Sediment as a Physical Water Quality Stressor on Macro-Invertebrates: A Contribution to the Development of a Water Quality Guideline for Suspended Solids

Report to the
Water Research Commission

by

AK Gordon, J Niedballa and GC Palmer

WRC Report No. 2040/1/13
ISBN 978-1-4312-0456-4

August 2013

Obtainable from
Water Research Commission
Private Bag X03
GEZINA 0031

orders@wrc.org.za or download from www.wrc.org.za

EXECUTIVE SUMMARY

BACKGROUND AND RATIONALE

Although the ecological consequences of high sediment loads in rivers have been long recognised, appropriate methods of managing instream particulates based on biological effects data have been problematic to develop. Exposure-response relationships derived from unspecified natural sediments have possessed unacceptably high variability making them unreliable predictors of biological effects. Recently, there has been recognition that specific characteristics of particulates can affect the severity of biological effects (e.g. particle size, shape, geochemical composition) and, consequently, that the exposure-response relationships used to produce water quality guidelines will have to be more situation-specific, aimed at a restricted combination of the above mentioned particle characteristics (see Chapter 2 for a literature review).

The Department of Water Affairs has undertaken to revise the South African water quality guidelines for fresh waters, and update the current guideline for instream particulates with one based on biological effects. Research undertaken in this report represents the start of work toward providing appropriate biological response data in this regard, and the beginning of investigations into improving the management of this aquatic ecosystem stressor.

AIMS

The aims of the project (and the chapter in which they are addressed) are presented below:

1. Establish the most appropriate sediment test material for exposure trials – **Chapter 2**
2. Test the effects of suspended particulates on selected macroinvertebrates at different levels of biological organization – **Chapter 3**
3. Generate an exposure-response relationship framework from data generated in Aim 2 and relevant data in the literature – **Chapter 4**
4. Attempt to relate exposure-response data developed in the laboratory to natural conditions in the field – **Chapter 5**
5. Develop and apply a detailed site-specific suspended solids risk assessment protocol with different levels of complexity – **Chapter 5**
6. Identify further research that will lead to the development of an effective and applicable guideline for instream particulates – **Chapter 6**

RESULTS AND DISCUSSION

AIM 1

Chapter 2 presents the rationale that an exposure-response relationship cannot be derived from a universal sediment test material for application over a wide geographical area. Furthermore, there is a discussion regarding the need for more specific guidelines based on biological responses to specific ranges of particle size, shape and geochemical composition.

AIM 2

There were several technical challenges encountered when undertaking stress-response experiments with suspended kaolin particles and indigenous macroinvertebrates: 1) maintaining particulates in suspension, and keeping the concentrations of those suspensions constant across replicates; and 2) the difficulty of observing responses of organisms in turbid treatments limited the type of biological endpoints that could be measured and how often measurements could take place.

All organisms exposed appeared to be very tolerant of the suspended kaolin, especially in terms of mortality which limited the generation of statistical point-estimates of effect. The most useful biological response measured was that of reproduction in shrimp *Caridina nilotica*. At a population endpoint level, however, the results were contradictory. Although there were fewer gravid females at 680 mg/L, the number of juveniles produced from this treatment were not statistically different from any other treatment. Furthermore, growth was not found to be a useful biological endpoint in *C. nilotica* as these organisms appeared to exhibit density dependent growth, growing larger at higher stress exposures as more individuals in the exposure vessel succumbed to mortality. An attempt was made to link mayfly kaolin exposure to a physiological response by linking damage to gills (either from abrasion or clogging) to two hypoxia biomarkers (lipid peroxidation and catalase activity). Unfortunately, a method could not be developed for viewing the mayfly gills under scanning electron microscopy (the gills were always damaged during the critical point drying process), and the biochemical responses exhibited no statistical exposure-response relationship. An important finding of these experiments was, however, that the biological consequences of settled particulates are much more pronounced than those of suspended solids.

AIM 3

Biological effects data were collated from an extensive search of the international scientific literature, with some additional indigenous South African data generated during the course of this project. Most of lethal point-estimate data were for marine species which appeared to be extremely tolerant of suspended kaolin particles. Nevertheless these data were applied to a species sensitivity distribution (SSD). There were very few studies reporting sub-lethal endpoints in the form of no observed effect concentration (NOEC) or sublethal point-estimate (ECx) data. Nevertheless, the available six NOECs and one EC25 data point (of varying exposure periods) were also applied to an SSD.

In the absence of sufficient traditional exposure-response data (point-estimates or NOECs) to derive reliable water quality guidelines for suspended solids, a number of attempts have been made by researchers to characterise the exposure-response relationship for freshwater and estuarine fishes as empirical models composed of three variables: suspended particulate concentration; duration of exposure; and severity-of-effect. The data collated as part of this project were applied to this type of exposure-response relationship too.

The exposure-response relationships generated for kaolin suggest that sub-lethal effects at concentrations below 30 mg/L, and lethal effects at concentrations below 55 mg/L, over extended periods of exposure are unlikely to occur for all but the most sensitive species. In terms of short-term exposures (< 24 hours), the freshwater macroinvertebrates tested were very tolerant (no mortalities at the highest concentration tested of 801 mg/L), but marine fish larvae were much more sensitive (mortality occurring at 30 mg/L in one species, but generally mortality occurring in remaining species tested when exposure concentrations exceeded 320 mg/L).

AIM 4 and 5

A suspended solids risk assessment framework was developed with the purpose of determining if instream suspended particulate matter concentrations at a site, or within a specific region, are having unacceptable effects on the resident biota. A three tiered approach was proposed:

• Tier 1. A 'desktop' approach in which the geospatial characteristics within a catchment are used to determine the potential for unacceptable biological effects from suspended particulate matter.

• Tier 2. A comparison of sediment load characteristics (turbidity and suspended solids concentrations) measured in the field with a relevant biological effects exposure-response relationship generated from laboratory data in order to infer the potential for unacceptable biological effects from suspended particulate matter.

• Tier 3. Site-specific biomonitoring of biota resident at a site in order to directly measure for unacceptable biological effects from suspended particulate matter.

This approach required that two assumptions to be met for Tier 1: 1) there is a link between a geospatial characteristic and a sediment load characteristic; and 2) there is a link between a particular sediment load characteristic and a macroinvertebrate assemblage response. It also required that two questions be answered for Tier 2 and 3 respectively: 1) which sediment load characteristic best predicts biological (macroinvertebrate) changes in the field (so allowing effort in developing a water quality guideline to focus on that particular sediment load characteristic); and 2) which biological response variable is a sensitive measure of particulates exposure and should therefore be employed in site-specific biomonitoring? Five hypotheses were designed to address these assumptions and questions, and were investigated in a field study described in Chapter 5.

The aim of the field study was to examine hypotheses that could be used to test and refine the application of the proposed suspended solids risk assessment protocol. Hypotheses 1 and 2 were undertaken to ensure that there were indeed differences between sites/catchments in terms of geospatial and sediment load characteristics respectively, allowing the 3rd hypothesis to specifically investigate assumption 1 of Tier 1: is there a link between a geospatial characteristic and a sediment load characteristic? Results indicated that during low flows (low rainfall season) the land class of 'cultivation', when close to the river (within 1-2 km), had a strong negative correlation with instream turbidity and TSS. At higher flows (during high rainfall season) the land classes of 'degradation' and 'cultivation', erosion gully measures and measures of higher population density had a strong and consistent positive correlation with instream turbidity and TSS. The 'natural' land class had a strong negative correlation with instream turbidity and TSS. During the higher flows, the number of significant correlations measured between geospatial and sediment load characteristics were considerably higher when a restricted subcatchment radius of 20 km from sampling sites for geospatial data was used, instead of using geospatial data from the whole catchment. Settled solids concentrations were positively correlated with erosion gullies occurring within 3 km of the river site. Consequently, the first assumption, that there is a link between certain geospatial characteristics and certain sediment load characteristics could be confirmed, although flow/rainfall season and the type of sediment characteristic chosen can affect the most suitable geospatial characteristic.

With Hypotheses 1 and 2 confirming there were indeed differences between sites/catchments in terms of geospatial and sediment load characteristics, Hypothesis 4 was undertaken, and also confirmed a difference between sites/catchments in terms of the macroinvertebrate assemblages (as measured by various macroinvertebrate biological stress response variables). These results then validated the use of the different catchments in the research area for examining Hypothesis 5: can differences in macroinvertebrate assemblage composition between the sites be attributed to a sediment load characteristic? The aim of Hypothesis 5 was three fold: firstly to investigate the 2^{nd} assumption governing the use of Tier 1: i.e. that there is a link between a particular sediment load characteristic and a macroinvertebrate assemblage response; secondly, to help refine Tier 2 by indicating which sediment characteristic best predicts biological (macroinvertebrate) changes in the field so allowing effort in developing a water quality guideline to focus on that particular sediment load characteristic; and lastly, the outcome of Hypothesis 5 would also provide useful information for Tier 3, indicating which biological response is a sensitive measure of particulates exposure and should therefore be employed in site-specific biomonitoring.

Results from Hypothesis 5 indicated that, according to the macroinvertebrate stress responses employed, the differences in macroinvertebrate assemblage composition between the sites could be attributed to sediment load characteristics. Secondly, that the overwhelming evidence suggested that settled solids concentration best explained changes in macroinvertebrate assemblage composition. Lastly, the most sensitive biological responses appeared to be % Trichoptera abundance (it had the most sensitive and consistent response to all sediment load characteristics), NMDS of all macroinvertebrate taxa and to a lesser degree NMDS of FFGs. Lastly, SASS Score and ASPT were consistently negatively correlated (and had high r^2 values) with settled solids concentration.

CONCLUSIONS

The limited number of macroinvertebrates samples collected during this study means that any conclusions should be viewed as promising leads for further investigation. Given this, further refinement of the risk assessment protocol should focus on the geospatial characteristics found significant in this study: the land cover classes of ‘natural’, ‘degradation’ and ‘cultivation’; measures of erosion gullies; and measures of population density. Furthermore, settled solids should be investigated in order to confirm the importance of this sediment load characteristic over suspended solids in causing aquatic ecosystem effects. Lastly, the biological responses mentioned in the paragraph above as being the most sensitive measures of excessive exposure to particulates should be further examined and validated.

RECOMMENDATIONS FOR FUTURE RESEARCH

In Chapter 6, a number of potential further research options emanating from the conclusions of this project are presented:

1) The need to identify the most accurate, or biologically realistic, method for measuring environmental concentrations of settled and suspended solids is discussed. This need emanates from the fact that settled solids are highly spatially variable over small areas (and probably temporally variable too), and suspended solids are known to be highly variable temporally. Undertaking numerous replicate measures can give an indication of the range of concentrations at a site, both spatially and temporally. What needs to be investigated is how best to compare this range of environmental concentrations to a possible water quality guideline (i.e. use the highest value, the mean, mode, or some quartile, etc.). Furthermore, the measurement of settled solids is not well standardised, and issues that need investigation include: what approach to use (qualitative or quantitative); how to collect the quantitative sample (equipment and technique); and where (what benthic habitat) in the river to sample.

2) Characterisation of the particulate solids in South African rivers is required as the development of water quality guidelines for aquatic particulates will necessarily be quite site-specific, requiring knowledge of the important components of the particulates at a high spatial resolution. Currently, very little is known regarding the particulate size ranges and geochemical composition of particulates in South Africa’s rivers.

3) Developing separate exposure-response relationships for settled and suspended solids may provide a useful management solution for dealing with the different biological stresses that instream particulates have when in these two different states. A number of specific tasks requiring investigation in this regard are detailed: including identifying which taxa are suitable for settled and which are more applicable to the suspended solids exposure-response relationship; and determining which particulate combination type (particle size, shape or geochemical composition) should be investigated first. This choice should probably be based on what the most abundant combination in South African rivers is, or what the combination in an important river is.

4) The risk assessment protocol develop in this project was refined through application in the Eastern Cape research area. Validation of this refined risk assessment protocol is now required. The protocol could be reapplied to the same research area in order to determine if the addition of more data alters the conclusions that led to its refinement. Alternatively the protocol could be applied to another research area. Lastly, as settled solids concentration has been identified as important stressor of aquatic organisms, this measure should be included in the risk assessment protocol and tested.

ACKNOWLEDGEMENTS

The authors would like to thank the Water Research Commission for the financial support that made this research possible. We also acknowledge the support and enthusiasm of the Chairman of this project's steering committee Mr Bonani Madikizela. We are grateful for the advice of the project's steering committee members: Dr Sebastian Jooste, Dr Randal Albertus, Prof Victor Wepener; Ms Christa Thirion and Ms Bridget Shaddock.

There were a number of contributors to the project: Ms Asiphe Sahula, Mr Stephen Bennett, Dr Charles von der Meden, Mrs Alexandra Holland, Mr Tarqyn Human, Ms Khaya Mgaba, Dr Sukhmani Mantel, Mr Bennie van de Waal, Prof Kate Rowntree, Mr Matthew Muller, Mr Hayden Jacobs, Mr Hugo Retief, Dr Neil Griffin, Mr Derick Forbanka, Dr Augustine Niba, and Dr Denise Schael.

Administrative and technical support was ably provided by Mrs Juanita Mclean, Ms Una Wium, Mrs Daksha Naran and Mr David Forsyth.

Support in undertaking fieldwork in the Maclear district was gratefully received from Phyll Sephton of Woodcliffe Farm, Rob Scholtz of Working for Water and Declan and Roleen Sparks.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

South African rivers, like those in many semi-arid countries, deliver large amounts of sediment to the ocean, with the Orange River considered one of the most turbid (in terms of sediment delivery) in the world (Bremner et al. 1990). The causes of high sediment loads in South African rivers are related to a combination of interacting factors such as rainfall erosivity which is linked to slope steepness and length, the high stream flow variability characterised by South African rivers (Grenfell and Ellery 2009), and to the high erosivity of our soils (Le Roux et al. 2008). The above are exacerbated by poor land management and associated land degradation.

The ecological consequences of high sediment loads in rivers have long been recognised (Ellis 1936) and will be briefly reviewed in the next chapter. However, appropriate methods of managing suspended solids based on biological effects data have been problematic to develop. Dunlop et al. (2008) suggest this due to the recognition that numerous site-specific factors can affect the severity of effect (e.g. particle size, shape, geology and hydrological regime). As a consequence, many studies have been undertaken using natural sediments but without quantifying sediment particle characteristics. When these biological data are combined to form exposure-response relationships, the range of sediment characteristics used in exposure tests cause unacceptably high variability making the exposure-response relationship an unreliable predictor of biological effect.

The Department of Water Affairs (DWA) has undertaken to revise the South African water quality guidelines (WQGs) for fresh waters, initially developed in 1996 (DWAF 1996). In the 1996 guidelines suspended solids was managed according to a percentage ‘departure from reference condition’ in which turbidity levels, measured in nephelometric turbidity units (NTU), at a downstream site should not increase by more than 10% of those at an unimpacted upstream or reference site. Recognition that suspended solids are an important potential stressor in South African fresh water resources, and that the new WQGs should be based on biological effects data and be more site-specific prompted the initiation of this Water Research Commission funded study.

The aims of the project and the chapter in which it is addressed presented below:

1. Establish the most appropriate sediment test material for exposure trials – **Chapter 2**

2. Test the effects of suspended particulates on selected macroinvertebrates at different levels of biological organization – **Chapter 3**

3. Generate an exposure-response relationship framework from data generated in Aim 2 and relevant data in the literature – **Chapter 4**

4. Attempt to relate exposure-response data developed in the laboratory to natural conditions in the field – **Chapter 5**

5. Develop and apply a detailed site-specific suspended solids risk assessment protocol with different levels of complexity – **Chapter 5**

6. Identify further research that will lead to the development of an effective and applicable guideline for instream particulates – **Chapter 6**

2 LITERATURE REVIEW

2.1 INTRODUCTION

There has been renewed interest in the role suspended particulates play in the aquatic ecosystem, with special focus on developing scientifically defensible and reliable tools for managing their impacts on aquatic biota. This has resulted in a number of very detailed, and recently produced, literature reviews. This report will provide an overall summary of the most important issues related to the management of suspended solids as discussed in these reviews. For more detailed information the reader is directed to the references listed in Table 2.1.

Table 2.1 Extensive literature reviews of the effects of suspended solids on aquatic biota.

Reference	Review topic
Wood and Armitage 1997	Biological effects of fine particulates in river systems
Berry et al. 2003	Biological effects of suspended solids in aquatic systems
Dunlop et al. 2005, 2008	Challenges in managing suspended solids
Bilotta and Brazier 2008	Influence of suspended solids on water quality and aquatic biota
Collins et al. 2011	Issues to be considered in managing sediment particulates
Kemp et al. 2011	Impact of fine sediment particulates on riverine fish
Jones et al. 2011	Relationship between fine sediment particulates and macrophytes in rivers
Jones et al. 2012	Impact of fine sediment particulates on freshwater macroinvertebrates

2.2 WHAT ARE SUSPENDED SOLIDS?

In general, suspended solids are the mass or concentration of organic and inorganic material held in the water column (Bilotta and Brazier 2008). More specifically, suspended solids are one of several particulates including organic matter, phytoplankton and mineral content which together make up the Total Suspended Solids (TSS) and are often a primary driver of turbidity (Dunlop et al. 2005). The precise physical nature of the sediment component of suspended solids is highly variable, with different particle size distributions and geochemical properties originating from different colluvial and alluvial sources of sediment production, each of which are affected by climate, catchment geology and topography, land use and type of vegetation cover (Wood and Armitage 1997; Dunlop et al. 2005). Suspended solids are usually defined as less than 2000 µm in size, and thus include sand (<2000 to >62 µm), silt (<62 to >4 µm), and clays (< 4 µm) (Wood and Armitage 1997). In this report, the focus is on the sediment component of suspended solids.

2.3 WHERE DO SUSPENDED PARTICULATES COME FROM?

There are two main sources of solids particulates present in a river: 1) in-channel sources, where, depending on the hydraulics, particulates can be derived from or re-deposited to the beds or banks of a stream; and 2) non-channel sources within the catchment (Grimshaw and Lewin 1980). Details of these sources are provided in Table 2.2.

Table 2.2 Sources of suspended particles (adapted from Wood and Armitage 1997).

In-channel sources of suspended particulates	Rivers banks subject to erosion
	Mid-channel bars subject to erosion
	Fine bed material from surficial or interstitial deposits
	Natural backwaters where particulates settle during low flows
	Fine particles trapped within aquatic macrophyte or marginal vegetation stands
	Biotic particles like phytoplankton and zooplankton
Catchment sources of suspended particulates	Exposed soils subject to erosion
	Disaster events like landslides or soil creep
	Urban areas (which alter volume and timing of runoff)
	Anthropogenic activities
	Little fall, especially vegetation adjacent to river
	Atmospheric deposition (aeolian processes or precipitation of airborne particles)

Although suspended solids, including suspended sediment, are naturally present in most aquatic environments, concentrations relative to natural or pre-industrial levels have commonly become enhanced through land use changes, anthropogenic activities such as logging, livestock farming and urbanisation, and through natural or anthropogenic disturbance (Walling and Fang 2003; Bilotta and Brazier 2008).

2.4 WHAT ARE THE CONSEQUENCES OF EXCESSIVE EXPOSURE TO SUSPENDED SOLIDS?

While suspended sediment is a fundamental and necessary part of aquatic ecosystems and their functioning, elevated sediment concentrations influence aquatic biota through both direct and indirect means, producing complex interactions affecting individuals, populations and whole communities (Dunlop et al. 2008). These authors provide a conceptual model showing how changes in habitat, and direct effects on individuals, lead to reduced mortality and reduced reproductive ability, which in turn reduce the size of populations, ultimately leading to the loss of sensitive species and changes at the community level (Table 2.3).

Table 2.3 Generalised conceptual model of effects of excessive suspended solids exposure to aquatic biota.

	Consequence of exposure at different levels of biological organisation		
	Individuals	**Populations**	**Community**
Direct effects →	Mortality		
	→	Decreased recruitment →	Loss of sensitive species
Indirect effects (changes to habitat) →	Reduced reproduction		

More explicit examinations of the direct and indirect effects of suspended particulates are undertaken for macroinvertebrates in Jones et al. (2012), for fish (Kemp et al. 2011), macrophytes (Jones et al. 2011) and more generally in Wood and Armitage (1997), Berry et al. (2003) and Bilotta and Brazier (2008). A synopsis of these reviews is discussed below. Direct effects across a variety of vertebrate and invertebrate taxa include clogging and abrasion of gills and filter-feeding organs. This causes damage to these organs and reduces their efficiency, increasing the energy expenditure of exposed organisms, resulting in impaired reproductive output, or in extreme conditions death. Macrophytes, too, can be negatively affected by direct abrasion and scour caused by particulates in fast flowing water. The reduction of light penetration can have a direct impact on the predator/prey relationship of vertebrates and invertebrates, and for periphyton, phytoplankton, algae and macrophytes can reduce photochemical processes. Indirect effects, for all taxa, stem largely from changes to habitat composition or quality, and to food availability, often changing the distribution of benthic and epibenthic organisms, and affecting whole populations or communities. For both fauna and flora, suspended solids can have additional indirect effects by transporting harmful or limiting nutrients and toxic compounds (Bilotta and Brazier 2008). The potential for indirect effects such as these means that cumulative or synergistic effects can often be introduced. For example fine suspended solids have been found to enable or increase the toxic effects of contaminants through changes in adsorption, transportation and bioavailability of the toxicant (Hartman and Martin 1984; Van den Hurk 1994; Wood and Armitage 1997).

Of course as in any ecosystem, changes, particularly to basal or key organisms, often has knock-on effects and it is no different with the impacts of suspended and deposited particulates. For example, changes to periphyton and other macrophytes through abrasion and reduced photosynthesis can have significant effects on within-stream hydraulic conditions and habitat diversity (Armitage 1995; Hearne and Armitage 1993 in Wood and Armitage 1997).

Deposited particulates are inextricably linked to suspended solids and can influence biota through similar mechanisms, usually related to smothering; mainly these are clogging of respiratory and feeding organs, changes to benthic food accessibility and effects on colonization, including prevention of attachment to the substratum of algal cells (Brooks 1986; Peeters et al. 2006; Bo et al. 2007). Deposition of fine particulates can also induce significant changes to several physico-chemical properties of the substrate and benthic environment. Most harmful of these effects is the reduction of percolation within the substrate, which, combined with high organic content can create anoxic or toxic conditions (Jones et al. 2012). A common incorrect assumption is that deposition only occurs under conditions of slow flowing water, when in fact, deposition occurs over a range of flows according to particle size – settling velocity relationships that are affected by physical features including particle shape, turbulence and water temperature (Wood and Armitage 1997). Predicting where and when such impacts may occur is, therefore, complex as these factors would need to be used to identify systems vulnerable to effects of deposition.

2.5 METHODS FOR QUANTIFYING SUSPENDED SOLIDS

Most commonly, suspended solids are quantified using a surrogate measure – turbidity (Bilotta and Brazier 2008). This is an integrated measure that simply reflects the optical clarity of water due to both suspended and dissolved material including effects of algal cells and dissolved minerals and humic substances (Dunlop et al. 2005; Bilotta and Brazier 2008). Specific measures of turbidity include quantification by Nephelometric Turbidity Units (NTU) or use of a Secchi disc. Other techniques separate suspended from dissolved solids, quantifying only the Total Suspended Solids (TSS) or Non Filterable residue (NFR) (Dunlop et al. 2005). While measuring turbidity has logistic advantages the method is not nearly as targeted as filtration methods since turbidity is a function of several factors other than suspended solids. In contrast, filtration methods are able to isolate and quantify directly the suspended solids in water samples and thus to provide specific information on particle size fractions and geochemical characteristics (Bilotta and Brazier 2008).

2.6 APPROACHES FOR MEASURING EFFECTS OF SUSPENDED SOLIDS ON AQUATIC BIOTA

The relative nature and intensity of the impacts that suspended particulates have on organisms are dependent upon several characteristics of exposure, of the particulate itself and of the organism exposed (Table 2.4). The first two factors are related to exposure (i.e. concentration and duration of exposure), and are well established principles in exposure-response relationships. The next four factors relate to characteristics of the particle: geochemical composition (Robinson et al. 2010), particle size (Kirk and Gilbert 1990; Swietlik et al. 2003; Awata et al. 2011), particle shape (Lake and Hinch 1999) and zeta potential of the particles (affects flocculation). Lastly, the behaviour, physiology, life cycle and the availability of refugia affect the organism’s response to the stressor. Of course, these factors can, and do, interact with each other, potentially resulting in very broad ranges of harmful concentrations (Berry et al. 2003).

Table 2.4 Characteristics affecting the biological severity of suspended solids exposure (Swietlik et al. 2003; Bilotta and Brazier 2008; Collins et al. 2011).

Category	Characteristic
Exposure	Stressor concentration
	Duration of exposure
Particulate	Size
	Geochemical composition
	Shape
	Zeta potential
Organism	Behaviour
	Physiology
	Life cycle
	Available refuge

For the reasons described above, traditional exposure-response relationships determined from laboratory experiments in which unspecified sediment particles have been used as the stressor provide a simplified and imprecise idea of biotic responses as they are not able to account for interactive or cumulative effects that would likely be found in the field (Bilotta and Brazier 2008; Collins et al. 2011). The consequent exposure-response relationships have very high variance and are not reliably predictive. Instead, stress-response tests should expose organisms to very precisely characterised particulates in order to build up a deeper understanding of the biological consequences of different size ranges, different shapes and geological composition. In the current study, Kaolin clay particles were chosen (see Chapter 3)

Laboratory based stress-response testing has been useful for determining and isolating specific lethal concentrations under very controlled conditions for continuous exposure to chemical toxicants. However, this type of response testing in a mortality context has not proven very useful in the case of suspended solids (Jones et al. 2012) as mortality is rarely recorded, even at very high TSS concentration exposures (Kefford et al. 2010). Furthermore, natural TSS concentrations vary considerably over time, and a rarely constant. Although lab based stress-response testing has provided some information on hazardous or effect concentrations for non-lethal effects (e.g. Rowe and Dean 1998; Broekhuizen et al. 2001; Larsen and Ormerod 2010), the extrapolation of such data to field conditions has been problematic, also due to concerns over the environmental realism of the test conditions (Collins et al. 2011).

One of the main concerns is the use of aggregated single species stress-response tests to protect communities in the field. Alternatives include field-scale experimental manipulations: either artificial streamside flow-through type setups with multiple species; or those that experimentally dose streams with sediment; or use real-time disturbances like mining or road construction as case studies. It is argued by Jones et al. (2012), that while in-situ flow-through experiments are an improvement on laboratory testing as they allow observation of more natural conditions and behavioural responses, the extent of their spatial resolution is usually limited by logistic constrains meaning fewer experiments are possible. A likely consequence of this limitation is a reduction in the applicability of results to different areas and taxa (Jones et al. 2012). Like the flow-through setups, controlled dosing experiments suffer from similar logistic constraints. Another approach that is able to provide information on stressor-biota relationships over large scales is that of correlation. Despite the proven capacity of this method to successfully relate biotic responses to chemical data, correlations cannot always distinguish between the effects of contributory factors or covariates, and they certainly do not identify causation (Jones et al. 2012). Very few of the multispecies or correlation experiments have been undertaken, and those that do exist have not adequately defined the particle characteristics listed in Table 2.4.

The way forward, according to Collins et al. (2011), is the development of catchment specific modelling toolkits which could link the current and future projected sediment load regimes with a database of biological response data from a range of taxa. The sediment load regime would be defined according to accepted characteristics (e.g. mass and hydraulics being used to derive the temporally variable suspended and settled particulate concentration regimes for the river system in question; and determination of particle size distributions and geochemical composition of particulates occurring in the river system). The biological response database would comprise exposure-response data to specific suspended particulate characteristics. Ultimately it would also include the biological effects of settled particulates. This would enable catchment-specific targets for suspended and settled solids. This is a very ambitious goal, and especially in South Africa as so little is known about the sediment load regimes and particulate characteristics of South African catchments. In addition, very little exposure-response data exist which sufficiently characterise instream particulates. However, in revising the approach to deriving water quality guidelines in South Africa, the Department of Water Affairs intends implementing a probabilistic risk assessment framework based on a Bayesian fault tree. This approach is compatible with the approach suggested by Collins et al. (2011). Consequently, the research undertaken in this report represents the start of work toward providing appropriate biological response data for instream particulates.

Jones et al. (2012) are at pains to point out that scale is an important aspect to consider in the use of exposure-response experiment results, or in field assessments of aquatic communities exposed to particulate stress. Exposure-response tests most often measure responses of individuals, of one species, in the laboratory and then infer effects on populations of single species or communities of multiple species in the environment. The complex interactions of populations and communities are not considered. This problem of scales in terms of biological organisation is well known in ecotoxicology. However, Jones et al. (2012) also point out a scale problem particularly pertinent to instream sediments. Due to hydraulic variability, a river system can be composed of depositional and erosional patches, which can change over very small distances. Thus, a response may be evident at one scale but sampling over a much larger or smaller scale may not identify any such impact. Given the interrelated nature of aquatic ecosystems, isolated effects, even at a low level of biological organisation, can instigate changes at higher levels which may often occur at different spatial scales. Consequently, it is important that the scale used to examine the potential effects of instream particulates is relevant to the ecosystem component being protected and goals of managers.

3 LABORATORY-BASED TESTS MEASURING RESPONSES OF SOUTH AFRICAN MACROINVERTEBRATES TO KAOLIN CLAY PARTICLES

3.1 INTRODUCTION

The potential for excessive sediment input to rivers and lakes to affect aquatic biota and water quality has long been known (Ellis 1936). However, it is only recently that there has been international recognition that suspended solids (sediment and organic particulates) have become a high priority potential stressor for freshwater biodiversity and ecosystems, requiring more active management (Bilotta and Brazier, 2008).

This has prompted research toward the development of guidelines aimed at defining thresholds at which suspended solids cause unacceptable biological effects within the aquatic ecosystem. However, results have shown little agreement between the environmental effects of suspended solids as a function of concentration and duration (Newcombe and MacDonald 1991) with the existence of very broad ranges regarding the exposure-response relationship and endpoints such as reduced feeding, impaired growth and mortality in fish and invertebrate species (Berry et al. 2003). It soon became apparent that, unlike chemicals which have a particular mode of toxic action governed only by concentrations and duration of exposure, particulates have many mechanisms of inducing adverse biological effects (Bilotta and Brazier 2008; Dunlop et al. 2008). In addition to concentration and exposure duration, organisms are affected directly by particle size, shape and geochemical composition, and indirectly by changes in predator-prey relationships and habitat alteration (Collins et al. 2011; Jones et al. 2012). Consequently, it may only be possible to attain more accurate dose-response relationships if biological endpoints are measured against specific defined particle characteristics (e.g. particle size, shape and geology). Any guidelines developed from these dose-response relationships will be, unavoidably, very site/situation specific.

In this chapter, the responses of three indigenous South African macroinvertebrates to suspended kaolin clay particles are reported. This particulate was chosen as commercial brands have a defined particle size range (<4 µm), known particle shape (laminar), and are chemically inert with a low toxicity to aquatic species (WHO 2005). Consequently, effects observed during exposure-response tests were likely due to the physical effects of the kaolin particles and not due to any toxic effect associated with the kaolin (Kefford et al. 2010).

3.2 MATERIALS AND METHODS

3.2.1 Exposure stressor

Purified kaolin clay was acquired from Sigma Aldrich Chemicals. The kaolin clay powder was added to a diluent of 'aged' dechlorinated tap water to constitute the exposure medium. The diluent was produced by running tap water though a commercial dechlorination unit and into a large blue drum above which a home-made charcoal filter further removes contaminants from the tap water while the water recirculates, and is aerated, for a period of at least three days.

3.2.2 Organisms exposed

Two mayfly nymph taxa (*Tricorythus discolor* and an Oligoneuridae sp.) and the fresh water shrimp *Caridina nilotica* were utilised in exposure-response trials. The mayfly nymphs were collected from an unimpacted site (32°33'47.2''S; 26°40'30.1''E) on the Balfour River in the Eastern Cape Province of South Africa. The method of collection is according to DWAF (2000) whereby nymphs were either washed, or removed from stones and rocks with a soft paintbrush, and placed in cooler boxes fitted with several layers of mesh to provide substrate. The cooler boxes were filled with river water, aerated through battery-operated pumps fitted with airstones and kept cool by means of freezer blocks. On arrival in the laboratory, early instar mayfly nymphs with no evidence of harm (i.e. broken feelers or tails) were placed randomly into the exposure vessels containing exposure diluent ('aged' dechlorinated water) and acclimated overnight. The next day any dead individuals were removed from the experiment and replaced by a living organism to ensure sufficient numbers in each vessel. Specific quantities of Kaolin clay (the stressor) were then added to each treatment and the experiment begun.

The shrimp were obtained from laboratory cultures maintained at the Institute for Water Research, Rhodes University. Individuals were removed from the culture system and placed in the exposure vessels containing exposure diluent for acclimation overnight. The remaining procedure was the same as that of the mayflies.

3.2.3 Exposure vessels

The mayfly nymphs were exposed in laboratory-based recirculating artificial streams (DWAF (2000). Streams consisted of 1 m polyvinylchloride (PVC) channels, each receiving exposure medium via 15 mm tubing from a submersible pump placed in a 25 L bucket – essentially acting as a sump. The channels were placed at an angle so that the exposure medium could flow through the channel and down into the sump (holding-bucket), from where the exposure medium was pumped back up to the channel's elevated end. The flow rate down the channel averaged 0.36 m/sec. A 1 mm mesh screen was placed at the bottom end of the channel to prevent organisms from being washed into the holding-bucket. A second submersible pump was placed into each bucket to agitate the exposure medium and prevent sediment from settling in the holding-buckets. The experiment was conducted in a controlled environmental room. Two trials with mayfly nymphs were conducted, the first using three stones placed along the length of the channel for substrate, while the second trial used three square ceramic tiles instead. More details regarding the differences between the two trials is described in the experimental design section.

As shrimp require lower flow rates compared to mayfly nymphs, 15 L rectangular glass tanks were used as the experimental vessels. Ten litres of experimental medium was used in each tank. Two air stones connected to an aeration pump were placed in opposite corners of each tank to provide oxygen for the organisms and to keep the suspended particulate exposure solutions in circulation. Vertical nets were suspended at the centre of each tank provided substrate for shrimp and allow avoidance of water flow created by the air stones. A stone was placed at the centre of each tank to act as additional substrate. An aquarium heater was placed in each tank to maintain the temperature between 24°C-26°C. Plastic cling wrap was placed over the tank openings to reduce excessive evaporation.

3.2.4 Measurement of water quality variables

The water quality variables pH, electrical conductivity (EC in mg/L), water temperature (°C) and dissolved oxygen (DO in mg/L) were measured daily (in the case of the mayfly trials and weekly during the longer shrimp experiment). Measurements of the actual exposure concentrations of suspended kaolin particles were undertaken using two methods: the more rapid surrogate measure of turbidity (nephelometric turbidity units – NTU); and the more laborious measure of total suspended solids (TSS in mg/L). To determine TSS, 250 mL of exposure medium from each channel was filtered through pre-weighed filter paper, which was then dried and re-weighed to determine the sediment concentration per litre of water. A relationship between TSS concentration and turbidity was then quantified.

An analysis of the size particle distribution of Kaolin clay used in the exposure-response tests was undertaken using a SediGraph 5100. The SediGraph utilised x-rays to scan a sample of the exposure medium and can analyse particles ranging from 0.1 to 150 micrometres. This analysis runs at constant temperature in order to increase the accuracy of the reading.

3.2.5 Experimental design

One shrimp and two mayfly exposure trials were undertaken:

Mayfly exposure trial 1

In this trial, 10 *T. discolor* individuals were placed in each of the 20 experimental channels and allocated into five treatments, including a control (i.e. four replicates per treatment). The experiment was conducted over a period of 10 days in an environmentally controlled room with air temperature set at 18°C and a light:dark cycle of 12:12 hours. Organisms were not fed for the duration of the trail. A wide range of kaolin exposure concentrations were used for the exposure treatments. However, these nominal suspended kaolin concentrations were seldom achieved in reality because of particle settling during the exposure trial. Consequently, only measured concentrations of suspended kaolin particles are reported.
The biological endpoint measured was mortality.

Mayfly exposure trial 2

In this trial, two mayfly taxa, *T. discolor* and an Oligoneuridae sp., were exposed together in the artificial streams. As in trial 1, there were five treatments (including a control) with four replicates each, and 10 individuals of each taxon in each channel. The experiment was conducted over 13 days and from day 5 onwards an attempt was made to feed the mayflies every second day using very finely ground Tetramin® fish flakes. These food particles were observed to recirculate repeatedly through the system allowing the mayflies repeated opportunity to encounter the food. Only measured concentrations of suspended kaolin exposures are reported.

In addition to mortality, the measurement of three sublethal biological endpoints was attempted: the physiological response of gill damage from sediment particle abrasion; and two biomarkers of hypoxia – lipid peroxidation and catalase activity. The methodologies used to determine these three sublethal biological endpoints are described later in the Materials and Methods section.

Shrimp exposure trial

This experiment was divided into two phases:

Day 0-17: Five females and five males were placed in each of the 15 glass tanks and allocated into five treatments (including a control) with three replicates each. After 17 days, the number of gravid females was counted and the adult mortality determined for each exposure treatment. Turbidity of the exposure medium prevented daily observations of mortality or determination of when females became gravid.

Day 17-71: Male shrimp were then removed from the tanks and the experiment was continued with the eggs from the gravid females being allowed to hatch. After 42 days of exposure, tanks were emptied and restarted with fresh exposure medium, while concurrently an initial count of the number of juveniles produced in each tank was conducted. After 71 days of exposure the final count of juveniles was conducted and the carapace length of juveniles measured. Once again, the turbidity of the exposure medium prevented more frequent observations of the numbers of juvenile shrimp produced over the course of the trial. Furthermore, as juvenile shrimp are very sensitive to handling it was decided not to capture and remove the shrimp from the tanks more frequently. During both phases of the trial shrimp were fed every second day with crushed Tetramin® flakes.

The growth of the shrimp was determined by measuring carapace length (Figure 3.1). As defined by Hart (1980), the carapace length is the mid-dorsal distance from the posterior margin of the eye orbits behind the insertion of the eyestalk to the posterior edge of the carapace. The carapace of each juvenile was measured using an eye microscope at 12x magnification.

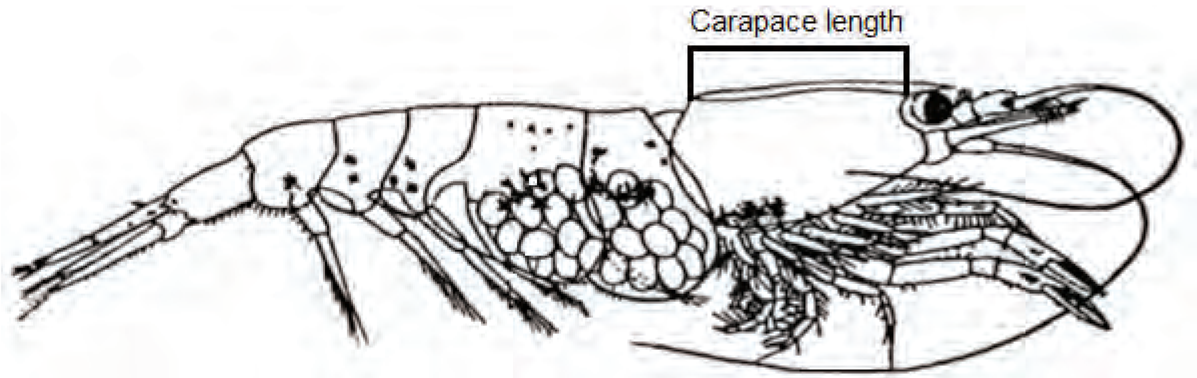


Figure 3.1 An indication of carapace length in a gravid *Caridina nilotica* shrimp (diagram adapted from Hart et al. 2001)

3.2.6 Scanning electron microscopy

An attempt was made to photograph the gills of exposed and control mayflies. Organisms underwent the standard preparation of critical point drying and gold splatter, before being viewed with the scanning electron microscope at Rhodes University (TESCAN Vega TS 5136LM).

3.2.7 Catalase activity assays

On completion of the exposure trial, surviving *T. discolor* and Oligoneuridae sp. individuals were collected from each replicate stream and immediately frozen in separate 1.5 mL Eppendorf microcentrifuge tubes with liquid nitrogen. Due to their small size, some *T. discolor* individuals from the same replicates were grouped into one tube for analysis. Whole samples were stored at -70°C until the assays were undertaken.

Samples were prepared using a modified method of Cohen et al. (1996). A volume of 500 µl phosphate buffer solution (PBS) was added to the sample, and the sample macerated using a micropestle. The mixture was sonicated for 10 s and then centrifuged in a refrigerated centrifuge at 13 000 g for 5 min. 50 µl aliquots were collected for protein determination using the Biorad DC Protein Assay (Biorad Laboratories, USA). A volume of 25 µl of 5% (w/v) Triton X-100 was added to the remaining solution which was first gently vortexed and then centrifuged at 13 000 g for 10 min in a refrigerated centrifuge. A 100 µl aliquot was removed for the catalase assay and the remaining sample kept for lipid peroxidation assay.

Catalase assay was performed using a modified method of Cohen et al. (1996). The assay was performed at 0°C, with 100 µl of sample being added to 750 µl PBS (pH 7.0, 0.05 M). Contamination by microorganisms was controlled by addition of 50 µl 10 mM sodium azide. The reaction was then started by adding 100 µl 60 mM H_2O_2 to the sample solution, which was then gently vortexed and incubated at room temperature. Duplicate aliquots of 10 µl were taken at 2 and 10 min and added to a mixture of 400 µl 0.6 N, H_2SO_4 and 100 µl 10 mM $FeSO_4$ to stop the reaction. Colour development was achieved by the addition of 40 µl 2.5 M potassium thiocyanate. Samples were covered with aluminium foil to protect them from direct sunlight. A volume of 250 µl was transferred to the microtitre plate and absorbance measured at 460 nm using Powerwave X microplate reader (Bio-Tek instruments with KC Junior software). Catalase units were determined using the following equation:

$$\text{Enzyme units} = [\ln (A1/A2)/t]/ \text{ protein concentration}$$

Where In is the natural log, A1 and A2 are mean absorbances at 2 and 10 min respectively, and t is the time difference between the time points.

3.2.8 Lipid peroxidation assay

One of the products formed during lipid peroxidation is malondialdehyde (MDA), which is used in the thiobarbituric acid (TBA) reactive substances test to infer the level of oxidative stress. The assay used was based on the method described by Ringwood et al. (2003), with some modifications. For example, in the present study the MDA activity was described in terms of protein concentration rather than in relation to whole body mass, and the assay was undertaken with half the quantities described by Ringwood et al. (2003), to allow the use of 1.5 mL Eppendorf tubes. Macerated samples in PBS and Triton X-100 (prepared during the catalase assay) were centrifuged at 4°C at 13 000 g for 10 min and 50 µL of the supernatant was transferred to a new 1.5 tube with 700 µL 0.375% TBA and 7 µL 2% butylated hydroxytoluene (BHT). Samples were then vortexed and both the samples and standards heated for 15 min at 100°C. Thereafter, samples and standards were centrifuged at 13000 g for 5 min at room temperature. 100 µL of sample or standard was placed in a well of a 96-well microtiter plate and read at 532 nM using a microtiter plate reader (PowerWave X, Bio-Tek Instruments, USA). Each sample and standard was analysed in quadruplicate (i.e. four wells per plate). MDA activity in µmol.L-1 was calculated for each sample, using the linear regression obtained from concentration versus absorbance of MDA standards and multiplied by four to represent the activity over an hour. Sample MDA activity was converted from µmol.L-1 to nmol.mL-1, which was divided by the protein concentration of the particular sample to produce MDA activity in nmol.hour.mg-1 protein.

3.2.9 Statistical analyses

The following procedure was followed when testing for significant differences in mortality, reproduction, growth and sublethal responses between exposure treatments and the control treatment. The data were tested for normality and equality of variances using the Shapiro-Wilk test and Levene's test respectively. The students t-test was applied to parametric data with equal variance, while the Mann-Whitney U test was applied to non-parametric data or data with unequal variance. All the above analyses (including correlation analysis) were undertaken using Statistica (version 9), with significance set at $P < 0.05$.

The calculation of LC50s for mortality data was attempted using the Probit method (developed by the US Environmental Protection Agency). In the event that the data were not suited to this parametric model, the non-parametric Trimmed Spearman-Karber (TSK) method was attempted.

3.3 RESULTS

The size particle distribution readings for the kaolin clay used in this study are summarised in Figure 3.2. This graph shows particle size range in terms of cumulative percentage of particles under a certain size range, and indicates that 80% of the kaolin particles are smaller than 1 µm, with the largest particles in the 3-4 µm range.

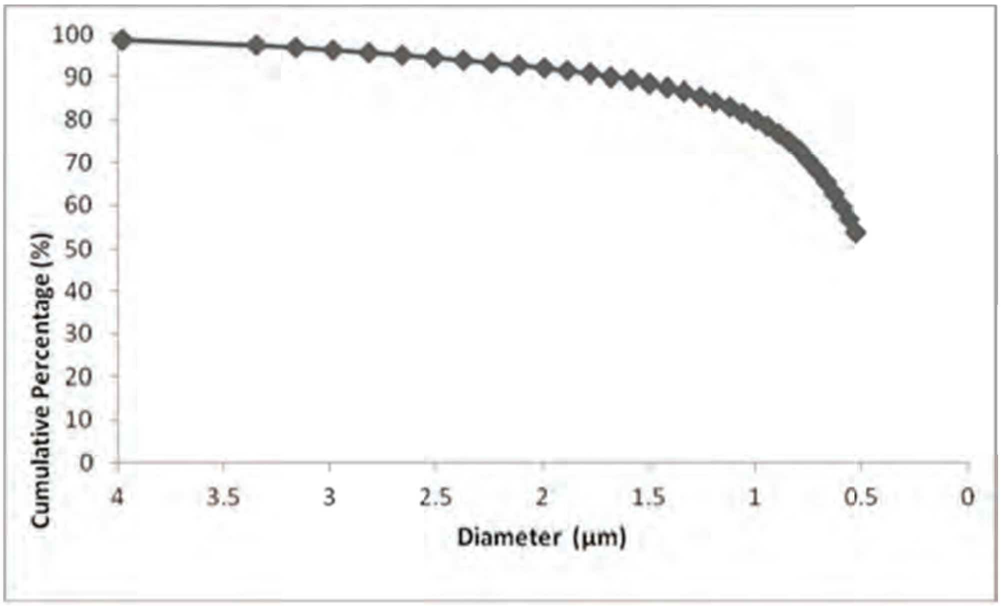


Figure 3.2 **Particle size distribution for kaolin clay utilised in this study.**

3.3.1 Mayfly exposure trial 1 – *T. discolor*

Settling of kaolin particles in some replicates resulted in high variability of exposure concentrations within some treatments as indicated by the high standard deviations in Table 3.1. The dechlorinated tap water, used as a diluent, was the source of the high EC values, with some variability in control and second lowest treatments. The remaining water quality variables were all very similar among the different treatments (Table 3.1).

Table 3.1 **Average suspended kaolin concentrations and water quality parameters (± standard deviation) per exposure treatment.**

Exposure concentration (mg/L)	pH	Water temperature (°C)	Electrical conductivity (mS/m)	Dissolved oxygen (mg/L)
Control (2 ± 3)	8.0 ± 0.1	17.7 ± 0.1	99 ± 21	9.1 ±0.1
60 ± 26	8.1 ± 0.0	17.8 ± 0.2	114 ± 2	9.1 ± 0.0
229 ± 89	7.9 ± 0.2	17.9 ± 0.1	90 ± 19	9.1 ± 0.1
325 ± 189	8.1 ± 0.0	18.0 ± 0.1	118 ± 1	9.1 ± 0.1
751 ± 117	8.1 ± 0.1	17.9 ± 0.1	117 ± 7	9.1 ± 0.1

Exposure to suspended kaolin particles for 4 days resulted in low percentage mortality in all exposure treatments and no mortality in controls (Figure 3.3). At 4 days the percentage mortality in exposure treatments was not determined to be significantly different ($p < 0.05$) from that of the control. However, between days 8-9 of the trial the majority of exposed mayflies died and mortality began occurring in the control treatment. By day 10 all treatments were significantly different to the control with >95% mortality across all kaolin concentrations and 23% in the control (Figure 3.3). The mayflies were not fed during this experiment and thus a possible explanation for the sudden mortality is the compounded stress effects of sediment exposure and lack of food. In addition, the stones placed along the bottom of channel to provide substrate became surrounded by settled kaolin particles resulting in mayflies using this substrate coming into direct contact with the settled clay. As control mayflies did not experience the same degree of mortality the exposed mayflies did, the effect of not having food alone does not seem a probable cause.

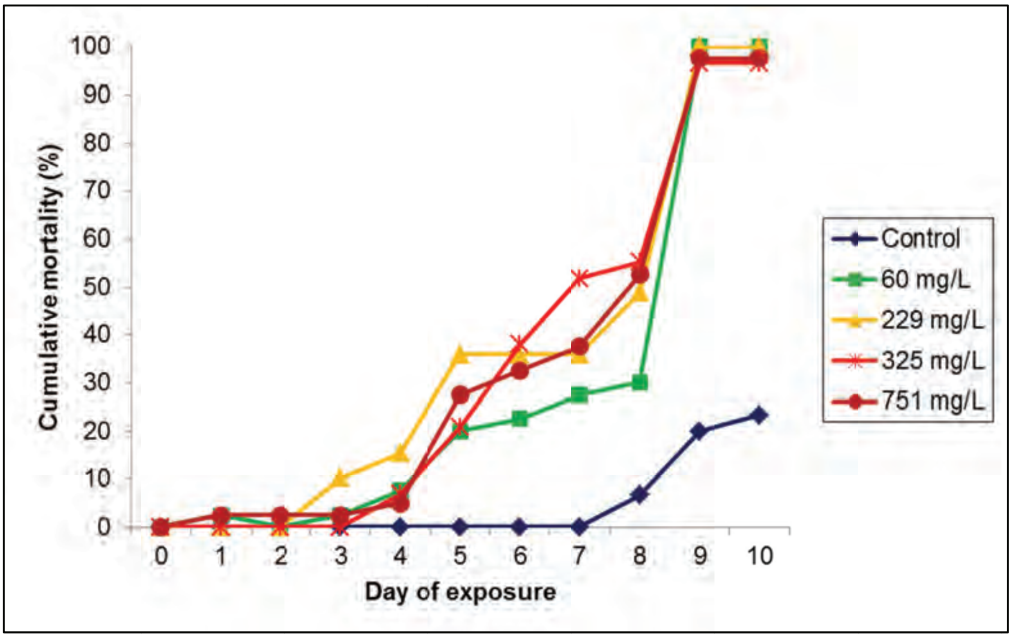


Figure 3.3 Percentage cumulative mortality curves for *Tricorythus discolor* exposed to increasing concentrations of suspended kaolin particles over a 10 day period.

3.3.2 Mayfly exposure trial 2 – *T. discolor* and an Oligoneuridae sp.

In this trial the variability in exposure concentration between replicates was much smaller than in trial 1, resulting in a clear separation between treatments (Table 3.2). In addition, the use of square tiles resting above the rounded bottom of the channel as substrate for the mayflies, instead of stone as in the previous trial, resulted in mayflies avoiding contact with the settled particles. The EC increased during the length of the trial in all treatments from approximately 34 to 60 mS/m due to evaporation from the artificial streams. The EC and all other measured water quality parameters were very similar for all treatments during the course of the experiment (Table 3.2).

Table 3.2 Average suspended kaolin concentrations and water quality parameters (± standard deviation) per exposure treatment.

Exposure concentration (mg/L)	pH	Water temperature (°C)	Initial Electrical conductivity (mS/m)	Final Electrical conductivity (mS/m)	Dissolved oxygen (mg/L)
Control (6 ± 3)	8.4 ± 0.1	17.4 ± 1.0	34.3 ± 0.4	59.1 ± 2.4	9.1 ± 0.1
55 ± 3	8.4 ± 0.1	17.5 ± 1.1	34.2 ± 0.6	59.5 ± 4.0	9.1 ± 0.0
174 ± 12	8.4 ± 0.1	17.4 ± 1.1	34.4 ± 0.2	60.7 ± 1.7	9.1 ± 0.1
383 ± 26	8.4 ± 0.1	17.5 ± 1.1	34.4 ± 0.2	60.6 ± 2.3	9.1 ± 0.1
801 ± 57	8.3 ± 0.2	17.4 ± 1.0	34.0 ± 0.4	60.9 ± 2.3	9.1 ± 0.1

After 4 days of exposure, mortality levels in *T. discolor* were below 10% for all treatments (Figure 3.4A). Mortality rate in *T. discolor* began to accelerate after approximately 9 days of exposure, however by the end of the trial the highest treatment mortality was only 50%, compared to the 100% measured in trial 1. There was no dose dependent response and no significant difference in mortality between control and exposed treatments (Figure 3.4A). An LC50 could not be determined.

The Oligoneuridae sp appeared to be more sensitive to kaolin exposure with between 10-20% mortality after 4 days (Figure 3.4B). In addition, morality rate began increasing from approximately day 4, earlier than for *T. discolor*, although once again by the end of the trial the highest treatment mortality was only approximately 50%. Unfortunately, the control mortality at the end of the trial was 25%. It is possible that the increased earlier mortality rate and higher control mortality may be related to the requirement of this mayfly species for fast water flows. In a study on the distribution of Ephemeroptera species in the Komati River, Matthew (1969) found the highest densities of two Oligoneuridae species (*Oligoneuriopsis lawrencei* and *Elassoneuria trimeniana*) at stream flows between 0.5-1.4 m/sec. The flow of water in the experimental stream was 0.36 m/sec. Although an LC50 could not be determined, the two highest kaolin concentrations were found to cause significantly higher mortality than the control, giving a no observed effect concentration (NOEC) of 174 mg/L (Figure 3.4B).

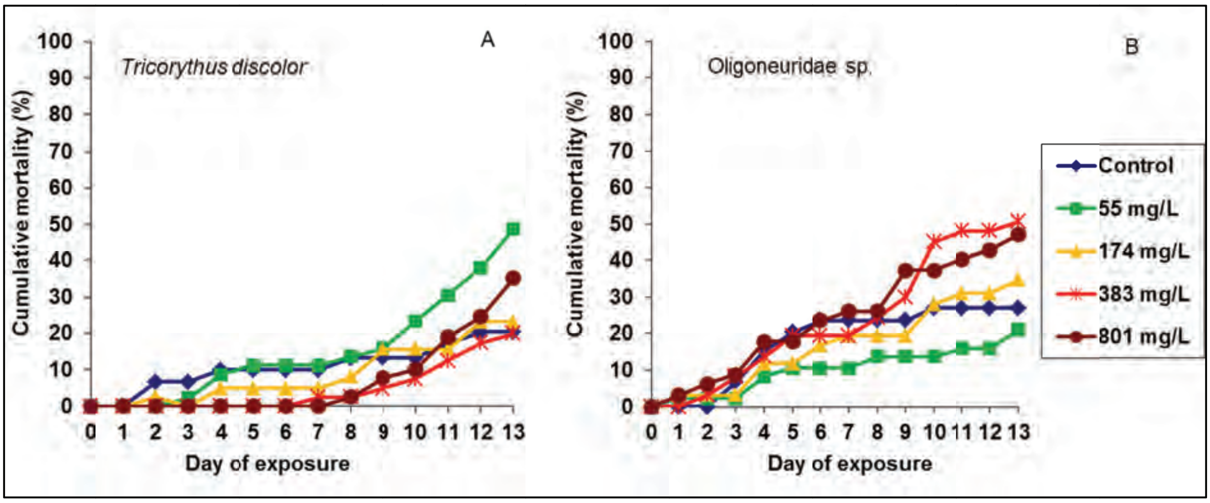


Figure 3.4 Percentage cumulative mortality curves for (A) *Tricorythus discolor* and (B) Oligoneuridae sp. exposed to increasing concentrations of suspended kaolin particles over a 13 day period.

An attempt was made to determine the effect of kaolin particles on the gills in both mayfly species by measuring roughness (i.e. damage) of gills using scanning electron microscopy (SEM). Unfortunately the gills of both exposed and control mayflies all appeared ‘crumpled’ (Figure 3.5), obscuring any evidence of possible damage. It is not clear what caused the gills to crumple as the standard fixation and critical point drying method for these types of organisms was followed. Technicians at the Scanning Electron Microscopy unit at Rhodes University were unable to provide a clear explanation either, suggesting that perhaps the sensitive structure of mayfly gills are not suited to the SEM process.

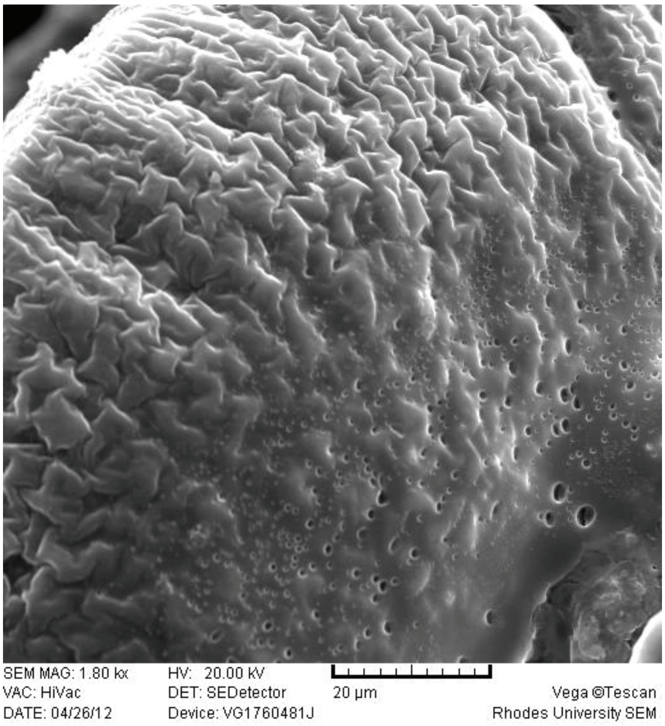


Figure 3.5 A scanning electron microscope picture of a *Tricorythus discolor* gill showing damage (crumpling of the gills) caused during the critical point drying process.

In an attempt to link the possible physical damage of suspended kaolin clay particles on mayfly gills to a subcellular response, two biochemical indicators of hypoxia were measured. Lipid peroxidation and catalase activity have been shown to be good indicators of exposure of hypoxic conditions in a number of organisms (Gorokhova et al. 2010). Lipid peroxidation in *T. discolor* appeared to increase at the lower exposure concentrations and then decrease at the higher kaolin concentrations after both 4 and 13 days of the trial, although this is not statistically significant (Figure 3.6A). Lipid peroxidation appeared to increase in this mayfly species over the course of the experiment with some individuals by day 13 experiencing high levels of cell damage in the exposed treatments (Figure 3.6A). There appeared to be a small dose dependent increase in lipid peroxidation in Oligoneuridae sp. after both 4 and 13 days of exposure, although this was again not statistically significant (Figure 3.6B). Unlike *T. discolour*, Oligoneuridae sp. showed no increase in lipid peroxidation between day 4 and 13 (Figure 3.6B).

In terms of catalase activity, *T. discolor* exhibited no dose dependent response during the trial (Figure 3.7A). After both 4 and 13 days of exposure, average catalase activities in Oligoneuridae sp. indicate a possible dose response, although this was not statistically significant (Figure 3.7B).

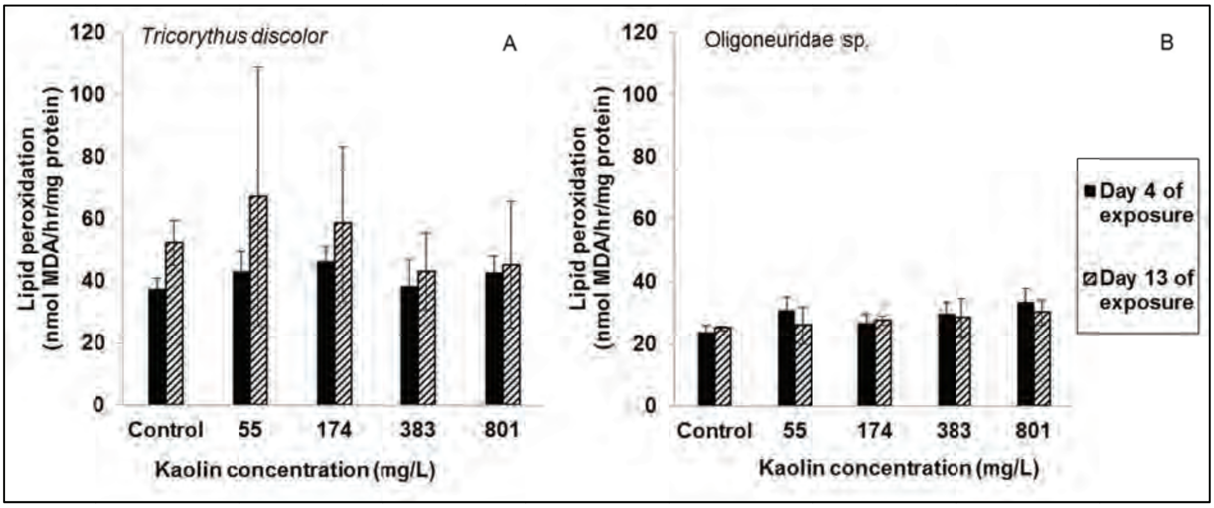


Figure 3.6 Lipid peroxidation as a potential biomarker of hypoxia in (A) *Tricorythus discolor* and (B) Oligoneuridae sp. after 4 and 13 days of exposure (n=4).

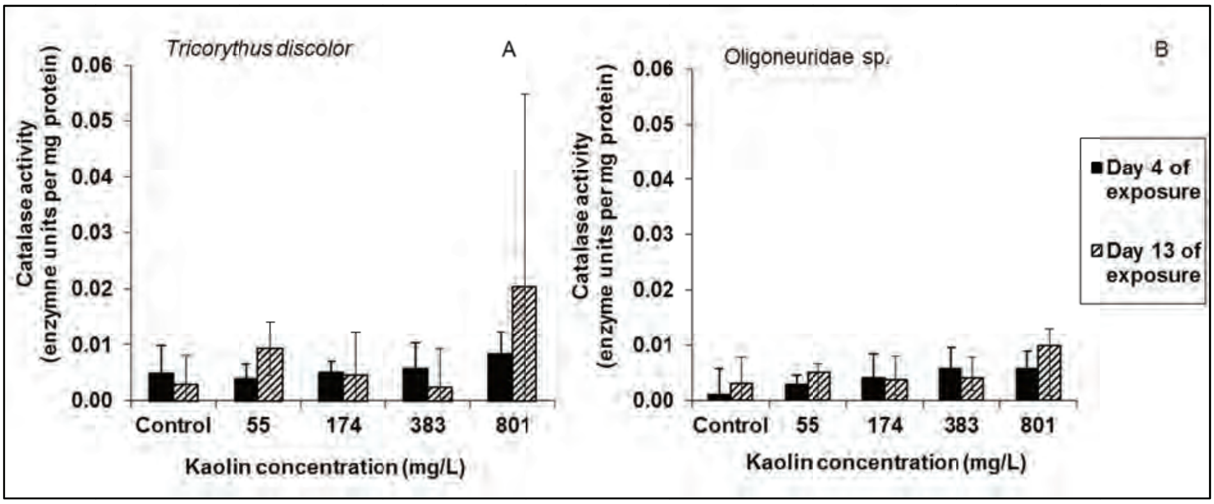


Figure 3.7 Catalase activity as a potential biomarker of hypoxia in (A) *Tricorythus discolor* and (B) Oligoneuridae sp. after 4 and 13 days of exposure (n=4).

3.3.3 Shrimp exposure trial

Phase 1: Day 0-17

Suspended kaolin concentrations were highly variable within treatments, reflecting the challenge of maintaining particles in suspension within the glass tank exposure vessels (Table 3.3). The EC increased during the length of the trial in all treatments from approximately 40 to 60 mS/m due to evaporation. In Phase 2 of the shrimp trial, cling film was placed over the tanks which successfully reduced evaporation. The EC and all other measured water quality parameters were very similar for all treatments during the course of the experiment (Table 3.3).

Table 3.3 Average suspended kaolin concentrations and water quality parameters (± standard deviation) per exposure treatment.

Exposure concentration (mg/L)	pH	Water temperature (°C)	Initial electrical conductivity (mS/m)	Final electrical conductivity (mS/m)	Dissolved oxygen (mg/L)
Control (2 ± 1)	8.1 ± 0.2	25.8 ± 0.6	40.7 ± 0.2	61.2 ± 0.9	7.2 ± 0.1
135 ± 43	8.3 ± 0.1	25.1 ± 0.7	40.3 ± 0.2	59.1 ± 1.1	7.2 ± 0.2
293 ± 81	8.3 ± 0.1	25.7 ± 0.4	40.5 ± 0.3	63.3 ± 1.8	7.2 ± 0.1
586 ± 161	8.4 ± 0.1	26.9 ± 0.7	40.5 ± 0.3	65.2 ± 3.5	7.0 ± 0.1
680 ± 166	8.3 ± 0.1	26.2 ± 0.8	40.3 ± 0.1	62.4 ± 2.4	7.2 ± 0.1

Mortality of *C. nilotica* over the 17 days of exposure was very low, with no mortality in the control treatment and less than 20% occurring at the highest kaolin concentration (Figure 3.8A). The average mortality at 680 mg/L was statistically higher than in the control. The average number of gravid females occurring in each treatment decreased with increasing exposure concentration, being statistically lower at the highest concentration of 680 mg/L (Figure 3.8B).

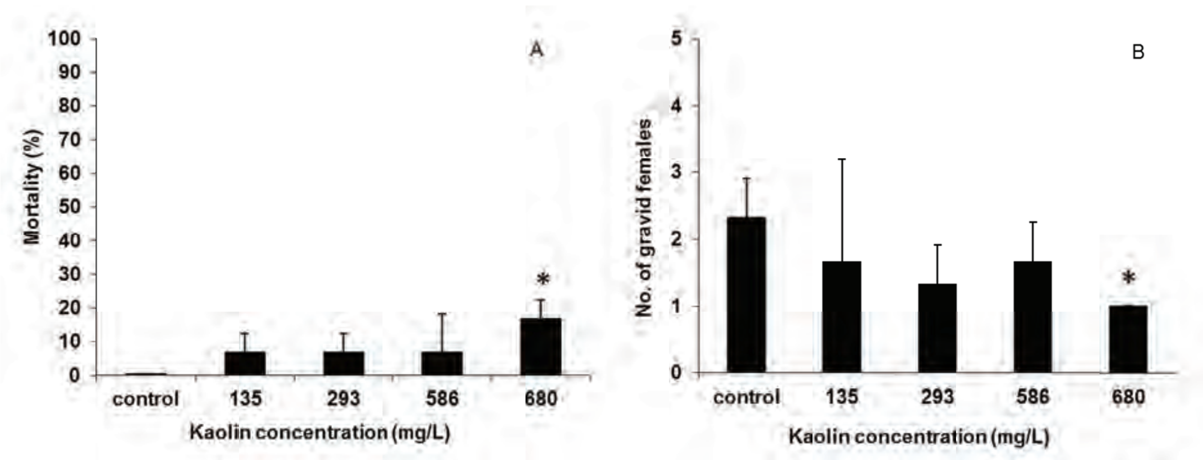


Figure 3.8 (A) Average percentage mortality, and (B) number of gravid females produced, during the 17 day exposure period of adult *Caridina nilotica* to increasing concentrations of kaolin clay particles.

Phase 2: Day 17-71

The issue of excessive settling of kaolin particles was monitored more closely in phase 2. Although actual suspended kaolin concentrations were always much lower than desired, indicating the occurrence of particle settling in all replicates to some degree, excessive settling occurred in three exposure vessels, indicated by the italicised numbers highlighted in Table 3.4. The reason for the excessive settling was not evident visually or from water quality parameters measured (Table 3.4) – although the zeta potential of the exposure medium was not measured which might have given some indication of why the suspended particles flocculated so readily in those particular tanks. The excessive settling was significant, as in those replicates where it occurred, far fewer juveniles shrimp survived compared to other replicates within the same treatment (Table 3.4). The removal of replicates with excessive settling from the dataset indicated that suspended kaolin particles at high concentrations did not negatively affect the number of juvenile shrimp produced, while settled particles did (Figure 3.9A). Growth of juveniles, measured as final carapace length, was also not statistically affected by increasing concentrations of suspended kaolin particles, or excessive particles settling (Figure 3.9B). However, an inverse relationship between the number of juveniles in each tank or exposure replicate and carapace length appears evident, suggesting density dependent growth for these organisms.

Table 3.4 Actual measured suspended kaolin concentrations (average ± standard deviation), with associated juveniles produced, per replicate over the exposure period.

Desired kaolin concentration (mg/L)	Measured kaolin concentration (mg/L)	No. of juveniles produced per replicate	pH	Water temperature (°C)	Electrical conductivity (mS/m)	Dissolved oxygen (mg/L)
0	2 ± 3	215	7.8 ± 0.3	25.5 ± 0.4	46.8 ± 6.9	7.0 ± 0.2
	3 ± 3	133	8.1 ± 0.3	25.5 ± 1.1	47.4 ± 7.0	7.2 ± 0.1
	2 ± 3	174	8.1 ± 0.3	25.6 ± 0.6	45.9 ± 5.6	7.2 ± 0.1
500	296 ± 75	149	8.3 ± 0.1	25.4 ± 0.4	44.7 ± 5.8	7.2 ± 0.1
	281 ± 68	257	8.3 ± 0.2	25.6 ± 0.8	45.6 ± 6.1	6.9 ± 0.2
	271 ± 92	151	8.1 ± 0.3	25.1 ± 1.1	44.1 ± 4.4	7.1 ± 0.3
1000	628 ± 225	266	8.3 ± 0.2	25.6 ± 0.3	45.0 ± 5.8	7.1 ± 0.1
	461 ± 246	*97*	8.3 ± 0.2	25.5 ± 0.4	43.7 ± 4.6	7.1 ± 0.1
	595 ± 268	127	8.2 ± 0.3	25.3 ± 0.5	45.2 ± 5.4	7.2 ± 0.1
1500	814 ± 335	168	8.3 ± 0.2	25.6 ± 0.4	44.9 ± 5.9	7.0 ± 0.3
	923 ± 261	133	8.2 ± 0.2	25.9 ± 0.6	43.6 ± 5.1	7.1 ± 0.1
	466 ± 174	*11*	8.2 ± 0.2	25.7 ± 0.7	44.7 ± 6.0	7.0 ± 0.2
2000	995 ± 419	209	8.3 ± 0.2	25.7 ± 0.4	46.1 ± 6.5	7.0 ± 0.1
	1265 ± 523	164	8.2 ± 0.2	25.9 ± 0.6	43.8 ± 5.3	7.0 ± 0.1
	576 ± 261	*0*	8.2 ± 0.2	25.7 ± 0.6	45.3 ± 6.1	7.2 ± 0.1

Note: highlighted values in italics indicate replicates with excessive kaolin settling

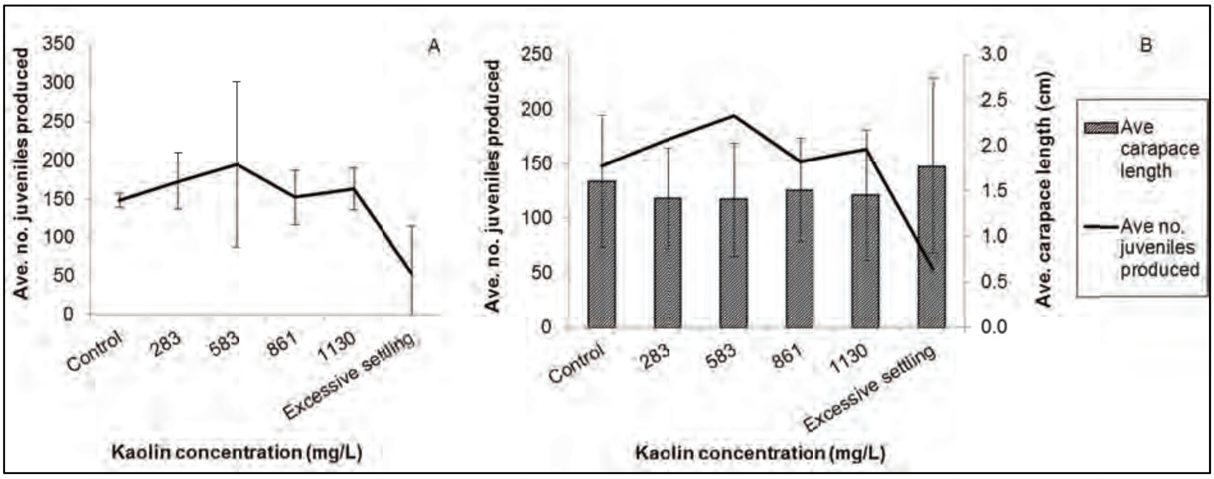


Figure 3.9 (A) Average number of juveniles produced per treatment (with standard deviation) – data from those replicates with excessive settling were not included treatment averages, but are presented separately. (B) Average carapace length (with standard deviation) per treatment is superimposed on the graph showing average number of juveniles produced – suggesting an inverse relationship between the two data sets (error bars for average number juveniles are not shown in this graph as they obscure those of average carapace length).

3.4 DISCUSSION

Obtaining the desired exposure concentrations for suspended kaolin particles and then also maintaining consistent actual concentrations over the exposure period proved a considerable challenge. In both the mayfly trials and the shrimp trial, actual concentrations were considerably lower than desired as the exposure medium could not be agitated enough to keep the particles in suspension while also keeping turbulence from negatively affecting the exposed organisms. Furthermore, suspended kaolin concentrations varied between replicates of the same treatment and within individual replicates over the course of the trial. It is recommended for future trials to keep large differences between treatment exposure concentrations so that variation of replicates within a treatment will not overlap with variation of other treatments. The increasing EC during mayfly trial 2 and phase 1 of the shrimp trial may have contributed to stress in all treatments. This might account for mortality in mayfly controls, although there were no mortalities in the shrimp trial phase 1. However, South African aquatic invertebrates are tolerant of high EC concentrations, with 55 mS/m still considered representative of good to fair water quality (DWA 2008).

The high mortalities of juvenile shrimp in those replicates where excessive settling occurred suggests that these organisms are more sensitive to the effects of settled rather than suspended particles. There were also higher mortalities of *T. discolor* in the first mayfly trial (where mayflies used stones as a substrate and would have had direct contact with settled kaolin particles) compared to the second trial (where square ceramic tiles resting on a rounded channel bottom kept the mayflies from coming into contact with the settled clay). A possible confounding factor in the mayfly trials is that exposed organisms were fed in the second trial but not in the first. However, the control treatment mortalities in both trials were very similar suggesting that feeding alone was not the driving factor causing mortality. Other research has also highlighted the sensitivity of organisms to settled over suspended particles. Kefford et al. (2010) found that two Coenagrionid, one Aeshnid and one Corixid species found in lowland Australian rivers were unaffected by suspended kaolin particle concentrations up to 601 mg/L, while the burial (1-5 mm) of *Physa acuta* (Gastropoda:Physidae), *Gyraulus tasmanica* (Gastropoda: Planorbidae) and *Chironomus cloacalis* (Diptera: Chironomidae) eggs with kaolin or sand reduced hatching significantly. Furthermore, in a review of the effects of fine sediment on macroinvertebrates, Jones et al. (2012) conclude that many of the impacts of fine sediments appear related more to the deposition of material than particles in suspension.

Indeed, when mayflies *T. discolor* and Oligoneuridae sp. were exposed in trial 2 to suspended particle concentrations as high as 801 mg/L for 13 days, the mortality incurred was only 50%. Mortality of adult *C. nilotica* shrimp over the 17 days of exposure was also very low, with no mortality in the control treatment and less than 20% occurring at the highest kaolin concentration of 680 mg/L. Although lethal concentration (LC) estimates could not be determined, a lethality NOEC for Oligoneuridae sp. was determined at 174 mg/L, and at 586 mg/L for adult *C. nilotica*. In terms of lethality, it appears that macroinvertebrates can be very tolerant of suspended kaolin particles. Kefford et al. (2010) reported less than 20% mortality for Coenagrionid *Ischnura aurora* exposed to suspended kaolin concentrations of 601 mg/L. Furthermore, McFarland and Peddicord (1980) showed that benthic marine invertebrates were exceedingly tolerant with 10% mortality or less at an exposure concentration of 117000 mg/L for sea urchin *Strongylocentrotus purpuratos*, Japanese clam *Tapes japonica*, hermit crab *Pagurus hirsutiusculus*, isopod *Sphaeroma pentodon*, mud snail *Nassarius obsoletus*, and blue mussel *Mytilus edulis* exposed between 5-12 days depending on species. Coast mussel *Mytilus californianus* was more sensitive with an LC50 of 96 000 mg/L, as was amphipod *Anisogammarus confervicolus* (LC50 = 78000 mg/L), polychaete *Neanthes succinea* (LC50 = 48000 mg/L) and tunicate *Ascidia ceratodes* (LC50 = 38000 mg/L). The most susceptible species to suspended kaolin exposure reported in the literature is *Daphnia magna* exposed over 7 days with an LC50 of 75 mg/L (with 95% confidence limits of 65-85 mg/L) (Robinson et al. 2010). The kaolin particles were shown to clog of the gut tract, causing decreased food uptake and assimilation. This is the likely mechanism of biological effect exerted by the suspended particles on all filter feeders (Robinson et al. 2010). Not surprisingly then, the marine filter feeders tested by McFarland and Peddicord (1980), the polychaete and tunicate, were also the least tolerant.

Mortality is, however, a course measure of effect, and suspended particles may be exerting sublethal effects on organisms that can still negatively affect the fitness of the species population and change the community structure (Adams et al. 2000; Wu et al. 2002). Consequently, sublethal effects at low levels of biological organisation were measured in mayflies *T. discolor* and Oligoneuridae sp. exposed in trial 2. One of the possible mechanisms of effect exerted by suspended particles on aquatic macroinvertebrates is damage to gill structures leading to reduced oxygen uptake. The biochemical responses of lipid peroxidation (Livingston 2001) and catalase activity have been shown to successfully reflect hypoxia in amphipod *Monoporeia affinis* (Gorokhova et al. 2010), midge *Chironomus riparius* (Choi et al. 2000) and estuarine fish (Ross et al. 2001). There was an increase, although not statistically significant, in average lipid peroxidation in *T. discolor* exposed to the two lowest suspended kaolin particle concentrations, with the response decreasing at the higher exposure concentrations (Figure 3.6A). The pattern was the same after for 4 and 13 days of exposure. This is a similar pattern to that reported by Wu et al. (2010) in the aquatic macrophyte *Hydrocharis dubis* exposed to a toxicant. Wu et al. (2010) suggest that, at lower toxicant concentrations, the plant's antioxidant enzyme system had not yet been activated and hence could not protect cells from membrane damage and consequent lipid peroxidation. However, catalase (an antioxidant enzyme) in the exposed *T. discolor* did not increase at the higher exposure concentrations (Figure 3.7A). The higher variability in lipid peroxidation after 13 days exposure suggests that perhaps some more sensitive individuals were beginning to experience high levels of stress with prolonged exposure time. The Oligoneuridae sp. exhibited slight, but not statistically significant, increases in both lipid peroxidation and catalase activity with increasing exposure concentrations, although there were no differences between exposure periods. These results suggest that either: lipid peroxidation and catalase activity may not be sensitive indicators of hypoxia in the two mayflies species tested; or that these species may have adapted to be particularly tolerant of high levels of suspended particles and like other benthic species such as molluscs and hypoxia-tolerant fish may have the ability to reduce oxygen consumption and metabolic rates (which could become as low as <10% of their normal levels – Stickle et al. 1989); or that these biochemical stresses may only be measureable at higher exposure concentrations and/or longer exposure periods. Lastly, low number of replicate samples and high within-treatment variability limited the ability of statistical tests to determine significant differences between treatments.

Two sublethal responses, at higher levels of organisation, were measured in *C. nilotica*: reproduction in terms of number of gravid females after 17 days of exposure and then the ultimate number of juveniles produced from the eggs of these gravid females and still surviving 54 days later; and growth in terms of final carapace length of juveniles. There were significantly fewer gravid females (one in each replicate) at the highest exposure concentration of 680 mg/L resulting in a NOEC of 586 mg/L (Figure 3.8B). However, there was no significant difference in the number juveniles produced in any of the treatments 54 days later (those replicates with excessive settling were not included in treatment averages as excessive settling appeared to greatly increase mortality of juveniles) (Figure 3.9A), perhaps suggesting density dependent competition in these organisms. Interestingly, average carapace length did not exhibit a dose response relationship either, but rather was correlated (-0.95) with the number of juveniles present in the replicate (Figure 3.9B), this time suggesting density dependent growth. This was observed in a similar experiment when juvenile shrimp were exposed to a laundry detergent chemical (Gordon 2012). In that study, as in this one, shrimp from all treatments were fed fish food flakes to satiation. A possible explanation, however, is that *C. nilotica* has the ability to feed on algae and bacteria growing on faecal pellets (Hart 1980) and other surfaces within the experimental vessel, resulting in potentially more nutritious natural food being available in experimental vessels with fewer individuals. These results suggest that *C. nilotica* may not be suitable for growth trials. In the only trial, to our knowledge, where reproductive or growth endpoints were measured in an invertebrate exposed to suspended kaolin particles, Robinson et al. (2010) report *D. magna* showed significantly lower growth at 30 mg/L but no change in the number of neonates (juveniles) produced. When *D. magna* were exposed to a 24 hour pulse of kaolin concentrations up to 400 mg/L and allowed a 20 day recovery period a NOEC of 50 mg/L for time to gravidity was determined, but as in the case of the *C. nilotica* results, there was no impact on the ultimate number of neonates produced.

3.5 CONCLUSIONS

There were technical challenges encountered when undertaking the stress-response experiments with suspended kaolin: 1) maintaining particulates in suspension, and keeping the concentrations of those suspensions constant across replicates; and 2) the difficulty of observing responses of organisms in turbid treatments limited the type of biological endpoint that could be measured and how often this measurement could take place.

All organisms exposed appeared to be very tolerant of the suspended kaolin, especially in terms of mortality which limited the generation of statistical point estimates of effect. The most useful biological response measured was that of reproduction in shrimp *C. nilotica*. However, from a population point level of view the results were contradictory. Although there were fewer gravid females at 680 mg/L, the number of juveniles produce from this treatment were not statistically different from any other treatment. Growth was not found to be a useful biological endpoint in *C. nilotica* as these organisms appeared to exhibit density dependent growth, growing larger at higher stress exposures as more individuals in the exposure vessel succumb to mortality. An attempt to link mayfly kaolin exposure to a physiological mode of action by linking damage to gills (either from abrasion or clogging) to two hypoxia biomarkers (lipid peroxidation and catalase activity). Unfortunately, a method could not be developed for viewing the mayfly gills under scanning electron microscopy (the gills were always damaged during the critical point drying process), and the biochemical responses exhibited no statistical exposure-response relationship.

Lastly, the experiments undertaken during this study suggest that the biological consequences of settled particulates are much more pronounced than suspended solids.

4 DEFINING THE EXPOSURE-RESPONSE RELATIONSHIP BETWEEN SUSPENDED KAOLIN PARTICLES AND AQUATIC ORGANISMS – WORK TOWARD DEFINING A WATER QUALITY GUIDELINE FOR SUSPENDED SOLIDS

4.1 INTRODUCTION

Exposure-response data are used to derive guidelines for protecting the aquatic ecosystem from specific stressors. To date, most of these endeavours have focused on chemical stressors. In this regard, sub-lethal response data (preferred to lethal responses) from long-term exposure (preferred to short-term exposures) from as large a number of aquatic organisms as possible (from a range of different trophic and taxonomic groupings) are collated and subjected to a derivation method (the statistically-based species sensitivity distribution being preferred to the assessment factor approach) in order to produce a specific concentration below which undesirable effects are not expected to occur). See Appendix 1 for a detailed explanation of the data requirements and processes currently being used internationally for undertaking water quality guideline derivations.

However, to date, due to insufficient biological effects data, water quality guidelines for suspended solids in South Africa (DWAF 1996) and in many states in the USA (Swietlik et al. 2003) rely on a percentage 'departure from reference condition' in which turbidity levels are measured in Nephelometric Turbidity Units (NTU). Slightly more advanced guidelines are those of Australia and New Zealand (ANZECC and ARMCANZ 2000). Under the ANZECC guidelines, trigger values (mostly in NTU, but also suspended solids concentrations when enough field data exists) are determined as a percentile of a reference system's distribution for one of three recognised possible ecosystem conditions: high ecosystem/conservation value; slightly to moderately disturbed ecosystems; and highly disturbed ecosystems. Additionally, 'default trigger' values have been calculated within the ANZECC guide to be used in situations where no reference conditions can be established. All trigger values are region specific and all regional trigger values are further divided under ecosystem types: upland rivers, lowland rivers, lakes and reservoirs. These ecosystem-specific quantitative trigger values are complimented by ecosystem modifier information and narrative statements in the guideline, aiding managers in decision-making for a given water body. In Canada (Singleton 1985; Caux et al. 1997), an attempt was made to calculate the amount of deviation from reference using empirical models of available effects-based data of fishes indigenous to the region. This approach is based on the work and data of Newcombe and Jensen (1996), who defined thresholds of effect for salmonids in North American rivers. This work is, however, site/situation specific as these organisms are particularly sensitive to suspended solids. Consequently, the guidelines aren't applicable to many of the water bodies found throughout the world and also not applicable to ecosystems in which salmonids do not exist or are not an ecologically or economically important species. Wilber and Clarke (2001) updated the work of Newcombe and Jensen (1996), correcting some data errors for estuarine fish species and including a marine shellfish, presenting the results as descriptive graphics of the exposure-response relationship rather than model equations. Berry et al. (2003) do, however, caution that the empirical models developed by Newcombe and Jensen

(1996) and the graphics developed by Wilber and Clarke (2001) have questionable scientific rationale and that their accuracy is yet to be determined. Lastly, although there is no quality standard for suspended or deposited solids under the Water Framework Directive in the United Kingdom (WWF UK 2007), the UK Technical Advisory Group on the Water Framework Directive (UKTAG) does provide a 'Guideline Standard' (rather than an Imperative Standard) through the Freshwater Fish Directive (FFD 2004/44/EC) of an annual mean suspended solids concentration of 25mg/L (UKTAG 2008). This guideline is based on the requirements of salmonid and cyprinid fish populations.

The 'departure from reference condition' approach has been criticised due to the difficulty in finding suitable reference sites, the unreliability of turbidity as a surrogate measure of sedimentation, and the difficulty in defining a suitable reference range that is representative of a number of different regions (Bilotta and Brazier 2008; Dunlop et al. 2008; Collins et al. 2011). It has been suggested that long-term solutions to generating reliable guideline for suspended solids will most likely be found through the use of exposure-response data and their application in a biological effect based guideline derivation process (Dunlop et al. 2008). However, a number of problems have been met when developing these types of guidelines for suspended solids. In addition to the usual factors which control biological responses to chemical stress exposure (e.g. stressor concentration, duration of exposure, organism life history stage), suspended solids have additional mechanisms of effect, i.e. geochemistry, particle size and possibly particle shape (Collins et al. 2011). Of the limited biological effects data currently available, most do not stipulate what these three additional characteristics of the exposure medium were – in most cases they were not measured or determined. Consequently, application of these currently available biological effects data in guideline derivation are likely to resulted in imprecise threshold levels due to high variations in the concentration-response relationship.

A possible solution is to develop more site-specific guidelines by generating biological effects data to suspended particles of a particular geochemistry and restricted size range. This could involve using natural sediment from the site of interest, sieved to a particular size range. Another option is to develop an understanding of the biological effects of commonly occurring particles. With this in mind, biological response data to kaolin clay particle exposure were collated from the scientific literature and from those generated in Chapter 4. Using various available methods, these data were used to generate a number of exposure response relationships describing aquatic organism responses to kaolin particle exposure. However, there were very few effect point estimates for lethality (LC50) (with all but one representing marine organisms), only one sub-lethal point estimate (ECx) and six no observed effect concentrations (NOECs) for both short and long term exposures (see next section for details). The difficulty in generating biological effects data is evident from the results in Chapter 4 and from the paucity of good quality data in the scientific literature. Consequently, this study is not intended to generate a robust water quality guideline for suspended solids in general or specifically for kaolin-like sediments in South Africa. Instead the aim is to define possible exposure-response relationships using available methods. These will be the first step in the process toward developing a guideline and this work is intended to generate debate and guide further refined research toward this goal.

4.2 AVAILABLE BIOLOGICAL EFFECTS DATA FOR SUSPENDED KAOLIN CLAY PARTICLES

Data were collated from an extensive search of the international scientific literature, with some additional indigenous South African data generated during the course of this project. Most of lethal point estimate data was for marine species which appear to be extremely tolerant of suspended kaolin particles (Table 4.1). The ages of the most tolerant marine species exposed were not stated, but the more sensitive *Oplegnathus fasciatus* and *Parapristipoma trilineatum* were marine larval fish. As there were so few data, results from both long-term and short-term exposures were included in the same table. The only freshwater organism represented was *Daphnia magna* (Table 4.1). This species was the most sensitive, with stress induced through kaolin particles blocking the gut ultimately leading to death (Robinson et al. 2010).

Table 4.1 Lethal point estimate data (lethal concentration affecting 50% of exposed organisms – LC50) available for aquatic organisms exposed to suspended kaolin clay particles.

Species name	Fresh water (FW) or salt water (SW)	Exposure duration (days)	LC50 (mg/L)	Source
Mytilus californianus [1]	SW	8.3	96000	A
Anisogammarus confervicolus [2]	SW	4.2	78000	A
Crangon nigromaculata [2]	SW	8.3	50000	A
Neanthes succinea [3]	SW	8.3	48000	A
Ascidia ceratodes [4]	SW	4.2	38000	A
Cancer magister [2]	SW	8.3	32000	A
Cymatogaster aggregate [5]	SW	4.2	6000	A
Oplegnathus fasciatus [5]	SW	0.5	710	B
Parapristipoma trilineatum [5]	SW	0.5	170	B
Daphnia magna [2]	FW	7	75	C

1 Bivalve; 2 Crustacean; 3 Annelida; 4 Tunicate; 5 Fish.

Sources: A) McFarland and Peddicord 1980; B) Isono et al. 1998; C) Robinson et al. 2010.

There were very few studies reporting sub-lethal endpoints in the form of NOECs or ECx data. The reproductive ability of the indigenous freshwater shrimp (*Caridina nilotica*) was only affected by suspended kaolin concentrations in excess of 586 mg/L (Table 4.2). The two freshwater larval fish species measuring avoidance response and feeding efficiency in the presence of suspended kaolin particles were the next two most tolerant sub-lethal endpoints (Table 4.2). The last four NOECs of 50 mg/L belong to *D. magna*, two bacteria species and a robust field mesocosm study examining the response of a mixed periphyton and macrophyte community to extended periods of suspended kaolin exposure. The reason all four NOECs were the same concentration (i.e. 50 mg/L) is a statistical artefact of the concentration ranges chosen for these studies (Chapman et al. 1996). In the case of *D. magna* and the mesocosm study, the actual no effect concentration could fall anywhere between 50 and 100 mg/L, and for the bacteria species between 50 and 200 mg/L (see supplementary data file containing details of these studies).

Table 4.2 Sub-lethal no observed effect concentration (NOEC) data and a point estimate datum (effect concentration affecting 25% of exposed organism – EC25) for freshwater organisms exposed to suspended kaolin clay particles.

Species name	Exposure duration (days)	NOEC or otherwise stated (mg/L)	Biological response	Source
Caridina nilotica [1]	17	586	Reproduction	A
Galaxias fasciatus [2]	0.014	EC25 = 133	Avoidance response	B
Morone saxatilis [2]	0.017	75	Feeding efficiency	C
Daphnia magna [1]	1	50	Reproduction	D
Bodo saltans [3]	Not stated	50	Population growth and abundance	E
Monosiga ovata [3]	Not stated	50	Population growth	E
Periphyton and macrophyte community [4]	63	50	Whole stream respiratory rate, periphyton biomass, percentage macrophyte cover	F

1 Crustacean; 2 Fish; 3 Bacteria; 4 Plant.

Sources: A) Current study; B) Breitburg 1988; C) Boubee et al. 1997; D) Robinson et al. 2010; E) Boenigk and Novarino 2004; F) Parkhill and Gulliver 2002.

In the absence of sufficient traditional exposure-response data (point estimates or NOECs) to derive reliable water quality guidelines for suspended solids, a number of attempts have been made to characterise the exposure-response relationship for freshwater and estuarine fishes as empirical models composed of three variables: suspended particulate concentration; duration of exposure; and severity-of-effect (Newcombe and Jensen 1996; Wilber and Clarke 2001). These studies provide graphical models and, additionally in the case of Newcombe and Jensen (1996), equations describing the potential consequences of suspended particulate exposure and allowing some estimation of risk. Appendix 2 lists all exposure-response data available in the scientific literature and generated for indigenous South African organisms exposed to suspended kaolin particles according to six severity-of-effect classes: no effect; sub-lethal effect; <20% mortality; 20-40% mortality; 41-60% mortality; and >60% mortality. In the next section, these data will be collated into graphical representations of the exposure-effect relationship.

4.3 APPROACHES FOR DESCRIBING THE EXPOSURE-RESPONSE RELATIONSHIP

Currently, the most internationally accepted approach for generating an exposure-response relationship is the species sensitivity distribution (SSD). A SSD is a statistical distribution describing the variation in biological effects of a range of species to particular concentrations of a stressor. From this statistical distribution an estimate of the percentage species affected by a specific concentration of the stressor can be made. The water quality guideline value chosen is based on a specific percentage of species effected, e.g. the concentration that protects 95% of species (i.e. protective concentration 95% – PC95), which is the same as the concentration that affects 5% of species (i.e. hazardous concentration 5% – HC5). The particular distribution utilised in this report belongs to the Burr Type III family and was utilised in the development of the Australian and New Zealand Water Quality Guidelines (ANZECC and ARMCANZ 2000) by applying the specially developed BurrliOZ software v1.0.9 CSIRO (Shao 2000).

There are a number of assumptions regarding the statistical distribution models used in SSDs. These pertain to the number of biological effects data employed in the distribution (the more data points the more likely the distribution will fit the data to provide an accurate estimation of percentage species affected) and the type of taxa represented in the data (the taxa should represent the species in the ecosystem of concern in order to cover the range of sensitivities of organisms occurring there). A detailed description of the various methods, particularly focussing on SSDs, used for deriving water quality guidelines internationally, and the specific data requirements required per jurisdiction, is presented in Appendix 1. As indicated earlier, there is no intension here to generate a water quality guideline for South Africa, however, in the absence of any other management tools (based on biological responses) for managing suspended solids it is still a useful exercise to generate exposure-response relationships with the data available in order to compare with other exposure-response relationships generated in the literature for other types of sediments, and also to compare with biological responses measured in the field (Chapter 5).

Due to the number of available biological effects data, results from all exposure durations were included in the respective SSDs. The SSD for the lethal point estimate (LC50) data (listed in Table 4.1) is presented in Figure 4.1. The SSD for sub-lethal data (listed in Table 4.2) is presented in Figure 4.2. The hazardous concentration (HCx) estimates for both sets of data are detailed in Table 4.3. The determination of a definitive no-effect level using SSDs, i.e. 0% species affected, is not possible as the extreme tails of the typical normal or logistic statistical distribution are extended to plus and minus infinity (the same is true for determining an absolute effect level, i.e. 100% species affected) (Van Straalen 2002). Consequently, there is a need to choose an arbitrary cut-off point in the distribution to express the risk of the chemical to the environment. The 5th percentile is the most commonly chosen value. It represents the concentration that is theoretically hazardous to 5% but protects 95% of species from the effect that the biological effects data represent and is termed the HC5. The ecological effect of 5% of species not being protected is considered acceptably small. Of course it needs to be remembered that the SSDs presented below are based on a limited number of data, incorporating short and long-term exposures, and in the case of the lethal estimate data, comprised of a mixture of freshwater and marine organisms exposed specifically to kaolin clay. Any interpretation of the HCx values require this to be kept in mind.

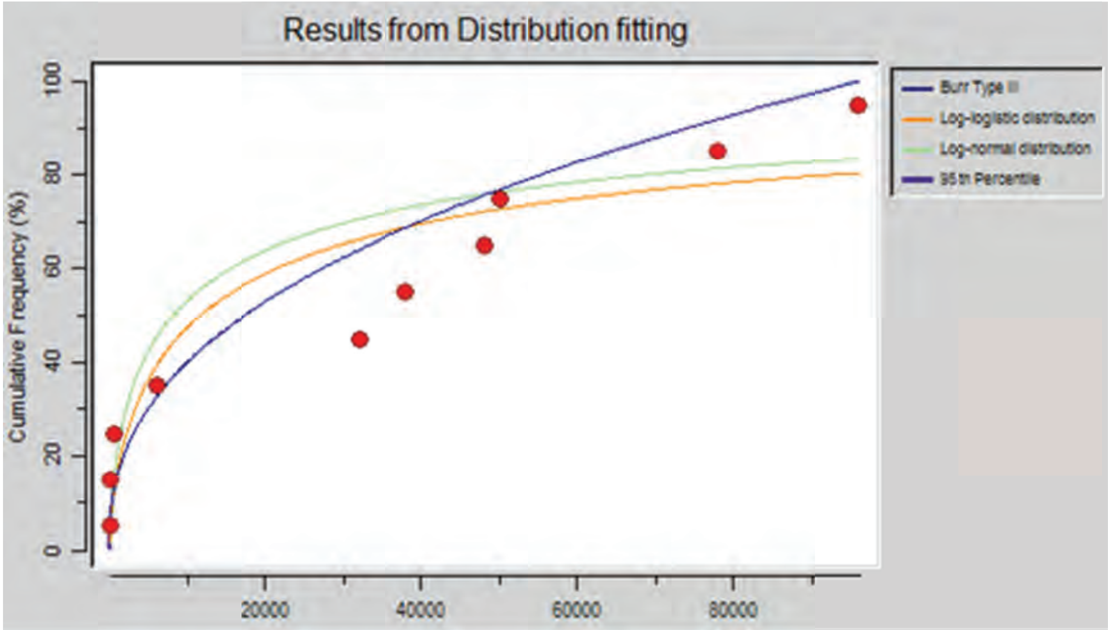


Figure 4.1 Species sensitivity distribution for lethal point estimate data (LC50) of long and short-term exposure to suspended kaolin. X axis is suspended kaolin concentration in mg/L.

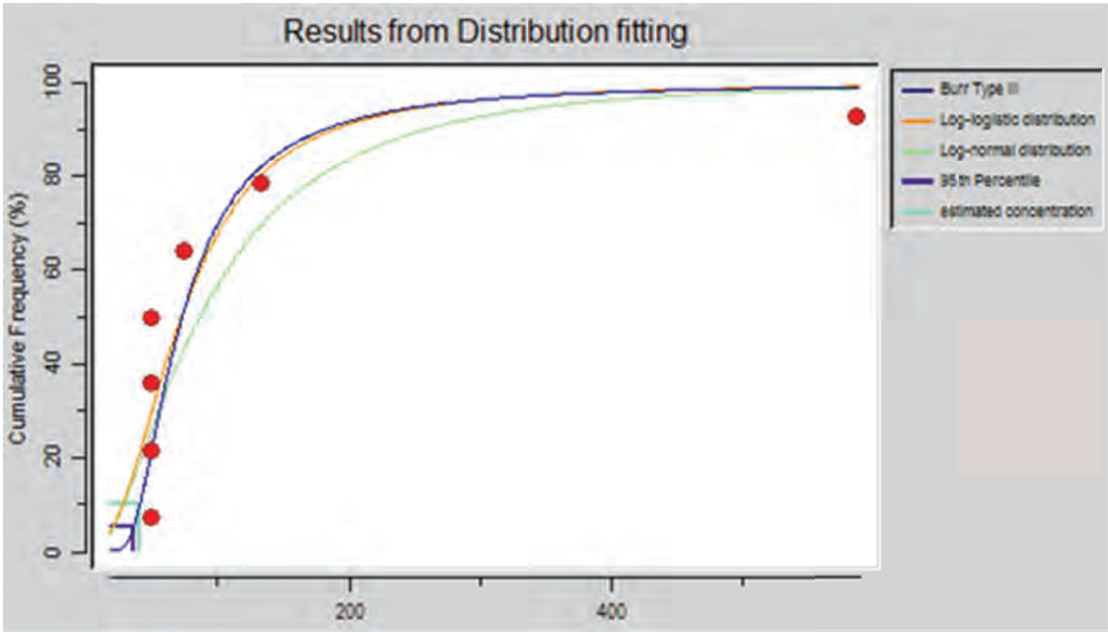


Figure 4.2 Species sensitivity distribution for sub-lethal of long and short-term exposure to suspended kaolin. X axis is suspended kaolin concentration in mg/L.

Table 4.3 Hazardous concentration (HCx) estimates for lethal and sublethal biological effects data for suspended kaolin exposure.

Hazardous concentration affecting x% of species	Lethal point estimate data (Table 4.1) (mg/L)	Sub-lethal data (Table 4.2) (mg/L)
HC5	58	36
HC10	322	41
HC20	1788	49
HC30	4878	56
HC40	9941	64
HC50	17269	73
HC60	27116	85
HC70	39711	101
HC80	55263	126
HC90	73965	181
HC95	84555	255

A second method of describing the exposure-response relationship for organisms exposed suspended kaolin involved a graphic representation of three variables: suspended kaolin concentration; duration of exposure; and severity of effect. The data used to generate these graphic models is listed in Appendix 2. The first graphic (Figure 4.3) depicts the exposure-response relationship for freshwater organisms only, showing that sub-lethal responses began at approximately 30 mg/L and lethal responses at approximately 55 mg/L when exposure periods were between 5-17 days. The three indigenous macroinvertebrates tested in Chapter 3, *Tricorythus discolor*, Oligoneuridae sp. and *Caridina nilotica* exhibited no mortality effects, in excess of that measured in the control treatments, for concentrations up to 801 mg/L during the first 5 days of exposure (Appendix 2 and Figure 4.3). When the exposure period was less than 12 hours the most sensitive sub-lethal response was approximately 100 mg/L (Figure 4.3).

The inclusion of marine biological effects data did not indicate a change in where sub-lethal or lethal effects began occurring for exposures of between 5-17 days (Figure 4.4). However, the additional short-term lethality trials with marine fish larvae did indicate that sensitive species began experiencing low percentage mortality from 30 mg/L, with all species affected by moderate percentage mortality from 320 mg/L. Regardless of exposure period, when concentrations exceeded 1000 mg/L even the most tolerant organisms begin showing lethal effects (Figure 4.4). The use of only freshwater macroinvertebrate data to generate a severity-of-effect model generally resulted in the same conclusions as detailed above (Figure 4.5). This last graphic will be used to predict possible effects on the macroinvertebrates of selected South African rivers in the field assessment section of this project (Chapter 5).

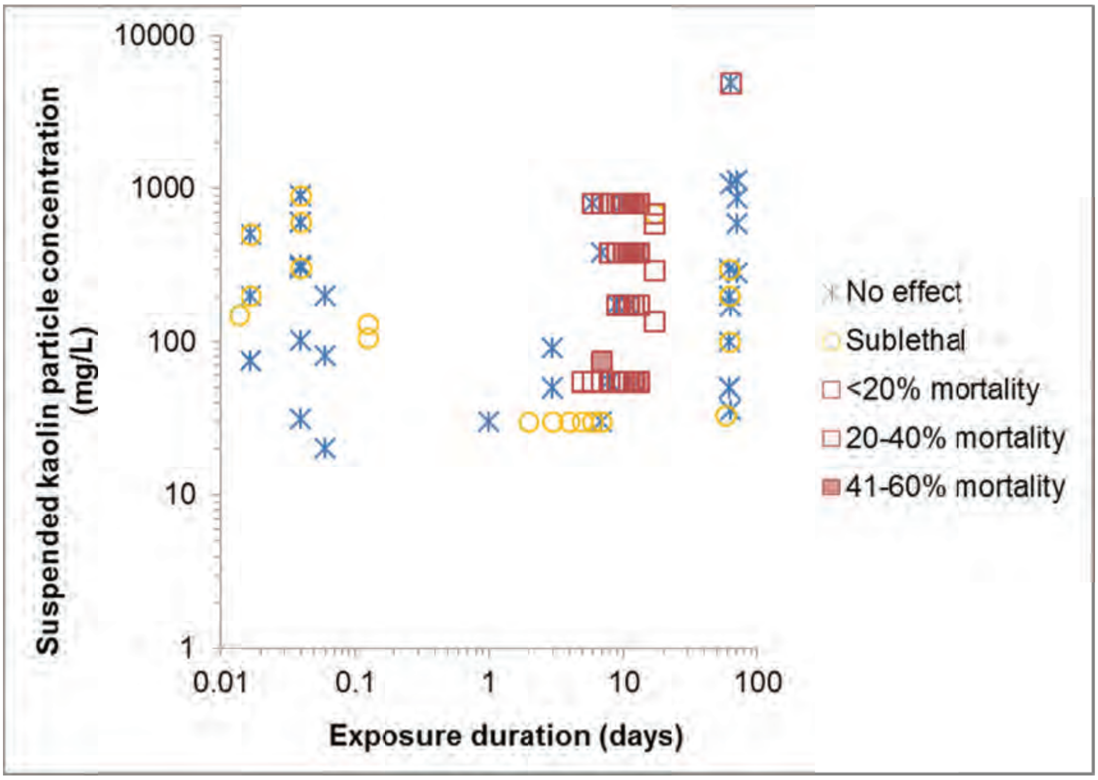


Figure 4.3 Graphic representation of the severity-of-effect of exposure of freshwater organisms to suspended kaolin concentrations.

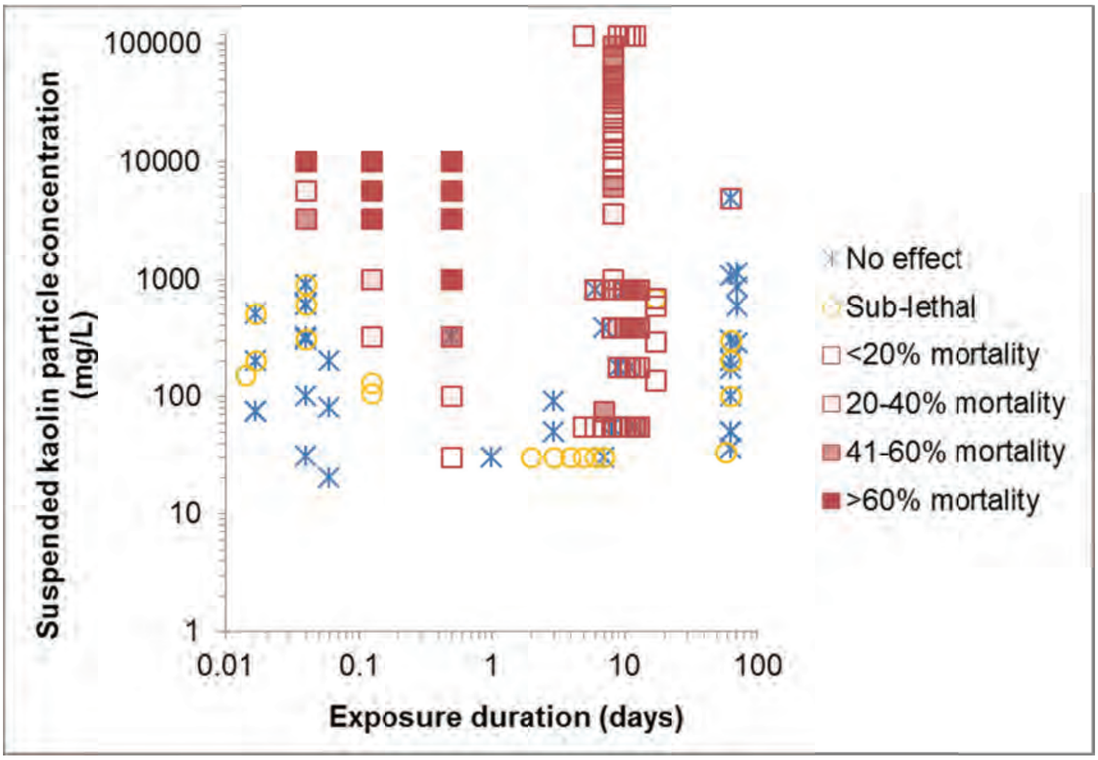


Figure 4.4 Graphic representation of the severity-of-effect of exposure of marine and freshwater organisms to suspended kaolin concentrations.

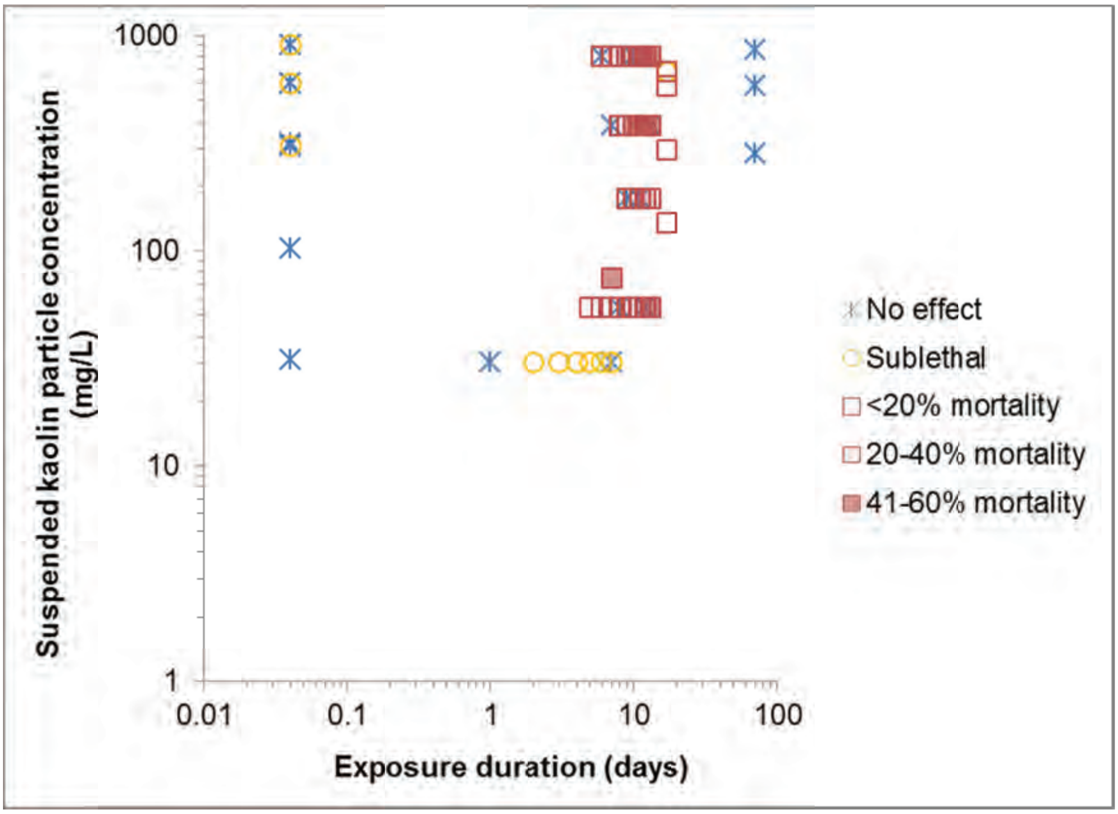


Figure 4.5 Graphic representation of the severity-of-effect of exposure of freshwater macroinvertebrates to suspended kaolin concentrations.

4.4 DISCUSSION

The SSD HC5 estimate for mortality response (58 mg/L) was very similar to the severity-of-effect graphic's most sensitive mortality effect class (<20%) concentration of 55 mg/L recorded in kaolin exposure experiments. For the sub-lethal data, estimates were also similar, with an HC5 of 36 mg/L and the most sensitive datum in the sub-lethal effect class being 30 mg/L. Although this might not be entirely unexpected as the same experimental results were used for both exposure-response relationship approaches, the SSD did only utilise a fraction of the available data (being the statistically derived LC50 for lethality and NOECs or EC25 for sub-lethality), whereas in contrast, the severity-of-effect graphics presented all the available data (including lowest observed effect data and mortality data that could not be used to derive the LC50). The above determined sub-lethal exposure-response relationships predicting very low or no effects at concentrations approximating 30 and 36 mg/L correspond well to the European Union Freshwater Fish Directive Guideline of an annual mean suspended solids concentration of 25mg/L (UKTAG 2008). They are less conservative than the Canadian guidelines for clear flow periods, however, which state that concentrations of suspended solids should not increase by more than 25 mg/L over background concentrations during any 24 hour period, and by no more than 5 mg/L over a period of a month (Caux et al. 1997).

The results from the SSDs relate to the effects of long-term exposure (although there were some short-term data utilised in the generation of the SSDs, the majority of the biological effect data came from long-term exposure experiments). There is only a little data to suggest how organisms might respond to the more environmentally realistic situation of higher suspended solids concentrations for shorter durations (reflecting high flow incidents such as floods). The situation is different for the freshwater and marine data collated for kaolin. The three indigenous freshwater macroinvertebrates investigated in Chapter 3 showed no mortality, beyond that experienced by organisms in the control treatments, at exposure concentrations up to 801 mg/L for 5 days. In contrast, mortality began at 30 mg/L for three larval marine species exposed for 12 hours, and intensified at concentrations above 320 mg/L for exposure periods of 1, 3 and 12 hours. These marine data could be applicable to freshwater resources if the assumption that the sensitivity of marine and freshwater fish larvae to suspended solids is unlikely to be very different.

Smit et al. (2008) derived SSDs of LC50 data for marine organisms exposed to suspended particles of barite and bentonite clay, reporting than the species tested were slightly more sensitive to bentonite, although this difference was not statistically significant. The determined HC5 for bentonite was 8 mg/L and for barite 18 mg/L (Smit et al. 2008). The SSD determined in this report for kaolin, predominantly from marine data, determined a HC5 of 58 mg/L, indicating that organisms appear less sensitive to kaolin. Results of a trial exposing *D. magna* to three types of clay: kaolin; a natural clay (consisting of 60% kaolin); and montmorillonite (the main constituent of bentonite) appear to corroborate this (Robinson et al. 2010). *Daphnia magna* were most sensitive to the montmorillonite (HC5 = 5 mg/L), then the natural clay (HC5 = 51 mg/L) and most tolerant of kaolin (HC5 = 75 mg/L). Although limited, the data suggest that suspended sediments consisting predominantly of kaolin will have fewer biological effects than bentonite dominated sediment.

4.5 Conclusion

The exposure-response relationships generated for kaolin suggest that sub-lethal effects at concentrations below 30 mg/L, and lethal effects at concentrations below 55 mg/L, over extended periods of exposure are unlikely for all but the most sensitive species. In terms of short-term exposures (< 24 hours), the freshwater macroinvertebrates tested were very tolerant (highest concentration tested was 801 mg/L), but marine fish larvae were much more sensitive (low mortality occurring at 30 mg/L in one species), but generally mortality occurring in all species tested when exposure concentrations exceeded 320 mg/L. An attempt will be made to test the relevance of these exposure-response relationships by examining the responses of macroinvertebrates at sites experiencing different concentrations of suspended solids (Chapter 5).

5 DEVELOPMENT AND APPLICATION OF A SITE-SPECIFIC SUSPENDED SOLIDS RISK ASSESSMENT FRAMEWORK

5.1 GENERAL INTRODUCTION

The purpose of the suspended solids risk assessment framework is to determine if instream suspended particulate matter concentrations at a site, or within a specific region, are having unacceptable effects on the resident biota.

In the context of this study, “unacceptable effects” refer to changes in the components of the macroinvertebrate assemblage, and the biota being investigated are macroinvertebrates. Every risk assessment is different and these particular issues need to be defined before a risk assessment is undertaken.

5.2 DEVELOPMENT OF A SITE-SPECIFIC SUSPENDED SOLIDS RISK ASSESSMENT FRAMEWORK

As site-specific risk assessments are expensive and time consuming, it is useful to have a tiered approach. In this study a three tiered approach is proposed:

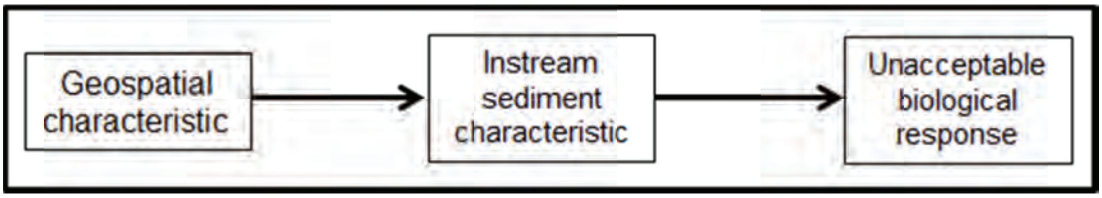
- **Tier 1** involves a ‘desktop’ approach in which the geospatial characteristics within in a catchment are used to determine if there is the potential for unacceptable biological effects from instream particulate matter.
- **Tier 2** involves comparing sediment load characteristics (e.g. turbidity, suspended solids concentrations) measured in the field with a relevant biological effects exposure-response relationship generated from laboratory data in order to infer the potential for unacceptable biological effects from suspended particulate matter.
- **Tier 3** involves site-specific biomonitoring of biota resident at the site of interest in order to directly measure for unacceptable biological effects from suspended particulate matter.

The cost and time needed to undertake the assessment increases from Tier 1 to Tier 3, but the likely accuracy of the methods utilised in each tier, in terms of reliably measuring an unacceptable biological response – i.e. confidence, also increase from Tier 1 to Tier 3 (where in Tier 3 direct measurements of the biota are undertaken). Consequently, the Tier 1 approach could be used to cover a larger number of potential sites at lower cost and in a shorter time than the more site-specific orientated Tier 2 and Tier 3 approaches. However, there are a number of assumptions or questions associated with each of the Tiers that need validation and acceptance before this approach can be considered a useful tool for managing suspended particulates.

Tier 1 assumptions

The two assumptions are: 1) there is a link between a geospatial characteristic and a sediment load characteristic and 2) there is a link between a sediment load characteristic and a biological response (see Box 5.1). What would need to be determined is which combination of geospatial characteristic, instream sediment load characteristic and biological response provide the most accurate/reliable link? Some of these assumptions are shared by the remaining two tier's.

Box 5.1 Tier 1 assumption



Tier 2 assumptions

The first assumption is that one of the instream sediment load characteristic being correlated with an unacceptable biological response. The second assumption for this tier is that the exposure-response relationship, based on laboratory organism exposure to the best correlated sediment load characteristic, provides an accurate prediction of an unacceptable biological response.

Tier 3 question

Which one of the biological response measures determined in the course of biomonitoring best reflects the effects of excessive sediment load?

The best way to investigate the usefulness of this risk assessment approach, and address the assumptions and questions raised, is through applying it in a case study where catchments are in close proximity (reducing the effects of other environmental factors on the instream biota) but have contrasting sediment load characteristics. Such a situation was found in the north east area of the Eastern Cape Province, and the application of the risk assessment protocol is described in section 5.3.

5.3 APPLICATION OF A SITE-SPECIFIC SUSPENDED SOLIDS RISK ASSESSMENT FRAMEWORK

5.3.1 Introduction

Five hypotheses were formulated to help examine the applicability of the risk assessment framework (Table 5.1). They were investigated in catchments with rivers of potentially contrasting sediment loads. The first two hypotheses investigate whether the catchments do indeed contrast in terms of: potential for sediment input to rivers; and measures of sediment load (turbidity; suspended and settled solids). Hypothesis 3 examines if there is a relationship between certain geospatial characteristics and instream sediment load characteristics/measures. This will address the first issue or assumption of the Tier 1 risk assessment: whether there a link between a geospatial characteristic and sediment load characteristic. Hypothesis 4 examines whether composition of the macroinvertebrate assemblage differs between catchments. If there is a difference in measured sediment characteristics and macroinvertebrate community characteristics, then Hypothesis 5 examines if there is a link between the macroinvertebrate response and a particular sediment characteristic. This then addresses the second issue or assumption of the Tier 1 risk assessment: whether there is a link between a sediment load characteristic and a biological response. It will also help refine Tier 2 by indicating which sediment characteristic best predicts biological (macroinvertebrate) changes in the field so allowing effort in developing a water quality guideline to focus on particular that sediment characteristic (e.g. it may be total suspended solids concentration, which would be useful as some biological effects data already exist for this measure, but if it is settled solids then many biological effects data will need to be created). Lastly, the outcome of Hypothesis 5 will also provide useful information for Tier 3, indicating which biological response is a sensitive measure of sediment load exposure and should therefore be employed in site-specific biomonitoring.

In this chapter, the materials and methods used to gather the geospatial, water physic-chemical, sediment load characteristics and macroinvertebrate data are described in the next section. Thereafter, a description of the sampling sites and catchments in which they occur is provided. Each hypothesis is then examined according to the data collated and the result provided. The chapter concludes with an assessment of the proposed risk assessment protocol and suggestions for possible refinement.

Table 5.1 Hypotheses to be examined and how they relate to the risk assessment protocol.

Null Hypothesis	Alternative Hypothesis	Risk assessment protocol issue addressed
H_1: there is no difference in land degradation between the catchments (specifically Pot and Luzi)	H_{A1}: there is a difference in land degradation between catchments	Is the study being undertaken in catchments that differ in terms of land degradation and sediment load characteristics?
H_2: there is no difference in sediment load characteristics between catchments	H_{A2}: there is a difference in sediment load characteristics between catchments	
H_3: there is no correlation between any geospatial catchment characteristic and sediment load characteristic measured	H_{A3}: there is a correlation between a geospatial catchment characteristic and the sediment load characteristic measured	Tier 1: link between geospatial data and sediment load characteristic?
H_4: there is no difference in the macroinvertebrate community between the sites according to various biological stress responses	H_{A4}: there is a difference in the macroinvertebrate community between the sites according to various biological stress responses	Does the macroinvertebrate community vary between site/catchments as measured by various biological stress responses?
if H_{A3} and H_{A4} apply		
H_5: the differences in macroinvertebrate assemblage composition between the sites cannot be attributed to a particular sediment load characteristic	H_{A5}: the differences in macroinvertebrate assemblage composition between the sites can be attributed to a sediment load characteristic	Tier 1: link between sediment load characteristic and biological response? Tier 2: which sediment load characteristic best predicts biological harm? Tier 3: most sensitive biological response for indicating unacceptable levels of sediment exposure?

5.3.2 Materials and methods

5.3.2.1 Geospatial data sources

The purpose of the spatial data analysis was to delineate the study catchments and then describe them in terms of spatial characteristics, geology, geomorphology and land cover, and relate these characteristics to processes of sediment delivery. First, the geospatial data sources are described. Table 5.2 provides an overview of the freely available and proprietary data sets obtained. In section 6.2.2, the methodology of the spatial data analyses is presented. ArcMap 10.0, SAGA GIS 2.08 and R 2.15.1 were utilised together with the geodata processing capabilities of R implemented in the packages sp, raster, rgdal and maptools.

Table 5.2 Overview of geospatial data sets utilised

Data set	Projection	Resolution / scale
ASTER DEM	WGS 1984 UTM 35S	29.07 m
LandSat GLS	WGS 1984 UTM 35N	30 m
SPOT 5	GCS WGS 1984	0.2 arcseconds
Orthophotos	WGS 1984 UTM 35S	0.5 m
MODIS NDVI	Sinusoidal	926.7 m
WorldClim	GCS WGS 1984	30 arcseconds [1]
LandScan Population Grid	GCS WGS 1984	30 arcseconds
National Land Cover	WGS 1984 Albers Equal Area	30 m
Geology	WGS 1984 UTM 35S	1:250000
Topographic Maps	Gauss-Conform, 29°E, WGS 1984	1:50000

Digital elevation model

The elevation model used was the Advanced Spaceborne Thermal Emission and Reflection Radiometer Global Digital Elevation Model Version 2 (ASTER GDEM v2) (LP DAAC 2011), the highest-resolution digital elevation model available at no costs. The tiles covering the research area were downloaded in geographic projection GCS WGS1984 and reprojected to UTM Zone 35S to make the linear unit meters. The resulting resolution was 29.07 m per pixel.

Slope

Slope steepness is an important factor contributing to soil erosion and its calculation is readily implemented in GIS software. It was computed in SAGA GIS using the method “fit 2nd degree polynoms” (Bauer et al. 1985). Output was in radians and was converted to degrees by:

$$1{:}\mathit{rad} = \frac{360^\circ}{2\pi} \approx 57.29578^\circ$$

Terrain Ruggedness Index

The Terrain Ruggedness Index (TRI) (Riley et al. 1999) expresses terrain heterogeneity as relative elevation difference between a raster cell of a digital elevation model (DEM) and its neighbours and is implemented in SAGA GIS. It was run using the standard settings: radius = 1; no distance weighting. Thus, only the eight surrounding cells of each raster cell, known as the Moore neighbourhood, were used. The resulting values were classified into seven ruggedness classes according to the class boundaries given by Riley et al. (1999), but as these were given for a 1x1 km^2 grid, they were corrected to represent the same relative elevation difference using the factor 29.07 m/1000 m.

[1] 30 arcseconds = 0.0083°, equals ca. 800x920 m

Stream network delineation

A synthetic stream network was derived from the DEM in ArcMap using the Spatial Analyst extension. Initially, internal sinks were filled, and afterwards flow direction and flow accumulation were computed. As cell values of the flow accumulation grid represent the number of upstream cells, the specific catchment area of each point can be calculated as:

$$catchment{:}area{:}[km^2]=cell{:}value\cdot(0.02907{:}km)^2$$

Thus, stream networks with varying degrees of detail could be defined by setting their minimum specific catchment areas to 1, 2, 5 and 10 km^2.

Sampling sites and catchment delineation

As catchments statistics were to be restricted to the specific catchment areas of our sampling sites, watershed delineation was a critical step. From the sampling site GPS coordinates (which were converted to decimal degrees and snapped to the nearest stream of the newly created stream network to create pour points) we calculated the specific catchment area of each sampling site using the ArcMap “Watershed” command. The resulting grid was converted to a polygon shapefile and checked for consistency and accuracy with the help of the 20 m contour lines in the 1:50000 topographic maps. Corrections to the catchment shapefiles were made manually where the calculated catchments boundaries deviated significantly from the catchment boundaries as indicated by the 20 m contour lines in the topographic maps. The resulting shapefile containing the corrected specific catchment areas of the six sampling sites was then used for restricting the geodata statistics to the individual catchments.

Geology

Geological maps of South Africa at a scale of 1:250000 are available as georeferenced GeoTIFFs Geological Survey (1981). The map sheets used were 3028: Kokstad and 3128: Umtata. The geological classes were vectorized manually in ArcMap to create a gapless polygon surface. The geology polygons were then converted to rasters with cell size and the extent set to match those of ASTER DEM.

1:50000 Topographic maps and extracted information

The highest level of detail available is given by the 1:50000 topographic maps (The National Directorate: Geo-spatial Information, 2011). Eight map sheets covered the research area: 3028CA Naudesnek; 3028CB Sethabathaba; 3028CC Elands Heights; 3028CD Mdeni; 3028DA Mount Fletcher; 3028DC Katkop; 3128AA Ugie; and 3128AB Maclear. Data extracted from these maps were:

Former Transkei border

The border of the former Transkei within the research catchments was extracted from the topographic maps.

River gradient

The river gradient was calculated by vectorizing the stream segments containing the sampling sites between two adjacent 20 m contour lines from the topographic maps and calculating the feature lengths. From the feature lengths and the 20 m elevation difference the river gradients were calculated as $\Delta h/l$.

Houses
Houses as indicated in the topographic maps were extracted to a point shapefile.

MODIS normalized differenced vegetation index (NDVI)
MODIS (Moderate Resolution Imaging Spectroradiometer) is an instrument aboard the NASA satellites Aqua and Terra. A time series of MOD13A3.5 data from the Terra satellite, the 1 km standard resolution monthly Normalized Differenced Vegetation Index (NDVI) data set, was downloaded from the USGS Global Visualization Viewer[2] in hdf4 file format. The NDVI is a simple indicator of land with live green vegetation. Each month's raster was converted to TIFF file format and analyzed in R (see section 6.2.2). A time series from 2001 to 2011, comprising 11 complete years, was analyzed. The NDVI is calculated as from the ratios of the red and near infrared (NIR) channels. The bandwidths used were 620-670 nm (red) and 841-876 nm (Huete et al. 1999). The Index is calculated as:

$$\mathrm{NDVI}=\frac{\mathrm{NIR}-\mathrm{Red}}{\mathrm{NIR}+\mathrm{Red}}$$

Satellite and aerial imagery
Landsat
Path 169, Row 81 and 82, was downloaded from USGS's Earth Explorer[3]. Entity IDs used and acquisition dates were:
Global Land Survey 2010:
LT51690812009107JSA00 17-APR-09
LT51690822009107JSA00 17-APR-09
Global Land Survey 2005
LE71690812005136ASN00 16-MAY-05
LE71690822005136ASN00 16-MAY-05
Global Land Survey 2000
P169R081_7X20010302 02-MAR-01
P169R082_7X20011215 15-DEC-01

Climate data
WorldClim global climate data
WorldClim[4] is a worldwide raster dataset of interpolated climate surfaces now in version 1.4. The highest resolution available is 30 arc-seconds, equivalent to approximately 1 km. Monthly precipitation and temperature data were downloaded as GeoTIFF in GCS WGS 84 geographical projection (tile 46: 30-60°S, 0-30° E), restricted to the catchments and values were extracted to allow for a general characterisation of climate. Extracted values were: minimum, mean and maximum temperature per month and catchment.

[2] glovis.usgs.gov/
[3] http://earthexplorer.usgs.gov/, accessed 21/10/2012
[4] www.worldclim.org, accessed 27.09.2012

World map of Köppen-Geiger climate classification

The relatively coarse (ca. 55 km in South Africa) world map of Köppen-Geiger climate classification was implemented in ArcGIS Online (Rubel and Kottek 2010) and provides a rough overview of classified observed and predicted climate in the world. The present climate classification was extracted from these maps.

Human population

Population was assessed using two different approaches. One was to calculate statistics for the LandScan 2007 data set, the other to digitize the houses within the specific sample site catchments from 1:50000 topographic maps. There was a discrepancy between these two population estimates. But as LandScan is a global dataset with relatively low spatial resolution, whereas topographic maps are much more detailed and trustworthy in this small-scale application, the latter was be given priority.

LandScan 2007 Population Grid

The LandScan 2007 gridded population of the world data set (Bright et al. 2008) in approximately 1 km^2 resolution.

Houses

Houses within the study catchments were digitized from 1:50000 topograhic maps, as this approach promised much more detail and potential for spatial analysis. Housing density was determined by dividing the number of houses total catchment area.

SANBI national land cover

The updated high resolution national land cover data set for South Africa, SANBI National Land Cover 2009, is made available by the South African National Biodiversity Institute (SANBI 2009, based on Fairbanks et al. 2000). It contains seven land cover classes, six of which are present in the research area: natural; cultivation; plantations; urban/built-up; waterbodies; and importantly, degraded land. The class 'mining' is not present in the research catchments. As the map shows land cover in 30 m resolution, it appeared to be sufficiently detailed to be used to link land cover and instream sediment load characteristics, which might be influenced by small-scale processes not readily visible in coarser resolution. Furthermore, it is the only land cover data set available that contains a high-resolution and ready-to-use “degraded” land cover class, thus avoiding the need to derive a measure of degradation from multispectral satellite imagery or other sources.

Erosion gullies

Le Roux et al. (2010) vectorized erosion gullies from SPOT 5 satellite imagery and created a shapefile of erosion gullies for the Eastern Cape Province. These data were used in the description of the sample site catchments.

Administrative areas

A shapefile of national, provincial and municipal borders was downloaded from GADM database of Global Administrative Areas v2.0[5]. The border of the former Transkei was extracted from 1:50000 topographic maps.

Protected areas

The World Database on Protected Areas[6] offers shapefiles of protected areas around the world. Data for South Africa were obtained, there are, however, no protected areas within the research catchments.

Land capability

An image of land capability was downloaded from the Agricultural Geo-Referenced Information System AGIS Natural Resources Atlas[7]. There was no downloadable shapefile available, thus the image was georeferenced and catchments compared visually.

5.3.2.2 Calculation of catchment characteristics from geospatial data

Two different approaches for calculating catchment statistics were followed. The first, and more basic approach, was to calculate statistics for the whole of each catchment. This approach was applied to the following data sets: elevation; slope; ruggedness; geology; land cover; houses; erosion gullies; and MODIS NDVI. For data sets with continuous values (i.e. elevation, slope, ruggedness), means and quantiles were used. For data sets with categorical values (geology, land cover), area and/or percentage of total area for each class was used.

The second, more elaborate, approach was to restrict the statistics to the land area near rivers (e.g. riparian buffer zones) or near sampling sites (circular upstream zones), and thus not include areas further away from the rivers (such as mountain ridges). This approach was applied to the following date sets: land cover; houses (density and number) and erosion gullies (percentage and area).

Correlation analyses were undertaken between the above mentioned geospatial dataset and the sediment load characteristics measured at sampling sites (e.g. turbidity during high and low flow. settled fine particulate solids during low flow. TSS during high and low flows). Owing to the very large number of pairs and the anticipated violation of assumptions of Pearson's correlation, Spearman rank correlation was utilised. The processes used to derive the whole and subcatchment characteristics are describe in further detail below.

Whole catchment analyses

Before extracting data from the layers, the catchment shapefile was reprojected to each thematic layer's projection in order to avoid data and information loss unavoidable in reprojecting and resampling raster data. The elevation, slope, ruggedness, land cover and

[5] www.gadm.org/, accessed 03.09.2012

[6] http://protectedplanet.net/, accessed 21.09.2012

[7] www.agis.agric.za/agisweb/agis.html, accessed 05/02/2013

MODIS NDVI time series raster data sets were cropped to each catchment polygon separately, the values were then extracted, saved and used for the calculation of catchment statistics. Polygon shapefiles (geology and erosion gullies) were rasterized (to the extent and cell size of ASTER DEM [30 m] in the case of geology and to the extent and half the cell size of the land cover map [15 m] in the case of erosion gullies) and once again values were extracted, saved and used for the calculation of catchment statistics.

In the point shapefile representing individual houses, the corresponding catchment was extracted for each point/house using the ArcMap "Extract by Points" function. Ultimately, a table containing statistics for all the previously mentioned data sets was created for use in correlation analysis.

Restricted area analyses

This approach used a set of parameters to control the size and shape of the mask for which values were extracted and statistics calculated for each type of restricted area. The first parameter was stream network, four different networks were used, each with a different level of detail (ranging from a network including small mountain streams to another showing main rivers only). Secondly, a number of riparian zone buffers were applied to each stream network, ranging from 100 to 1000 m in 7 steps (100, 200, 300, 400, 500, 750 and 1000 m). Thirdly, a number of circular zones were applied upstream of the sampling sites (1, 2, 3, 4, 5, 7.5, 10, 15, 20 km). The possible combination of these parameters (4x7x9=252) was calculated at each sampling site (6 sites x 252 buffer/area combinations = 1512) for each type of geospatial data (land cover class, density and number of houses, percentage and area of erosion gullies). Consequently, an enormous number of correlations (thousands) were generated, with the resulting concern that although some might be meaningful, some might be random. In order to address this issue, a threshold of 5% was applied, i.e. out of the 252 correlations that were calculated for every pair of sediment load and geospatial catchment characteristic (252 buffer combinations) at least 5% or 13 'combinations' were required to be significant in order for that restricted area zone statistic to be considered. Following this approach it was hoped that false positives would be excluded while false negatives would not be greatly increased.

5.3.2.3 Climate and Flow Data

Precipitation and evaporation

Meteorological and hydrological data were obtained from the Department of Water Affairs Hydrological Services – Surface Water[8]. The meteorological station closest to the research catchments is T3E003 (Stockenstroom @ Sheeprun) with a continuous time series of monthly precipitation and evaporation readings from 08/1963 to 08/1980 (evaporation from A Class pan, 11-12/1977, 6-11/1978, 1-4 and 7-11/1979 and 1-7/1980 were missing) (Table 5.3). Monthly and annual mean precipitation and evaporation values were extracted from these time series. Precipitation values from T2E003 Roodeheuvel @ Mtata Dam and T3E001 Tsolo @ Matatiele were used for regression analysis with MODIS NDVI values.

[8] www.dwaf.gov.za/hydrology, accessed 2012-09-26

Table 5.3 Meteorological Stations near the study area (precipitation and evaporation).

Station	Name	Period	Elevation (masl)
T2E002	Umtata	12/1957-01/1999	710
T2E003	Roodeheuvel @ Mtata Dam	07/1980-present	700
T3E001	Tsolo @ Matatiele	07/1937-present	1570
T3E002	Kokstad	12/1959-09/1980	1350
T3E003	Stockenstroom @ Sheeprun	08/1963-09/1980	1440
T3E004	Padock @ Belfort Dam	10/1992-02/1995	1570

Temperature

As no time series of temperature readings could be found, monthly mean temperatures for the catchments were extracted from the WorldClim monthly average temperature grids.

Runoff and river regime

Time series of average daily flow values (m^3/s) are obtainable from the Department of Water Affairs – Surface Water[9]. There are no flow gauges within the catchments sampled, and the closest gauge with comparable catchment size is T3H009 (Mooi River @ Maclear, elevation 1260 m asl) a few kilometers south of the Pot River catchment. At a catchment size of 307 km^2 it is around 30% smaller than the catchments of the lower sites on Pot and Luzi rivers, but can still give an estimate of flow within the research catchments. Two other nearby flow stations of comparable catchment size have been omitted for want of rating curves (only water level data available), while two more stations with rating curves present had catchment areas almost an order of magnitude larger than those of the research catchments (Table 5.4.

From the daily flow values, monthly means were calculated for each year separately, omitting months with at least one missing daily value. Thereafter, the long-term monthly means were calculated by aggregating years. In addition, long-term annual mean runoff values were calculated, again omitting years with at least one missing daily flow value. The river regime was described using the Pardé coefficient, the quotient of mean monthly runoff of month i and mean annual runoff:

$$PC_i = \frac{MQ_i}{MQ_{annual}}$$

[9] www.dwaf.gov.za/hydrology/HyStations.aspx? Region=T&StationType=rbRiver, accessed 2012-10-11

Table 5.4 Flow Stations near the study area.

Station	Name	Catchment Area (km^2)	Elevation (masl)	Comment
T3H003	Tsitsa River @ Halcyon Drift	482	1230	no rating curve
T3H009	Mooi River @ Maclear	307	1260	09/1964-present
T3H001	Mabele River @ Gladstone	134	1470	no rating curve
T3H005	Tina River @ Mahlungulu	2597	830	01/1951-present
T3H002	Kinira River @ Kinira Drift	2101	1350	08/1949-present

5.3.2.4 Assessment of Land Degradation

Land degradation within the catchments was assessed by means of three different data sets: Erosion gullies; National Land Cover map with land cover class ‘degraded’; and MODIS NDVI statistics (see section 5.3.2.1 for details on the data sets and data preparation)

The number of erosion gullies, their total area, percentage relative to total catchment area and density (number divided by total catchment area) within each catchment was calculated from the gully shapefile created by Le Roux et al. (2010). Similarly, the percentage of ‘degraded’ land cover class from the National Land Cover map (SANBI 2009) was extracted. As both of these data sets could only provide singular values per catchment, they could not be tested for significant differences.

Only the MODIS NDVI data could be tested for significance. The two main data sets used were normal catchment NDVI and catchment NDVI without *Pinus* plantations (which maintain high NDVI values throughout the year, especially during the dry season). In ArcMap, a polygon was created representing the outline of plantations and forests from LandSat Global Land Survey 2010, and compared to GLS 2005 and 2000 (this was mainly applicable the Lower Pot catchment where forestry occurred). The polygon was rasterized to a TRUE/FASLSE raster mask matching the MODIS rasters, and the plantation raster cells removed from the statistical analysis. Thereafter, the monthly mean values from the 11-year time series (2001-2011) were aggregated by year, providing annual mean NDVI values for each catchment or subcatchment. In addition, a subset for growing season (Oct-Mar) was created and aggregated in the same way. These data were generated for 1) whole catchments (Pot full, Luzi full, Upper Tsitsa Tributary full); and for 2) subcatchments (Upper Luzi, Lower Luzi, Upper Tsitsa Tributary, Upper Pot, Upper Little Pot, Lower Pot). Statistical analysis for trends was tested using linear regression models. For whole catchments, comparisons of the mean values were tested via one-way analysis of variance (ANOVA). The assumptions of the linear model, normality of residuals and homogeneity of variances, were tested using the Shapiro-Wilk test and Bartlett’s test. The Tukey HSD post-hoc test was used to determine pairwise differences of group (catchment) mean values. For subcatchment analysis, the assumption of independence of observations is violated if the upper catchment and the lower catchment (which contains the upper catchment) are compared. In this case, means were tested for differences with the Kruskal-Wallis one-way analysis of variance, and pairwise differences computed with the Multiple Comparison Test implemented in R package “pgirmess”.

Relationship between NDVI and precipitation

An attempt was made to establish a causal relationship between NDVI and precipitation using data from two nearby meteorological stations that have recorded values up to the present day: T3E001 Tsolo @ Matatiele and T2E003 Roodeheuvel @ Mtata Dam (Table 5.3). The distances of these stations from the research catchments are similar (ca. 75 km). Their geographical setting, however, differs considerably, with T3E001 being situated in the mountains below the escarpment and T2E003 almost 900 m lower in less mountainous surroundings.

Linear regression models were used to examine the relationship between each catchment's mean annual NDVI and precipitation data from the meteorological stations. In the regressions, the same rainfall data for each scenario was used for all catchments. The different scenarios calculated for various rainfall sums from both stations were:.

- $\sum$ *rain* $_{\textit{same year}}$
- $\sum$ *rain* $_{\textit{preceding year}}$
- $\sum$ *rain* $_{\textit{same year, growing season}}$
- $\sum$ *rain* $_{\textit{preceding year, growing season}}$
- $\sum$ rain $_{\text{last summer (Oct−Dec preceding year + Jan−Mar same year)}}$

For each scenario, eight different regression models were calculated (one for each subcatchment). Each model's regression parameters, the significance of the parameters (most importantly: slope), and the coefficient of determination were computed. In order to assess the validity of the models, the assumption of normality of residuals was tested using the Shapiro-Wilk test. The best scenario was chosen based on the averaged coefficients of determination of the catchments. Additionally, autocorrelation function of the precipitation time series and NDVI were calculated and tested for significance.

5.3.2.5 Field collected data

Water physico-chemical parameters

Temperature, pH, electrical conductivity (mS/m), dissolved oxygen (mg/L) were measured on-site using portable field meters. Water samples of 250 ml were also collected on-site, preserved with 5 ml of 10% HCl and analyzed spectrophotometrically in the laboratory for ammonium, nitrite, nitrate and phosphate. Nitrogen data were summed to obtain a concentration for total inorganic nitrogen (TIN). Algal biomass, measured as phytoplankton (chlorophyll-*a* in water per unit volume) and periphyton (chlorophyll-*a* per unit area – top of a flat stone), was determined following methods described by Holm-Hansen and Riemann (1978). Phytoplankton and periphyton samples were filtered through Whatman glassfibre filters with a Mityvac hand vacuum pump, the filters stored in the dark in refrigerated acetone (90%) and analyzed spectrophotometrically in the laboratory.

Sediment load characteristics

Fine settled particulate solids were sampled by placing a plastic bucket (with the bottom removed) into the river-bed sediment of a riffle-run. The sediment was agitated with 10 vigorous stirs with a stout wooden stick, after which a sample of the water in the bucket was quickly taken. The water level in the bucket was kept similar at each site in order to keep the dilution of settled solids the same. This sample was filtered through sieve with 500 µ*m* mesh size in order to remove any large plant material. 100 mL of the sample was then filtered (with pre weighed 0.45 µ*m* glass filters), the filters dried and re-weighed. The weight of the filtered river solids was converted to concentration per unit area (mg/m^2) using the area of the bucket.

The process for determining the total suspended solids (TSS) concentration was similar to that of settled solids. The only differences were the sample of river water was collected at half of depth of the river, and once filtered through the 500 µ*m* sieve, 250 mL was filtered through 0.45 µm glass filter. The weight of the TSS was expressed as mg/L. Turbidity (ntu) was measured on-site using portable field meter.

Determination of the sediment particle size composition of suspended and settled solids samples was attempted. After sonicating the samples in an ultrasonic bath, the analysis was undertaken with the Micromeritics SediGraph 5100 Particle Size Analyzer. However, the samples were too diluted to be measured with this machine. Another approach attempted was to cut out small sections of the glassfibre filters that had been used to determine settled and suspended solids concentration and take photographs under the scanning electron microscope at Rhodes University (TESCAN Vega TS 5136LM). However, a particle size distribution could not be generated as particles either appeared or disappeared from view as the magnification was changed to view larger or smaller particles.

Macroinvertebrates

Sampling of aquatic macroinvertebrates was undertaken using the South African Scoring System Version 5 (SASS5) rapid bioassessment index methodology (Dickens and Graham 2002) Using a collection net (300mm x 300mm), three biotopes were sampled individually: stones in-and-out-of-current; vegetation (marginal and aquatic if available), and gravel/sand/mud (GSM). According to the sampling protocol: stones were kicked for two minutes while holding the net downstream to collect dislodged organisms; two meters of marginal vegetation was swept; and GSM was stirred with the sampler's feet and swept with the net for one minute. After each biotope was sampled, the contents of the net were emptied into a tray, cleaned of leaves and twigs, and macroinvertebrates identified and recorded on the SASS5 score sheet. The SASS5 rapid bioassessment index assigns scores to taxa, based on perceived sensitivity to water quality impairment. The highest score (15) represents especially sensitive taxa and the lowest score (1) represents especially tolerant taxa. The SASS score is the sum of the sensitivity scores of taxa identified from the sample. A further metric is obtained by dividing the SASS score by the total number of taxa sampled in order to obtain the Average Score per Taxon (ASPT) (Dickens and Graham 2002). Once the SASS evaluation was complete, a further two samples from each of the biotopes were collected (replicate samples) and all samples were preserved in 80% ethanol. All samples were further enumerated in laboratory, providing accurate counts for each of the taxa (see

Appendices 2 and 3 for the list of taxa collected) for use in the assessment of the macroinvertebrate assemblage composition using various structural taxon-based metrics and functional trait-based metrics (Table 5.5).

Table 5.5 Macroinvertebrate stress responses measured in this study: adapted from Barbour et al. (1996), Baptista et al. (2007) and Brossett et al. (2010).

Biological stress response	Predicted response to chemical stress (when compared to reference site)	Description
Structural taxon-based metrics		
Measures of macroinvertebrate taxa tolerance		
SASS5	Decrease	Measures of the sensitivity of taxa to contaminant stress. Baetidae / Ephemeroptera and EPT / Chironomidae represent a ratio of abundance of organisms from these taxa to one another
Baetidae / Ephemeroptera	Increase	
EPT / Chironomidae	Decrease	
Measures of macroinvertebrate community composition		
% EPT abundance	Decrease	Indicates changes in percentage abundance of indicator species (sensitive or tolerant) to contaminant stress
% Ephemeroptera abundance	Decrease	
% Trichoptera abundance	Decrease	
% Diptera abundance	Increase	
NMDS of all macroinvertebrate taxa	Differentiation	
Measures of macroinvertebrate community richness		
Number of EPT families	Decrease	High richness is often used to indicate good habitat availability (water physicochemical, substrate, food resources)
Shannon-Weiner Diversity Index	Decrease	
Margalef's Taxon Richness Index	Decrease	
Functional trait-based metrics		
Measures of trophic level representation in macroinvertebrate community		
NMDS of functional feeding groups	Differentiation	Potential measure of changes in trophic dynamics of water resource by simplifying macroinvertebrate into trophic guilds according to feeding strategy

Community structure, measured as taxonomic richness and diversity, has been the approach most readily investigated and applied (Peru and Doledec 2010). Taxonomic richness can include all taxa at a site, or focus on pollution-sensitive groups such the Ephemeroptera, Plecoptera and Trichoptera (EPT), or pollution-tolerant groups such as Chironomidae (Peru and Doledec 2010). Biotic indices can also be based on the relative tolerances of macroinvertebrates to water quality degradation (an extensive review is provided by Ollis et al. 2006). Such an index is the South African Scoring System (SASS), now in Version 5 (Dickens and Graham 2002), the most popular macroinvertebrate rapid bioassessment tool in use in South Africa. Chapman (2002) suggests that protecting ecosystem structure should, in general, protect ecosystem function. However, it may also be possible to utilise biotic measures of functional diversity to provide an indirect measurement of ecosystem functioning (Peru and Doledec 2010). A functional guild is a group of taxonomically unrelated species that perform similar functions or use similar resources within the community (Simberloff and Dayan 1991). One of the most widely used measures of functional diversity is the assessment of macroinvertebrate functional feeding groups (FFG), defined by the size and type of food particle consumed and the mode of feeding, which reflects the trophic functioning of stream ecosystems (Downes et al. 2002). The applicability of the above measures to a physical stressor such as suspended particulates is not well known. Wagenhoff et al. (2012) and Buendia et al. (2013) have shown that in certain circumstances some of the metrics from both groups are useful in detecting the impacts of fine sediment exposure. Consequently, in this study all the metrics are applied to the field collected macroinvertebrate data, allowing some indication of which are most reflective of the impacts of suspended solids on South African macroinvertebrates.

The classification of macroinvertebrates into functional feeding groups (FFG) was developed by Cummins (1973, 1974) to describe macroinvertebrates that use similar resources in similar ways (i.e. feeding strategies). These guilds are ecologically meaningful units that facilitate an understanding of organic matter processing in streams (Vannote et al. 1980). The designation of macroinvertebrates into FFG can be based on feeding apparatus morphology, behaviour, and/or gut contents. A number of studies indicate that the use of FFG designations for North American taxa, as described in Merritt and Cummins (1984), is inappropriate for other geographical regions (King et al. 1988; Tomanova et al. 2006). In addition, macroinvertebrate taxa are flexible in feeding strategy and their grouping into a single FFG can be idealistic. There also appears to be a common affinity among taxa for fine detritus (Palmer et al. 1993a; Tomanova et al. 2006). Consequently, the assignment of taxa to FFG needs to be undertaken on a site-specific basis, involving determination of gut contents, a study of mouthpart morphology, and observations of feeding behaviour. The response of macroinvertebrate FFGs to water quality indicators in the Buffalo River (in the Eastern Cape Province of South Africa) was determined by Palmer et al. (1996). The designations of selected taxa to FFG were determined through gut contents analysis (Palmer et al. 1993a) and studies on functional feeding morphology and behaviour (Palmer et al. 1993b). As the species within the Baetidae and Caenidae were found to consist of both gatherers and filterers (Palmer et al. 1996), and the macroinvertebrates in the current study were identified to family level only, these groups were not included in the FFG trait analyses. The functional feeding group designations adopted for use in this report are presented in Table 5.6.

Table 5.6 Functional feeding group designations and associated family-level taxa utilised in this study.

Functional feeding group designation	Family-level taxon	Reference
Filterer	Simuliidae Hydropsychidae	Palmer 1996 Palmer 1996
Brusher	Leptophlebiidae	Palmer 1996
Scraper	Heptageniidae Burnupia	Palmer 1996 Palmer 1996
Predator	All Odonata Perlidae Planariidae	Cummins 1973,1974 Palmer 1996 Palmer 1996

Statistics used to analyse field collected data

Field collected water physico-chemical data and sediment load characteristics were analysed for normality using the Shapiro-Wilk W test. For comparisons between sites, normally distributed data were statistically analysed using the Student's t test, while non-normally distributed data were analysed using the Kruskal-Wallis ANOVA and Median test or Mann-Whitney U test when comparing only two variables. Correlation analyses undertaken between the geospatial data and the field collected water physico-chemical data or sediment load characteristics utilised Spearman's rank correlation.

Statistical comparisons of macroinvertebrate taxon and trait-based metrics used the same tests and procedures as listed for the water physico-chemical parameters in the paragraph above. Correlation analyses between biological and sediment load characteristics was undertaken in Statistica®. Macroinvertebrate assemblage composition analyses involving principle component analysis and cluster analysis utilised R vegan package. Further multivariate statistical analysis was undertaken using non-metric multi-dimensional scaling (NMDS) provided for in the PRIMER V5 programme (Clarke and Warwick 2001). The NMDS ordination plot represents the similarity of abundances of family-level taxa, or FFG guilds, between samples. To statistically analyse for these similarities, an analysis-of-similarity (ANOSIM) test was undertaken. In addition to the significance value, the Global R value indicates the degree to which the samples are similar or dissimilar. An R value of 1 indicates complete separation of groups, whereas an R value near 0 implies little or no segregation.

5.3.3 Sampling site and catchment description

5.3.3.1 General

The research catchments are located in the north eastern part of the Eastern Cape Province, mainly within the Magisterial Districts of Maclear and Mt Fletcher (Figure 5.1). They are part of the Umzimvubu River Basin which in total comprises 19,900 km². This area of the country was chosen because a previous study by Madikizela and Dye (2003) showed that the rivers here possessed high suspended sediment loads, with few other potential stressors in the upper catchments.

5.3.3.2 Sampling sites

Initially, four catchments (of as similar size as possible) were chosen a-priori to represent different sediment inputs to the river, with the Pot and Little Pot rivers having little visual evidence of land degradation and erosion in the catchment, while the Luzi River and Tsitsa tributary catchments appearing more degraded with more erosion alongside the river (Table 5.7). The final sites were chosen in terms of available macroinvertebrate habitat (although with so many physical factors to consider this was often challenging), resulting in the exclusion of the lower sites of the Little Pot and Tsitsa rivers (Figure 5.1, Table 5.8). None of the rivers upstream of the chosen sites were hydrologically regulated.

Table 5.7 Sampling site and catchment descriptions and graphics

Little Pot River

Flows through privately owned farmland (limited cattle grazing). Little evidence of erosion. Chosen as an example of a low turbidity river. The upstream site is on the farm Woodcliffe. The downstream site was adjudged not to have enough suitable habitats (instead consisting of mostly bedrock and boulders).

Little Pot River – Upstream site	Little Pot – River Downstream site
	

Pot River

Also flows through privately owned farmland (limited cattle grazing). Little evidence of erosion although there is cultivation upstream of the lower site. Chosen as an example of a low turbidity river. The upstream site is located at Pot River Pass and downstream site on the farm Hulley Hill.

Pot River – Upstream site	Pot River – Downstream site
	

Tsitsa River

The Tsitsa catchment is a mixture of private and communal land uses. There is evidence of increased erosion and consequently it was chosen as an example of a river with higher sediment load. The upstream site was located on a tributary of the Tsitsa at Mhontlo with a number of homesteads in its catchment. Sand mining also occurs at this site. The downstream site, located near the disused weir upstream from the Tsitsa Falls was adjudged not to have enough suitable habitats (consisting mostly of bedrock).

Tsitsa River – Upstream site	Tsitsa River – Downstream site
	

Luzi River

The Luzi River catchment is dominated by communal land use with evidence of over grazing and consequent erosion. In the upper reaches, where the upstream site is located, there is a low population density and the effect of overgrazing is less evident. However, between the upstream site and downstream site there is extensive evidence of erosion. The downstream site is located at the R56 Bridge (top picture shows low flows and bottom picture shows turbid high flows).

Luzi – Upstream site	Luzi – Downstream site
	 

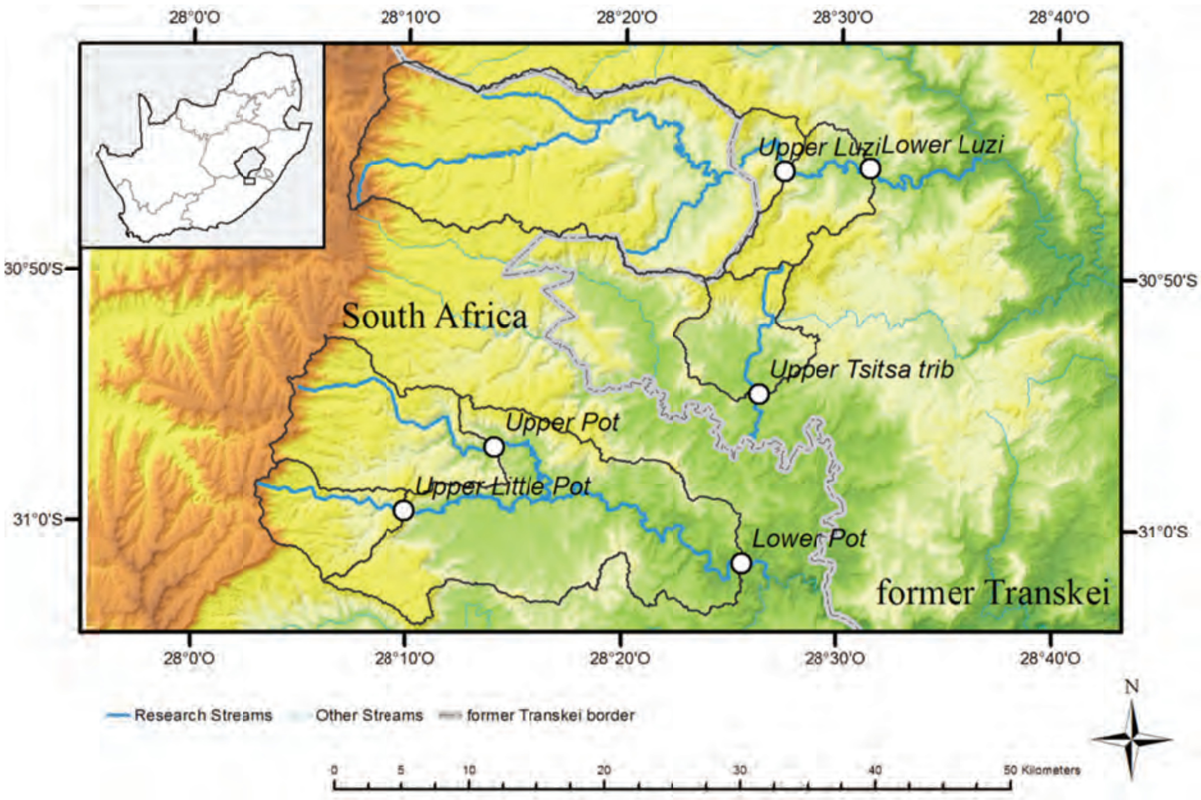


Figure 5.1 Selected monitoring sites on the four headwater tributaries of the Umzimvubu River.

Table 5.8 Spatial details of the chosen sampling sites

Site	Latitude (S)	Longitude (E)	Catchment area (km^2)	Elevation (meters a.s.l.)
Upper Luzi	30° 45’ 51.8”	28° 27’ 11.8”	349.6	1330
Lower Luzi	30° 45’ 39.8”	28° 31’ 21.0”	409.7	1300
Upper Tsitsa trib	30° 54’ 43.6”	28° 26’ 14.3”	64.2	1260
Upper Pot	30° 56’ 57.0”	28° 14’ 02.4”	120.0	1310
Upper Little Pot	30° 59’ 32.9”	28° 09’ 55.5”	56.7	1370
Lower Pot	31° 01’ 28.4”	28° 25’ 33.4”	438.8	1140

The sampling sites were visited on five occasions in 2012/13 (Table 5.9). However, sudden heavy rains during two field trips (March 2012 and October 2012) prevented biomonitoring, while a particularly wet summer resulted in higher than usual flows in February 2013 also preventing biomonitoring. This means that biomonitoring data are only available for two occasions limiting rigorous statistical analysis. Standard water physico-chemical parameters, turbidity and TSS were collected on each sampling trip, but settled solids concentration could only be collected during the two low flow occasions (Table 5.9).

Table 5.9 Date of site visit and description of sampling activities.

Date of site vist	March 2012	May 2012	October 2012	November 2012	February 2013
Description of sampling activities	Unexpected heavy rain caused flooding and biomonitoring could only be undertaken at the upper site on the Luzi River. Suspended solids and turbidity were measured at all sites	Low water. Biomonitoring, water quality parameters, turbidity, suspended and settled solids measured	Rainfall during the site visit increased the flow at sites preventing biomonitoring. Water quality parameters, turbidity and suspended solids measured	Low water. Biomonitoring, water quality parameters, turbidity, suspended and settled solids measured	Low water. Biomonitoring, water quality parameters, turbidity, suspended and settled solids measured

5.3.3.3 Geology

The general picture of catchment geology is that the highest areas around the escarpment are made up of basaltic lava from the Drakensberg Formation (Jurassic), below which are strata of Triassic Sandstones and Mudstones (Figure 5.2). The topmost of these is fine-grained sandstone from the Clarens Formation, followed by Mudstone from the Elliot Formation and coarse- to fine grained sandstone of the Molteno Formation. In lower areas some Dolerites occur as slim bands. Some quaternary alluvium is present to a small extent. Basalts are more dominant in the Luzi catchment than in the Pot catchment and absent from the lower lying parts of the catchments and the whole of the Upper Tsitsa Tributary catchment. The Clarens sandstone forms a band several kilometers wide in the Pot catchment, whereas in the Luzi catchment it is a slim band only a few hundred meters wide. Most sampling sites are located within Molteno Sandstone, only Upper Pot and Upper Little Pot are in Elliot mudstone (which is more erodible than the sandstone – K. Rowntree pers. comm.) (Table 5.10).

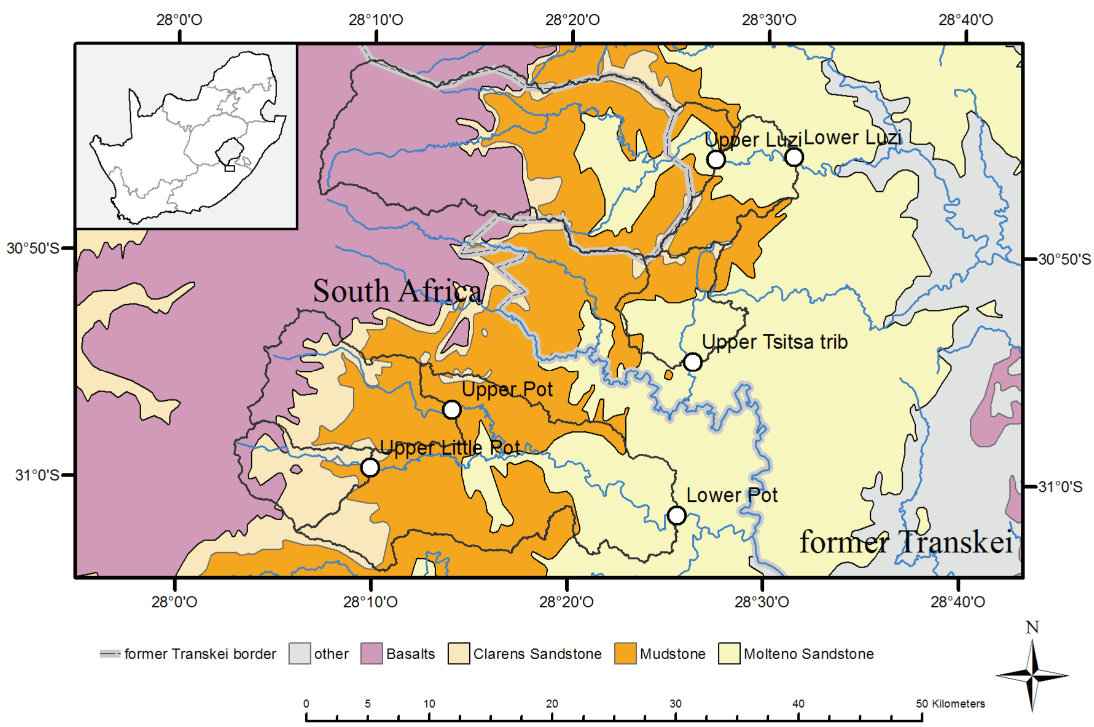


Figure 5.2 Simplified geology of the research area (low percentage occurrence of dolerite and alluvium not show).

Table 5.10 Catchment size and percentages of geological formations.

Catchment	Area (km^2)	Basalts	Dolerite	Mudstone	Sandstones	Alluvium
Upper Luzi	349.6	43%	2%	33%	21%	1%
Lower Luzi only	60.1	-	5%	38%	57%	-
Upper Tsitsa Trib.	64.2	-	2%	33%	65%	-
Upper Pot	120.0	28%	-	40%	31%	-
Upper Little Pot	56.7	27%	-	7%	66%	-
Lower Pot only	262.1	-	8%	44%	43%	5%
Luzi full	438.8	37%	2%	34%	26%	1%
Pot full	409.7	11%	5%	38%	43%	3%

5.3.3.4 Topography

Elevation

The catchments are in a very mountainous region and vary considerably in elevation. The highest points of Pot and Luzi catchments are at 2600 and 2720 meters, that of Upper Tsitsa Tributary at 2140 meters, and the sampling sites at around 1100-1300 meters (Table 5.11).

Slope

The Upper Pot and Upper Little Pot catchments have steeper slopes than the other catchments. Upper and Lower Luzi have almost identical mean slope values. As a whole, though, Pot and Luzi catchments have very similar slope values. Slopes are result of catchment geology and steepest on escarpment and where strata of sedimentary rocks of different erosion resistance meet (Table 5.11).

Ruggedness

Similarly to slope, Upper Pot and Upper Little Pot have higher mean ruggedness values, higher percentages of land classified as rugged and less level or nearly level land. Differences between Luzi and Pot catchments are small and Upper Tsitsa Tributary catchment is similar (Table 5.11).

Table 5.11 Topographic parameters for selected catchments.

Catchment	Mean elevation (m)	Mean slope (degrees)	Level/nearly level terrain (%)	Moderately-extremely rugged (%)
Upper Luzi	1790	13.9	30	31
Lower Luzi only	1630	14.1	31	32
Upper Tsitsa Trib.	1520	13.0	30	27
Upper Pot	1780	17.7	16	47
Upper Little Pot	1810	19.1	13	53
Lower Pot only	1440	12.3	33	25
Luzi full	1770	14.0	30	31
Pot full	1580	14.7	26	34

5.3.3.5 Soils

In general, soil depth is limited on steep slopes in upper catchments, and deeper in colluvial and alluvial sediments on footslopes and floodplain areas. Consequently, once vegetation over is removed, sediment on steeper slopes becomes highly erodible due to its unconsolidated nature (Dollar and Rowntree 1995). Based on the K-factor soil erodibility index (Schulze 2007), with expert-based classification with regard to contribution to formation of erosion gullies for the research area (Le Roux and Sumner 2011), it can be concluded that the research catchments are for most part moderately to highly erodible. The upper third of Luzi catchment has low erodibility, but the lower two thirds are high. The Pot River is more dominated by moderately erodible soils.

5.3.3.6 Climate and Runoff

Climate classification and overview

According to the world map of Köppen-Geiger climate classification the catchment areas are located between climate zones Cwb and Cfb, describing warm temperate climate, either fully humid (Cfb, towards the coastal areas) or winter dry climate (Cwb, along the escarpment towards northeast), both with warm summers. The more detailed WorldClim data set shows a distinct seasonality in both rainfall and temperature within the catchments. The area receives most rainfall during the summer months (mostly October to March) and has dry winters.

Precipitation and Evaporation

According to station T3E003 (Stockenstroom @ Sheeprun) in Lower Pot River catchment, mean annual precipitation (of the period from 1964 to 1979) was 825 ±1 43 mm, minimum and maximum annual precipitation values in these years being 530 mm and 1142 mm, respectively. Although there can be snowfall in the mountains in winter, there is rarely with lasting snow cover, and thus generally low precipitation in winter.

Mean potential annual evaporation from an A Class pan between 1964 and 1976 was 1442 ± 112 mm, minimum and maximum were 1283 mm and 1649 mm.

Temperature

Monthly mean temperatures, as derived from the WorldClim monthly temperature data set, indicate 7°C in winter and 19°C in summer, although there is high intraday variation.

5.3.3.7 River flow

During the 47 complete years of observations since 1965 the mean monthly flow at station T3H009 (Mooi River @ Maclear) was between 0.36 m^3/s (July) and 10.1 m^3/s (February). Mean discharge was 3.2 m^3/s, which at a catchment area of 307 km^2 results in a catchment runoff yield factor of 10.4 $L/(s \cdot km^2)$. There are no flow gauges on the research streams, but as catchment size, geology, topography are comparable, the patterns are expected to be similar.

Flow measured at each of the sites during sampling visits with a portable flow meter are presented in Table 5.12.

Table 5.12 Field measured flow (m/s) at sampling sites.

Site	March 2012	May 2012	October 2012	November 2012	February 2013
Pot River – Upper	1.4	0.4	1.1	0.6	1.3
Pot River – Lower	1.4	0.2	1.2	0.6	0.7
Little Pot River – Upper	-	0.5	0.8	0.5	0.9
Luzi River – Upper	0.9	0.2	0.9	0.4	1.6
Luzi River – Lower	0.8	0.2	0.8	0.6	1.0
Tsitsa Tributary	0.8	0.2	0.5	0.6	1.3

5.3.4 Results

5.3.4.1 Hypothesis 1 – Land Degradation Assessment

Land Cover

There were considerable differences between catchments in terms of percentages of land cover classes. The Upper Little Pot was classified 100% natural, with the Upper Pot very similar (99%) (Table 5.13, Figure 5.3). The highest degradation (34%) and least natural (55%) occurred in the Lower Luzi catchment. In general, natural landscape occurred mostly in the upper catchments, with degraded, cultivated and urban/built-up areas more prominent in lower areas. The Tsitsa Tributary subcatchment had the highest cultivation (24%), with Lower Luzi and Lower Pot being similar (8.0 and 8.7% respectively). The share of the remaining categories was small with 'urban' exceeding 1% only in Lower Luzi and Tsitsa Tributary. Plantations contributed 4% to the Lower Pot catchment, and were insignificant in the Luzi catchment and absent from the Tsitsa Tributary catchment. In terms of whole catchment values, the Luzi catchment was almost 9 times more degradation than in Pot catchment (8% vs. 0.9%) (Table 5.13, Figure 5.3).

The assessment of population density showed low density in the upper reaches of the catchments and denser population in lower areas (Table 5.13, Figure 5.3). The population in the former Transkei area was much more concentrated than in what was then South Africa (where houses tended to be more evenly distributed with larger distances between them). Clustered population, especially rural populations with high numbers of livestock, can put severe pressure on the land and lead to increased degradation in these areas.

Table 5.13 Land cover and population statistics

Catchment	Natural	Cultivation	Degraded	Number of Houses
Upper Luzi	91.9%	4.4%	3.5%	199
Lower Luzi only	55.4%	8.0%	34.3%	321
Upper Tsitsa Trib.	73.6%	24.0%	1.3%	397
Upper Pot	99.4%	0.2%	0.4%	10
Upper Little Pot	100.0%	0.0%	0.0%	0
Lower Pot only	83.8%	8.7%	1.3%	171
Luzi full	86.5%	5.0%	8.0%	520
Pot full	90.2%	5.2%	0.9%	181

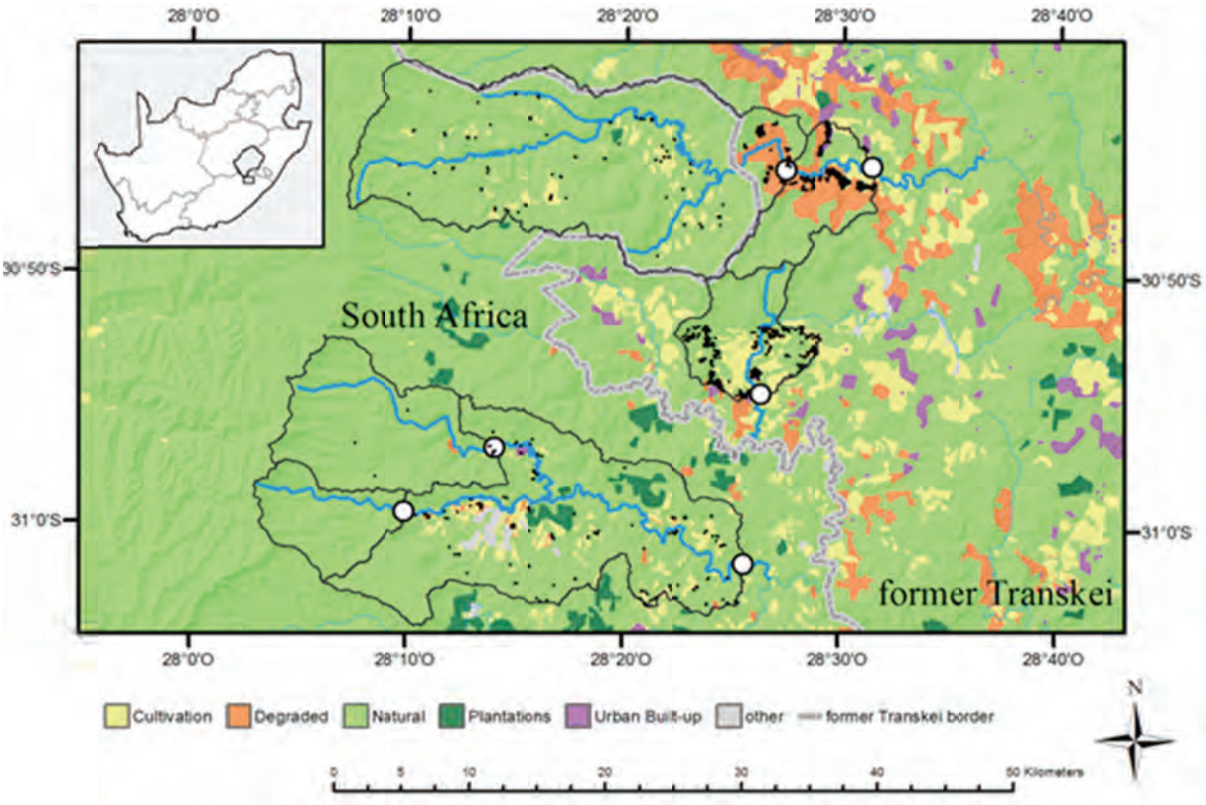


Figure 5.3 Land cover and houses (black dots) within the subcatchment areas of sampling sites.

Erosion gullies

No erosion gullies were mapped within the Upper Little Pot catchment, with only a few small gullies occurring in Upper Pot (Figure 5.4, Table 5.14). The Lower Pot catchment had the next lowest number with the number and percentage gullies per area catchment being similar to that of the Upper Luzi. The Lower Luzi had the highest proportion of erosion gullies (2% of total area of subcatchment – i.e. excluding the Upper Luzi catchment). Many of these were incised erosion gullies ending directly in the river. The percentage of erosion gullies in this subcatchment was 10 times higher than in Upper Luzi and 18 times higher than in Pot catchment. While the largest single erosion feature in the Lower Luzi measured more than 18 ha, the average size was below 1 ha in all catchments, but ca. 50% higher in Luzi than in

Pot catchments as a whole. Data for the Tsitsa catchment were somewhere in between the Luzi and Pot catchments (Figure 5.4, Table 5.14).

There is a conspicuous discrepancy between the erosion gully map and the SANBI land cover map in certain places, namely in Upper Tsitsa and a small portion of Lower Luzi catchment, where gullies were mapped in areas designated “natural”. It is beyond the scope of this project to validate or ground truth these data sets, but is nevertheless worth noting. These data sets do not represent sheet erosion, this is instead dealt with in the next section though the NDVI index.

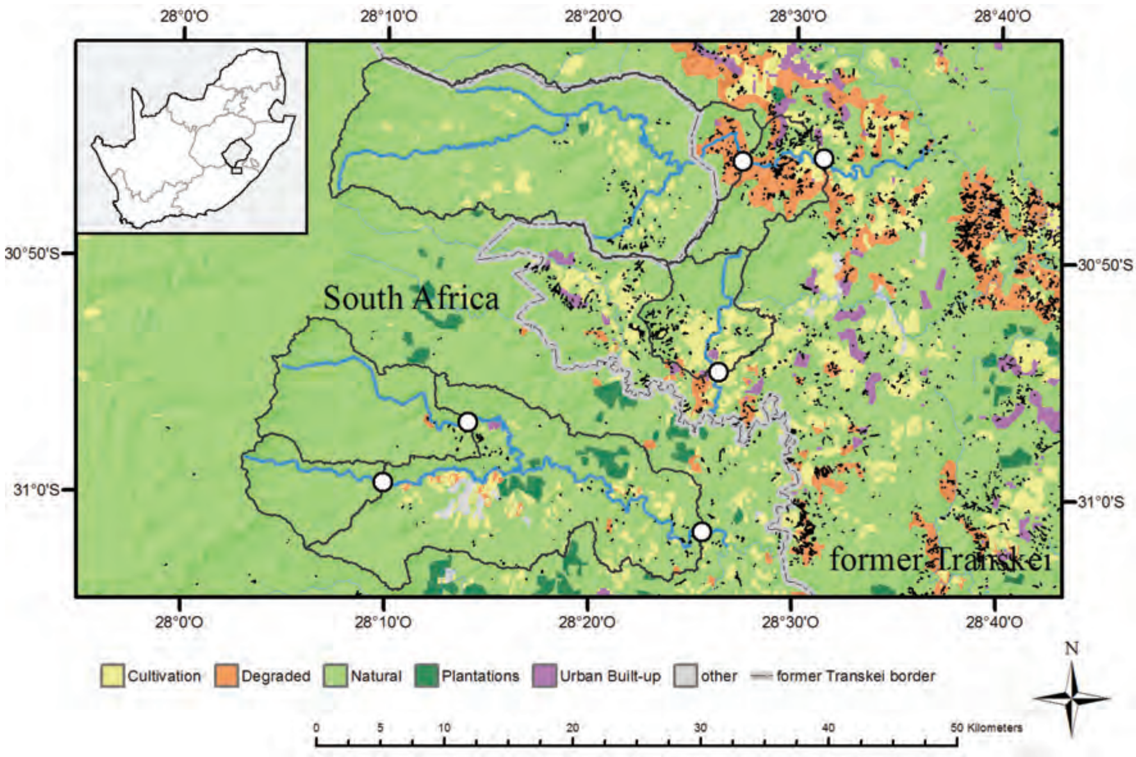


Figure 5.4 Land cover and erosion gullies superimposed in black dots within the catchment areas

Table 5.14 Different measures of land degradation: Erosion Gullies, mean NDVI 2001-2011.

Catchment	Erosion Gullies					Mean NDVI ± Std. dev.
	No.	Total area (km^2)	Total area (ha)	% of catchment	Number per km^2	
Upper Luzi	124	0.69	0.55	0.20	0.35	0.496 ± 0.014
Lower Luzi only	145	1.26	0.87	2.10	2.41	0.448 ± 0.014
Upper Tsitsa trib.	102	0.69	0.68	1.08	1.59	0.481 ± 0.012
Upper Pot	18	0.07	0.38	0.06	0.15	0.535 ± 0.011
Upper Little Pot	0	0.00	-	-	-	0.544 ± 0.012
Lower Pot only	89	0.44	0.49	0.17	0.34	0.534 ± 0.014
Luzi full	269	1.95	0.72	0.48	0.66	0.489 ± 0.013
Pot full	107	0.51	0.48	0.12	0.24	0.536 ± 0.012

Normalized differenced vegetation index (NDVI)

Comparison of long-term annual means of the NDVI (a simple indicator of land with live green vegetation) shows that the whole Pot, Upper Pot and Upper Little Pot are very similar to each other, possessing the highest values (0.535-0.544) (Table 5.14). The mean NDVI of these catchments is 10% higher than that of the full Luzi catchment. The Lower Luzi (shown to be the most degraded, and with the highest number and density of erosion gullies) shows lowest NDVI (0.448), 10% lower than upper part of Luzi catchment and 17% lower than Pot subcatchments. The Tsitsa catchment has the second lowest mean NDVI (0.481) (Table 5.14). When the NDVI for the wet growing season (Oct-Mar) is assessed separately, very similar results are produced.

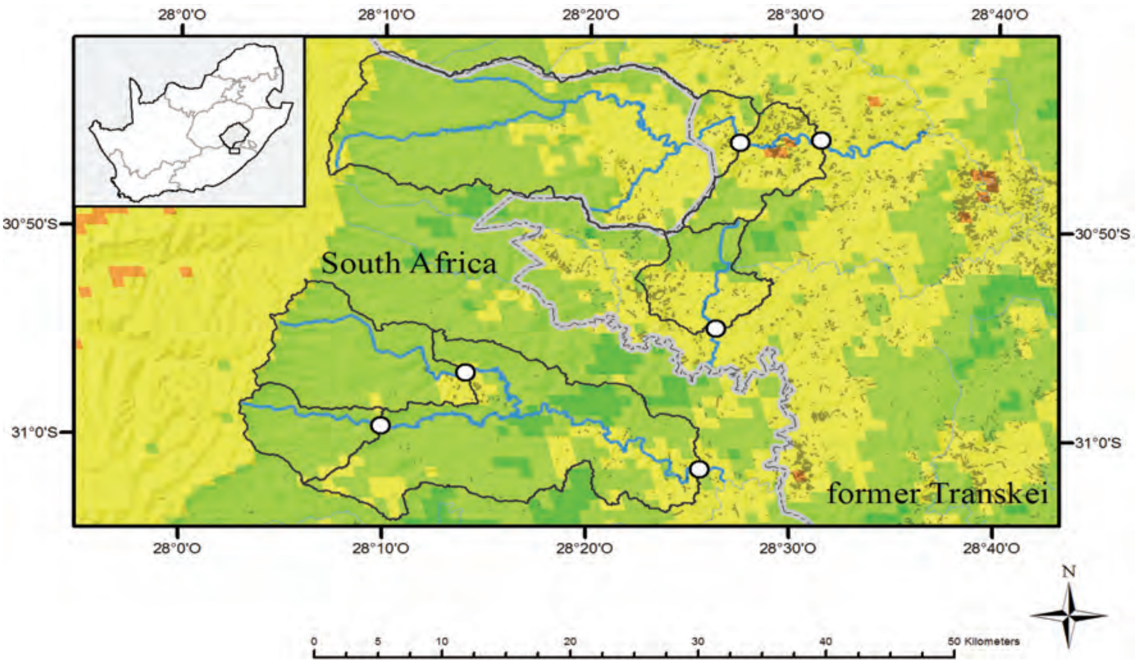


Figure 5.5 Normalized differenced vegetation index lowered vegetation index (high values in green, moderate in yellow and low in orange).

Statistical analysis of the NDVI means were undertaken separately for the whole catchments (Luzi full, Pot full, Upper Tsitsa), and then for all subcatchments (i.e. excluding Pot full and Luzi full, on account of autocorrelation between complete catchment and related subcatchments). For the whole catchments, annual mean NDVI values were found to be significantly different (ANOVA) from one another, both when plantations were included ($F_{2.30}$ = 60.93, p < 0.001) and excluded ($F_{2.30}$ =63.60, p < 0.001) (Table 5.15). For the comparison of subcatchments ANOVA was not valid for every month and thus Kruskal-Wallis one-way analysis of variance was used for six of the months. There were significant differences in annual means between all months, both when plantations were included and excluded (Table 5.15).

Table 5.15 Comparison of MODIS NDVI monthly means between full catchments (Luzi full, Pot full and Upper Tsitsa Trib) and all subcatchments (excluding full catchments). Full catchments analysed with one-way ANOVA, but for subcatchments Kruskal-Wallis test was undertaken for six of the month analyses.

Month	Full catchments				All subcatchments			
	with plantations		without plantations		with plantations		without plantations	
	$F_{2.30}$		$F_{2.30}$					
J	37.85	***	40.33	***	53.70	***	53.99	***
F	20.97	***	22.66	***	49.19	***	49.44	***
M	45.54	***	48.76	***	55.20	***	55.47	***
A	12.52	***	13.16	***	40.63	***	41.04	***
M	22.41	***	23.07	***	47.70	***	47.82	***
J	31.68	***	32.27	***	49.34	***	50.03	***
J	52.00	***	53.88	***	52.10	***	53.03	***
A	131.09	***	140.41	***	55.02	***	55.02	***
S	27.89	***	27.17	***	47.531	***	47.37	***
O	7.99	**	7.68	**	25.59	***	25.45	***
N	5.49	**	5.53	**	27.13	***	27.32	***
D	7.50	**	7.87	**	35.95	***	35.82	***

Note: * = p < 0.05; ** = p < 0.01, *** = p < 0.001

When a comparison of monthly mean values was undertaken, there were significant differences between every month for the full Pot and Luzi catchments, with the most pronounced being in late winter (August, before the onset of rainy season) (Table 5.16). The Pot catchment had significantly higher mean values than Luzi every month, and higher than Tsitsa Trib for all months except November. The Luzi catchment values were higher than the Tsitsa Trib during the summer months and August (winter) (Table 5.16). Results when plantations were included or excluded were very similar.

Table 5.16 Tukey Honest Significant Differences in MODIS NDVI for complete catchments (omitting plantations).

Catchment comparisons	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
Luzi full – Upper Tsitsa	***	*	***					**				
Pot full – Upper Tsitsa	***	***	***	***	***	***	***	***	***		*	**
Pot full – Luzi full	***	**	***	**	***	***	***	***	***	**	*	*

Note: * = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$

There were also highly significant differences between certain subcatchments every month (Table 5.17). The Lower Luzi subcatchment differs significantly from the Upper and Lower Pot catchments almost every month, but not from Tsitsa Trib, and only from Upper Luzi in January. The Lower and Upper Pot catchments did not differ significantly from one another (Table 5.17). In general, differences are most pronounced in winter months, with the least difference in spring/summer (October and November), possibly due to beginning of vegetation growth as a result of increased rainfall and warmer temperatures. There were no significant differences in the mean NDVI of catchments between years nor significant trends over the annual time series 2001-2011 (linear regression models, p values of slopes: 0.38-0.80).

Table 5.17 Significance of multiple comparison test of MODIS NDVI values between subcatchments per month using Kruskal-Wallis.

Sub catchment comparison	J	F	M	A	M	J	J	A	S	O	N	D
Lower Luzi only-Lower Pot only	***	***	***	***	***	***	***	***	***	**	**	**
Lower Luzi only-Upper Little Pot	***	***	***	***	***	***	***	***	***	**	***	***
Lower Luzi only-Upper Luzi	*											
Lower Luzi only-Upper Pot	***	***	***	***	***	***	***	***	***		**	***
Lower Luzi only-Upper Tsitsa Trib												
Lower Pot only-Upper Little Pot												
Lower Pot only-Upper Luzi							*	*	***	*		
Lower Pot only-Upper Pot												
Lower Pot only-Upper Tsitsa Trib				*	**	*	**	***	*			
Upper Little Pot-Upper Luzi			*		*	**	**	*	**	*		
Upper Little Pot-Upper Pot												
Upper Little Pot-Upper Tsitsa Trib	***	***	***	**	**	***	***	***				**
Upper Luzi-Upper Pot						*			*			
Upper Luzi-Upper Tsitsa Trib												
Upper Pot-Upper Tsitsa Trib	***	***	***	**	**	**	**	**				*

Note: * = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$

Effects of variation in rainfall on NDVI values

In most scenarios, station T3E001 Tsolo @ Matatiele provided better explanation of variation in NDVI than T2E003 Roodeheuvel @ Mtata Dam. The mean of all catchment's r^2 values per scenario ranged from 0.01 to 0.48, thus the best models explained almost half of the variability in inter-annual NDVI with rainfall. Top models were:

- $\sum$ *rain* $_{\text{same year during growing season}}$: +ve correlation; r^2 = 0.484; all catchments significant (*p*= 0.004−0.042, except ULP at *p*=0.058)
- $\sum$ *rain* $_{\text{preceding year during growing season}}$: -ve correlation, ; r^2 = 0.477 , all catchments significant (*p* = 0.009−0.021)
- $\sum$ *rain* $_{\text{same year}}$: +ve correlation, r^2 = 0.439, six of eight catchments significant (*p* = 0.007−0.044).

There was high variability of rainfall between 2000-2011 with inter-annual coefficient of variation being 21%, and deviations from mean ranging from -26 to +39%. There was significant negative autocorrelation of the rainfall time series (2000-2011) at lag τ = 1, and correlation coefficients alternating between negative and positive (yet not significant) up to τ = 5, i.e. sequence of years alternating between above and below average rainfall. This explains the counter-intuitive negative correlation of mean NDVI with the preceding year's rainfall sum, and therefore is not a causal relationship. The NDVI of all catchments shows the same autocorrelation pattern, alternating between positive and negative correlation coefficients for 5 years. At lag τ = 1 there is significant negative autocorrelation in the full Luzi, Upper Luzi and Upper Pot catchments. In the other catchments similar negative, albeit not significant, autocorrelations were observed. Thus, the deviation of each catchment's annual mean NDVI from long-term mean is best explained by same year's growing season rainfall sum. Nevertheless there were consistent and highly significant differences in mean NDVI between the catchments that rainfall could not account for.

Conclusion for Hypothesis 1

There were considerably more erosion gullies (and a higher percentage of gullied land), houses, and nearly 10 times the 'degraded' land, and a significantly lower NDVI mean value in the Luzi catchment compared to the Pot catchment. The difference between the Lower Luzi subcatchment and Upper and Lower Pot, and the Little Pot subcatchments was even more extreme. Rainfall sums have been found to explain the variation of mean NDVI between the years well, but rain does not compensate for the systematic and highly significant differences in mean NDVI between the catchments, which must therefore be attributed to the effects of land degradation. There being no trends in mean catchment NDVI values either suggests that the degraded land has reached some stability (i.e. land degradation did not worsen between 2001-2011).

In conclusion, the null hypothesis is rejected and the alternate hypothesis "there is a difference in land degradation between catchments" accepted.

5.3.4.2 Hypothesis 2 – do the studied catchments differ according to sediment load characteristics?

Three different sediment load variables were assessed: settled solids (measured in May and November 2012), turbidity and TSS (measured on all 5 sampling trips). The data from March 2012 were collected as flood-like conditions began and the instream sediment parameters are very high, perhaps reflecting upper limits for these sites (Table 5.18). The data from May were collected during low flow conditions (Table 5.12) and reflect the lower limits of the parameters measured. Although flow was higher in November 2012 than May 2012 the sediment load variables were similar, suggesting sampling occurred toward the end of the flood peak. The data from October 2012 and February 2013 reflect sediment load characteristics between these two extremes and were collected during moderate flow. Madikizela and Dye (2003) also report high temporal variations in TSS in the rivers of this region.

The data show that the upper sites of the Pot and Little Pot rivers experience low suspended solids concentrations and turbidity, while at the Upper Luzi River site (situated in a more degraded catchment) these parameters are higher. The lower site on the Pot River had very high TSS concentrations and turbidity, similar – although lower – than those at the Lower Luzi River site.

Settled solids concentration

Regarding settled solids concentration, only one replicate was collected in May 2012, and these data were considered difficult to interpret and unreliable because of very high variance observed in November 2012 when three replicate samples of settled solids concentration were collected (Table 5.18). For the November 2012 data, no significant differences between sampling sites was observed (Kruskal-Wallis: $H_5 = 8.135$, $p = 0.149$) due to high variance between replicates at each site. If these data were aggregated by river system (i.e. separately for the Pot and Luzi rivers) they were still not significant (W = 36, p-value = 0.33), despite both Luzi sites having highest mean concentrations. Aggregation by upper or lower site did, however, produce a significant result (W = 7, p-value = 0.0176). As an aside, at both the Upper and Lower Luzi sites the highest settled solids concentration was 2x the maximum concentrations at the other sites, indicating patchiness of settled solids distribution. When comparing of mean concentrations for all sites from both sampling occasions, settled solids were significantly lower in May 2012 (106 ± 39 g/m^2) than November 2012 (232 ± 100 g/m^2) (two-sample $t_{6.504} = -2.84$; $p = 0.027$). This is possibly explained by more higher rainfall in spring/summer causing more sediment delivery to the rivers and subsequent settling.

Turbidity and suspended solids concentration

The variation in concentration between sites of these two characteristics during sampling occasions was large, with lower sites having the highest values (Table 5.18). Concentrations also varied by season, related to the rainfall events (Table 5.18). Due to these highly variable values, each set of measurements (turbidity and TSS for each sampling occasion) were rank-transformed (Table 5.19). There were significant differences in mean rank of turbidity measurements between sites ($p < 0.001$), between Luzi and Pot rivers ($p < 0.01$), and between upper and lower sites ($p < 0.001$) (Table 5.20). In terms of pairwise comparisons: Lower Luzi was different from Upper Pot and Upper Little Pot ($p = 0.01$). This was almost identical for TSS (between sites = $p < 0.001$; between Luzi and Pot rivers = $p < 0.05$, between upper and lower sites = $p < 0.001$.). In terms of pairwise comparisons: Lower Luzi was different from Upper Pot and Upper Little Pot ($p < 0.05$); and Lower Pot from Upper Pot and Upper Little Pot ($p < 0.05$) (Table 5.20).

Multivariate analyses of sediment load characteristics

Principle component analysis considered all the sediment load characteristics together in determining the relationship between sites. There was a strong segregation between upper and lower sites (Figure 5.6). The upper sites of different river systems were very different from one another, but as one moved downstream this difference decreased, with the Lower Luzi and Lower Pot sites being much more similar (Figure 5.6). The Tsitsa tributary was quite different from the other sites measured in terms of the sediment load characteristics.

Table 5.18 Sediment load characteristics measured on sampling occasions

River	Site	March 2012			May 2012			October 2012			November 2012			February 2013		
		Suspended solids conc (mg/L)	Turbidity (ntu)	Settled solids (g/m^2)	Suspended solids conc (mg/L)	Turbidity (ntu)	Settled solids (g/m^2)	Suspended solids conc (mg/L)	Turbidity (ntu)	Settled solids (g/m^2)	Suspended solids conc (mg/L)	Turbidity (ntu)	Settled solids (g/m2) mean ± std dev	Suspended solids conc (mg/L)	Turbidity (ntu)	Settled solids (g/m^2)
Pot	Upper	111.0	80.6	Could not measure	1.4	0.8	57.9	6.4	9.8	Could not measure	0.4	1.7	172.3 ± 85.5	11.0	8.9	Could not measure
	Lower	729.0	484.0		2.8	5.4	85.3	16.0	21.2		2.8	4.1	281.3 ± 18.1	44.0	57.3	
Luzi	Upper	432.0	412.0		1.6	2.4	149.3	14.4	21.5		2.6	5.3	287.3 ± 273.0	29.5	23.7	
	Lower	620.0	727.0		3.0	6.3	127.0	20.0	28.8		2.8	6.7	379.7 ± 209.3	40.0	30.9	
Tsitsa trib.	Upper	268.0	342.0		2.2	5.4	145.8	10.0	22.4		3.8	9.7	132.0 ± 35.2	48.5	55.9	
Little Pot	Upper	336.0	144.0		1.1	3.6	73.0	6.8	3.5		0.0	0.8	134.3 ± 81.0	4.0	2.0	

Table 5.19 Mean ranks of sites for five measurements of turbidity and suspended solids concentration.

	Upper Luzi	Lower Luzi	Tsitsa trib. – Upper	Upper Pot	Lower Pot	Upper Little Pot
Turbidity	3.4	5.4	4.8	1.6	4.2	1.6
Suspended solids	3.4	5.1	4.2	1.6	5.1	1.6

Table 5.20 Comparison of turbidity and suspended solids ranks between: all sites; the three river systems; Luzi and Pot rivers only; and upper and lower sites (omitting Tsitsa) (Kruskal-Wallis ANOVA / Mann-Whitney U tests were applied)

	All sites		Luzi, Pot, and Tsitsa rivers		Luzi and Pot rivers		Upper and lower sites	
	H_5		H_2		W		W	
Turbidity	21.58	***	10.80	**	25	**	140	***
Suspended solids	21.48	***	5.04	ns	37.5	*	150	***

Note: * = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$

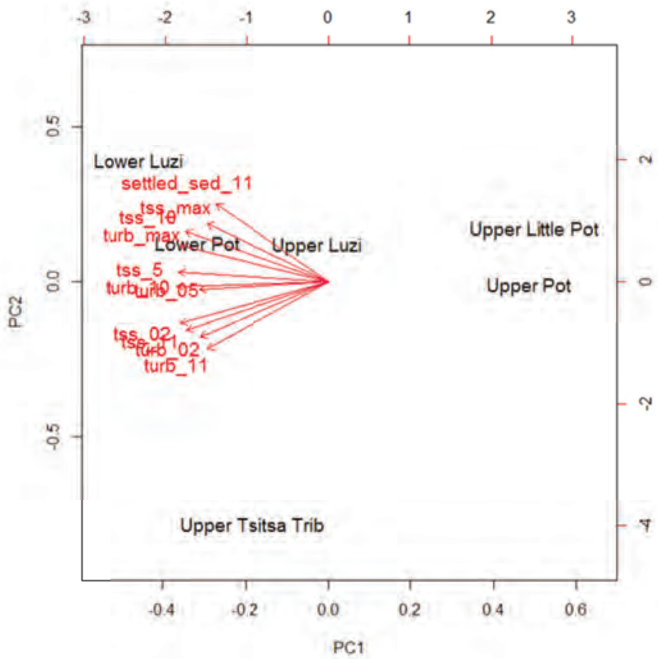


Figure 5.6 Principle component analysis of sediment load characteristics measured at the six sampling sites. Note: tss = suspended solids conc; the numerals indicate month sampled and 'max' indicates March sampling occasion.

Conclusion for Hypothesis 2

A comparison of turbidity and suspended solids concentration ranks for sampling sites indicates that, for these two variables, there are significant differences between the Pot and Luzi river systems as a whole. However, differences between the up and downstream sites of these river systems are even more pronounced. The principle component analysis confirms this, showing that the upper sites of different river systems sampled are very different from one another, but as one moved downstream these differences decrease.

In conclusion, the null hypothesis can be rejected and the alternate hypothesis "there is a difference in sediment load characteristics between catchments" accepted, albeit with the caveat that this difference appears to decrease with distance downstream.

5.3.4.3 *Hypothesis 3 – relationship between geospatial catchment characteristics and the sediment load characteristics*

Hoping to gain new insight into sediment delivery to the rivers and in an attempt to understand the mechanisms behind this we correlated the percentages of the different land cover classes within the catchments with sediment load characteristics measured at the sampling sites. Two different approaches for calculating geospatial catchment characteristics were followed: 1) statistics for the whole of each catchment were calculated; and 2) statistics were restricted to the land area near rivers (e.g. riparian buffer zones) or near sampling sites (circular upstream zones). The second approach was undertaken in case the catchment characteristics from areas closer to the river or sampling sites correlated more closely with sediment load characteristics than when the catchment as a whole was considered.

Whole catchment analyses

The TSS and turbidity data were frequently intercorrelated (3 out of 5 sampling occasions), and on the other two sampling occasions there was a high rank correlation coefficient (ϱ), with lack of significance probably related to the small number of observations. Nevertheless, the TSS results and the turbidity results were analysed separately. Settled solids concentration was not correlated with any geospatial characteristic.

Turbidity and TSS were negatively correlated with the 'Natural' land class on three of the sampling occasions, while only turbidity was negatively correlated with mean NDVI (Table 5.21). Despite the "Urban/Built-up' land class exceeding just 1% of the land area in the Lower Luzi subcatchment, and comprising a smaller proportion in the other catchments, it was the most consistently and strongly correlated catchment characteristics with turbidity and TSS. 'Cultivation' was slightly more strongly correlated with TSS than turbidity. Interestingly, the 'Degraded' land class was correlated on only two occasions with turbidity and not correlated with TSS. In contrast, the various independent measures of degradation (erosion gullies and population – houses) exhibited stronger correlations with turbidity, and to a lesser extent with TSS, than the 'degraded' land class dataset did (Table 5.21). No significant correlations were found between turbidity or TSS and river gradient, or the main geology classes (basaltic, sandstone, mudstone), catchment area size, or population density as extracted from LandScan map.

There were a number of correlations among the geospatial catchment characteristics themselves (Appendix 5). Number and density of houses, as a proxy of human population density, were correlated, among others, to natural land cover (-), cultivation (+), density and percentage of gullies (+) and mean catchment NDVI (-). Mean NDVI was in turn correlated to the percentage of natural landscape (+) and the density of erosion gullies (-) and the percentage of the catchments that possessed erosion gullies (-).

Table 5.21 Correlation analysis of geospatial catchment characteristics with turbidity and TSS. The "number" column indicates number of significant correlations (*p* ≤ 0.05) out of five sampling trips.

Geospatial catchment characteristic	Turbidity			Suspended solids		
	number	ϱ sign	significance	number	ϱ sign	significance
Urban/Built-up	4/5	+	*/**	3/5	+	*/**
Natural	3/5	-	*/**	3/5	-	*/**/***
Cultivation	2/5	+	*/**	2/5	+	***
Degraded	2/5	+	*/**	0/5		
Gullies: number	2/5	+	*	2/5	+	*
Gullies: % of catchment	2/5	+	**/***	2/5	+	*
Gullies: total area [km^2]	2/5	+	**/***	2/5	+	*
Gullies: density [km^{-1}]	2/5	+	**/***	2/5	+	*
Houses: number	2/5	+	**/***	2/5	+	*
Houses: density [km^{-1}]	2/5	+	**/***	2/5	+	*
NDVI mean	2/5	-	*/**	0/5		

Note: * = $p < 0.05$; ** = $p < 0.01$, *** = $p < 0.001$

Restricted area analyses

In most cases where the whole catchment statistics yielded significant correlations between geospatial characteristic and sediment load characteristic, the restricted area data did too, with only natural land cover class having one less significant correlation (Table 5.22). For the cultivation land cover class the restricted areas approach revealed three more significant correlation, for the degraded land class two more, for % gullies and number of houses three more each, and for gully and house density one more each (Table 5.22).

Table 5.22 Overview of correlations between restricted area geospatial characteristics and sediment load characteristics according to different flow events

Measure	Sampling date	Correlations
Turbidity low flow	May ‘12	+ ‘cultivation’ within 1-2 km radius of site
Sus solids low flow		+ ‘cultivation’ within 1-2 km radius of site + % gullies within 2-20 km radius of site, 100-500 m riparian zone + house density within 5-20 km radius from site, 100-300 m riparian zone + house numbers within 5-20 km radius from site, 100-750 m riparian zone
Turbidity moderate flow	Nov ‘12	+ ‘cultivation’ when within 7.5-10 km radius from site, and within 100-400 m riparian zone - natural when within 7.5-20 km radius from site + % gullies within 5-20 km radius from site + house density within 2-20 km radius from site, 200-1000 m riparian zone + house numbers within 2-20 km radius from site, 200-1000 m riparian zone
Sus solids moderate flow		+ ‘cultivation’ when within 3-20 km radius from site - natural when within 7.5-20 km radius from site + % gullies within 4-20 km radius from site + area gullies within 4-20 km radius from site, 100-1000 m riparian zone + house density within 3-20 km radius from site, 200-1000 m riparian zone + house numbers within 3-20 km radius from site, 200-1000 m riparian zone
Settled solids		+% gullies within 1-3 km radius from site, 200-1000m riparian zone + area gullies within 1-3 km radius from site, 200-1000m riparian zone
Turbidity high flow	Mar ‘12	+ ‘degraded’ when within 7.5-20 km radius from site + % gullies within 3-10 km radius from site + area gullies within 3-10 km radius from site, 200-1000 m riparian zone + house numbers within 5-20 km radius from site, 100-300 riparian zone
	Oct ‘12	+ ‘degraded’ when within 7.5-20 km radius from site + “cultivation’ when within 7.5-10 km radius from site, and within 100-500 riparian zone - ‘natural’ when within 5-20 km radius from site + % gullies within 5-20 km radius from site + area gullies within 4-20 km radius from site + house density within 2-20 km radius from site, 200-1000 m riparian zone + house numbers within 2-20 km radius from site, 200-1000 m riparian zone
	Feb ‘13	+ ‘cultivation’ when within 7.5-20 km radius from site - ‘natural’ when within 0-20 km radius from site + house numbers within 3020 km radius from site, 100-300 m riparian zone
Sus solids high flow	Mar ‘12	No correlations
	Oct ‘12	+ ‘degraded when within 7.5-20 km radius from site + % gullies within 3-10 km radius from site + area gullies within 3-10 km radius from site, 200-1000 m riparian zone + house numbers within 5-20 km radius from site, 100-3000 m riparian zone
	Feb ‘13	+ ‘cultivation’ when within 3-20 km radius from site - ‘natural’ when within 0-20 km radius from site + housing density when within 10-20 km radius from site, 100-1000 m riparian zone

Note: Correlation was included in table if at least 5% of buffer/zone correlations were significant – see section 6.2.2 for methods explanation.

During low flow, turbidity and TSS were positively correlated to the percentage of cultivation close to the sampling site (1-2 km circular buffer), and only TSS was correlated with % gullies when they occurred from 2-20 km from the site (Table 5.22). This suggests that during the low rainfall season some sediment delivery from cultivated land is possibly occurring, but only if it is very close to the river. There is also some evidence of sediment input from erosion gullies occurring from fairly close to the river (2 km).

During moderate and high flows there is a positive correlation between turbidity / TSS and cultivation occurring at moderate to large distances from the sampling site. There is a similar relationship between the sediment load characteristics and the degraded land class. The density of erosion gullies and their percentage of the catchment are also regularly correlated with all sediment load characteristics. This suggests that higher rainfall runoff and flows are transporting particulate solids larger distances. The negative correlation with the natural land class occurring at high flows shows the importance of good land management in preventing erosion during the high rainfall periods (Table 5.22).

When settled solids concentrations were measured in November 2012, they were positively correlated with density of erosion gullies and their percentage, but only when those gully statistics were very close the river (1-3 km radius) (Table 5.22). The number and density of houses were almost always correlated with turbidity and TSS, regardless of season. The lack of correlation with settled solids concentration may only be an artefact of the low number of samples collected for this characteristic.

Conclusion for Hypothesis 3

During moderate and high flows (rainfall season) there were significant correlations between turbidity / TSS and population density (as measured by urban land cover class and housing estimates) and measures of degraded land (erosion gully estimates and the degraded land cover class) occurring within a 20 km radius of the site. During the low rainfall season, only cultivation, and perhaps erosion gullies, close to the river explain sediment load characteristics the best. The restricted area analyses of geospatial characteristics are much more likely to identify rivers with the potential to suffer from excessive sediment load. This is especially the case during low rainfall seasons.

In conclusion, the null hypothesis can be rejected and the alternate hypothesis ‘there is a correlation between specific geospatial catchment characteristics and sediment load characteristics’ can be accepted.

5.3.4.4 *Hypothesis 4 – investigates whether the macroinvertebrate community indeed varies between sites/catchments according to various biological stress response measures?*

Biological stress responses were measured at different levels of organisation: three measures of macroinvertebrate tolerance to water quality impairment; five measures of community composition; three measures of community richness; and a surrogate measure of ecosystem function (functional feeding groups) (Table 5.5). In Table 5.5, an indication of the generally acknowledged response of the measured variables to chemical stress exposure is provided. Whether the same responses can be expected for a physical stressor like particulates is not generally known, although Wagenhoff et al. (2012) and Buendia et al. (2013) have investigated the response of EPT metrics to sediment exposure.

Although five sampling trips were undertaken to the research area, biological monitoring was only possible on two occasions. This limited dataset reduces the statistical robustness of analyses and makes interpretation of results tentative. Consequently, the results reported here should not be considered as definitive, but can give an indication of the possible relationship between macroinvertebrates and the sediment load characteristics they are exposed to. Further data collection should be undertaken in order to confirm the results reported here. Lastly, an unfortunate incident in the laboratory resulted in the destruction of a number of macroinvertebrate replicate samples from the Upper Little Pot site, and one replicate from Upper Pot and Lower Luzi. Where the absence of these data interfered with the statistical analyses, all data from this site were then omitted. A table of the trait data is provided in Appendix 6.

SASS5 – structural taxon-based metric for taxa tolerance

For the SASS5 metrics there were only two replicates per site (Table 5.23). Despite these limited data, an ANOVA identified the SASS Scores of Upper and Lower Luzi to be significantly different to the other sites. Number of Taxa and ASPT were not found to be significantly different, but the ASPT figures presented in Table 5.23 show a similar pattern to that of the SASS Score, with the Luzi River sites always being lower than the Pot River sites. A decrease in a SASS5 metric is often associated with a water quality stress (Table 5.5). Although instream habitat can also affect the SASS5 metrics, the sites in this study were specifically chosen to have very similar instream habitat.

Table 5.23 SASS5 metric results from Autumn and Summer sampling of research sites.

Site	Sampling season	SASS score	Number of taxa	ASPT
Luzi River – Upper	Autumn	123	21	5.86
	Summer	113	21	5.38
Luzi River – Lower	Autumn	90	15	6.00
	Summer	101	18	5.61
Tsitsa trib. – Upper	Autumn	178	26	6.85
	Summer	162	27	6.00
Little Pot River – Upper	Autumn	157	21	7.48
	Summer	167	27	6.19
Pot River – Upper	Autumn	187	27	6.93
	Summer	136	20	6.80
Pot River – Lower	Autumn	193	24	6.66
	Summer	149	23	6.48

Baetidae/Ephemeroptera ratio – structural taxon-based metric for taxa tolerance

The ratio of Baetidae to Emphemeroptera was significantly higher in summer compared to autumn. When analysed by season, sites in autumn were not significantly different from one another, while in summer the ratio at Lower Luzi was significantly lower the at Lower Pot and the Tsitsa Tributary. An increase in this ratio is usually used to indicate chemical stress exposure (Table 5.5), however in this case the lowest ratio was measured at the Lower Luzi which generally has the highest turbidity, TSS and settled solids concentrations (Table 5.18), indicating that perhaps this ratio is not a suitable measure of instream sediment load stress. However, there are too few replicate samples to be conclusive.

EPT/Chironomidae ratio – structural taxon-based metric for taxa tolerance

The ratio of EPT to Chironomidae taxa was significantly higher in summer compared to autumn. When analysed by season, sites were not significantly different from one another for either autumn or summer.

% EPT abundance – structural taxon-based metric for community composition

The percentage of EPT individuals of all taxa sampled was significantly higher in summer compared to autumn. When analysed by season, sites in autumn were not significantly different from one another. However, in summer the Upper and Lower Pot were significantly higher than the Tsitsa Tributary, the Lower Pot was significantly higher than the Upper Luzi, and the Lower Luzi was significantly higher than the Upper Luzi and Tsitsa Tributary. There was no difference between the Upper Pot and the Lower Pot and Lower Luzi, both the latter experiencing some of the highest instream sediment loads sampled. When the EPT richness alone is considered, the total number of EPT taxa at the Upper Pot site was significantly higher than the Lower and Upper Luzi and Tsitsa Tributary, and considerably higher than the Lower Pot, generally matching the higher instream sediment loads of the latter four sites (Table 5.18).

% Ephemeroptera abundance – structural taxon-based metric for community composition

The percentage Ephemeroptera of all taxa sampled was significantly higher in summer compared to autumn. When analysed by season, sites were not significantly different from one another.

% Trichoptera abundance – structural taxon-based metric for community composition

The percentage Trichoptera of all taxa sampled was significantly higher in autumn compared to summer. In Autumn the % Trichoptera at the Upper Little Pot were significantly higher than at the Lower Pot, Tsitsa Tributary, Upper and Lower Luzi. In addition, the Upper Pot was significantly higher than the Lower Luzi. In summer, % Trichoptera at the Tsitsa Tributary was significantly higher than at the Lower Pot and Lower Luzi. In the autumn results, sites which generally have higher sediment loads also had a lower % Trichoptera, but in summer this trend is not as clear, with the highest % Trichoptera at the Tsitsa Tributary site which has a higher sediment load than the Upper Pot (Table 5.18).

% Diptera abundance – structural taxon-based metric for community composition

The percentage Diptera of all taxa was significantly higher in autumn compared to summer. However, when analysed by season, sites in autumn were not significantly different from one another. In summer the % Diptera at the Lower Pot site was significantly lower than at the Upper Luzi and Tsitsa Tributary. In addition, the Lower Luzi was significantly lower than the Upper Luzi.

NMDS of macroinvertebrate sample data – structural taxon-based metric for community composition

The macroinvertebrate community composition was found to differ significantly by season (Figure 5.7). When autumn data were analysed separately, the Upper Luzi and Tsitsa Tributary sites were the most distinct (within group similarity was > 60%), with the remaining sites being similar (> 60%) to each other (Figure 5.8). In summer the macroinvertebrate community was highly segregated according to site (> 70%) (Figure 5.9), with the Pot River sites being on the lower part of the ordination, and the Luzi River and Tsitsa Tributary sites being on the upper part. Further analysis revealed no difference between up and downstream sites during each season. When sites were aggregated by river (separately for each season), all three rivers were significantly different from one another (autumn: Global R = 0.36; summer: Global R = 0.77).

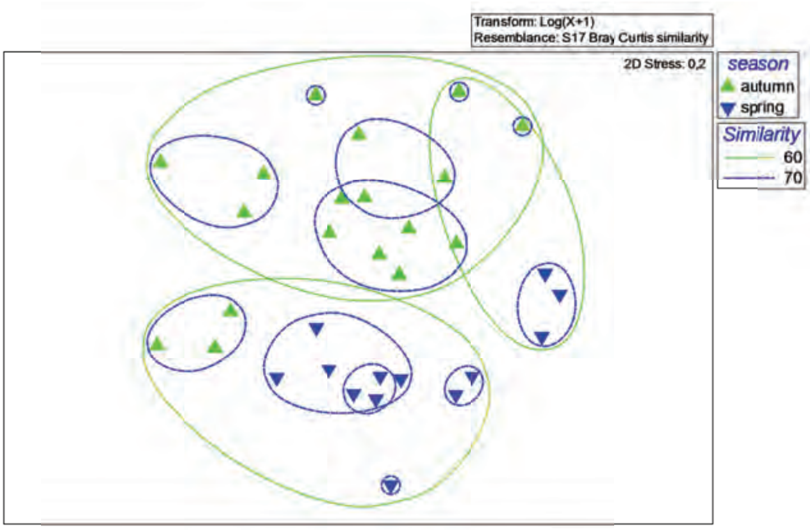


Figure 5.7 NMDS ordination of enumerated macroinvertebrate data as measures of community composition for autumn and summer 2012. Seasons were significantly different (ANOSIM: Global R = 0.44; p ≤ 0.05).

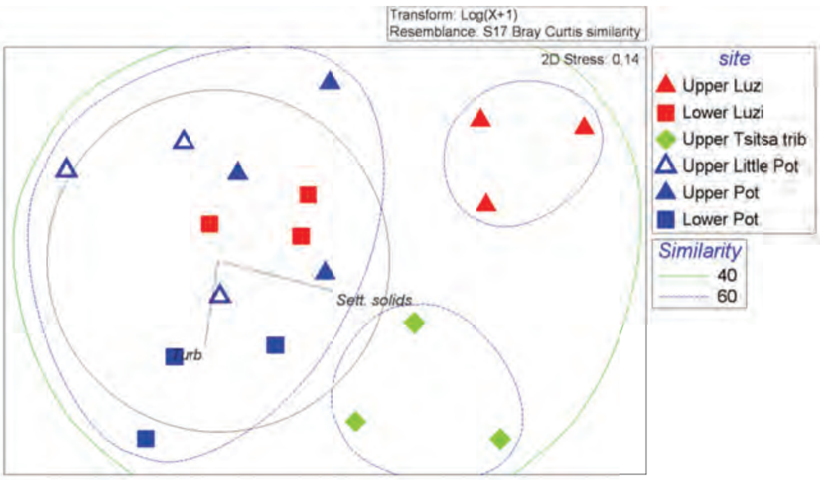


Figure 5.8 NMDS ordination of enumerated macroinvertebrate data as measures of community composition for autumn 2012 (ANOSIM: Global R = 0.72; p ≤ 0.05).

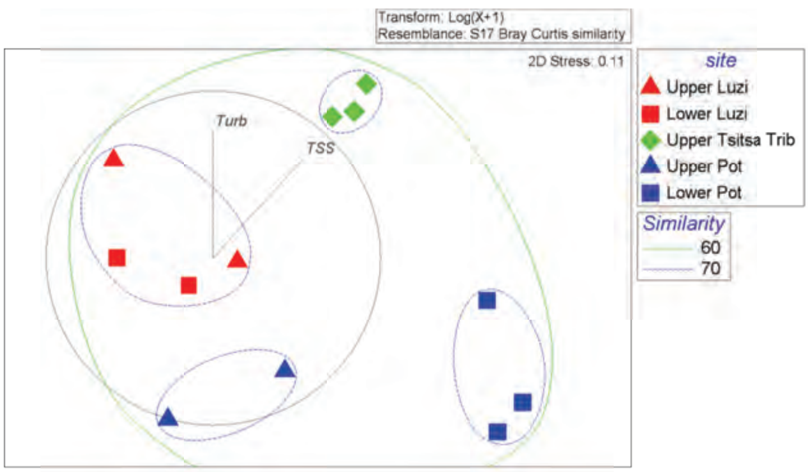


Figure 5.9 NMDS ordination of enumerated macroinvertebrate data as measures of community composition for summer 2012 (ANOSIM: Global R = 0.99; p ≤ 0.05).

Number of EPT families – structural taxon-based metric for community richness

The number of EPT families was significantly lower at the Upper Luzi site compared to the Upper and Lower Pot and Tsitsa Tributary sites. In addition, the Lower Luzi was significantly lower than the Lower Pot site.

Shannon-Weiner Diversity Index – structural taxon-based metric for community richness

This index was significantly higher in autumn compared to summer, but when analysed by season none of the sites were significantly different from one another.

Margalef's Taxon Richness Index – structural taxon-based metric for community richness

This index was significantly higher in summer compared to autumn. The difference between which index is different in which season is likely due to the fact that the Margalef's Index includes the number of individuals as well as the number of taxa in its computation. The higher number of individual organisms in summer is likely to have caused a higher value for Margalef's Index in summer. When analysed by season, sites in autumn were not significantly different from one another, but in summer the Lower Luzi was significantly lower than the Tsitsa Tributary.

NMDS of functional feeding groups – functional trait-based metric for trophic-level community composition

Although not visually obvious from the ordination of all FFG data, there was a significant difference between seasons (ANOSIM: Global $R = 0.44$; $p \leq 0.05$) (Figure 5.10). When autumn data were analysed separately, the Upper Luzi site was distinct from the remaining sites (Figure 5.11). When summer data were analysed, sites were much more segregated from one another, the most distinct group being Lower Luzi, with both Pot River sites together and the Upper Luzi and Tsitsa Tributary grouping out together (Figure 5.12).

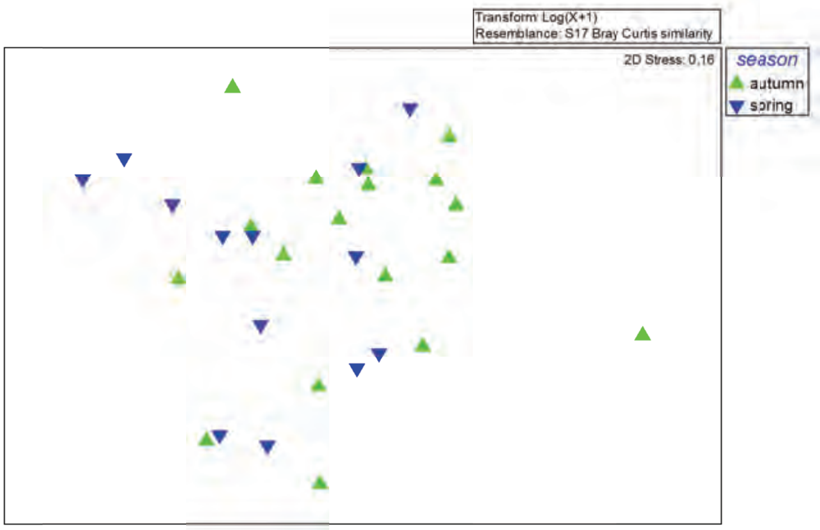


Figure 5.10 NMDS ordination of enumerated functional feeding groups data for autumn and summer 2012. Seasons were significantly different (ANOSIM: Global R = 0.44; p ≤ 0.05).

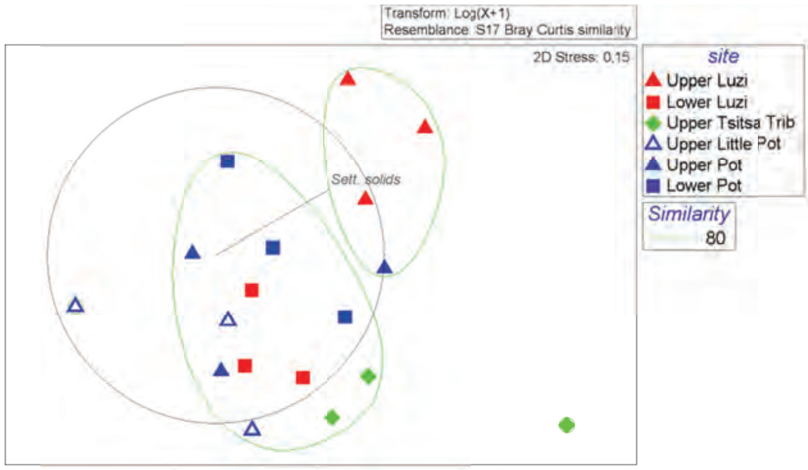


Figure 5.11 NMDS ordination of enumerated macroinvertebrate functional feeding groups data for autumn 2012 (ANOSIM: Global R = 0.49; p ≤ 0.05).

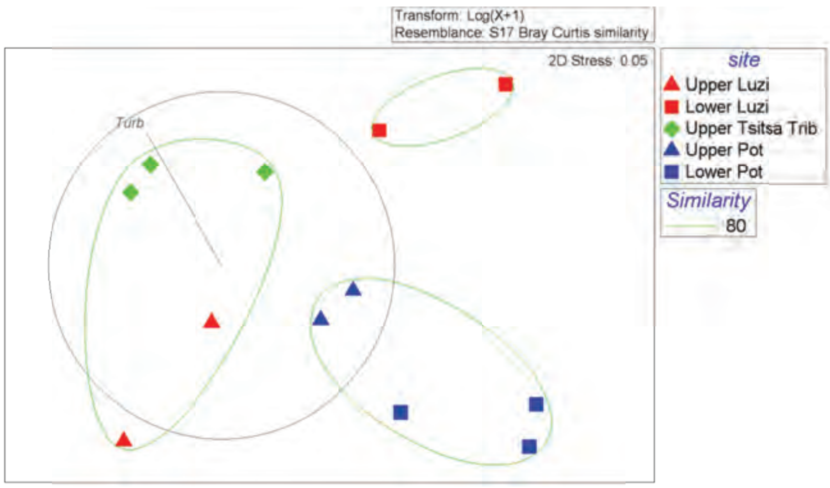


Figure 5.12 NMDS ordination of enumerated macroinvertebrate functional feeding groups data for summer 2012 (ANOSIM: Global R = 0.93; p ≤ 0.05).

Conclusion for Hypothesis 4

The low number of invertebrate samples collected reduced the power of the statistical analyses. Consequently, any conclusions drawn are tentative. Despite this, this report has the potential to highlight potential important relationships which can be further examined after additional data collection. The NMDS plots of all macroinvertebrate data and FFGs showed that in autumn the Upper Luzi site (and Tsitsa Tributary to a slightly lesser extent) was different from the other sites (Figure 5.8 and Figure 5.11). In summer, for NMDS of all macroinvertebrate data, all sites were different from the Luzi River sites (and different among themselves too) (Figure 5.9), and for the FFGs the Lower Luzi and Lower Pot were different from all other sites (Figure 5.12). The remaining biological stress measures employed showed varied relationships between sites, which have been summarised in Table 5.24. In this table the number of significant differences the between sites as determined during statistical analysis of the biological stress measures is recorded. The issue of redundancy should be acknowledged, however, as a number of biological stress responses measure similar aspects of the macroinvertebrate community (e.g. the EPT metrics, % Ephemeroptera and % Trichoptera). Nevertheless, the overwhelming weight of evidence suggests there is a difference between sites, particularly sites from different catchments. In addition, the traits of % Trichoptera and FFG NMDS indicate differences between up and downstream sites within the same river system, especially where the sediment loads of up and downstream sites are quite different (e.g. Pot River system). This observation appears to correlate well with that observed for settled solids which also differ significantly between up and downstream sites.

In conclusion, the null hypothesis can be rejected and the alternative hypothesis that "there is a difference in macroinvertebrate community between the sites according to various biological stress responses" accepted.

Table 5.24 Matrix indicating the number of times a significant difference was found between sites by the biological stress response measures employed (NMDS analyses excluded).

	Little Pot Upper	Pot Upper	Pot Lower	Tsitsa Trib.	Luzi Upper	Luzi Lower
Little Pot Upper						
Pot Upper	1					
Pot Lower	1	0				
Tsitsa Trib.	1	2	5			
Luzi Upper	2	3	6	2		
Luzi Lower	2	3	5	5	2	

5.3.4.5 *Hypothesis 5 – Are differences in macroinvertebrate assemblage composition between the sites attributable to a sediment load characteristic?*

It was only possible to collect corresponding macroinvertebrate data and sediment load characteristics on two occasions (May and November 2012). Any conclusions drawn in this study regarding correlations between macroinvertebrate assemblage composition and sediment load characteristics should be regarded as tentative, and only an indication of possible relationships that require further investigation.

Results and discussion

Statistically significant correlations between the various biological stress response variables, employed to represent different measures of the macroinvertebrate assemblage composition, are detailed in Table 5.25. Correlations were determined for all data, and separately for each season. A strikingly large number of correlations involved settled solids concentration, with turbidity also correlating fairly regularly with the macroinvertebrate stress response measures, and TSS only featuring twice. This finding supports the assertions of Jones et al. (2012) that many of the impacts of fine sediments on macroinvertebrates appear related to the deposition of material. Although Von Bertrab et al. (2013) show that it might not necessarily be the quantity of fine sediment driving the composition of benthic macroinvertebrate assemblages, but rather the chemical composition (carbon to nitrogen ratio) of the sediment (and in their study the instream dissolved oxygen was also a significant driver). The poor correlation of TSS to biological responses contradicts the commonly held belief that it is a more precise way of measuring the effects of suspended solids than turbidity (Bilotta and Brazier 2008). However, this result may only pertain to the rivers investigated in this study.

Both SASS5 Score and ASPT were negatively correlated with settled solids concentration when both seasons were combined, while in summer only the SASS5 score was negatively correlated with settled solids concentration (Table 5.25). As part of this research project, an investigation of concurrently measured SASS5 metrics and turbidity data recorded in the River Health Programme's database was undertaken (turbidity was the only measured sediment load characteristic available in the database). All three SASS metrics (SASS score, Number of Families and ASPT) were found to be significantly negatively correlated with turbidity, although the respective R^2 values (0.12, 0.10 and 0.08) were weak suggesting very high variability of the data (Appendix 7). Although neither turbidity nor TSS were correlated with the SASS metrics measured in the current study, the correlation with settled solids concentration was also consistently negative. In the case of the River Health Programme database investigation, electrical conductivity was also identified as a significant driver in SASS Score and Number of Families, but not for ASPT. A positive correlation between turbidity and electrical conductivity data was also determined in that study (Appendix 7). In the current study however, it is unlikely that electrical conductivity would have affected macroinvertebrates as the values recorded during sampling (Table 5.26) were well below the guideline for natural rivers of < 30 mS/m (DWA 2008). In fact it is unlikely that any of the water physico-chemical parameters measured would have impacted the macroinvertebrate community, with all but total inorganic nitrogen (TIN) and periphyton chlorophyll-a being classified as being in a natural state (Table 5.26). TIN concentrations in February 2013 (Table 5.26) would have been classified as a large change (1-4 mg/L) from a natural condition, while the remaining sampling occasions would have been classified as natural. Periphyton chlorophyll-a was sometimes classified as a moderate (12-21 mg/m^2) or small (1.7-12 mg/m^2) (DWA 2008) change from natural state (Table 5.26).

Table 5.25 Statistically significant correlations between different biological stress response variables representing measures of macroinvertebrate assemblage composition and correspondingly measured sediment load characteristics (p < 0.05).

Biological stress response measures	Sampling season		
	Combined	**autumn**	**summer**
SASS5 Score	Sett. Solids -0.60		Sett. Solids -0.85
SASS5 ASPT	Sett. Solids -0.64		
Baetidae / Ephemeroptera			Sett. Solids -0.61
EPT / Chironomidae	Sett. Solids +0.60		
% EPT abundance	Sett. Solids +0.46	Sett. Solids -0.56	Sett. Solids +0.59 Turb. -0.63
% Ephemeroptera abundance	Sett. Solids +0.73		Sett. Solids +0.69 Turb. -0.71
%Trichoptera abundance	Sett. Solids -0.75	Sett. Solids -0.51 TSS -0.75 Turb. -0.51	Sett. Solids -0.73 Turb. +0.73
% Diptera abundance	Sett. Solids -0.36		
NMDS of all taxa	Sett. Solids vector Turb. vector	Sett. Solids vector Turb. vector	Turb. vector TSS vector
Number of EPT families		Sett. Solids -0.49	
Shannon-Weiner Diversity Index			
Margalef's Taxon Richness Index			
NMDS of FFGs	Sett. Solids vector Turb. vector	Sett. Solids vector	Turb. vector

Notes: the vectors for NMDS measures can be seen in Figure 5.8; Figure 5.9; Figure 5.11; Figure 5.12.

The Baetidae to Ephemeroptera ratio was negatively correlated with settled solids concentration in summer. However, the other measure of taxa tolerance, EPT to Chironomidae ratio, was positively correlated to settled solids concentration (Table 5.25). These responses are the opposite of what is expected of these biological measures when macroinvertebrates are exposed to chemical stress (Table 5.5). In fact, % EPT abundance and % Ephemeroptera abundance should also exhibit negative correlations with chemical stress (Table 5.5), and Wagenhoff et al. (2012) show that % EPT abundance was negatively correlated with deposited fine sediment in a stream mesocosm. However, these two measures were inconsistent, being positively and negatively correlated with settled solids concentration depending on the season. This inconsistency might be related to the very high numbers of Ephemeroptera sampled in summer compared to autumn. Increasing the number of seasons sampled may help establish a clearer trend.

Table 5.26 Water physico-chemical parameters measured on sampling occasions.

May 2012

River and site	Temp (°C)	pH	DO (mg/L)	EC (mS/m)	Ammonium (mg/L)	Nitrites (mg/L)	Nitrates (mg/L)	TIN (mg/L)	Phosphates (mg/L)	Phytoplankton Chl *a* (ug/L)	Periphyton Chl *a* (ug/cm^2)
Pot - Upper	11.5	6.6	6.8	4.4	<0.1	<0.01	<0.1	<0.1	<0.1	0.03	18.1
Pot - Lower	10.5	6.4	7.2	5.2	<0.1	<0.01	<0.1	<0.1	<0.1	0.07	13.2
Luzi - Upper	11.5	7.7	7.1	8.1	<0.1	<0.01	<0.1	<0.1	<0.1	0.03	14.3
Luzi - Lower	12.0	7.1	7.1	8.3	<0.1	<0.01	<0.1	<0.1	<0.1	0.11	14.0
Tsitsa trib	15.8	6.4	7.1	2.7	0.12	<0.01	<0.1	0.12	<0.1	0.27	7.0
Little Pot - Upper	9.0	7.5	7.1	4.0	<0.1	<0.01	<0.1	<0.1	<0.1	0.07	18.8

October 2012

River and site	Temp (°C)	pH	DO (mg/L)	EC (mS/m)	Ammonium (mg/L)	Nitrites (mg/L)	Nitrates (mg/L)	TIN (mg/L)	Phosphates (mg/L)	Phytoplankton Chl *a* (ug/L)	Periphyton Chl *a* (ug/cm^2)
Pot - Upper	13.0	6.6	8.3	3.3	<0.1	<0.01	<0.1	<0.1	<0.1		
Pot - Lower	15.5	6.5	7.7	3.3	<0.1	<0.01	<0.1	<0.1	<0.1		
Luzi - Upper	14.0	6.9	8.1	5.3	<0.1	<0.01	<0.1	<0.1	<0.1	Unable to sample due to	
Luzi - Lower	15.0	7.1	8.1	5.1	<0.1	<0.01	<0.1	<0.1	<0.1	high water	
Tsitsa trib	14.0	6.1	8.3	2.0	<0.1	<0.01	<0.1	<0.1	<0.1		
Little Pot - Upper	12.0	6.8	8.3	3.3	<0.1	<0.01	<0.1	<0.1	<0.1		

November 2012

River and site	Temp (°C)	pH	DO (mg/L)	EC (mS/m)	Ammonium (mg/L)	Nitrites (mg/L)	Nitrates (mg/L)	TIN (mg/L)	Phosphates (mg/L)	Phytoplankton Chl *a* (ug/L)	Periphyton Chl *a* (ug/cm^2)
Pot - Upper	16.5	7.5	8.6	6.7	<0.1	<0.01	<0.1	<0.1	<0.1	0.04	13.5
Pot - Lower	23.0	7.4	8.0	6.7	<0.1	<0.01	<0.1	<0.1	<0.1	0.06	7.4
Luzi - Upper	17.5	7.4	8.1	11.5	<0.1	<0.01	<0.1	<0.1	<0.1	0.05	8.0
Luzi - Lower	23.0	7.2	7.9	11.1	<0.1	<0.01	<0.1	<0.1	<0.1	0.06	6.6
Tsitsa trib	19.0	6.7	8.5	3.4	<0.1	<0.01	<0.1	<0.1	<0.1	0.03	17.6
Little Pot - Upper	16.0	6.6	8.7	6.5	<0.1	<0.01	<0.1	<0.1	<0.1	0.09	21.2

February 2013

River and site	Temp (°C)	pH	DO (mg/L)	EC (mS/m)	Ammonium (mg/L)	Nitrites (mg/L)	Nitrates (mg/L)	TIN (mg/L)	Phosphates (mg/L)	Phytoplankton Chl *a* (ug/L)	Periphyton Chl *a* (ug/cm^2)
Pot - Upper	16.5	7.3	7.6	5.5	<0.1	0.03	<0.1	<0.1	<0.1	0.02	6.6
Pot - Lower	18.5	6.7	5.4	4.9	<0.1	0.09	<0.1	<0.1	<0.1	0.03	-
Luzi - Upper	18.0	7.4	6.7	8.1	<0.1	0.13	1.28	1.41	<0.1	0.03	-
Luzi - Lower	17.5	7.3	6.4	8.0	<0.1	0.14	1.46	1.60	<0.1	0.03	-
Tsitsa trib	15.5	6.8	6.5	3.0	<0.1	0.21	1.86	2.07	<0.1	0.03	1.3
Little Pot - Upper	16.0	7.0	6.2	4.5	<0.1	0.02	<0.1	<0.1	<0.1	0.01	1.1

The % Trichoptera abundance measure was significantly correlated with all three sediment load characteristics (depending on season) (Table 5.25). The correlations were consistently negative, with only turbidity in summer being positively correlated. Wagenhoff et al. (2012) report the responses of two Trichoptera genera *Oxyethira* and *Psilochorema* to fine deposited sediment was also negative. The % Trichoptera appears to be one of the more sensitive and consistent indicators of excessive instream particulate exposure. Two other promising measures are NMDS of all macroinvertebrates sampled and of FFGs (Table 5.25; for vectors see Figure 5.8; Figure 5.9; Figure 5.11; Figure 5.12). Buendia et al. (2013) argue the merit of functional trait-based metrics like FFGs over structural taxon-based metrics. However, our results indicate that % Trichoptera abundance and NMDS of all macroinvertebrate data show as much promise (although it could be argued that Trichoptera represent the functional trait of “filter feeders”). The remaining structural taxon-based metrics of Shannon-Weiner Diversity Index and Margalef’s Taxon Richness Index were found to have no correlations with any sediment load characteristic, with % Diptera abundance and number of EPT families only slightly better, being negatively correlated (but having low r^2 values) with settled solids concentration.

Conclusion for Hypothesis 5

The low number of samples makes this conclusion a tentative one. Despite the likely low power of statistical analyses undertaken on the limited data, once again there was overwhelming weight of evidence suggesting that settled solids concentrations are a very important sediment load characteristic in explaining macroinvertebrate structure and function, and that surprisingly TSS had a very low correlation with the macroinvertebrate response measures employed.

In conclusion, the null hypothesis can be tentatively rejected and the alternate hypothesis "the differences in macroinvertebrate macroinvertebrate assemblage composition between the sites can be attributed to a sediment load characteristic".

5.3.5 Overall conclusion and refinement of risk assessment protocol

The aim of the field study undertaken in this chapter was to examine hypotheses that could be used to test and refine the application of the proposed suspended solids risk assessment protocol. Tier 1 of the risk assessment is a desktop approach, with the objective to use catchment or subcatchment geospatial data to determine the likelihood of negative biological consequences from excessive particulate solids input to the river (in this study macroinvertebrates were used as the biological response). This approach requires two assumptions to be met: 1) there is a link between a geospatial characteristic and sediment load characteristic; and 2) there is a link between a particular sediment load characteristic and macroinvertebrate assemblage response. Hypotheses 1 and 2 were undertaken to ensure that there were indeed differences between sites/catchments in terms of geospatial and sediment load characteristics, allowing the 3rd Hypothesis to specifically investigate assumption 1 of Tier 1: is there a link between a geospatial characteristic and a sediment load characteristic? Results indicate that during low flows (low rainfall season) the land class of 'cultivation', when close to the river (within 1-2 km), has a strong negative correlation with instream turbidity and TSS. At higher flows (during high rainfall season) the land classes of 'degradation' and 'cultivation', erosion gully measures and measures of higher population density had a strong and consistent positive correlation with instream turbidity and TSS. The 'natural' land class had a strong negative correlation with instream turbidity and TSS. During the higher flows, the number of significant correlations measured between geospatial and sediment load characteristics was considerably higher when a restricted subcatchment radius of 20 km from sampling sites for geospatial data was used, instead of using geospatial data from the whole catchment. Settled solids concentrations were positively correlated with erosion gullies occurring within 3 km of the river site. Consequently, the first assumption, that there is a link between certain geospatial characteristics and certain sediment load characteristics could be confirmed, although flow/rainfall season and the type of sediment characteristic chosen can affect the most suitable geospatial characteristic. The limited number of samples collected during this study means that any conclusions should be viewed as promising leads for further investigation. Given this, refinement of the risk assessment protocol should focus on the geospatial characteristics found significant in this study: the land cover classes of 'natural', 'degradation' and 'cultivation'; measures of erosion gullies; and measures of population density.

With Hypotheses 1 and 2 confirming there were indeed differences between sites/catchments in terms of geospatial and sediment load characteristics, Hypothesis 4 was undertaken, and also confirmed a difference between sites/catchments in terms of the macroinvertebrate assemblages (as measured by various biological stress response variables). These results then validated the use of the different catchments in the research area for examining Hypothesis 5: can differences in macroinvertebrate assemblage composition between the sites be attributed to a sediment load characteristic? The aim of this Hypothesis 5 was three fold. Firstly to investigate the 2nd assumption governing the use of Tier 1: i.e. that there is a link between a particular sediment load characteristic and a macroinvertebrate assemblage response. Secondly, to help refine Tier 2 by indicating which sediment characteristic best predicts biological (macroinvertebrate) changes in the field so allowing effort in developing a water quality guideline to focus on that particular sediment load characteristic (be it TSS, turbidity or settled solids). Lastly, the outcome of Hypothesis 5 will also provide useful information for Tier 3, indicating which biological response is a sensitive measure of particulates exposure and should therefore be employed in site-specific biomonitoring.

Results from Hypothesis 5 indicated that, according to the macroinvertebrate stress responses employed – which consisted of both structural taxon-based metrics and a functional trait-based metric – the differences in macroinvertebrate assemblage composition between the sites could be attributed to sediment load characteristics. Secondly, that the overwhelming evidenced suggested that settled solids concentration best explained changes in macroinvertebrate assemblage composition. Lastly, the most sensitive biological responses appeared to be % Trichoptera abundance (it had the most sensitive and consistent response to all sediment load characteristics), NMDS of all macroinvertebrate taxa and NMDS of FFGs (although problems associated with the allocation of taxa to specific guilds due to flexible in feeding strategies means that the wide spread application of this approach in South Africa requires more investigation of taxon feeding strategies and stomach contents). Lastly, SASS Score and ASPT were consistently negatively correlated (and had high r^2 values) with settled solids concentration. Attempts to refine of the risk assessment protocol should consider the above information for further investigation.

Thus, results from this chapter show that the approaches suggested for Tiers 1 and 3 are valid and with proper refinement are potentially useful management tools. The application of Tier 2, comparing field measured sediment load concentrations to a laboratory defined exposure-response relationship was problematic. The exposure-response relationship that was defined in Chapter 3 was for kaolin clay particles measured as TSS. However, the geochemical composition of the instream sediment at the field sites could not be determined and, furthermore, it appears that the most important sediment load characteristic is settled solids concentration, not TSS. Consequently, application of the laboratory defined exposure-response relationship for kaolin would likely lead to erroneous conclusions. Suggestions on how to develop more appropriate exposure-response relationships for application in Tier 2 of the risk assessment protocol are detailed in Chapter 6.

6 CONCLUSIONS AND FUTURE RESEARCH

Generating exposure-response data for particulates is fraught with difficulty (Kefford et al. 2010). It is now apparent that a universal guideline for defining the ecological effects of particulates will be inadequate, being far too imprecise (Bilotta and Brazier 2008; Collins et al. 2011). Instead, guidelines will have to be more site-specific, addressing particular narrow ranges of size and shape of particulates of specific geochemical composition. In attempts to generate biological effects data from indigenous South African macroinvertebrates exposed to suspended kaolin particles highlighted a number of challenges: 1) maintaining particulates in suspension, and keeping the concentrations of those suspensions constant across replicates; 2) the difficulty of observing responses of organisms in turbid treatments limited the type of biological endpoint that could be measured and how often this measurement could take place; and 3) mortality is not a suitable experimental endpoint for suspended solids. Regarding the latter two challenges, reproductive endpoints appeared to be the most sensitive and reliable endpoints for determining the individual and population level effects of suspended particulate exposure in the organisms tested in this study. Regarding the first challenge, problems encountered in keeping particulates suspended at similar concentrations across experimental treatments highlighted an important conclusion, that in those vessels where excessive settling of particulates occurred, a significantly greater biological response was measured (see shrimp *C. nilotica* results). The issue that many of the impacts of fine sediments on macroinvertebrates appear related to the deposition of material, and that these effects occur at lower sediment loads has been raised by Dunlop et al. (2008); Kefford et al. (2010); and Jones et al. (2012). Further evidence of this was determined during the correlation analyses of sediment load characteristics (turbidity, total suspended solids [TSS] and settled solids) with macroinvertebrate assemblage responses that were collected from a field study of catchments with contrasting sediment loads described in Chapter 5. It was overwhelmingly evident than changes in the macroinvertebrate assemblage at sites was more correlated to settled solids that to TSS or turbidity. Although many benthic macroinvertebrates and benthic-associated fish are likely to be negatively affected, either directly, or through habitat change caused from deposited solids, there are also pelagic invertebrates and fish likely to be directly affected by suspended solids (and aquatic flora indirectly affected due to changes in light penetration). In order to manage these two different states in which particulates occur in rivers, a possible approach is to develop separate exposure-response relationships for each state (Dunlop et al. 2008). For example, species sensitivity distributions (SSD) could be developed for settled solids using stress responses from benthic organisms or organisms with a benthic life stage (e.g. fish which spawn over cobble beds), and also for pelagic organisms (e.g. daphnia, some fish and organisms with a planktonic life stage). See Box 6.1 detailing future research opportunities. However, as shown by Buendia et al. (2013) and Smit et al. (2008), chemical composition and particle size do still have to be considered when dealing with the biological effects of settled solids.

A site-specific risk assessment protocol for suspended solids was developed in Chapter 5 consisting of three tiers. Tier 1 of the risk assessment was a desktop approach, aiming to use catchment or subcatchment geospatial data to determine the likelihood of negative biological consequences from excessive particulate solids input to the river (this was a low spatial resolution option). For this approach to work, two assumptions had to be met: 1) there is a link between a geospatial characteristic and a sediment load characteristic; and 2) there is a link between a particular sediment load characteristic and a macroinvertebrate assemblage response. These assumptions were tested in a field study detailed in Chapter 5 and found to be valid. Regarding assumption 1, Chapter 5 showed that during low flows (low rainfall season) the land class of 'cultivation', when close to the river (within 1-2 km), had a strong positive correlation with instream turbidity and TSS. At higher flows (during high rainfall season) the land classes of 'degradation' and 'cultivation', erosion gully measures and measures of higher population density had a strong and consistent positive correlation with instream turbidity and TSS. Settled solids concentrations were positively correlated with erosion gullies occurring within 3 km of the river site. However, as settled solids were only collected once, the lack of correlation with other geospatial characteristics might be misleading and requires further investigation. Regarding assumption 2, evidenced suggested that settled solids concentration best explained changes in macroinvertebrate assemblage composition and that the most sensitive biological responses appeared to be % Trichoptera abundance, NMDS of all macroinvertebrate taxa, NMDS of functional feeding group guilds, and the SASS metrics. Although it should be noted that unusually high rainfall in the research area over the course of the study reduced the number of macroinvertebrate samples collected to two seasons. This limited the statistical robustness of the conclusions drawn from this data. Further data would need to be collected to validate these conclusions.

The aim of Tier 2 of the risk assessment protocol was to compare sediment load characteristics measured in the field with a relevant biological effects exposure-response relationship generated from laboratory data in order to infer the potential for unacceptable biological effects. A tentative exposure-response relationship was developed for kaolin particles and TSS in Chapter 4. However, it could not be applied in Chapter 5 as the geochemical composition and size range of particulates in the rivers of the research area could not be determined. Furthermore, the exposure-response relationship was determined according to TSS and results from the field assessment in Chapter six suggested that settled solids concentration best explained changes in macroinvertebrate assemblage composition. Consequently, future research should focus on determining appropriate exposure-response relationships (see Box 6.1 – research options 1, 2 and 3).

Tier 3 involves site-specific biomonitoring of biota resident at a site in order to directly measure for unacceptable biological effects from instream particulate matter. When applied to the research site studied in this report (Chapter 5), this approach successfully used macroinvertebrates to separate sites with excessive sediment load from those with less. Results suggested that the most promising biological response variables to use in this tier were % Trichoptera abundance, NMDS of all macroinvertebrate taxa, NMDS of functional feeding group guilds, and the SASS metrics. As mentioned earlier, the limited number of macroinvertebrate samples collected in this study limited the statistical robustness of these conclusions. Future research should be undertaken, and with the benefit of more data, these conclusions can be re-examined, perhaps as part of a reapplication of the risk assessment protocol (see Box 6.1, Option 4, for details).

Box 6.1 Future research options

1) Identify the most accurate, or biologically realistic, method of measuring environmental concentrations of settled and suspended solids
    a. Settled solids are highly spatially variable over small areas (and probably temporally variable too). Suspended solids are known to be highly variable temporally. Undertaking numerous replicate measures can give an indication of the range of concentrations at a site, both spatially and temporally. What needs to be investigated is how best to compare this range of environmental concentrations to a possible water quality guideline (i.e. use the highest value, the mean, mode, or some quartile, etc.).
    b. Although the measures of suspended solids (TSS and turbidity) are fairly well standardised, the measurement of settled solids is not. Measures range from qualitative to unstandardized quantitative assessments. Issues that need to be investigated include: what approach to use (qualitative or quantitative); how to collect the quantitative sample (equipment and technique); and where (what benthic habitat) in the river to sample.

2) Characterise the particulate solids in South African rivers
    a. Very little is known regarding the particulate size ranges and geochemical composition of particulates in South Africa's rivers. These are likely to vary across the country and down the longitudinal profile of the river depending on the underlying geology of the area and hydraulics of the river system. As the development of water quality guidelines for aquatic particulates will necessarily be quite site specific, knowledge of the important components of the particulates will have to be at a high spatial resolution.
    b. A better understanding of the high temporal variability of suspended and settled solids in South African rivers would aid less site-specific assessments (such as described for Tier 1 and 2 in Chapter 5).

3) Develop separate exposure-response relationships for settled and suspended solids
    a. Identify which taxa are suitable for settled and which more applicable to the suspended solids exposure-response relationship. This relates to how their respective life cycles interact with either settled or suspended solids.
    b. Determine which particulate combination type (particle size, shape and geochemical composition) should be investigated first. Perhaps this choice should be made on what the most abundant combination in South Africa is, or what the combination in an important river is. This step requires that future research option 3 be undertaken.

4) Validate the refined risk assessment protocol described in this report
    a. The protocol could be reapplied to the same research area in order to determine if additional data alters the conclusions that led to its refinement in this report. Or the protocol could be applied to another research area, ensuring sufficient environmental sampling is undertaken.
    b. As settled solids concentration has been identified as important stressor of aquatic organisms, this measure should be included in the risk assessment protocol.

REFERENCES

Adams SM, Greeley MS and Ryon MG (2000) Evaluating effects of contaminants on fish health at multiple levels of biological organisation: Extrapolating from lower to higher levels. *Human and Ecological Risk Assessment* 6: 15-27.

ANZECC and ARMCANZ (Australian and New Zealand Environment and Conservation Council, Council and Agriculture and Resource Management Council of Australia and New Zealand). *National water quality management strategy, Australian and New Zealand Guidelines for fresh and marine water quality*. Canberra, Australia, 2000.

Armitage PD (1995) Faunal community change in response to flow manipulation. Pages 59-78 in D M Harper and AJD Ferguson (eds.). *The ecological basis for river management*. Wiley. Chichester.

Awata S, Tsuruta T, Yada T, Iguchiet K (2011) Effects of suspended sediment on cortisol levels in wild and cultured strains of ayu *Plecoglossus altivelis*. Aquaculture 314: 115-121.

Baptista DF, Buss DF, Egler M, Giovanelli A, Silveira MP, Nessimian JL (2007) A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. *Hydrobiologia* 575: 83-94.

Barbour MT, Gerritsen J, Griffith GE, Frydenborg R, McCarron E, White JS, Bastian ML (1996) A framework for biological criteria for Florida streams using benthic macroinvertebrates. *Journal of the North American Benthological Society* 15: 185-211.

Bauer J, Rohdenburg H, Bork H-R (1985) Ein Digitales Reliefmodell als Vorraussetzung fuer ein deterministisches Modell der Wasser- und Stoff-Fluesse. Landschaftsgenese und Landschaftsoekologie, H.10, Parameteraufbereitung fuer deterministische Gebiets-Wassermodelle. *Grundlagenarbeiten zu Analyse von Agrar-Oekosystemen*, (Eds.: Bork H-R, Rohdenburg H.): p1-15

Berry W, Rubinstein N, Melzian B, Hill B (2003) *The Biological Effects of Suspended and Bedded Sediment (SABS) in Aquatic Systems: A Review*. US Environment Protection Agency, National Health and Environmental Health Effects Laboratory, Rhode Island, Internal Report, 58.

Bilotta GS, Brazier RE (2008) Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research* 42: 2849-2861.

Bo T, Fenoglio S, Malacarne G, Pessino M, Sgariboldi F (2007) Effects of clogging on stream macro-invertebrates: An experimental approach. *Limnologica* 37: 186-192.

Boenigk J, Novarino G (2004) Effect of suspended clay on the feeding and growth of bacterivorous flagellates and ciliates. *Aquatic Microbial Ecology* 34: 181-192.

Bond NR, Downes BJ (2003) The independent and interactive effects of fine sediment and flow on benthic invertebrate communities characteristic of small upland streams. *Freshwater Biology* 48: 455-465.

Boubee JAT, Dean TL, West DW, Barrier RFG (1997) Avoidance of suspended sediment by the juvenile migratory stage of six New Zealand native fish species. *New Zealand Journal of Marine and Freshwater Research* 31: 61-69.

Breitburg DL (1988) Effects of turbidity on prey consumption by Striped Bass larvae. *Transactions of the American Fisheries Society* 117: 72-77.

Bremner JM, Rogers J and Willis JP (1990) Sedimentological aspects of the 1988 Orange River floods. *Transactions of the Royal Society of South Africa* 47: 247-94

Bright EA, Coleman PR, King AL, Rose AN, Urban ML (2008) *LandScan 2008*. Oak Ridge National Laboratory, Tennessee, USA.

Broekhuizen N, Parkyn S, Miller D (2001) Fine sediment effects on feeding and growth in the invertebrate grazers *Potamopyrgus antipodarum* (Gastropoda, Hydrobiidae) and Deleatidium sp (Ephemeroptera, Leptophlebiidae). *Hydrobiologia* 457: 125-132.

Brooks A (1986) Response of aquatic vegetation to sedimentation downstream from river channelization works in England and Wales. *Biological Conservation* 38: 352-367.

Brossett MP, Hughes DL, Olson JR, Gore JA (2010) Rapid bioassessment materials and methods. pp 17-34. In: *Rapid bioassessment of stream health*. Hughes DL, Brossett MP, Gore JA, Olson JR (eds.). CRC Press, Taylor and Francis Group, Boca Raton, USA.

Buendia C, Gibbins CN, Vericat D, Batalla, RJ, Douglas A (2013) Detecting the structural and functional impacts of fine sediment on stream invertebrates. *Ecological Indicators* 25: 184-196.

Caux P-Y, Moore DRJ, MacDonald D (1997) *Ambient water quality guidelines (criteria) for turbidity, suspended and benthic sediments.* Technical appendix prepared for the Ministry of Environment, Land and Parks, British Columbia, Canada.

Chapman PM (2002) Integrating toxicology and ecology: putting the “eco” into ecotoxicology. *Marine Pollution Bulletin* 44: 7-15.

Chapman PM, Caldwell RS and Chapman PF (1996) A warning: NOECs are inappropriate for regulatory use. *Environmental Toxicology and Chemistry* 15: 77-79.

Choi J, Roche H, Caquet T (2000) Effects of physical (hypoxia, hyperoxia) and chemical (potassium dichromate, fenitrothion) stress on antioxidant enzyme activities in *Chironomus riparius* Mg. (Diptera, Chironomidae) larvae: potential biomarkers. *Environmental Toxicology and Chemistry* 19: 495-500.

Clarke KR and Warwick RM (2001) *Change in marine communities: an approach to statistical analysis and interpretation*. 2nd edition. PRIMER-E, Plymouth.

Cohen G, Kim M, Ogwu V (1996). A modified catalase assay suitable for a plate reader and for the analysis of brain cell cultures. *Journal of Neuroscience Methods* 67: 53-56.

Collins AL, Naden PS, Sear DA, Jones JI, Foster IDL, Morrow K. 2011. Water quality targets for catchment sediment management: international experience and prospects. *Hydrological Processes* 25: 2112-2129.

Cuker BE (1987) Field experiment on the influences of suspended clay and P on the plankton of a small lake. *Limnology and Oceanography* 32: 840-847.

Cummins KW (1973) Trophic relations of aquatic insects. *Annual Review of Entomology* 18: 183-206.

Cummins KW (1974) Structure and function of stream ecosystems. *BioScience* 24: 631-641.

Dickens CWS, Graham PM (2002) The South African Scoring System (SASS) version 5 rapid bioassessment for rivers. *African Journal of Aquatic Science* 27: 1-10.

Dollar ESJ, Rowntree KM (1995) Hydroclimatic trends, sediment sources and geomorphic response in the bell river catchment, Eastern Cape Drakensberg, South Africa. *South African Geographical Journal* 77: 21-32.

Downes BJ, Barmuta LA, Fairweather PG, Faith DP, Keough MJ, Lake PS, Mapstone BD and Quinn GP (2002) *Monitoring ecological impacts: concepts and practice in flowing waters*. Cambridge University Press, Cambridge. 446pp.

Dunlop J, McGregor G, Horrigan N (2005) *Potential impacts of salinity and turbidity in riverine ecosystems: Characterisation of impacts and a discussion of regional target setting for riverine ecosystems in Queensland*. Aquatic Ecosystem Health Unit Water Quality and Monitoring Natural Resource Sciences, Australia.

Dunlop JE, Kefford BJ, McNeil VH, McGregor GB, Choy S, Nugegoda D (2008) A review of guideline development for suspended solids and salinity in tropical rivers of Queensland, Australia. *Australian Journal of Ecotoxicology* 14: 129-142.

DWA (Department of Water Affairs) (2008) *Methods for determining the Water Quality component of the Ecological Reserve. Department of Water Affairs, South Africa*. Second Draft. Prepared by P-A Scherman of Scherman Consulting.

DWAF (Department of Water Affairs and Forestry) (1996) *South African water quality guidelines. Volume 7: Aquatic ecosystems*. DWAF, Pretoria, South Africa.

DWAF (2000) *A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems. Version 1.* Produced by Scherman P-A and Palmer CG for DWAF, Pretoria, South Africa.

Eaton L, Sloman KA (2011) Subordinate brown trout exaggerate social signalling in turbid conditions. *Animal Behaviour* 81: 603-608

Ellis MM (1936) Erosion silt as a factor in aquatic environments. *Ecology* 17: 29-42.

Fairbanks D, Thompson M, Vink D, Newby T, Van den Berg H, Everard D (2000) The South African Land-cover Characteristics Database: a synopsis of the landscape. *South African Journal of Science* 96: 69-82

Goldes SA, Ferguson HW, Moccia RD, Daoust P-Y (1988) Histological effects of the inert suspended clay kaolin on the gills of juvenile rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* 11: 23-33

Gordon AK (2012) *Assessing the effect of a laundry detergent ingredient (LAS) on organisms of a rural South African river*. PhD thesis, Rhodes University.

Gorokhova E, Lof M, Halldorosson HP, Tjarnlund U, Lindstrom M, Elfwing T, Sundelin B (2010) Single and combined effects of hypoxia and contaminated sediments on the amphipod *Monoporeia affinis* in laboratory toxicity bioassays based on multiple biomarkers. *Aquatic Toxicology* 99: 263-274.

Grenfell SE, Ellery WN (2009) Hydrology, sediment transport dynamics and geomorphology of a variable flow river: The Mfolozi River, South Africa. *Water SA* 35: 271-282.

Grimshaw DL, Lewin J (1980) Source identification for suspended sediments. *Journal of Hydrology* 47: 151-162.

Hart RC (1980) Embryonic duration and post-embryonic growth rates of the tropical freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae) under laboratory and experimental field conditions. *Freshwater Biology* 10: 297-315.

Hart RC, Stewart BA, Bickerton IB (2001) Decapoda. In: Day JA, Stewart BA, De Moor IJ, Louw AE (ed.), *Guides to the freshwater invertebrates of Southern Africa. Volume 4: Crustacea III. Bathynellacea, Amphipoda, Isopoda, Spelaeogriphacea, Tanaidacea and Decapoda.* Water Research Commission Report No. TT 141/01, Pretoria.

Hartman WA, Martin DB (1984) Effect of suspended bentonite clay on the acute toxicity of glyphosate to *Daphnia pulex* and *Lemna minor*. *Bulletin of Environmental Contamination and Toxicology* 33:355-361.

Hearne JW, Armitage PD (1993) Implications of the annual macrophyte growth cycle on habitat in rivers. *Regulated Rivers Research and Management* 8: 313-322.

Holm-Hansen O, Riemann B (1978) Chlorophyll-a determination: Improvements in methodology. *Oikos* 30: 438-447.

Huete A, Justice C, Van Leeuwen W. (1999) *MODIS Vegetation Index (MOD 13) – Algorithm Theoretical Basis Document*. Technical Report. http://modis.gsfc.nasa.gov/data/atbd/atbd_mod13.pdf

Isono RS, Kita J, Setoguma T (1998) Acute effects of kaolinite suspension on eggs and larvae of some marine teleosts. *Comparative Biochemistry and Physiology Part C* 120: 449-455.

Jones JI, Collins AL, Naden PS, Sear DA (2011) The relationship between fine sediment and macrophytes in rivers. *River Research and Applications* 28: 1006-1018.

Jones JI, Murphy JF, Collins AL, Sear DA, Naden PS, Armitage PD (2012) The impact of fine sediment on macro-invertebrates. *River Research and Applications* 28: 1055-1071.

Kefford BJ, Zalizniak L, Dunlop JE, Nugegoda D, Choy SC (2010) How are macroinvertebrates of slow flowing lotic systems directly affected by suspended and deposited sediments? *Environmental Pollution* 158: 543-550.

Kemp P, Sear DA, Collins AL, Naden PS, Jones JI (2011) The impacts of fine sediment on riverine fish. *Hydrological processes* 25: 1800-1821.

King JM, Day JA, Hurly PR, Henshall-Howard M-P and Davies BR (1988) Macroinvertebrate communities and environment in a southern African mountain stream. *Canadian Journal of Fisheries and Aquatic Sciences* 45: 2168-2181.

Kirk KL, Gilbert JJ (1990) Suspended clay and the population dynamics of planktonic rotifers and cladocerans. *Ecology* 71: 1741-1755.

Lake RG, Hinch SG (1999) Acute effects of suspended sediment angularity on juvenile coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* 56: 862-867.

Larsen S, Ormerod SJ (2010) Low-level effects of inert sediments on temperate stream invertebrates. *Freshwater Biology* 55: 476-486.

LP DAAC (2011) NASA Land Processes Distributed Active Archive Center. ASTER Global DEM v2.

Le Roux JJ, Morgenthal TL, Malherbe J, Pretorius DJ, Sumner PD (2008) Water erosion prediction at a national scale for South Africa. *Water SA* 34: 305-314.

Le Roux JJ, Nkambule VT, Mararakanye N, Pretorius DJ (2010) *Provincial mapping of gully erosion at the field scale using high resolution satellite imagery (SPOT 5)*. Technical Report Spot 5, Department of Agriculture, Forestry and Fisheries.

Le Roux JJ, Sumner PD (2011) Factors controlling gully development: Comparing continuous and discontinuous gullies. *Land Degradation & Development* 23: 440-449.

Livingstone DR (2001) Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin* 42: 656-666.

Madikizela BR, Dye AH (2003) Community composition and distribution of macroinvertebrates in the Umzimvubu River, South Africa: a pre-impoundment study. *African Journal of Aquatic Sciences* 28: 137-149.

Matthew J (1968) 'n Ondersoek na die verspreiding van sekere Ephemeroptera (Insecta) in die Komatirivierstelsel, Oos-Transvaal. MSc Thesis, Instituut vir Dierkundige Navorsing, Departement Dierkunde, Potchefstroomse Universiteit vir C.H.O, Potchefstroom.

McFarland VA, Peddicord RK (1980) Lethality of a suspended clay to a diverse selection of marine and estuarine macrofauna. *Archives of Environmental Contamination and Toxicology* 9: 733-741.

Merritt RW and Cummins KW (eds) (1984) *An introduction to the aquatic insects of North America*. Kendall/Hunt, Dubuque, Iowa.

Newcombe CP, MacDonald DD (1991) Effects of suspended sediments on aquatic ecosystems. *North American Journal of Fisheries Management* 11: 72-82.

Newcombe CP, Jensen JOT (1996) Channel suspended sediment and fisheries: A synthesis for quantitative assessment of risk and impact. *North American Journal of Fisheries Management* 16: 693-727.

Ollis DJ, Dallas HF, Esler KJ, Boucher C (2006) Bioassessment of the ecological integrity of river ecosystems using aquatic macroinvertebrates: an overview with a focus on South Africa. *African Journal of Aquatic Science* 31: 205-227.

Palmer CG, O’Keeffe JH, Palmer AR, Dunne T and Radloff S (1993a) Macroinvertebrate functional feeding groups in the middle and lower reaches of the Buffalo River, Eastern Cape, South Africa. 1. Dietary variability. *Freshwater Biology* 29: 441-453.

Palmer CG, O’Keeffe JH and Palmer AR (1993b) Macroinvertebrate functional feeding groups in the middle and lower reaches of the Buffalo River, Eastern Cape, South Africa. 2. Functional morphology and behaviour. *Freshwater Biology* 29: 455-462.

Palmer CG, Maart B, Palmer AR and O'Keeffe JH (1996) An assessment of macroinvertebrate functional feeding groups as water quality indicators in the Buffalo River, eastern Cape Province, South Africa. *Hydrobiologia* 318: 153-164.

Parkhill KL, Gulliver JS (2002) Effect of inorganic sediment on whole-stream productivity. *Hydrobiologia* 472: 5-17.

Peeters ETHM, Brugmans BTMJ, Beijer JAJ, Franken RJM (2006) Effect of silt, water and periphyton quality on the survival and growth of the mayfly *Heptagenia sulphurea*. *Aquatic Ecology* 40: 373-380.

Peru N, Doledec S (2010) From compositional to functional biodiversity metrics in bioassessment: A case study using stream macroinvertebrates communities. *Ecological Indicators* 10: 1025-1036.

Riley SJ, DeGloria SD, Elliot R (1999). A Terrain Ruggedness Index That Quantifies Topographic Heterogeneity. *Intermountain Journal of Science* 5: 23-27.

Ringwood AH, Hoguet J, Keppler CJ, Gielazyn ML, Ward BP and Rourk AR (2003) *Cellular Biomarkers (lysosomal destabilization, glutathione & lipid peroxidation) in three common estuarine species: A methods handbook*. Marine Resources Research Institute, South Carolina Department of Natural Resources. South Carolina, USA.

Robinson SE, Capper NA, Klaine SJ (2010) The effects of continuous and pulsed exposures of suspended clay on the survival, growth, and reproduction of *Daphnia magna*. *Environmental Toxicology and Chemistry* 29: 168-175.

Ross SD, Kramer DA, Christensen BL (2001) Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. *Comparative Biochemistry and Physiology – Part C: Toxicology & Pharmacology* 130: 289-303.

Rowe DK, Dean TL (1998) Effects of turbidity on the feeding ability of the juvenile migrant stage of six New Zealand freshwater fish species. *New Zealand Journal of Marine and Freshwater Research* 32: 21-29.

Rubel F, Kottek M (2010) Observed and projected climate shifts 1901-2100 depicted by world maps of the Köppen-Geiger climate classification. *Meteorologische Zeitschrift* 19: 135-141.

SANBI (South African National Biodiversity Institute) (2009) *National Land Cover Map*. Pretoria, South Africa.

Schulze RE (2007) *South African Atlas of Climatology and Agrohydrology*. Water Research Commission, Pretoria, RSA, WRC Report 1489/1/06.

Shao Q (2000) Estimation for hazardous concentrations based on NOEC toxicity data: an alternative approach. *Environmetrics* 11: 583-595.

Simberloff D, Dayan T (1991) The guild concept and the structure of ecological communities. *Annual Review of Ecology and Systematics* 22: 115-143.

Singleton HJ (1985) *Water Quality Criteria for Particulate Matter: Technical Appendix*. Ministry of the Environment, Lands, and Parks, Victoria, B.C. pp. 1-82. [web: http://www.env.gov.bc.ca/wat/wq/BCguidelines/turbidity/turbidity.html]

Smit MGD, Holthaus KIE, Trannum HC, Neff JM, Kjeilen-Eilertsen, Jak RG, Singsaas I, Huijbregts MAJ, Hendriks AJ (2008) Species sensitivity distributions for suspended clays, sediment burial, and grain size change in the marine environment. *Environmental Toxicology and Chemistry* 27: 1006-1012.

Stickle WB, Kapper MA, Liu LL, Gnaiger E, Wang SY (1989) Metabolic adaptations of several species of crustaceans and molluscs to hypoxia: tolerance and microcalorimetric studies. The Biological Bulletin 177: 303-312.

Swietlik W. 2003. *Developing water quality criteria for suspended and bedded sediment (SABS): Potential Approaches*. U.S. EPA Science Advisory Board Consultation. US EPA Office of Water, Office of Science and Technology. [web: http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/pollutants/sediment/upload/2004_08_17_criteria_sediment_sab-discussion-paper.pdf].

Tomanova S, Goitia E and Helesic J (2006) Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. *Hydrobiologia* 556: 251-264.

UKTAG (UK Technical Advisory Group on the Water Framework Directive) (2008) *UK environmental standards and conditions*. Water Framework Directive, www.wfduk.org.

Van den Hurk P (1994) Effects of natural sediment properties on test results in bioassays with oyster larvae (*Crassostrea gigas*) on sediment elutriates. *Journal of Aquatic Ecosystem Health 3*: 185-191.

Vannote RL, Minshall GW, Cummins KW, Sedell JR and Cushing CE (1980) The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 130-137.

Van Straalen NM (2002) Threshold models for species sensitivity distributions applied to aquatic risk assessment for zinc. *Environmental Toxicology and Pharmacology* 11: 167-172.

Wagenhoff A, Townsend CR, Matthaei CD (2012) Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. *Journal of applied ecology* 49: 892-902.

Walling DE, Fang D (2003) Recent trends in the suspended sediment loads of the world's rivers. *Global and Planetary Change* 39: 111-126.

Wilber DH, Clarke DG (2001) Biological effects of suspended sediments: A review of suspended sediment impacts on Fish and Shellfish with relation to dredging activities in estuaries. *North American Journal of Fisheries Management* 21: 855-875.

WHO (World Health Organisation) (2005) *Bentonite, Kaolin and Selected Clay Minerals*. Environmental Health Criteria 231. International Programme on Chemical Safety (IPCS), World Health Organisation, Geneva.

Wolfe JE, Lind OT (2008) Influence of suspended clay on phosphorus uptake by periohyton. *Hydrobiologia* 610: 211-222.

Wood PJ, Armitage PD (1997) Biological effects of fine sediment in the lotic environment. *Environmental Management* 21: 203-217.

Wu RSS (2002) Hypoxia: from molecular responses to ecosystem responses. *Marine Pollution Bulletin* 45: 35-45.

Wu Z, Yu D, Li J, Wi G and Niu X (2010) Growth and antioxidant response in Hydrocharis dubis (BI.) Backer exposed to linear alkylbenzene sulfonate. *Ecotoxicology* 19: 761-769.

WWF-UK. 2007. Review of UKTAG proposed standard for suspended solids. APEM Scientific Report 410242. Web: [http://www.scotlink.org/files/policy/PositionPapers/JointLINKReportUKTAGSusSoild.pdf].

APPENDICES (SEE ACCOMPANYING CD)

Appendix 1: A review of the issues to be considered in the derivation of water quality guidelines.

Appendix 2: Exposure-response data available for aquatic organisms exposed to suspended kaolin particles classified according to six severity-of-effect classes.

Appendix 3: Enumerated macroinvertebrate families samples in autumn (May 2012).

Appendix 4: Enumerated macroinvertebrate families samples in summer (November 2012).

Appendix 5: Spearman rank correlation coefficients and significance levels of sediment characteristics and environmental variables.

Appendix 6: Data of the functional taxon-based metrics.

Appendix 7: The relationship between concurrently measured SASS and turbidity data archived in the South African River Health Programme's Rivers Database.

Appendix 8: Capacity building report.

Appendix 9: Enumerated macroinvertebrate species level data for autumn (May 2012). Data not analysed for this report.

Appendix 10: Enumerated macroinvertebrate species level data for summer (November 2012). Data not analysed for this report.