensitivity to changes in the environment, has made them useful indicators of environmental quality (Grosse & Pauley, 1989). Amphipods have been found to be sensitive to significant organic matter increases as well as other pollution such as metals and hydrocarbons (de-la-Ossa-Carretero *et al.,* 2009). Increased amphipod abundances have been found with distance from discharge points which suggest that these distant sites were less stressed environments due to the sensitivity of amphipods to environmental stresses compared with other marine organisms (de-la-Ossa-Carretero *et al.,* 2009).

Amphipods play an important role in marine ecosystem processes like nutrient cycling, secondary production, and dispersion and benthic amphipods having several important ecological roles in the environment (Thomas, 1993; Ellingsen, 2002). They are abundant, dominating many communities and are more sensitive to numerous contaminants than many other invertebrates. Amphipods are one of the major benthic components in biomass and diversity (6000 species) of marine systems (Grosse & Pauley, 1989; Thomas, 1993; Costa *et al.,* 1998). Many amphipods are an invaluable food source for numerous economically valuable fishes. Some species have not only shown sensitivity to acute and chronic exposure to pollutants, but also behavioural responses such as avoidance of sub-lethal concentrations of metals and organic pollutants (Thomas, 1993; Linton & Warner, 2003; Wiklund *et al.,* 2006). At chronic contaminant levels, amphipods were seen to demonstrate avoidance behaviour by decreasing in numbers as the concentration of the contaminant increased, thereby showing that the concentration of the toxicant influenced avoidance (Wiklund *et al*., 2006). Decreasing numbers were due to behavioural responses with mortality being low across concentrations. The absence of a particular species or group of organisms can still indicate levels of pollution and can be used as an indicator. These would be negative indicators as was discussed earlier.

Amphipods are used worldwide as indicators in pollution studies, sediment testing and as metal biomonitors (Marsden & Rainbow, 2004). They have been suggested as bioindicators of chemical changes which include single chemical aspects such as pH and single metal pollutants such as cadmium (Hodkinson & Jackson, 2005). Some species, particularly those from the families Ampeliscidae, Pontoporeidae, Melitidae and Gammaridae, have shown both positive and negative responses to significant increases in organic matter as well as metals and hydrocarbons (Dauvin & Ruellet, 2007). Some show high sensitivity to toxins in the sediment, especially Polychlorinated Biphenyls (PCBs), pesticides, metals and Polycyclic Aromatic Hydrocarbons (PAHs) (Gomez Gesteira & Dauvin, 2000). Some species have been shown to be influenced by sediment type, salinity and depth and are abundant near sewage outfalls where organic pollution is high (Grosse & Pauley, 1989; Bat *et al.,* 1998). Other species are commonly used in metal biomonitoring as they feed on decaying macroalgae, thereby taking up metals in solution from the water and food. These amphipods have been used as biomonitors for copper and zinc pollution in coastal waters (Rainbow & White, 1989; Moore *et al.*, 1991). Amphipods are used as primary bioindicators of sewage outfalls in many parts of the world (Thomas, 1993; Linton & Warner, 2003; Bach *et al*, 2009).

1.5. Rationale for this study

South Africa has legislation that allows the controlled discharge of effluents into the ocean via deep-sea outfalls. There are guidelines in place that are meant to be adhered to and monitoring programmes to ensure compliance with these guidelines (Gregory *et al.,* 2005). eThekwini Municipality has been discharging sewage and industrial wastes via two deep-sea outfalls along the Durban coastline since 1969 (McClurg, 2004). This study followed on from yearly monitoring programmes of these outfalls carried out by the CSIR, Durban. Identification of the entire benthic communities at the outfalls and reference sites were carried out, and chemical analyses of sediment were performed.

The CSIR's data show that amphipods are abundant and permanent, and are easy to identify compared with other benthic organisms found in the study area (McClurg & Newman, 2008). Studies have shown that amphipod species composition, abundance and biomass increase and decrease inter-annually (McClurg & Newman, 2008). It is important to determine the reasons for these changes in this study area as the outcomes may not only be of use to the CSIR in their yearly monitoring programmes, but could also be implemented in monitoring programmes in other parts of the country. It would reduce time spent on laboratory work by enabling the identification of only amphipods, rather than the entire benthic communities, to provide information about the status of the region in and around the outfalls and reference sites. This would allow for individuals being trained in the identification of amphipods rather than macrobenthic organisms in general. In addition, it would also result in more accurate identification of amphipods in particular rather than perfecting taxonomic skills in many different groups of organisms.

In this light, the purpose of this study was to determine i) whether sediment grain size is responsible for driving amphipod community structure along the KZN coast; ii) to relate the accumulation patterns of the grain sizes with other environmental variables; and iii) establish how amphipods respond to sediment grain size and the selected environmental variables. While amphipods have proven to be good bioindicators in other parts of the world it cannot be taken for granted that they will be useful in South Africa due to geographical and ecological differences. Different habitats are influenced by chemical and physical factors such as light gradient, wave strength and water temperature as well as substrate composition which differs from soft to hard thereby creating further differences in the underwater environment (Casazza *et al.*, 2002). If relationships between amphipods and the environmental variables are identified, the usefulness of amphipods as indicators will be comfirmed.

In order to understand what drives differences between years, a suite of environmental variables such as hydrodynamics, interannual changes in discharge rates, storms, etc., would be needed. These supplementary data are not available and therefore comparing years was not possible. This study therefore uses four years' of data, with each set of yearly data serving as one replicate in the analysis; this allows the overall biotic response to be determined at the conclusion of many years' of long-term exposure. This study will enable a link between environmental variables and amphipod communities to be emphasised, and the possible use of amphipods as bioindicators of the KwaZulu-Natal coast identified.

The objectives of this study were to:

1. Assess the distribution of sediment grain size over a portion of the KwaZulu-Natal coastline that is influenced by ocean waste-water outlets, with the objective of determining whether variables at impacted (effluent discharge) sites are different from those at reference sites.

2. Evaluate the distribution of selected physico-chemical variables over the portion of coastline alluded to in 1), above, with the objective of determining whether variables at impacted sites are different from those at reference sites.

3. Evaluate trends in amphipod community structure over the portion of coastline alluded to in point 1), above, with the objective of determining whether the communities at impacted sites are different from those at reference sites.

4. Evaluate whether amphipod community structure over the portion of coastline mentioned in 1), above, is influenced by a) sediment grain size, and/or b) other selected physico-chemical variables.

This study is designed to test the following null hypotheses:

Abiotic data:

A. Sediment grain size is homogenously distributed across the study area.

B. The concentrations of selected metals and TKN and COD in the sediment are homogenously distributed across the study area.

C. The spatial patterns of sedimentary metal concentration, TKN and COD are not modified by sediment grain size distribution (i.e interaction of A.i with B).

Biotic data:

D. Amphipod community structure is not influenced by i) sediment grain size distribution, ii) metal concentration, iii) TKN, and/or iv) COD.

2. Materials and Methods

2.1. Study area

The Central Works and Southern Works Outfalls discharge effluent into the sea off the Durban coastline (Figure 1). Central Works is a wastewater treatment facility that deals with predominantly sanitary wastewater; that is, wastewater that is flushed down toilets or rinsed into drains in houses and commercial facilities (McClurg & Newman, 2008). Southern Works handles both sanitary and industrial wastewater. Southern Works and Central Works treatment plants apply only primary treatment (McClurg & Newman 2008). Table 2 contains the specifications for the Central Works Outfall and Southern Works Outfall.

2.2. Wastewater treatment and outfall specifications

Primary treatment begins with raw sewage which has high ammonium, potassium, nitrate and phosphorus concentrations, a high conductivity (due to high solute content) and high alkalinity (Stander, 1973; Bramryd, 2002). There are also large amounts of suspended constituents such as paper, rags, faecal matter, plastic bottles, etc. (Stander, 1973). Sewage is screened, de-gritted and de-greased. Screening may be done manually on hand-raked screens, by mechanically-raked screens or the cutting up of coarse matter (Stander, 1973), and further screening prevents rags and large objects from reaching the sedimentation tanks. Detritus is then removed in the detritus channel. The presence of detritus can cause mechanical damage to equipment and can settle to form semi-solid banks of sludge which can clog pipes. Settlement of particles of organic matter then takes place (Stander, 1973). This isolates and removes insoluble, fine particles of organic matter from the bulk of the water, resulting in tank effluent. The settled sewage on the bottom of the tank is known as raw sludge. This is regarded as the first step in the purification process because organic matter has been removed from the liquid. The liquid phase then goes into the secondary treatment phase where it is treated to selected effluent quality standards and discharged into a stream or outfall (Stander, 1973; Howard *et al.,* 1997). Sewage at the Central Works and Southern Works Outfalls does not undergo secondary treatment and therefore it is the product at the end of the primary treatment phase that goes out to sea.

2.3. Sampling design

Sampling of the two outfall sites was carried out annually during May 2003 – 2006 with Mdloti, Cooper Light, and Amanzimtoti selected as reference sites (Figure 2a – f; Figure 3a – d). Due to oceanographic and local conditions off the KwaZulu-Natal coast, as well as the possibility of other anthropogenic sources of pollution such as those from rivers, it is difficult to select a site which can be defined as a control area in the strict definition. Therefore, sites that are as representative as possible of unimpacted areas were used as reference sites. The reference sites that were chosen were not identical to impacted sites but permitted the examination of spatial trends due to their spatial segregation.

Table 2. Specifications of the Southern Works and Central Works Outfalls (Livingston 1990; McClurg TP, June 2008, pers. comm.[1]).

	CENTRAL WORKS OUTFALL	**SOUTHERN WORKS OUTFALL**
Commissioned	1969	1968
Effluent type	Sanitary waste	Sanitary & industrial waste
Distance from shore (m)	3 200	4 200
Depth of diffuser section (m)	42 – 53	54 – 64
Main diameter (m)	1.32	1.37
Length of diffuser section (m)	450	290
Number of ports	18	34
Design capacity (m^3 per day)	135 000	230 000
Average volumes discharged during 2006 (m^3 per day)	75 000	147 000

These reference sites allow the direction of change, if any, in various biological variables to be detected through comparisons between surveys. All data for the years 2003, 2004, 2005 and 2006 were provided by the CSIR in order to analyse possible changes and trends in amphipod community structure and environmental variables with each year serving as replicates. The analyses for metals, sediment grain size, total Kjeldahl nitrogen (TKN) and Chemical Oxygen Demand (COD) were carried out by the CSIR (Durban).

Methods and protocols in this study were designed by the CSIR (Durban) for the purposes of their research and as part of a long-term monitoring programme (McClurg, 2004). In order to have a long term dataset from which to assess patterns, site selection, sample design and methodology were not altered for the purposes of this study (changing the sampling design was also not permitted by the CSIR). Each outfall site consisted of 15 stations, while the three reference sites off Mdloti, Cooper Light and Amanzimtoti comprised nine stations each. More stations were sampled at the outfalls due to the need for establishing detailed spatial patterns in these areas compared to the reference sites. The reference stations were placed at approximately the same depth as that of the outfall stations in order to ensure uniformity (Table 3). Stations were spaced 200 m apart from each other.

[1] Mr TP McClurg, CSIR Natural Resources and the Environment, 359 King George V Avenue, Durban

Table 3. Depths of reference and outfall sampling grids at all sites.

SITE	DEPTH (m)
Mdloti	56.2 – 59.8
Central Works Outfall	48.4 – 58.2
Cooper Light	51.1 – 60.0
Southern Works Outfall	57.0 – 62.5
Amanzimtoti	47.3 – 53.4

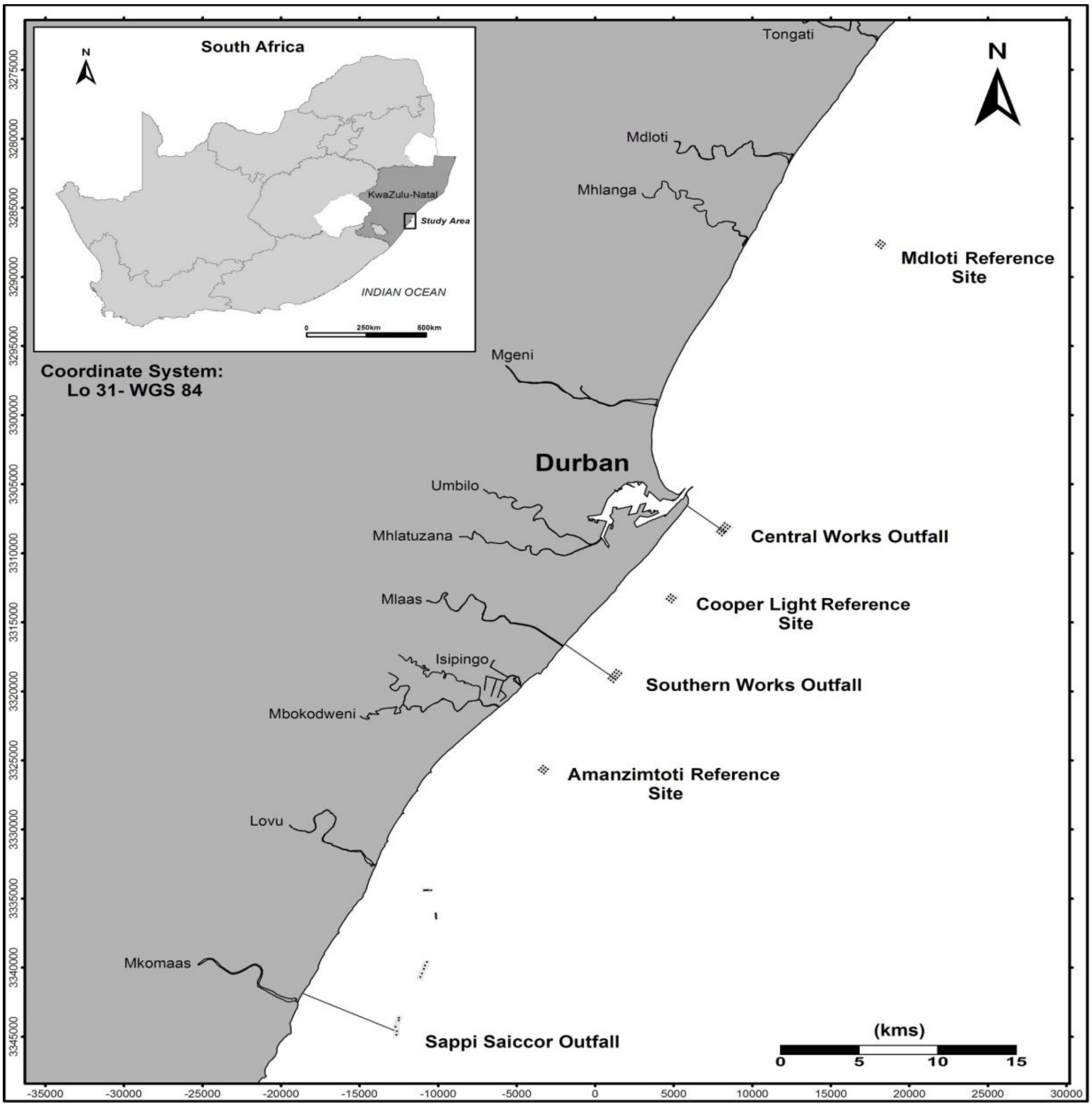

Figure 1. Locality map showing the Southern Works Outfall, Central Works Outfall and reference sites along the Durban coastline. The Sappi Siaccor outfall was not a part of this study.

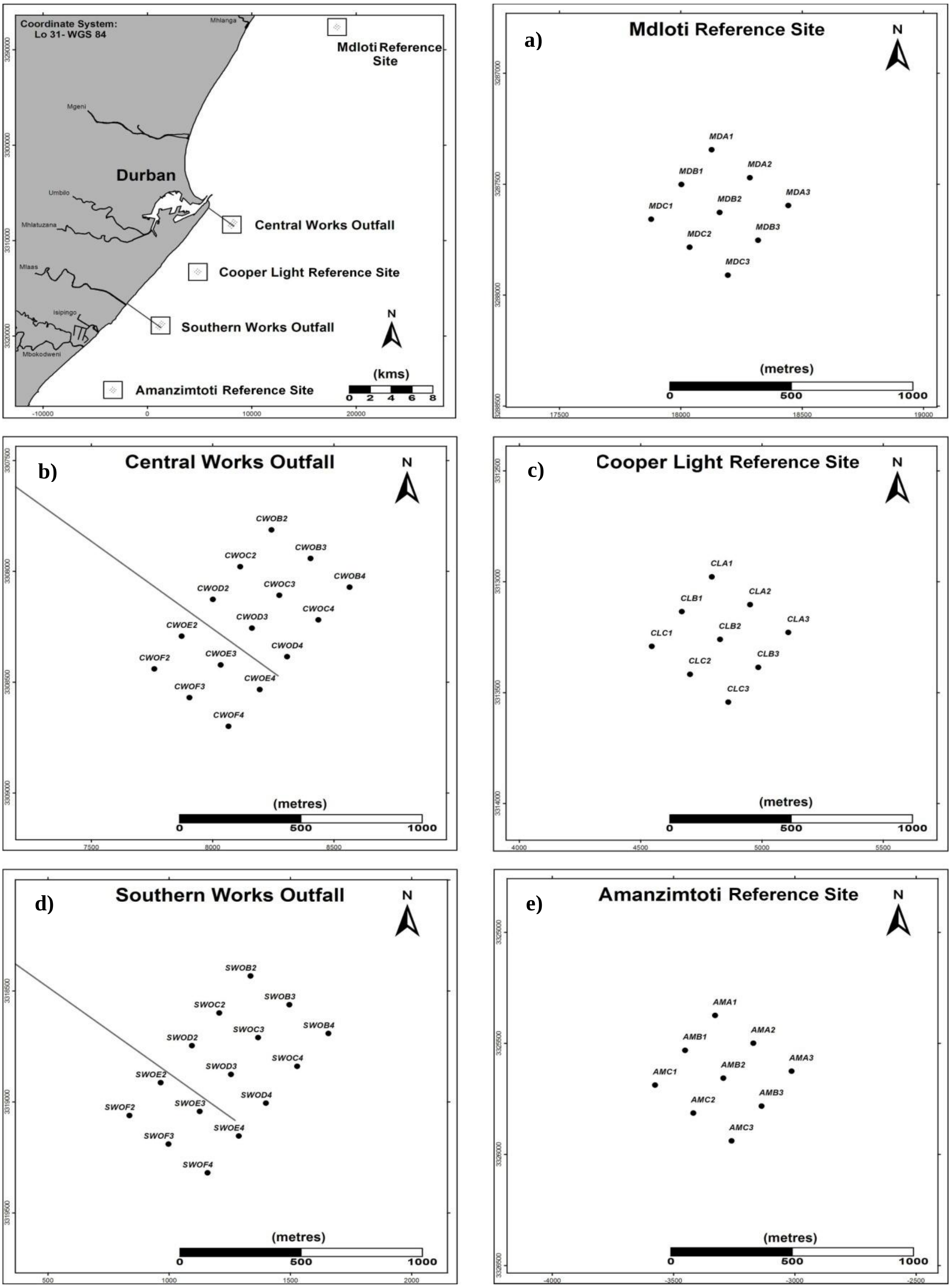

Figure 2a – e. Biological, sediment and chemical sampling stations at the Southern Works Outfall, Central Works Outfall and reference sites.

2.4. Biological and sediment sampling

Sampling was carried out in May on a yearly basis. At each station, a Day grab (0.25 m^2) was used to sample the seafloor. Three 30 cm^3 aliquots of sediment were collected for sediment grain size, COD, TKN and metal analyses. These samples were kept on ice while in the field, and frozen in the laboratory until further analysis. A separate sediment sample was collected per station, washed through a 1 mm sieve, and fixed in 4% formaldehyde for biological analysis. In total, 60 samples were collected per impacted site per year (4 samples x 15 stations) and 36 samples per reference site per year (4 samples x 9 stations). In the field, environmental data (water depth, distance from outfall, pH and temperature) were also collected at each station.

2.5. Sample processing

2.5.1. Biological sample preparation

The fixed samples were eluted with freshwater in the laboratory through a 235 μm sieve to yield the lighter organisms and then microscopically examined to remove denser material with fine forceps. Composite fauna at each station were preserved in 70% ethanol. These samples were then separated into taxonomic groups. Amphipods from each site were identified to species or genus level where possible, counted and preserved in 70% ethanol. This was carried out for each sample per station over the 4 year period.

2.5.2. Sediment grain size processing

One sediment sample per station was used for grain size analysis. Processing of sediment samples for grain size analysis involved wet sieving and dry sieving (Southwood & Henderson, 2000). Sediment was sieved into seven grain size classes according to the Wentworth Scale. These classes were mud (<0.063 mm), very fine sand (0.063 – 0.125 mm), fine sand (0.125 – 0.250 mm), medium sand (0.25 – 0.50 mm), coarse sand (0.5 – 1.0 mm), very coarse sand (1.0 – 2.0 mm) and gravel (>2.0 mm). The size classes were expressed as a fraction of sample dry mass. Sediment grain sizes were converted into Φ (phi) values by using the method explained in Morgans (1956). The Φ scale substitutes a logarithm for the diameter of the particle in millimetres. This allows the unequal class intervals of the Wentworth scale to be translated into equal intervals. The x-axis value that corresponds to the 50% value on the y-axis is read on the Φ scale and is converted into millimetres using a conversion chart (Morgans, 1956).

2.5.3. Metal analysis

One frozen sediment sample per station was defrosted, homogenised and approximately 1 g aliquots were placed into a high-pressure digestion vessel. A 10% nitric acid solution was used to digest the sediment with the aid of microwaves. Milli-Q deionised water was used to dilute the digests and concentrations of aluminium, iron, arsenic, cadmium, copper, chromium, nickel, lead, mercury and zinc were determined and quantified using a VISTA-PRO, CCD Simultaneous Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Reference sediment samples were digested and analysed along with all field samples. Corrections for extraction efficiency were carried out by running four blanks (concentrated nitric acid and deionised water) together with the reference sediment samples and field samples. No internal standard was used.

2.5.4. Total Kjeldahl Nitrogen (TKN)

One 30 cm^3 sediment sample was used to carry out the TKN and COD analyses. An ammonium stock standard solution was made using ammonium sulphate which was dried at 105 ºC for two hours prior to use. An organic nitrogen stock standard was prepared by dissolving 1547 g bactopeptone in 1000 ml distilled Milli-Q water with a resistivity of 18 MΩ.cm, and this was used to prepare the required series standards of concentrations 0.1 x 10^{-6}, 1 x 10^{-6} and 10 x 10^{-6} mg L^{-1}. The following reagents were used: 50% sulphuric acid for digestion; 153.5 g of NaOH was dissolved in 500 mL Milli-Q water, cooled and made up to 5 L for the sodium hydroxide neutralising solution; 5 mL of phenol red indicator solution was added to the NaOH solution; 1 mol L^{-1} sulphuric acid solution was prepared by diluting 14 g in 500 ml of Milli-Q distilled water for the sulphuric acid neutralising solution.

For the digestion of samples, 20 mL of the standards and samples were measured into digester tubes. Four to six bumping stones and 2 mL of the digestion were added to each sample and mixed well, and allowed to digest in a Lachat BD 46 block digester at 150 ºC for approximately 1.5 hours in order for the water to evaporate. The temperature was then increased to 340 ºC where it was maintained for 30 minutes after which the samples were removed and allowed to cool.

The preparation of samples for ammonia analysis on a Bran+Luebbe AutoAnalyser 2 included adding 40 mL of the sodium hydroxide neutralising solution to each test tube and mixed well. The colour changes to a yellow due to the phenol red indicator solution. The results were reported as µg g^{-1} (NH_3–N).

2.5.5. Chemical Oxygen Demand mn (COD)

Aliquots of sediment weighing 0.1 – 1.0 g were placed into a 250 mL Erlenmeyer flask. To this, 100 mL of de-ionised water, 0.5 mL of a 33% sodium hydroxide (NaOH) m/v solution and 10 mL of potassium permanganate ($KMNO_4$) solution at a concentration of 0.0125 mol L^{-1} were added. The samples were heated in a water bath for 30 min, 25 mL of manganese sulphate ($MnSO_4$) added and then rapidly cooled to room temperature. Approximately 1 g of potassium iodide (KI) was added and this mixture and titrated with 0.01 mol L^{-1} sodium thiosulphate (Na_2SO_3) using starch as an indicator (blue to clear end point). The results were reported as µg g^{-1} COD.

2.6. Data analysis

All data were pooled, un-averaged, with the years acting as replicates. Data tables were constructed per station per site per year. Intra-annual comparisons were not possible due to the lack of replication per year (sampling was carried out only once a year). Uni-variate and multi-variate statistical techniques were used to analyse data.

2.6.1. Abiotic data

PRIMER v6 was used to perform multivariate statistical analyses. Principal component analyses (PCA) were performed on the environmental data, including sediment grain characteristics and chemical composition and characteristics (metals, TKN, COD). The data were normalised (zero mean ± 1 standard deviation) and ranked, and the correlation-based PCA computed on Euclidian distance measures. Sediment granulometry data were not transformed, but the chemical composition data were *log* transformed. Pairwise correlation analyses were carried out for sediment grain size and the selected metals, TKN and COD in R 2.7.1 (Ihaka and Gentleman, 1996).

Multi-Dimensional Scaling (MDS) uses a two-dimensional scatter plot to examine relative similarities between stations based on environmental and biological variables (Clarke & Warwick, 1994). These ordinations were used to spatially assess the similarities and differences of sediment grain size and the physico-chemical variables distributions across sites. BIOENV analysis was performed on the full dataset (based on the Spearman rank correlation), which was 4th root transformed; BIOENV aided the identification of environmental variables that best explain the observed community patterns. This resulted in a list of the environmental variables that seemed to be driving the community structures, and helped link biological (amphipod abundances) and environmental data (sediment type, metals, COD, TKN and depth). Bubble plots for sediment grain sizes and the physico-chemical parameters were plotted, based on the results from the correlation analyses (i.e. patterns were only searched for if the pairwise correlations revealed strong correlations between variable pairs). Bubble plots are valuable 2D representations of the data where symbol size is proportional to the magnitude of an environmental variable ie. the larger the bubble the greater the value of the variable. This is a visualisation feature of MDS plots that superimpose specific variables onto the MDS as circles of differing sizes (Clarke & Warwick, 1994).

Analysis of similarity (ANOSIM) were used to determine whether significant difference occurred between impacted and reference sites based on the sediment grain sizes and physico-chemical parameters.

2.6.2 Biotic data

The DIVERSE function in PRIMER v6 was used to calculate Shannon-Weiner diversity (*H'*, as log to base *e*), species richness (*S*) and number of species (*N*) at each sample site. These indices provided biodiversity information which is important in pollution studies (Magurran, 1988).

PRIMER v6's Multi-Dimensional Scaling (MDS) ordinations were used to visually assess spatial differences or similarities in amphipod communities in the study area (Clark & Warwick, 1994). MDS ordinations were based on Bray-Curtis similarities after 4^{th} root transformations and standardisation of abundance data. Fourth-root transformations were used because they down-weigh abundant species and considers both mid-range and rare species in its analysis (Clark & Warwick, 1994). In order to identify the adequacy of MDS ordinations, stress values were calculated. Stress increases with reducing dimensionality and increasing quantity of data with values being defined from <0.05 – >0.3 (Clark & Warwick, 1994). Bubble plots for the biology were based on the results of the BIOENV analyses explained above

In this type of study it was important for the rare species to be considered in order to determine any patterns being observed over the years. The SIMPER function was used to identify dominant species based on percentage contributions of each species per site for each year. This function shows the most abundant species in each site, and which species were responsible for clustering, and dissimilarities between the clusters. The results of SIMPER were tabulated for all years per site, focussing only on species contributing >3% of the abundance (Field *et al*., 1982). This allowed determining which species were most representative of a site. For specific details of the PRIMER processes refer to Clarke & Warwick (1994).

3. Results

3.1 Sediment grain size

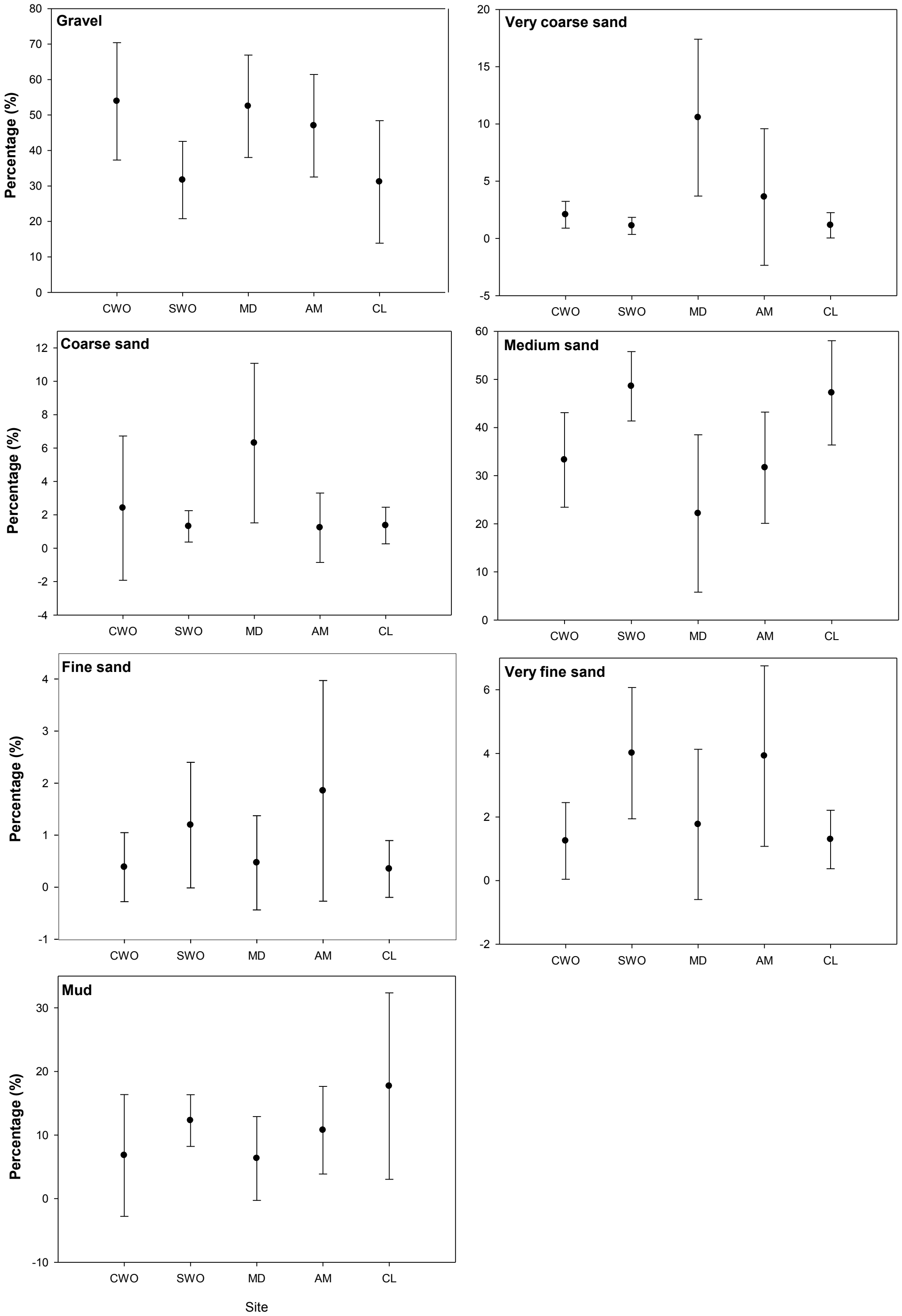

Figure 3. Percentage contributions of each grain size (gravel, very coarse sand, coarse sand, medium sand, fine sand, very fine sand and mud) across all five sites over a four-year period. Error bars represent ± standard deviations of the mean. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

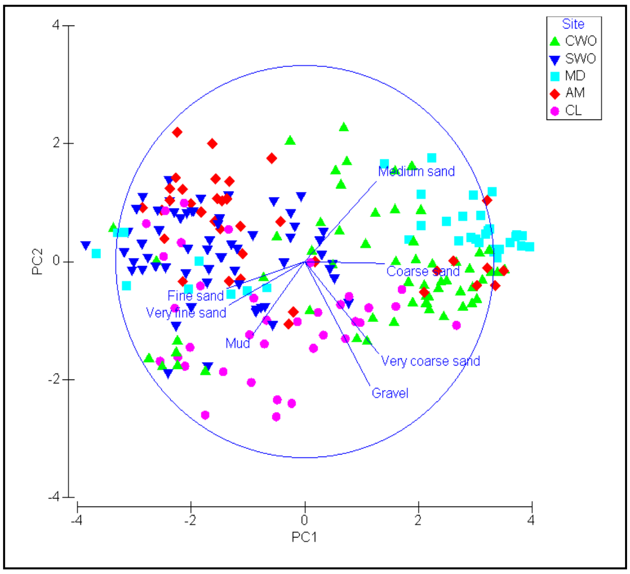

Figure 4. PCA of sediment grain sizes across all sites. PCA 1 and PCA 2 are shown. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

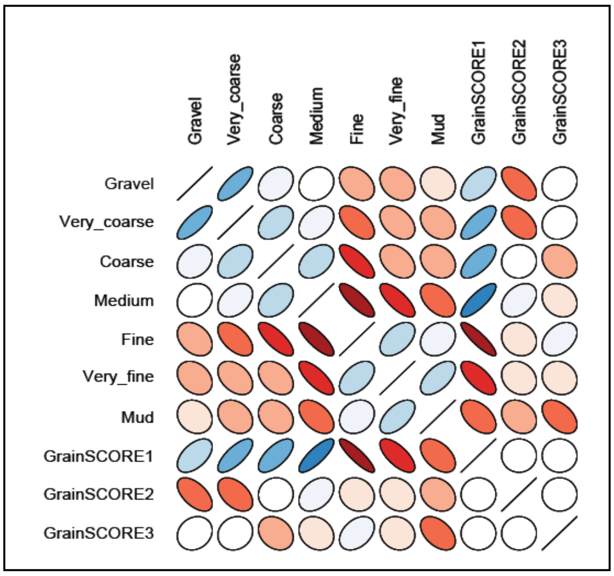

Figure 5. Correlations between sediment grain size classes across all sites. Narrower ellipses and more intense colours indicate strong correlations, while rounder ellipses and lighter colours show weaker correlations. Blue and red indicate that the relationships are positive and negative, respectively. GrainSCORE1, GrainSCORE2 and GrainSCORE3 are the scores along the three PC axes.

For the sediment data, Figure 3 shows a dominance at the Central Works Outfall and Mdloti reference sites of coarse-grained sediment, and the Southern Works Outfall and Amanzimtoti reference sites being dominated by fine-grained sediments. The Cooper Light reference site consists primarily of medium grain sizes.

The first three Principal Component (PC) axes were retained as together they explain 89.8 % of the variation (PC axes 1, 2 and 3 accounted for 66.1, 12.6 and 11.0 % of the variation, respectively). The first two PC axes are displayed in Figure 4 where it can be seen that replicates taken from the same site (i.e. replicates meaning data pooled over 4 years and all stations within each site) *generally* cluster together (of course, there are notable exceptions, where sometimes sub-clusters per site are visible – e.g. more so for Mdloti and Amamzimtoti – but no temporal or spatial influence could be discerned in these cases). Since the PC axes are interpretable as linear combinations of the influencing variables along the abscissa and ordinate directions (Equation 1), the influence of these variables is easy to interpret as causing the difference among sites. Moving from left to right along PC 1 represents an increase in gravel, very coarse, coarse, and medium sand, while fine and very fine sand and mud decrease. Large numbers (see Equation 1, below) have vectors that are more aligned (parallel) with the PC axis in question; the smaller the number, the more perpendicular they become with respect to the axis:

PC 1 = + 0.345_{gravel} + $0.390_{very\ coarse\ sand}$ + $0.420_{coarse\ sand}$ + $0.380_{medium\ sand}$ – $0.414_{fine\ sand}$ – $0.400_{very\ fine\ sand}$ – 0.278_{mud} ……. Equation 1

Similarly, from Figure 5 it can be seen that the first PC axis (denoted by GrainSCORE1) is influenced by grain sizes from medium and coarser in a positive manner (narrower blue ellipses), while sediment fractions finer than these negatively influence this component axis (narrow red ellipses). The narrowness of the ellipses suggests that the influence of those variable is large (i.e. in combination they explain 66.1 % of the variation); in contrast to this are the narrower ellipses in PC 2 and PC 3 indicate weaker correlations resulting from the grain size variables.

Central Works and Mdloti therefore tend to be dominated more by coarser sediment fractions (Figure 4), while Amanzimtoti and Southern Works tend to be dominated more by the finer sediment fractions. PC 2 on the other hand indicates that, moving from bottom to top in Figure 4, the dominance of all grain fractions (except fine sand) decreases (Equation 2). This influence is most strongly exerted on Cooper Light, which is situated nearer the bottom of the PC 2 axis, a region in PC space where sediment fractions with a negative sign (see Equation 2, below) dominate. The largest influence is by gravel, very coarse sand, and medium sand:

PC 2 = – 0.634_{gravel} – $0.469_{very\ coarse\ sand}$ – $0.009_{coarse\ sand}$ + $0.411_{medium\ sand}$ – $0.137_{fine\ sand}$ – $0.221_{very\ fine\ sand}$ – 0.376_{mud} ……. Equation 2

There is a strong positive correlation between gravel and very coarse sand, a strong negative correlation between find sand and coarse sand, fine sand and medium sand, and between very fine sand and medium sand (Figure 5). These relationships are verified in the PCA plot (Figure 4), with the PC vectors denoting very coarse sand and gravel positioned nearly parallel with each other, and the vectors indicating the negative correlations occurring on opposite sides of the plots. With regards to the sites, significant differences occur between all sites based on grain sizes (ANOSIM, global $R = 0.383$, $p < 0.001$).

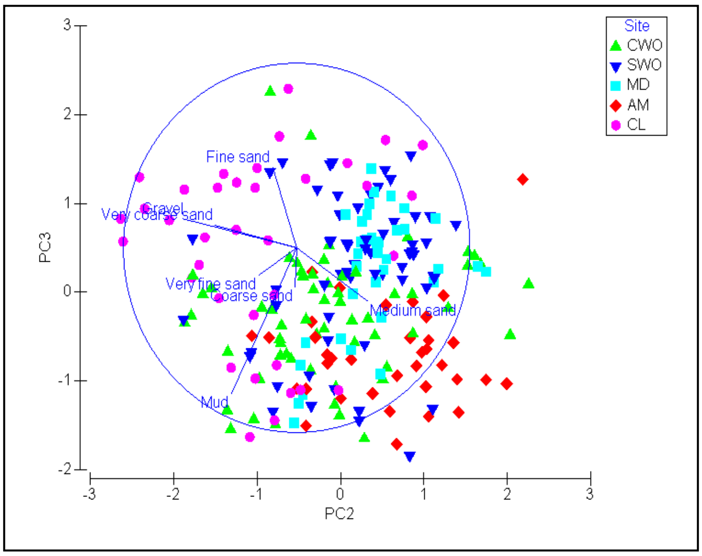

Figure 6. PCA of the different sediment grain sizes across all sites; PC 2 and PC 3 are shown. CWO = Central Works outfall, SWO = Southern Works outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

Figure 6 represents the PC axes 2 and 3. Grain size has very little influence along PC 2, with only medium sand exerting an influence in the positive direction, and chiefly gravel, very coarse sand, and mud separating the sites in PC space along the negative direction; consequently it is Amamzimtoti and Cooper Light that are separated from the other sites along PC 2 by these sediment grain size characteristics. Along PC 3 the influence of grain size if even weaker, with mud driving differences among sites in the negative direction, and fine sand pulling the sites apart in the positive direction (Equation 3). Once again the influence is most strongly seen on Amamzimtoti and Cooper Light, with the former having more mud and the latter more fine sand:

$$PC\ 3 = +\ 0.152_{gravel} + 0.121_{very\ coarse\ sand} - 0.212_{coarse\ sand} - 0.290_{medium\ sand} + 0.434_{fine\ sand} - 0.150_{very\ fine\ sand} -0.789_{mud} \ldots\ldots\ \text{Equation 3}$$

Gravel, very coarse sand and coarse sand (Figure 7) show similar patterns to each other and seem to be driving the patterns visible in the grain size MDS plot. These grain sizes appear to have a stronger influence on the Central Works Outfall, the Mdloti and Cooper Light reference sites, while having less influence on the Southern Works Outfall and the Amanzimtoti reference site. However, medium sand shows some influence on Central Works Outfall and the Amanzimtoti reference site. The finer grain sizes, fine sand and very fine sand, are definite drivers of Southern Works Outfall and Amanzimtoti reference sites. These finer sediments seem to have some influence on the Cooper Light reference site as is visible in the bubble plots. All the patterns observed in Figure 7 correlate with those found in Figures 4 and 5.

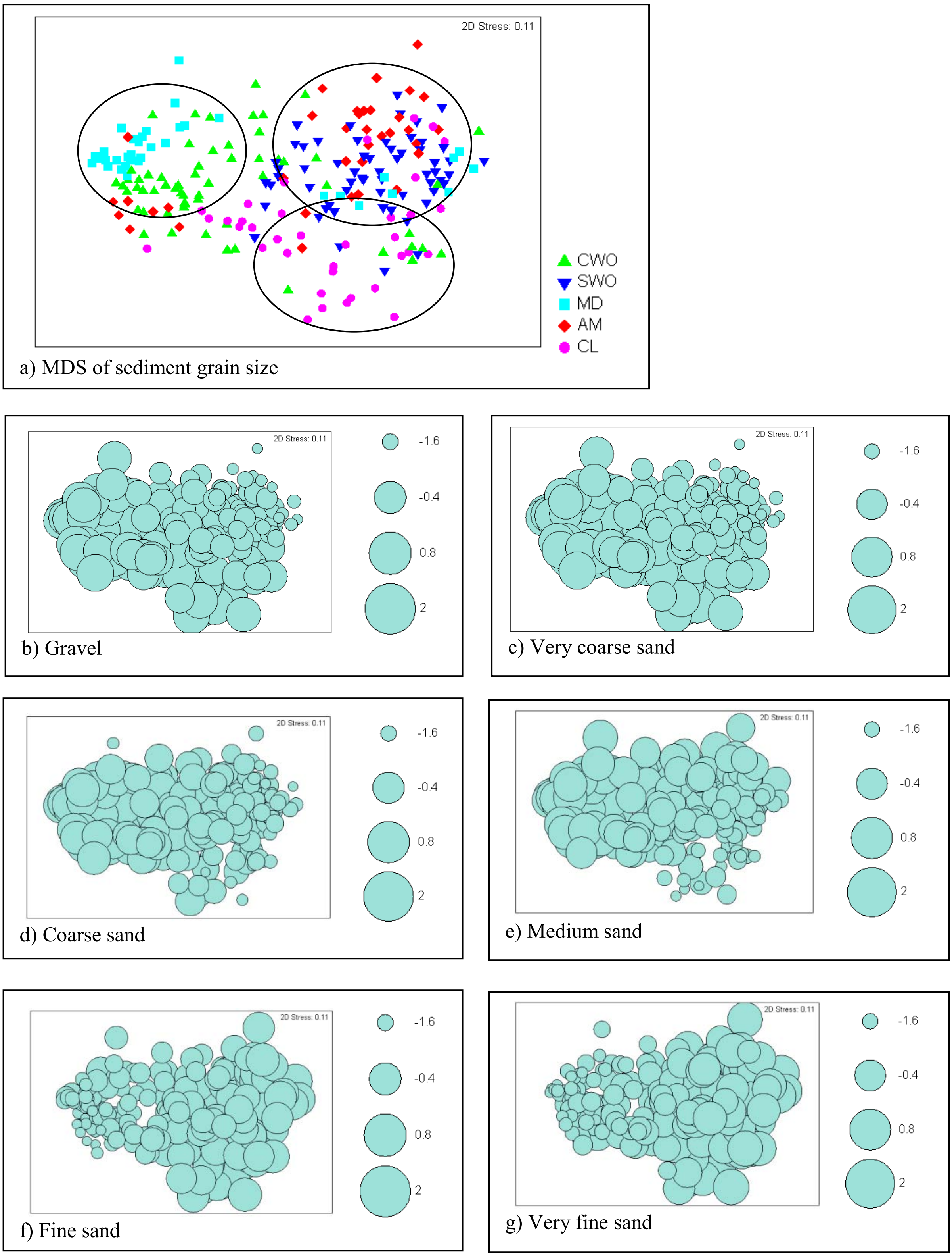

Figure 7. a) MDS of sediment grain size distribution at the five sites. The bubble plots represent the same MDS but with each grain size (b) gravel, c) very coarse sand, d) coarse sand, e) medium sand, f) fine sand, g) very fine sand) of the sampling locations represented by superimposed circles. The grain sizes used here were those identified in the correlation analysis in Figure 3. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

3.2 Metals, TKN and COD

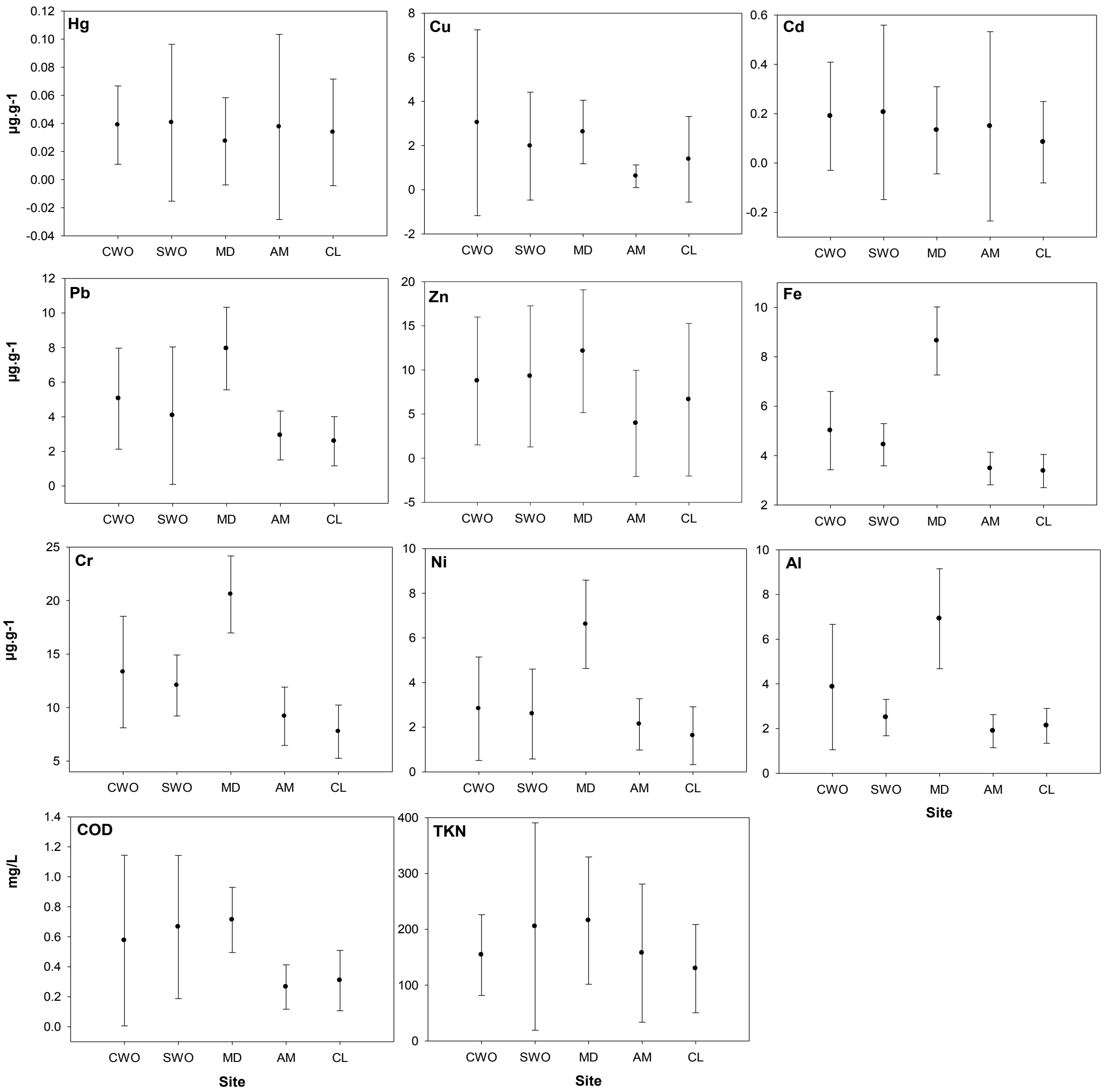

Figure 8. Concentrations of heavy metals (Hg, Cu, Cd, Pb, Zn, Fe, Cr, Ni, Al), COD and TKN at each site over a four-year period. Error bars represent ± standard deviations of the mean. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

Moving from left to right along PC 1 shows that Mdloti is dominated mostly by sediments with high concentrations of Cr, Cu, Fe, Pb, Al, Ni, and with a higher COD (Equation 4). Central Works Outfall shows few similar patterns to Mdloti with some dominance by Cu and Pb (Figure 8, 9). Central Works and Southern Works Outfalls exhibit dominance by Cd (Figure 8).

$$PC\ 1 = + 0.031_{Hg} + 0.316_{Cu} + 0.093_{Cd} + 0.370_{Pb} + 0.288_{Zn} + 0.387_{Fe} + 0.398_{Cr} + 0.351_{Ni} + 0.371_{Al} + 0.301_{COD} + 0.103_{TKN}$$ ……. Equation 4

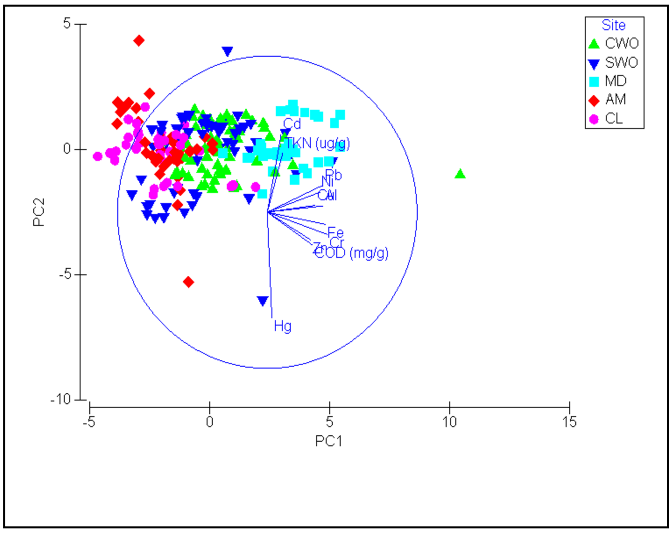

Figure 9. PCA showing the relationship between all metals, COD and TKN across all sites for all years. PC axes 1 and 2 are shown. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Cooper Light.

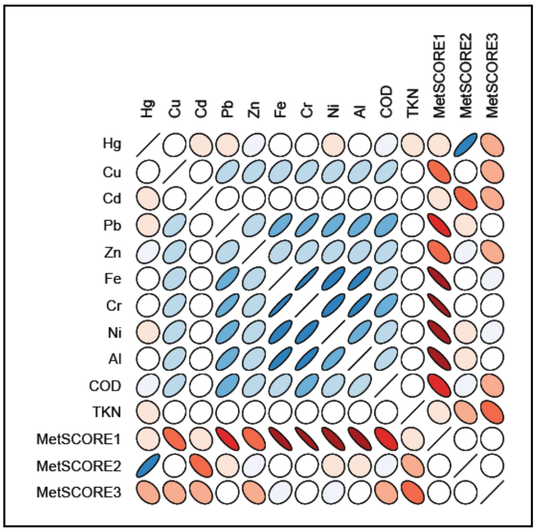

Figure 10. Correlations between metals, COD and TKN across all sites. Refer to Figure 2 for additional details.

For the metal data, the first three PC axes were retained as together they explain 71.9 % of the variation (PC axes 1, 2 and 3 accounted for 48.6, 13.5 and 9.7 % of the variation, respectively). The first two PC axes are displayed in Figure 9 and show more-or-less the same patterns as the sediment data. Again, it can be seen that replicates taken from the same site (i.e. replicates implying data pooled over 4 years and all stations within each site) generally cluster together. There are some exceptions to this clustering as can be seen from the five outliers. Clustered sites tend to be situated closer to one another along the top half of the PC 2 axis.

Hg, Cd and TKN explain most of the separation of the sites along the PC 2 axis (Equation 5), although their „power' of separating the sites is small compared to along PC 1 (PC 2 explains only 13.5 % of the variation, therefore very little separation compared to PC 1). Toward the top of Figure 9 the concentrations of TKN and Cd increase; the opposite is true for Hg. The largest separation happens between the two sub-groups of SWO, and the two outliers at the bottom.

$$PC\ 2 = -0.677_{Hg} + 0.043_{Cu} + 0.498_{Cd} + 0.172_{Pb} - 0.176_{Zn} - 0.079_{Fe} - 0.142_{Cr} + 0.127_{Ni} + 0.040_{Al} - 0.201_{COD} + 0.378_{TKN} \ \ldots\ldots. \text{ Equation 5}$$

A very strong positive correlation occurs between Fe and Cr (Figure 10). Additionally, strong positive correlations are observed between Pb, Fe, Cr, Ni and Al, COD, Zn and Cr. TKN does not show a strong correlation with any other variable. These correlations are verified in Figure 9 with the vectors indicating the PCA scores of the metals that are positively correlated occurring close (i.e. almost parallel) to one another on the plot. Significant differences occur between all sites based on the environmental variables (ANOSIM, global $R = 0.353$, $p < 0.001$).

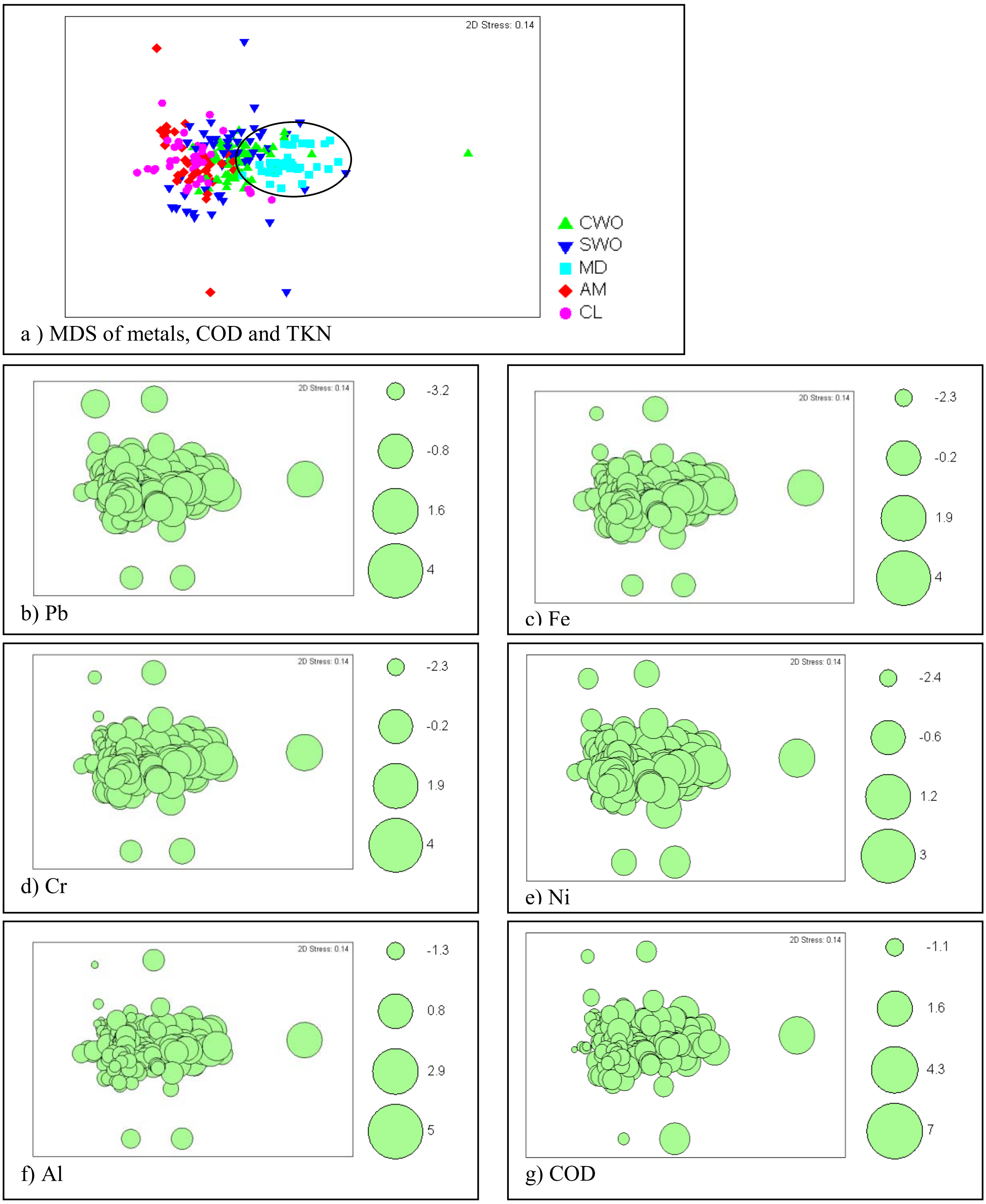

Figure 11. a) MDS of the environmental variables at the five sites. The bubble plots represent the same MDS but with each variable: b) lead, c) iron, d) chromium, e) nickel, f) aluminium, g) COD) of the sampling locations represented by superimposed circles. The environmental variables used here were those identified in the correlation analysis in Figure 8. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

All the variables shown in the bubble plots in Figure 11 exhibit similar patterns, with all variables dominating the Mdloti reference site. These patterns mirror those seen in PCA (Figure 9). However, the distinct clumping of bubbles across all sites shows some influence of these variables on the remaining four sites.

3.3 Relationship between biological communities and environmental variables

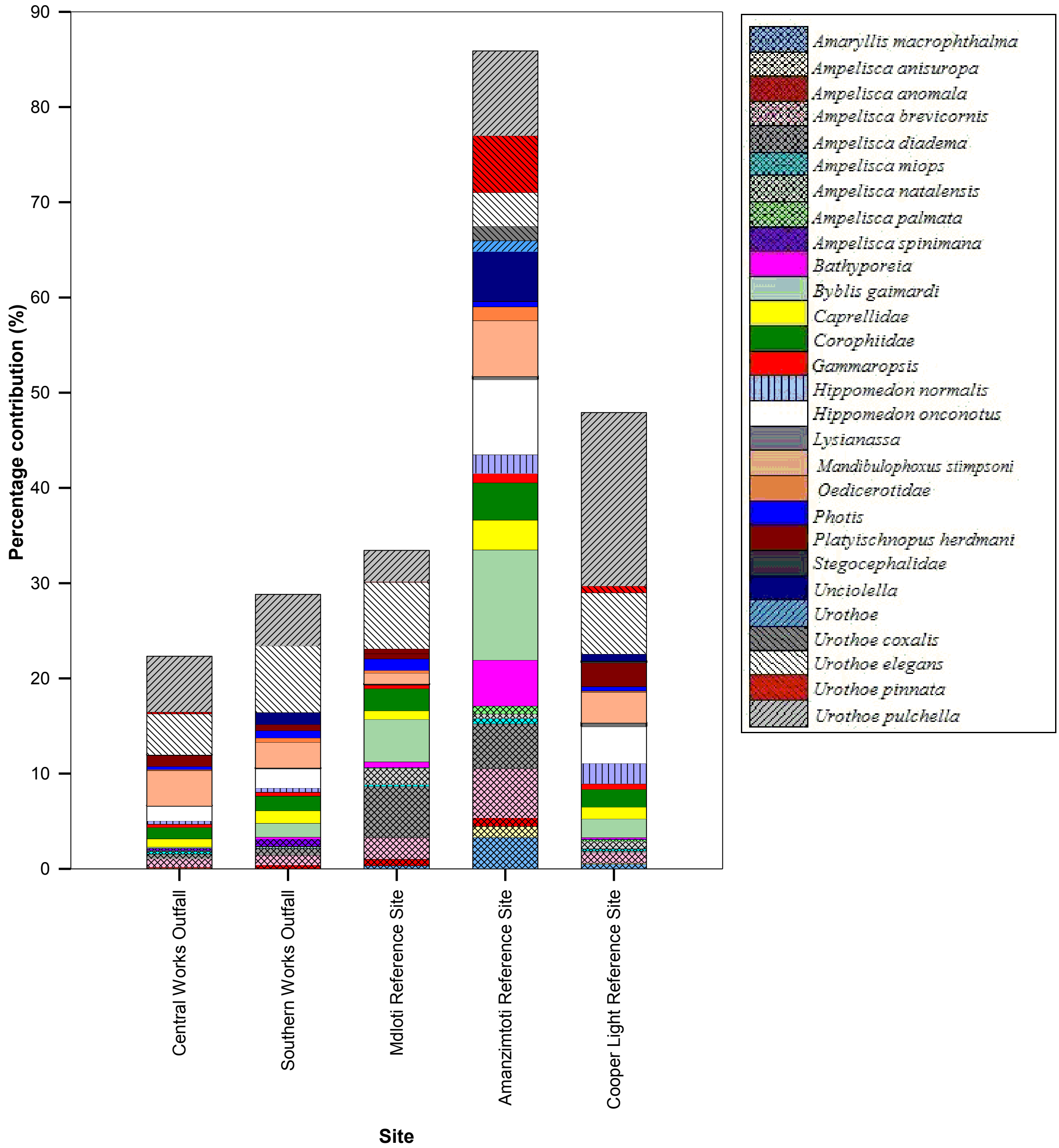

Figure 12. Cumulative percentage contributions of amphipods for the Central Works Outfall, Southern Works Outfall, Mdloti reference site, Amanzimtoti reference site and Cooper Light reference site as identified through a SIMPER analysis. Species percent contribution cut-off was set at 3 %.

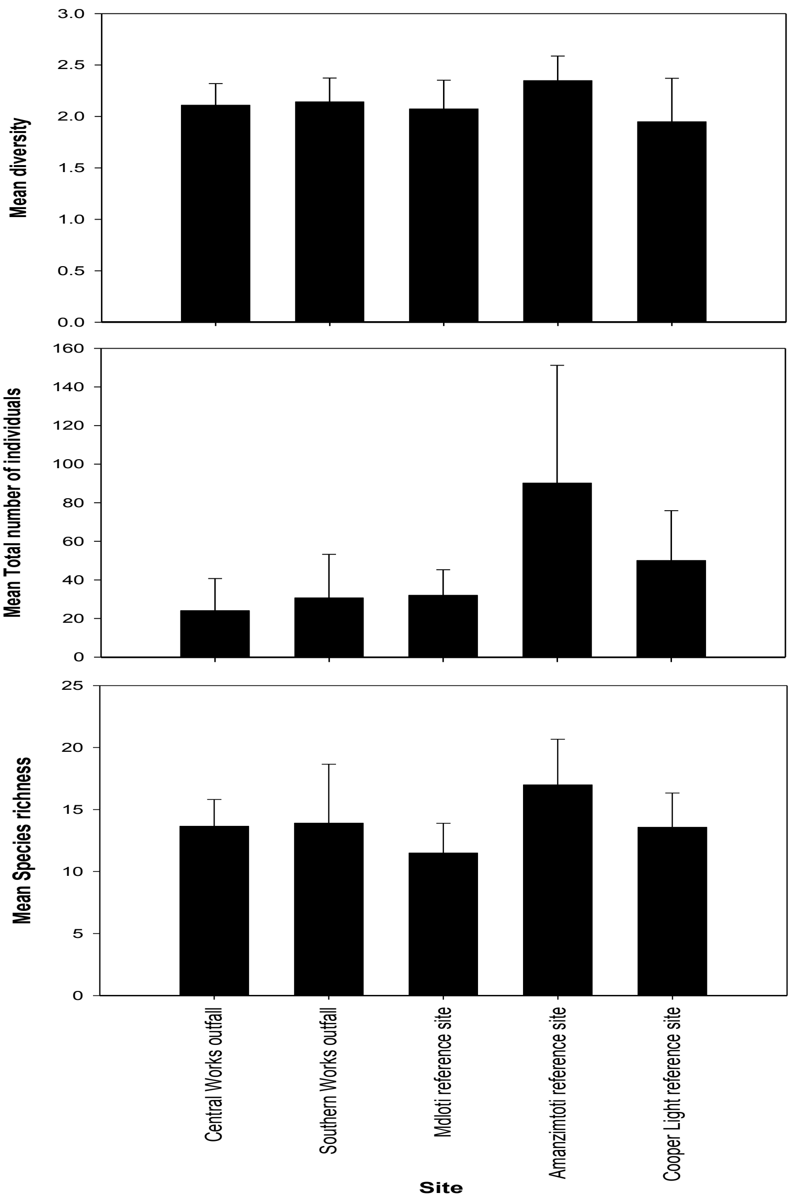

Figure 13. Variation in the (a) diversity, (b) total number of individuals and (c) species richness of amphipods in the Central Works Outfall, Southern Works Outfall, Mdloti reference site, Amanzimtoti reference site and Copper Light reference site. Bars and error bars represent means and are standard deviations of the mean.

A total of 65 Amphipod species was found across all sites. However, a SIMPER analysis identified 28 species contributing 3% or more to the dataset. All sites are dominated by species of *Ampelisca* and *Urothoe* (Figure 12) and no species was found solely in one site. The Amanzimtoti reference site has the highest species richness, total number of individuals and diversity of all the sites (Figure 13). The two outfalls and the Mdloti reference site have a very low number of individuals compared with Amanzimtoti and Cooper Light reference sites. Mdloti was found to have elevated metal concentrations (Figure 8) exhibiting similar diversity patterns as the impacted sites.

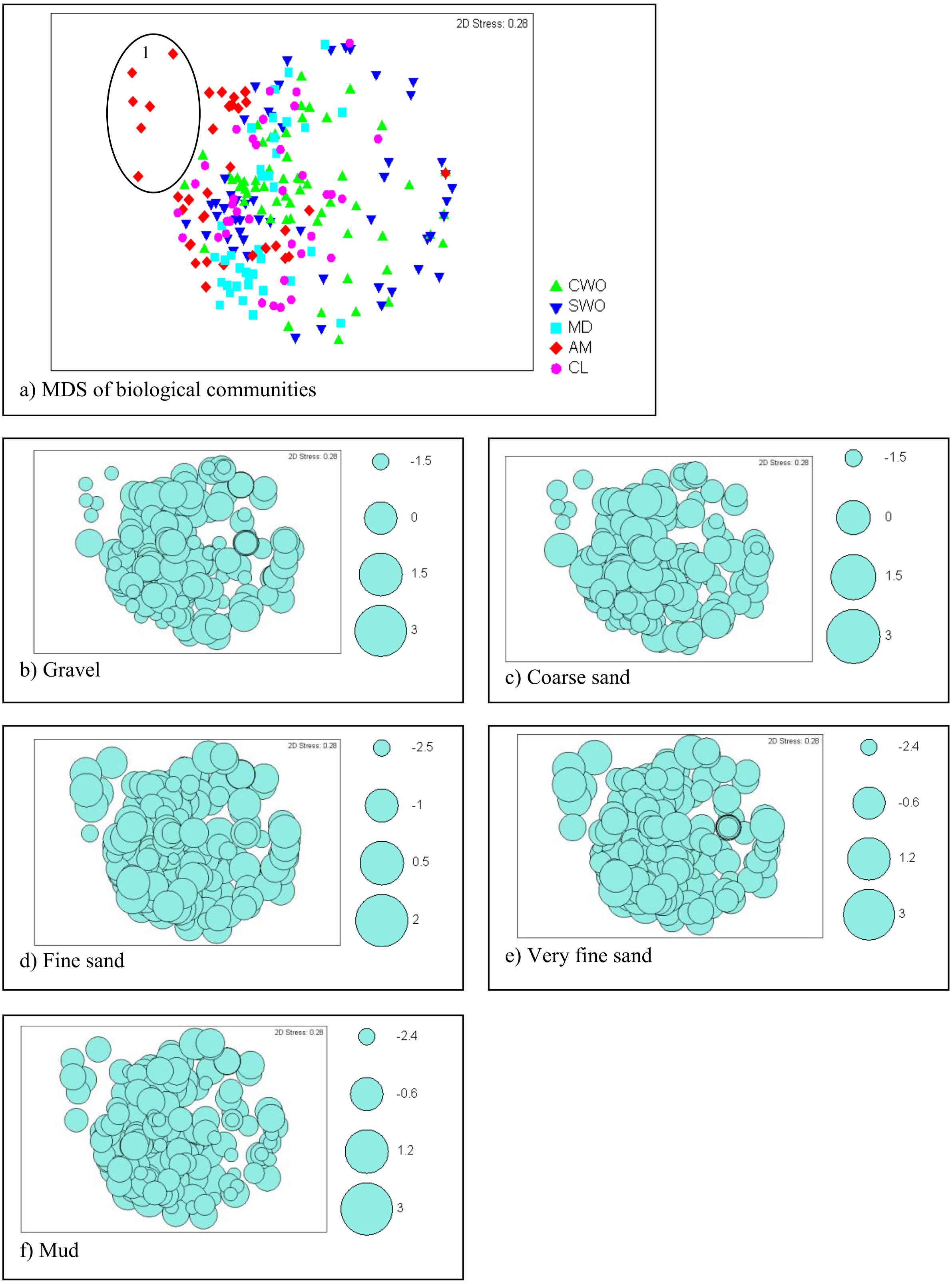

Figure 14. a) MDS of species abundances at the five sites. Turquoise bubble plots represent grain sizes (b) gravel, c) coarse sand, d) fine sand, e) very fine sand, f) mud). The grain sizes represented in these bubble plots were identified as driving factors of the communities in the BIOENV analysis. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

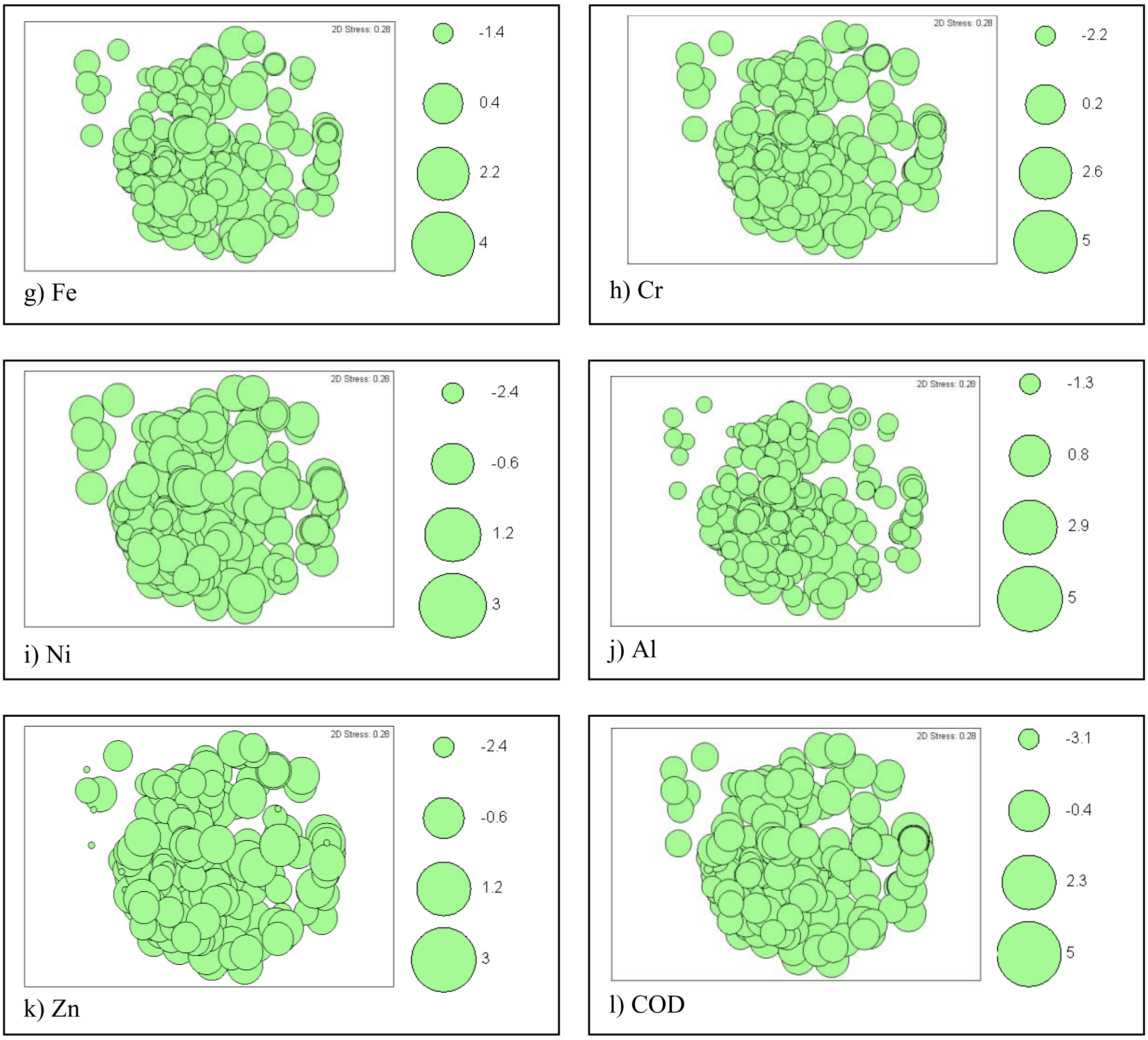

Figure 14 continued. Green bubble plots represent metals (g) Fe, h) Cr, i) Ni, j) Al), k) Zn and l) COD). These variables were superimposed on the species abundance MDS (a). The environmental variables represented in these bubble plots were identified as driving factors of the communities in the BIOENV analysis. CWO = Central Works Outfall, SWO = Southern Works Outfall, MD = Mdloti, AM = Amanzimtoti, CL = Copper Light.

BIOENV results identified gravel, coarse sand, fine sand, very fine sand, mud, Zn, Fe, Cr, Ni, Al and COD as the environmental factors driving the observed communities with ρ = 0.820. This value shows a strong correlation between these variables and the biotic data. PCAs (Figure 4 and Figure 9) both identify the same grain sizes and environmental variables as driving factors in community structure. Bubble plots representing the BIOENV results were superimposed on the biological MDS (Figure 14). Group 1 on the MDS (Figure 14) appears to be driven by fine sand, very fine sand, mud and Ni (larger bubble sizes compared with those for the other variables). Central Works Outfall, Southern Works Outfall, Mdloti and Cooper Light seem to be driven by all the variables except mud, Cr and Al. The community structure represented by the

MDS does not show any distinct grouping of sites apart from those stations in Amanzimtoti that pulled away from the central clump. This is probably due to the presence of the finer grain sizes. These finer grain sizes could also be linked to Amanzimtoti exhibiting higher species richness, diversity and density compared with the other sites (Figures 12 – 13). It is the common environmental drivers identified in the BIOENV analysis together with the bubble plots that are responsible for the general lack of community grouping.

4. Discussion

Marine pollution is continuously increasing due to elevated human activity such as human settlement, resource use, infrastructural development, construction, agricultural activities, urbanisation and tourism (Islam & Tanaka, 2004). Due to this anthropogenic input of chemicals, studies of long-term effects on the marine environment are imperative. The availability of long term datasets is invaluable in monitoring and controlling marine pollution. This study in particular focuses on the ability of different sediment grain sizes to accumulate metals and organic pollution, and its effects on marine benthic communities.

4.1. Sediment grain size and metals

Sediments provide a temporally and spatially integrated indication of the pollution in an area. However, due to naturally occurring metals in sediment, it can be difficult to determine whether metal concentrations identified are as a result of anthropogenic sources. The natural ranges of metals in the ocean are shown in Table 4 together with the values found in this study. It can be seen that some metals were found in higher concentrations at the impacted and reference sites in comparison to what is expected to be found naturally. This shows that it is not only the impacted sites that are accumulating metals and that the possibility of general pollution effects along the KZN coast does exist.

The accumulation of effluents on the seafloor is dependant on several natural and anthropogenic factors. These include the proportion of fine-grained sediment (primarily mud) naturally present on the seafloor, the type and concentration of contaminants in the effluent, and the dispersal/deposition of effluent particulate matter (Schropp *et al*., 1990; Newman & Mudaly, 2008; Cuclic *et al*., 2009). Numerous studies have shown the relationship between sediment grain size and metal accumulation. Lin *et al.* (2002) showed that grain size was a controlling factor in the spatial variations in metal distribution. Increases in the percentage of fine-grained sediments showed an increase in aluminium, iron, manganese, zinc, copper and lead concentrations. Similarly, a study on coastal embayment sediments off the coast of Spain by

Rubio *et al*. (2000) found aluminium, copper, iron and zinc to be almost seven times higher, lead and chromium almost four times higher, and nickel, cobalt and arsenic almost double the concentration in muddy compared to sandy sediments. Cadmium however, was found to have the same average concentration in both sediment types. This affinity of metals towards fine-grained sediments is partly due to the high specific surface of the smaller particles resulting in enrichment due to surface adsorption and ionic attraction. Organic matter coatings are also common in fine-grained sediments, binding to various trace elements (Plumb, 1981; Power & Chapman, 1995; Rubio *et al.,* 2000; Hartwell & Hameedi, 2007). The discharge of effluents has been shown by Gray (1997) to alter the grain size distribution of sediment on the seafloor around outfalls. This is due to the addition of fine organic matter in the area of deposition, and this is likely to result in altered patterns of metal distribution in outfall-affected sediments.

Table 4. Natural ranges of metals found in this study as well as actual ranges found at each study site. The grain sizes found in this study per site is also shown.

Metal	Natural range (µg/g)	Central Works Outfall (µg/g) Coarse sediment	Southern Works Outfall (µg/g) Fine sediments	Mdloti reference site (µg/g) Coarse sediment	Cooper Light reference site (µg/g) Mixed sediments	Amanzimtoti reference site (µg/g) Fine sediments
Mercury [a]	0.04 – 0.15	0.00 – 0.1	0.00 – 0.34*	0.00 – 0.12	0.00 – 0.15	0.00 – 0.37*
Copper [a]	2.8 – 31	0.71 – 28.9	0.01 – 10.8	0.02 – 6.28	0.02 – 6.47	0.02 – 1.89
Cadmium [a]	0.1 – 1.4	0.02 – 0.92	0.02 – 2.54*	0.02 – 0.51	0.02 – 0.91	0.02 – 2.29*
Lead [a]	2.7 – 12	2.37 – 24.5*	0.02 – 31.1*	5.13 – 15.5*	0.34 – 6.69	1.59 – 9.87
Zinc [a]	9.8 – 62	0.02 – 46.9	0.47 – 38.3	1.98 – 42.8	0.02 – 35.7	0.02 – 32.6
Iron [b]	0.76 – 1.38	3.22 – 15.6	3.17 – 7.30	6.42 – 11.6	2.4 – 5.91	2.05 – 4.89
Chromium [a]	6.5 – 43	7.38 – 48.3*	7.20 – 22.4	13.2 – 29.9	4.23 – 13.9	4.37 – 14.2
Nickel [a]	1.6 – 35	0.02 – 15.5	0.02 – 12.7	2.91 – 14.2	0.1 – 5.01	0.57 – 4.87
Aluminium [c]	0.005 – 0.0055	1.85 – 23.9*	1.09 – 4.21*	1.11 – 3.38*	1.11 – 3.99*	0.88 – 3.4*

[a] Parsons & Connell, 2000
[b] Johnson *et al.*, 1997
[c] Hydes & Liss, 1977
[*] Values in the study higher than the natural metal values

In the present study, a pattern of finer sediments is visible in the Southern Works Outfall and the Amanzimtoti reference site. Central Works Outfall and the Mdloti reference site exhibited dominance by coarser sediments (refer to Figure 4). These grain size patterns are verified by the MDS analyses (refer to Figure 5). The Mdloti river system tends to have coarser sediment grain sizes as well, thereby showing the possible influence of the river on the actual reference site

(Forbes & Demetriades, 2008). The Cooper Light reference site is a mixture of both grain sizes. Since muddy sediment is suitable for pollutant accumulation it would be expected that the Southern Works and Amanzimtoti sites should exhibit the highest metal concentrations. However, contrary to this expectation, it is Mdloti that seems to be most influenced by the different metals present. Mdloti shows elevated levels of lead, zinc, iron, chromium, nickel and aluminium. Possible reasons for this elevation at Mdloti could be due to flooding, extreme dry or wet years or even river run-off. However the likelihood of such stochastic events, which occur infrequently resulting in the distinct patterns found in Mdloti, can be questioned. The elevated metals, however, may not be linked to anthropogenic sources and may have originated naturally through the weathering of rocks and precipitation (Cuculic *et al.,* 2009). A combination of these factors could have resulted in the Mdloti reference site having the highest metal concentration from all the sites tested. The movement of the metals from Mdloti to the other sites would be minimal due to the location of Mdloti being far away from the remaining sites. The State estuarine ranking system, which is based on health and functionality of the different systems, has the following classifications: excellent, good, fair, poor and highly degraded (Forbes & Demetriades, 2008). The Mdloti sites are located near the heavily polluted Mdloti river mouth which is a source of anthropogenic influences. The overall health status of the Mdloti system is rated to be poor with habitat loss, eutrophication, freshwater diversions, sewage, sea level rise and chemical contamination being threats to the system (Forbes & Demetriades, 2008). The Mgeni and Umhlanga rivers (refer to Figure 1) could have also resulted in the entire region, which included Mdloti reference site, to be polluted above the naturally expected range. The Umhlanga river overall health status is poor with habitat loss, eutrophication, freshwater diversions, sea level rise and sewage threatening the environment. The Mgeni river system has been given the status highly degraded with habitat loss, eutrophication, freshwater diversions, sewage, sea level rise, chemical contamination, litter and overexploitation all being major threats to this system (Forbes & Demetriades, 2008). Generally, the KZN coast is heavily polluted and therefore no proper reference site can be identified. Since the choice of reference sites has been detemined by the CSIR at the inception of the monitoring programme, it could not be changed for this study, which had to proceed according to the sampling design initially implemented.

The two outfalls have higher concentrations of all metals in comparison to the Amanzimtoti and Cooper Light reference sites. Again, the expected pattern when considering grain sizes is that Southern Works and Amanzimtoti should accumulate the most metals due to the dominance of fine grained sediments at these sites. The lack of this pattern in Amanzimtoti may be attributed to the lack of anthropogenic influence and confirmation of its use as a reference site. However, when compared to Mdloti and the Central Works Outfall, the Southern Works Outfall has lower metal concentrations which could be due to the actual treatment of the effluent, rapid dilution of the effluent once it is pumped to sea or the dynamics of the KwaZulu-Natal coastline. The Agulhas Current, and its interaction with the shelf along the KwaZulu-Natal coast, plays a role in the distribution of the effluent, thereby possibly preventing the settlement of effluent in and around the outfall deposition zone (McClurg, 1988). The Agulhas Current comes closer inshore here as well compared with Mdloti where the shelf is wider. This region is also characterised by the Durban return eddy, which moves water from the Scotborough region back up onto the Natal Bight (Smit AJ, March 2010, pers. comm.[2]). However, it must be noted that not much is known as yet about the role that the Agulhas Current on sediment redistribution and pollution accumulation in the KZN benthic regions.

4.2. Total Kjeldahl Nitrogen (TKN) and Chemical Oxygen Demand (COD)

TKN is a measure of the sum of dissolved inorganic nitrogen and digestible organic nitrogen in the sediment. According to Alloway & Ayres (1997), TKN levels in marine sediments should be approximately 300 mg l^{-1}, but values ranging from 0 – 2222 mg l^{-1} have been reported (Darwish *et al.,* 2005; Gawad *et al.,* 2008). High TKN concentrations in the Sydney Harbour have been attributed to anthropogenic human activities and sewage overflows (Birch *et al.,* 1999), and in some instances TKN values at this locality were up to 45 times the acceptable Australian guidelines level. Savage *et al.* (2004) found that coastal ecosystems removed ~25% of nitrogen via permanent burial in sediments. Nitrogen removal via denitrification can range between 21 and 30% in coastal waters, while denitrification in estuaries can remove between 40 and 50% of the nitrogen entering as dissolved inorganic nitrogen (Savage *et al*., 2004). This can result in decreased exportation of nitrogen to the ocean (Gardner *et al*., 1987; Cabrita & Brotas, 2000). Levels of TKN in the current study ranged from approximately 35 – 550mg l^{-1}. Patterns found between sites were not very strong. However, Mdloti did once again show a slightly higher average TKN compared with other sites. The definite reason for this increase is unknown, but it could be due to increased nutrients in the sediment via anthropogenic sources. River inputs (as discussed previously) to the Mdloti site could be the contributing factor to the elevated TKN values due to the increased usage of artificial nitrogen fertilisers being used (Gruber & Galloway, 2008). Rivers have large influences on the coastal environments due to their input onto the coast.

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This widespread introduction of anthropogenic substances causes great difficulties for pollution studies like this, as pinpointing a specific source of an impact becomes challenging. In addition, reference sites that are closely located to rivers become less ideal in their use as unimpacted sites due to the possibility of anthropogenic influences form the rivers.

A study by Kenis *et al.* (1972) showed that ocean mixing is sufficiently strong to prevent oxygen depletion due to sewage that is dumped in the ocean. In addition, during the treatment of this sewage, anaerobic treatment can reduce chemical oxygen demand (COD) by 90% (Barker *et al.,* 1999). COD levels in the present study ranged from 0.01 $mg.l^{-1}$ to 4.53 $mg.l^{-1}$. The Mdloti reference site exhibited the highest COD values with the Central Works and Southern Works Outfalls again being lower than Mdloti but higher than Amanzimtoti and Cooper Light. Significant differences were found between Mdloti and Amanzimtoti, Mdloti and Cooper Light, Southern Works and Amanzimtoti, Southern Works and Cooper Light, Central Works and Amanzimtoti, Central Works and Cooper Light based on COD (ANOSIM, global $R = 0.353$, $p < 0.001$). There were no significant differences found between Mdloti and Southern Works and Central Works. The patterns observed in Mdloti were usually similar to that of impacted sites, if not often with higher concentrations of chemicals than the impacted sites. The MDS plot (Figure 11) confirms the patterns explained above with the dominance of COD being in the Mdloti reference site. High levels of COD generally suggest an impact associated with effluent discharge. This possible influence of effluent in impacted sites could be the reason for the high COD levels identified. However, this does not explain the elevated levels of COD in the Mdloti reference site. The Mdloti site is the furthest away from all the other sites, and therefore possible influence from the impacted sites was unlikely to occur only at the Mdloti reference site. Possible impacts on the Mdloti reference site that could result in increased COD are breaching, sewage from Umhlanga or the movement of low-oxygen bottom-water to the shelf.

4.3. Amphipod community structure and composition

An important part of a pollution study is to determine whether ecological impacts occur. Benthic macrofauna often fill this role of determining the possible repercussions of pollution around marine outfalls (McClurg, 2008). The diversity of amphipods on the KwaZulu-Natal coast is high and is structured by numerous natural factors. Sediment and water conditions, as well as biotic factors such as predation, competition and the availability of food influence the communities (McClurg, 2008). According to Haring (2005), benthic organisms are driven more by natural sources that anthropogenic ones. Therefore, in order to identify possible anthropogenic influences it is important to have reference sites against which comparisons can be made. A wide range of different amphipod species were found in this study with a distinct dominance by the families Ampeliscidae (9 different species) and Urothoidae (5 different species). The reference sites do show a higher percentage contribution of the dominant species when compared with the impacted sites. *Byblis gaimardi*, Caprellidae and *Hippomedon oncontus* make up relatively large percentage contributions at all sites.

The family Ampeliscidae, comprised mostly of detritus-feeding species, is one of the more diversified amphipod families in the ocean (Dauvin & Bellan-Santini, 1996; Griffiths, 1976). This family is widely distributed with species inhabiting from the intertidal to abyssal zone with a preference to mud and fine sand sediments. They form an important part of soft-bottom amphipods worldwide and construct tubes in fine sands and mud. They gather organic materials from the sediment surface of filter feed from the water column (Griffiths, 1976). The genus *Ampelisca* tend to be shallow water inhabitants while *Byblis* is generally found in deep waters (Dauvin & Bellan-Santini, 1996). Urothoids are marine gammaridean amphipods that inhabit shallow waters and are scarce in deep sea habitats (Jaume & Sorbe, 2001). They form an important part of burrowing fauna of intertidal and shallow subtidal sands. *Urothoe* species are well adapted to burrowing (Griffiths, 1976). Due to both *Ampelisca* sp. and *Urothoe* sp. having preferences to shallow water it was unexpected for these groups to be so dominant and have a high percentage contribution in a deep sea study.

Amphipods are benthic fauna and would therefore be influenced by physical and chemical sediment characteristics. Sediment grain size would be expected to structure amphipod communities with certain grain sizes being preferred over others due to differences in their burrowing ability and food availability (Sanders, 1958; Biernbaum, 1979). In addition, since sediment is a sink for contaminants, and grain size plays an important role in contaminant accumulation, these factors would be expected to affect benthic community structure (Reynoldson, 1987).

Sediment heterogeneity and silt and clay fractions have been found to positively influence benthic community structure and tend to exhibit higher diversity in comparison with sediment homogeneity (Grebmeier *et al*., 1989; Mancinelli *et al.,* 1998; Ellingsen, 2002). The type of substratum may influence the distribution of some amphipods which have a preference to mud or muddy-sand (Meadows, 1964). Meadows (1964) found that sediment grain size may influence the distribution of *Corophium volutator* due to their preference of mud and muddy sand compared to coarse sand. Marques & Bellan-Santini (1993) found high amphipod species diversity in medium to fine sand regions, suggesting that substrate grain size may be a controlling factor in the biodiversity and development of large amphipod communities. These sediment particle size distributions may also play a significant role in determining the nutrient quality and quantity for amphipods. In the present study, Amanzimtoti exhibits the highest percentage contribution of amphipods, mean diversity, total number of individuals and species richness than any of the other sites. Amanzimtoti also exhibited the lowest values for metals, TKN and COD. These low concentrations of environmental variables may be attributable to the high number of amphipods found at this location. Mdloti, with its high metal concentrations, does show lower amphipod diversity indices; however, these are not greatly different from both impacted sites. The MDS in Figure 14 shows these patterns well, with Amanzimtoti forming a distinct group away from the remaining sites and being driven by fine grain sizes.

The dominant species in Marques & Bellan-Santini's (1993) study were *Ampelisca* spp., which tended to have preferences for sandy sediments. However, in this study *Ampelisca* spp. were found across all sites with the highest percentage contribution being Amanzimtoti which was dominated by fine sediment grain fractions. This lack of similarity with Marques & Bellan-Santini's (1993) study maybe explained by Bat & Raffaelli (1998), who state that amphipods are useful in bioassays and sediment toxicity tests due to their low sensitivity to natural variability in sediments such as grain size and organic content. The surface deposit-feeding ampeliscid amphipod, *Byblis gaimardi*, has been found to dominate course grained sediment environments in the northeastern Chukchi Sea. This however contrasts findings carried out in the Bering Sea where ampeliscid amphipods were not common in the sandy-gravel sediment substrate (Feder *et al.,* 1994). The amphipod communities present either prefer certain sediments and therefore have inhabited these areas for that reason or do not have preferences and therefore are able to inhabit any sediment type.

Metal accumulation is one of the factors that can be responsible for the alteration of benthic communities. Some amphipods have been found to be useful indicators of metals due to the accumulation capacities of these metals from their food and solution. Some species are indicators of zinc and copper in particular, accumulating these metals through food and water (Rainbow, 1995a). In a study by Bat *et al.* (1998), the amphipod *Corophium* sp. has shown a decrease in survival rate and burrowing activity with increased sediment metal concentrations. More *Corophium* sp. individuals chose to burrow in clean sediment than metal contaminated sediment. However, some individuals did burrow in low metal contaminated sediment. This was attributed by Bat *et al.* (1998) to be due to either the possible tolerance of some individuals to low metal concentrations, or a reduced toxicity of metals in sediment due to oxidation by constant aeration, or that some *Corophium* sp. individuals were forced to inhabit metal contaminated sediment due to clean sediment being fully inhabited by majority of the *Corophium* sp. population. Ultimately, the effect of metals on community structure is important to identify. Studies have shown that a correlation between increasing concentrations of metals in sediment and decreasing species numbers and an altered abundance and the composition fauna exists (Philips & Rainbow, 1994; Warwick, 2001; Balthis *et al.,* 2002; Marsden & Rainbow, 2004). The results of this study do not show any clear link between metal effects and amphipod communities. As can be seen in Figure 12, all sites except for Amanzimtoti show similar patterns of community distribution irrespective of whether some metals were higher in concentration than others. This lack in pattern between metals and communities is possible due to a lack of metal effects on these communities. Although metals are identified, their presence is probably at a concentration at which amphipods can survive and if not, flourish.

Metal bioaccumulation is dependent on and driven by many different internal and external factors. Marsden & Rainbow (2004) extensively described amphipod metal uptake, accumulation, survival and community structure effects. They found that there are numerous internal and external factors that affect bioaccumulation in amphipods, and that metal uptake rates can vary considerably. Internal factors include individual variability (accounted for by physiological processes such as moulting), body size, gender, breeding condition, brooding, moulting, growth and behaviour. External factors affecting bioaccumulation are dissolved metal concentration, physicochemistry of metal absorption routes across membranes, dissolved oxygen, metal interactions, sediment, food, seasonal effects, geographical differences and adaptation. Infaunal amphipods are in direct contact with sediment, and hence sediment grain size, organic content and the presence of metals are important in the bioavailability of trace metals (Marsden & Rainbow, 2004). The bioavailability of metals that are ingested in sediment by burrowing amphipods is affected by organic and inorganic sediment constituents. This can affect the binding affinities for the metals in the food. Interaction between different metals that may share biological uptake pathways due to similar chemistries can also affect the accumulation of those metals. The presence of two such metals together can either increase or decrease their bioaccumulation. Exposure of amphipods to metals via sediment occurs through the release of metals from sediment into interstitial or burrows water. Bioavailability of metals in sediments is affected by the amount of metal present, as well as the relative strength of metal binding in the sediment and the organism's digestive processes (Marsden & Rainbow, 2004). Sediment grain size, together with sediment organic content, the presence of other metals and environmental conditions, affects metal binding. The burrowing of infaunal amphipods can increase surface sediment oxygen levels and redistribute metals. It has been found that species that have been exposed to increased metal concentrations over years may have evolved biochemical and physiological mechanisms that aid in the reduction of effect of these toxins (Marsden & Rainbow, 2004). It is thought that this could be selected for in brooding amphipods with limited mobility.

Note that metal accumulation is naturally driven by sediment grain size; however, in this study it was the Mdloti reference site which was dominated by coarse sediments that showed the highest concentrations of metals. Studies have shown that increased sediment metal contamination correlates with a decreased number of benthic species, and highly contaminated sediments have shown a decrease in certain species (Bryan, 1971; Bat & Raffaelli, 1998; Beltman *et al.,* 1999; Thompson *et al.,* 1999; Dauvin, 2007). However, it has been difficult to attribute these changes solely to metal contamination. There are studies in which distinct benthic communities occur at impacted sites (Marsden & Rainbow, 2004); however, in this study, communities at the references and impacted sites did not differ significantly although higher concentrations of metals were identified in the Mdloti reference site. Following on from that, amphipod community composition can be driven by metal contamination and natural increases in metals, COD and TKN from natural aerosols, river run-off, etc could have occurred which resulted in the patterns identified at Mdloti. Figure 12 shows the percentage contribution of amphipods at each site. The species composition at both impacted sites and Mdloti are very similar to each other while Amanzimtoti and Cooper Light exhibit similar communities. The impacted sites and Mdloti do not have *Urothoe coxalis* present at all. Table 5 summarises the species that were present and absent at each site based in the SIMPER results. The two outfalls combined have 10 amphipod species that are absent in those communities when compared with the reference sites. Of the 10 species, 6 are absent only at the impacted sites. The three reference sites lack between 3 – 4 species in total from the overall list. The reference sites also have species that are lacking solely from either impacted site. However, the lack or presence of species at both impacted and reference sites cannot be attributed only to pollution exposure, or lack thereof. The communities could be driven by many other factors such as the sediment type, bathymetry and benthic rugosity, presence of food, and/or competition.

It is clear from the literature that sediment grain size alone does not necessarily have a significant impact on benthic communities. Relationships between factors like COD, TKN, organic matter content, grain size and sediment accumulation together contribute to differences found in communities (Wu & Shin, 1997; Mancinelli *et al.*, 1998). It is these interactions between variables that result in the difficulty often experienced in identifying specific drivers of pollution impacts and effects on communities.

Table 5. Presence and absence of all species identified by the SIMPER analysis for each impacted and reference site. X – Species not present; √ - Species present.

	Central Works	Southern Works	Mdloti	Amanzimtoti	Cooper Light
Amaryllis macrophthalma	X	X	√	√	√
Ampelisca anisuropa	X	X	√	√	√
Ampelisca anomala	√	√	√	√	X
Ampelisca brevicornis	√	√	√	√	√
Ampelisca diadema	√	√	√	√	√
Ampelisca miops	√	X	X	√	√
Ampelisca natalensis	X	√	√	√	√
Ampelisca palmata	X	X	√	√	√
Ampelisca spinimana	√	√	X	X	X
Bathyporeia	√	√	√	√	√
Byblis gaimardi	X	√	√	√	√
Caprellidae	√	√	√	√	√
Corophiidae	√	√	√	√	√
Gammaropsis	√	√	√	√	√
Hippomedon normalis	√	√	√	√	√
Hippomedon oncontus	√	√	X	√	√
Lysianassa	√	X	X	√	√
Mandibulophoxus stimpsoni	√	√	√	√	√
Oedicerotidae	√	√	√	√	√
Photis	√	√	√	X	√
Platyischnopus herdmani	√	√	√	X	√
Stegocephalidae	X	X	√	√	√
Unciolella	√	√	√	√	√
Urothoe	X	X	√	√	X
Urothoe coxalis	X	X	√	√	X
Urothoe elegans	√	√	√	√	√
Urothoe pinnata	√	√	√	√	√
Urothoe pulchella	√	√	√	√	√

5. Conclusion

The primary aim of this study was to determine whether sediment grain size influences the amphipod community structure along the KwaZulu-Natal coast together with looking at potential impacts of two wastewater outfalls on these benthic communities. The results were contradictory when compared with previous studies carried out on the role of grain size on community structure and pollutant accumulation. The sites that were dominated by fine grain sediments were not those that exhibited the highest concentrations of metals. It was Mdloti, which showed dominance by coarse sediments that had the highest metals, TKN and COD levels. In terms of community structure, Amanzimtoti had the highest diversity indices levels and percentage contributions. Sites with the same grain sizes exhibited similar community structures thereby highlighting the role that grain size plays in benthic communities.

The use of amphipod communities in this study was identified to determine patterns and possible impacts of pollution. Although communities did not seem to be affected by the outfalls, differences in communities were observed. Further studies can be carried out in order to identify the specific roles amphipods play at these sites and their use as bioindicators of the anthropogenic influences from the outfalls.

An important point that was highlighted in this study is the difficulty in the accessibility of suitable reference (unimpacted) sites. In this study the use on Mdloti as a reference site was questioned. The influence of rivers on the integrity of reference sites was identified. Although the Mdloti reference site was positioned away from the impacted sites, the anthropogenic impact from rivers would result in an impact being identified. Generally it appears that although there are obvious differences among sites in terms of community structure, there does not seem to be much support for the environment in shaping the patterns observed.

The outcomes of the null hypotheses of this study were as follows:

Abiotic data:

A. Sediment grain size is homogenously distributed across the study area: rejected.

B. The concentrations of selected metals and TKN and COD in the sediment are homogenously distributed across the study area: rejected.

C. The spatial patterns of sedimentary metal concentration, TKN and COD are not modified by sediment grain size distribution: rejected.

Biotic data:

D. Amphipod community structure is not influenced by i) sediment grain size distribution, ii) metal concentration, iii) TKN, and/or iv) COD: accepted.

The lack of information specific to KwaZulu-Natal in terms of sediment grain size, its role in pollutant accumulation and the effects of marine pollution on amphipod communities was highlighted during this study. This lack of specific information particularly in recent years resulted in references often being made to older studies. There is a need for studies on amphipods in particular, considering their widespread use as bioindicators worldwide. This would allow more informed decisions to be made in terms of the requirements and restrictions of effluent discharge thereby minimising possible effects on the environment.

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