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Uses of benthic ecology in the assessment of anthropogenic impacts in the marine environment.

By

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A thesis submitted in partial fulfilment of the requirements of Edinburgh Napier University, for the award of Doctor of Philosophy

July 2009

NAPIER UNIVERSITY
EDINBURGH

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Abstract

The ability to detect and manage anthropogenic disturbances in the marine environment is more important than ever, given increasing pressure from a range of sources and the growing awareness of the sensitivity of some marine habitats. The main aims of this study were to ascertain if intensity and type of disturbance were important factors to consider during the assessment of these disturbances. Throughout, various techniques were used and assessed, e.g. primary, derived, multivariate and biological indices, as tools capable of indicating changes within benthic communities. A methodology of selecting appropriate indices linked to the perturbation of interest was also trialled. Finally, the behaviour of meiofauna and macrofauna towards *in situ* burial was investigated. The effects of disturbance were found to be type, as well as, site-dependent. In some cases, the intensity of disturbance was found to have non-linear effects. Site and disturbance-specific species and trophic group responses were also observed. The method used to select appropriate indices raised important questions. How can it be ensured that observed changes in indicator values are part of a cause-effect relationship? And, how do we identify / choose which of the potential impacts of the disturbance in question to use as a pressure indicator? Community-specific responses and sensitivities of meiofauna and macrofauna to the physical disturbance associated with *in-situ* burial highlight the importance of using both faunal types in the assessment of the effects of seabed disturbance in the marine environment. It is clear that no simple method exists for detecting disturbance which is applicable to all sites and situations. Hence, careful consideration, informed by ecological knowledge of sites and species, needs to be given to each case.

Table of contents

List of figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Acknowledgments

I would like to show my gratitude to Cefas for funding this study and providing the flexibility which has allowed me to further develop my scientific career. I would like to thank my supervisors Mark Huxham and Michaela Schratzberger for all their support, time and confidence that I could undertake this study. I would also like to thank a very special group of Cefas colleagues, especially Stefan Bolam who first introduced me to the pleasures of wading through waist deep mud, Hubert Rees who provided much wisdom and knowledge, Michaela again this time for revealing the wonderful world of nematodes and everybody else who has helped conceptually as well as physically in the field. Last but by no way least I would like to thank my friends and family and dedicate this thesis to Jax for her love and support, Martha for keeping me young, Mum for being Mum, Malc for being Dad and Kate for being little Sis.

Chapter one

Uses of benthic ecology in the assessment of anthropogenic impacts in the marine environment

1 General introduction

In ecological science, disturbance can be defined as “any discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability or the physical environment” (Pickett and White 1985). Infaunal benthic community structure has been predominantly used in the assessment of disturbance within the marine habitat (Gee et al., 1992). Community attributes including species diversity and abundance are the most commonly employed parameters for determining the impact of disturbance events (Schratzberger et al., 2000). Historically macro-benthic infauna (defined as organisms living within sedimentary systems that are retained on a 500μm mesh sieve) have been employed as an indicator of disturbance within soft-bottom sediments in both inshore and offshore environments (Warwick 1986). This fraction of the benthic community has been utilised as they can easily be counted and identified. They are also relatively immobile so must adapt to local conditions or perish.

Until recently, the importance of small zoobenthic organisms within the marine benthic environment has been underestimated ((Platt and Warwick 1980). Although their biomass may be insignificant compared to larger invertebrates, they have been shown to contribute disproportionately to benthic production (Platt and Warwick 1980, Kuipers et al., 1981, Raffaelli and Hawkins 1996). This complex small food web, consisting of bacteria, microfauna, meiofauna, temporary meiofauna and small macrofauna is characterised by the small size of individuals, a high turnover rate, and relatively short life spans (Kuipers et al., 1981). Kuipers et al., (1981) also showed that, in intertidal environments, these groups contribute to the production of food for juvenile stages of commercially viable carnivores such as shrimp, crab and fish. Other functions include the consolidation of sediments through the construction of mucous burrows; the mopping up of nutrients, and the breakdown of pollutants that would otherwise lead to the degradation of the coastal environment. However, the links between and within this 'small food web' are still poorly understood. Nevertheless, the value of meiofaunal nematode communities as indicators of environmental disturbance is now being realised (Moore and Bett 1989, Coull and Chandler 1992, Bongers and Ferris 1999, Kennedy and Jacoby 1999). This is due to their relatively sessile life style, ubiquitous distribution, short life cycle, intimate association with the sediment, high densities per sample and key position within the benthic food web (Coull and Chandler 1992, Bongers and Ferris 1999).

Numerous studies have been undertaken to assess the impacts of various types and intensities of disturbance on meiofaunal nematode and macrofaunal communities (Pearson and Rosenberg 1978, Heip 1980, Rees 1982, Zajac and Whitlach 1982, Hall 1994, Coull and Chandler 1992, Austen et al., 1998, Schratzberger and Warwick 1999, Huxham et al., 2000). These studies have included investigations into disturbances created by fishing (Jennings and Kaiser 1998, Lindegarth et al., 2000, Frid et al., 2000, Hansson et al., 2000, Schratzberger et al., 2002a), dredge disposal (Essink 1993, Essink and Romeyn 1994, Somerfield et al., 1995), organic enrichment (Gee et al., 1985, Moore and Pearson 1986, Schratzberger and Warwick 1998, Osterling & Pihl 2001, Mirto et al., 2002), sewage sludge disposal (Whomersley et al., 2007) and oil contamination (Gee et al., 1992, Daan et al., 1994, Schratzberger et al., 2003). To assess the extent of disturbance, infaunal meio and/or macrofauna communities are usually analysed. Results from the above studies have established that benthic community structure is affected in a number of ways within the disturbed area, and that these responses can vary depending on the type of disturbance and the type of receiving assemblage.

Fishing disturbance has several effects on the benthic community. These include injury and capture of the target species and shallowly buried infaunal bycatch (De Groot 1984, Frid et al., 2000,). The impact of the fishing gear (trawls and dredges) on the sea bed changes the habitat by increasing sedimentation and breaking up structures within the sediment such as polychaete tubes. (Tuck et al., 1998). Secondary effects include an increase in organic input created by the decaying bodies of dead and damaged organisms. Several community changes caused by fishing have been documented and are thought to depend on the duration and intensity of the disturbance (Underwood 1989). Changes include a reduction in biomass and production of macrofauna (Schratzberger et al., 2002), a reduction in abundance (Kaiser et al., 1998, Hansson et al., 2000), especially of fragile organisms such as *Echinocardium cordatum* and an increase in opportunistic species (*Capitella sp* and *Notomastus sp.*). This is thought to occur due to changes in competitive interactions and altered food availability (Frid et al., 2000). Lindegarth et al. (2000) also found increased spatial and temporal variability of the impacted benthic communities due to fishing disturbance.

The disposal of dredged material at sea primarily impacts the seabed. Many studies have examined the effects on macrofaunal communities (e.g. Rees et al., 1992;

Harvey et al., 1998). They have demonstrated that macrofauna does not appear to respond in a predictable manner to dredged material disposal. This may be due to the different types of sediments disposed and synergistic effects of components within different dredged materials. The general reported effects of dredgings disposal appear to be the burial and subsequent reduction of sensitive species, which include mucous tube and labial palp deposit feeders and an increase in opportunistic species (Rees et al., 1992, Somerfield et al., 1995). The recolonisation of an area after a disposal event is governed by the particle size and amount of sediment disposed (Hartnoll 1983), the presence of larval recruits within the water column and lateral migration from un-impacted sites. (Rees 1982).

Organic enrichment as a disturbance comes in many forms (e.g. aquaculture, fertiliser run off from agricultural land and sewage disposal etc.). Artificial inputs of organic material from the sources mentioned above are capable of raising the background levels of organic matter by up to 6 times (Eleftheriou et al., 1982). Pearson and Rosenberg (1978) showed that in an area of increased organic input the number of species would fall as the community became dominated by just a few pollution tolerant opportunistic species resulting in an increase in community abundance and biomass values. In transitional areas between impacted an un-impacted areas increased species diversity and abundances were also observed. Secondary effects of organic enrichment include increased algal mat growth (Pihl 1999). Algal mats may either cause an increase or decrease in the abundance of macrofaunal organisms depending on the extent of algal cover. Factors which may have a positive effect on the macrofaunal community are increased food availability, increased larval settlement and a reduction in predation due to the algal mat acting as a barrier to predators (Hull 1987). Factors which may have a negative effect on the macrofaunal community include reduced oxygen penetration leading to anoxia (Bolam and Fernandes 2002), a build up of toxic ammonium ions due to reduced flushing and an increase in silt deposition (Hull 1987). Changes in functional feeding groups have also been demonstrated. Osterling and Pihl (2001) showed that the abundance of suspension feeders and surface detritivores was reduced during the cover of algal mats.

Offshore oil installations have been extensively studied using macrozoobenthos as indicators of disturbance (Addy et al., 1984, Kingston 1992, Daan et al., 1994). Effects on the macrofaunal community have been shown to vary depending on the kind of lubricant used in the drilling process. Where oil based muds have been used numbers of locally abundant species decreased by up to 50% while in areas where water based lubricants had been used no real effects were observed (Daan et al., 1994). Studies based around the Beryl Alpha and Ekofisk platforms in the North Sea showed that the main coloniser of drill cuttings piles was the opportunistic polychaete species *Capitella capitata.* This study also showed that recolonisation of the cuttings pile began within 1-2 years after drilling had ceased (Westerlund et al., 2001).

Pollution studies utilising meiofauna (predominantly nematodes) as indicators have yielded varying results (Coull and Chandler 1992). Nematodes are thought to be resistant to physical disturbances such as fishing because they are more likely to be resuspended into the water column rather than be damaged or killed by the fishing gear (Schratzberger et al., 2002). Studies that have examined the effects of dredgings disposal on nematode communities have demonstrated that, in general, there is a reduction in diversity (Moore and Pearson 1986). The increased presence of non-selective deposit feeders *Sabatieria pulchra* grp and *Daptonema tenuispiculum* has also been reported as an effect of dredgings disposal on nematode communities (Somerfield et al., 1995). In additional studies Schratzberger et al., (2000a,b) examined the effect on nematode communities of simulated deposition of dredged material and the role of burial and contamination. It was found that newly deposited sediment was colonised by the upward migration of nematodes. Contaminated sediment was shown to have species specific effects depending on the kind of contamination and frequency of deposition. Studies investigating oil pollution (Moore et al., 1987, Danovaro et al., 1995, Ansari and Ingole 2002) have revealed that there is a reduction in abundances, species diversity and richness within the meiofaunal nematode community. Schratzberger et al., (2003) found that the strongest indicator (a measure, index or model used to estimate the current state and future trends in physical, chemical, biological, or socio-economic conditions of the environment (Fisher 2001, Rees et al., In Press) of change caused by oil pollution within a nematode community was species evenness. This was due to a decrease in the dominant species and loss of rare low abundant species. Nematode communities have also been shown to recover within weeks of the initial disturbance (McGuinness 1990). Studies of nematode communities underneath fish farms showed that densities of nematodes and species diversities were reduced when compared with reference conditions (Mazzola et al., 2000, Mirto et al., 2002).

The ability to assess the true effects of a known disturbance is vital to environmental managers and scientists alike. A method of assessing these impacts is the Before After Control Impact (BACI) design (Green 1979). This design observes interactions between two sites, an impacted and an un-impacted control / reference site, which can then be attributed to the disturbance being studied. However, in the original design, the ability to be certain that the interactions observed were not due to natural community variability at just one of the sites was difficult to discount. Later modification e.g. increased spatial and temporal replication of control / reference sites (Underwood 1992, 1994) further increased the designs capability in identifying true interactions and therefore the effects of human activities. Therefore there is a need for robust survey designs (Underwood 1994). However, the assessment of historic disturbances such as sewage sludge and dredge material disposal may result in the use of a control impact design due to data not being available before the activity began. The use of several control / reference sites may also not be practical due to finite resources and time.

A plethora of benthic indices have been developed to assess the effects of anthropogenic disturbance on the benthic environment (ICES 2008). These include primary and derived univariate indices, number of individuals (N) species number (S), species richness (Margalef, d), species diversity (Shannon-Wiener H') and taxonomic distinctness and diversity (Clarke and Warwick 2001). Multivariate techniques include multi-dimensional scaling (MDS), similarity percentage (SIMPER) and the RELATE procedure (Clarke and Gorley 2006). In addition, there are biological metrics which take into account the pollution tolerances and ecological strategies displayed by individual benthic faunal species e.g. the Azti Marine Biological Index (AMBI) (Borja et. al., 2003) and the Infaunal Trophic Index (ITI) (Word 1979, Maurer et al*.,* 1999). Multimetrics such as the Benthic Quality Index (BQI) (Rosenberg et al., 1994) and the biotic index, BENTIX (Simboura and Zenetos 2002), which are a combination of several discrete indices, have also been employed in an attempt to describe complex ecosystems. (Dauvin et al., 2007) (For individual formula see table 1.1.)

A renewed impetus for the further development and derivation of new indices has arisen from the global initiatives on sustainable development (UN WSS 2002), climate change (IPCC 2007) and regulatory frameworks such as the Water Framework and Marine Strategy Directives (Rees In Press). The main aim of indices are to summarize environmental quality to a number which can then be used within ecosystem based management structures. (Borja et al., 2007).

Table 1.1 Indices and associated formula

Indices	*Formula*
Number of species (S)	
Number of individuals (N)	
Peliou Evenness	$J = H/\ln S.$ Where J = Evenness Index, H = diversity index (see below), and S number of species. J will be minimum (=0) if all individuals are of one species, and maximum (= 1) if the number of species equals the number of individuals.
Margelef species Richness	$R = (S-1)/\ln(N).$ Where R = Species Richness, S = number of species, and N= total number of individuals.
Shannon-Wiener Diversity (H')	$H' = -\Sigma_i\, p_i \log(p_i)$ Where p_i is the proportion of the total count arising from the *i*th species.
Average Taxonomic Diversity (Δ)	$\Delta = [\Sigma\Sigma_{i<j}\, \omega_{ij} x_i x_j] / [N(N-1)/2]$ Where the double summation is over all pairs of species *i* and *j* (*i,j* =1, 2, …, *S*; (*i*<*j*), and $N=\Sigma_i\, x_i$, the total number of individuals in the sample.
Average Taxonomic Distinctness (Δ*)	$\Delta^* = [\Sigma\Sigma_{i<j}\, \omega_{ij} x_i x_j] / [\Sigma\Sigma_{i<j}\, x_i x_j]$
Infaunal Trophic Index (ITI)	$ITI=100-\left[33\frac{1}{3}\left(\frac{0n_1+1n_2+2n_3+3n_4}{n_1+n_2+n_3+n_4}\right)\right]$ Where n_1-n_4 are the number of individuals in Feeding Groups 1-4 ITI = <30 (Degraded) 30-60 (Changed) >60 (Normal)
AZTI Marine Biotic Index (AMBI)	$BC=\{(0 \text{ x } \%GI)+(1.5 \text{ x } \%GII)$ $(3 \text{ x } \%GIII)+(4.5 \text{ x } \%GIV)$ $(6 \text{ x } \%GV)\}/100$ 0.0<BC≤0.2=Unpolluted, 1.2<BC≤3.3=Slightly Polluted 3.3<BC≤5.0=Meanly Polluted, 5.0<6.0=Heavily Polluted >6.0=Azoic
BENTIX	BENTIX = {6x%G1 + 2 x (% G2 +% G3)} /100 G1 = Sensitive, G2 = Tolerant, G3 = 1st order oppurtunistic
BQI Benthic Quality Index	$BQI = (\sum_{i=1}^{n} Ai/totA\ x.ES50_{\ 0.05i}))x^{10}Log(S+1)$ A = Abundance, ES50 = Sensitivity, S = Species richness (per sample)

The need for advice relating to human impacts in the marine environment has led to an increased need for indices capable of detecting the impacts in question. However, there are many problems associated with the detection of these impacts (Underwood 1994). These problems include; identifying what type of change will occur within the biological community, hence which indices to use; where the change will occur in relation to the impact being studied; and the inherent natural spatio-temporal variability of biological communities and the physical environment in which they exist. (Underwood 1994, Smith 2002). Selecting the right indicator can also be difficult due to the variety of disturbances types and ecosystem characteristics that must be assessed. Indictor frameworks such as the Driving Force/Pressure/State/Impact/Response (DPSIR) model can aid this process by focussing where the indicator is needed within the framework (Rees et al., In Press) as follows:

D ***Driving forces*** are underlying factors influencing a variety of relevant variables. Example: the need to dredge channels to allow ports to receive large cargo vessels.

P ***Pressure*** indicators describe the variables which directly cause (or may cause) environmental problems. Example: the disposal of dredged material at sea.

S ***State*** indicators show the current condition of the environment. Example: the diversity of the benthic community at the dredge disposal site.

I ***Impact*** indicators describe the ultimate effects of changes of state. Example: the loss of diversity due to the disposal of the dredged material.

R ***Response*** indicators demonstrate the efforts of environmental managers (i.e. decision-makers) to solve the problems. Example: reduce the amounts of disposed material.

In addition to the indices previously discussed, a number of conceptual models have also been developed to describe and predict the effects of disturbance on macrofaunal community structure and dynamics. Two of the best-supported theories are the organic enrichment ‘Successional Model’ (Pearson and Rosenberg 1978) and the ‘Intermediate Disturbance Hypothesis’ (Grime 1973, Connell 1978). The successional model describes a reversible continuum of faunal change from an un-perturbed species-rich community to a perturbed species-poor one. The intermediate disturbance hypothesis describes a predictable unimodal relationship between the intensity and frequency of the disturbance and the species richness of a perturbed community. Underlying these models, biological mechanisms thought to drive community change include competition, facilitation, inhibition, tolerance and random colonisation (Whitlach 1980, Hall et al., 1994). The applicability of these models to particular communities depends in part on their history of disturbance (Dernie et al., 2003, Wurff et al., 2007). The ‘normal’ disturbance regime (whether natural, anthropogenic or both) that prevails at a site will help determine how that community responds to novel disturbance by establishing the ‘starting point’ for the models considered above. In addition, a history of disturbance may enhance community resistance (defined as the ability to withstand further disturbance without significant effect; Loreau 2000, Bengtsson 2002, Bolam and Rees 2003). The diversity of a community can also be considered an indication of the physical conditions that a community has developed in. A community that has developed within an ecosystem with a high degree of constancy in physical parameters would contain a greater number of species when compared with an ecosystem with varying unpredictable physical parameters. (Sanders 1968, Thistle 1983, Bolam and Rees 2003). The order that species are lost from a community e.g. community disassembly and their functional roles needs also to be considered when attempting to describe and predict effects of disturbance (Petchy et al., 2004).

Meiofaunal and macrofauna form vital links within food webs of marine and estuarine environments. The community structure and function of these assemblages are therefore likely indicators of the amount and extent of damage caused by man-made impacts within these ecosystems (Gee et al., 1992). At present, the study of benthic assemblages involves the identification of organisms to species level. This process is not only time-consuming but involves a high level of expertise. The procedure is made more difficult when dealing with meiofauna due to the perceived difficulty of meiofaunal identification. However, this problem has been eased over the past 20 years with the publication of taxonomic keys which provide a useful tool to aid the taxonomic identification. Much natural history information now exists for both meiofaunal nematode and macrofaunal communities that permits the allocation of functional and biological traits based on morphological, physiological, behavioural and trophic criteria (Weiser 1953, Fauchald and Jumars 1979, Moore and Bett 1989, Bongers 1990, Diaz 1990, Roth 1998, Bremner et al., 2003, Davic 2003, Schratzberger et al., 2007). These methods complement traditional measures of diversity, can simplify complex food webs, and allow the comparison of similar communities that contain different species but the same functional groups (Gaston et al., 1998). Such comparisons may lead to a better understanding of community dynamics, therefore allowing the effects of disturbance to be examined at a functional level.

2 Aims

In the proceeding chapters two macrofaunal community datasets spanning thirteen years and eight years respectively were used in the assessment of the ‘real world’ anthropogenic impacts of sewage sludge disposal and dredgings disposal. To further investigate the associated impacts of these and similar disturbances at varying intensity in a more controlled environment a long-term (one year) field experiment was carried out at two locations within the UK. The two locations provided very similar habitats (intertidal mudflat) and species composition, providing the opportunity to assess common model assumptions on the behaviour of benthic communities during disturbance events. The utility of meiofaunal nematode and macrofaunal communities as indicators of disturbance were also investigated.

The main aims of this study are to ascertain whether intensity and type of disturbance are important factors to consider during the assessment of the effects of anthropogenic perturbations in the marine environment. Throughout, various techniques are used and assessed e.g. primary, derived, multivariate and biological indices as tools capable of indicating changes within benthic communities. A methodology of selecting appropriate indices which are linked to the perturbation of interest will also be suggested and trialled. Finally, the behaviour of meiofauna and macrofauna towards *in situ* burial will be investigated and an assessment made on the merits of the two faunal types as indicator communities.

3. Outline and contribution to proceeding analytical chapters

3.1 Chapter 2.

The use of time-series data in the assessment of macrobenthic community change after the cessation of sewage-sludge disposal in Liverpool Bay (UK)

Published in: Marine Pollution bulletin (2007) 54 32-41

P.Whomersley, M. Schratzberger, M. Huxham, H. Bates, H. Rees

Time-series data were used in the evaluation of the near-field effects of the disposal of sewage sludge on the resident macrobenthic biota and their habitat. This data also provided an opportunity to examine community responses after the cessation of disposal. The data thus provide an opportunity to follow potential community recovery, in large-scale disturbance experiment. The null-hypotheses addressed in this study were:

- Macrobenthic communities at the reference and disposal-site stations do not differ in terms of univariate (density, species richness) and multivariate (community structure) attributes.
- The cessation of sewage-sludge disposal has no effect on macrobenthic communities, or the abundance of indicator species at the disposal site.

As lead author my contribution to this work included sampling of the benthic fauna post 2001. The retrieval and composition of the time-series data set and total responsibility for carrying out all biological analyses and the direction of the study

3.2 Chapter 3.

Biological indicators of disturbance at a dredged material disposal site in Liverpool Bay, UK: an assessment using time-series data

Published in: ICES Journal of Marine Science (2008) 65(8) 1414-1420.

P.Whomersley, S.Ware, H. Rees, C. Mason, T. Bolam, M. Huxham, H. Bates

Many selection criteria have already been suggested and used to evaluate metric performance in varying circumstances. In the current study, criteria selected were representative of those The aim of this study was to apply these recommendations in the evaluation of a suite of potential metrics using a large data set derived from the monitoring of a major dredge disposal site. The data used came from annual (1996-2003) macro-invertebrate infaunal surveys of Liverpool Bay dredged disposal site.

The main questions that were addressed are:

- What measurable environmental impacts associated with dredged material disposal can be linked with/correlated to a faunal response?
- Should an individual metric or a suite of metrics be employed when assessing dredged material disposal in Liverpool Bay?
- What are the wider lessons for indicator application?

As lead author my contribution to this work included sampling of the benthic fauna post 2001. The retrieval and composition of the time-series data-set and total responsibility for carrying out all biological analyses and the direction of the study. Chemical and sediment data were provided by Thi Bolam, and C.Mason respectively.

3.3 Chapter 4.

Response of intertidal macrofauna to multiple disturbance types and intensities. An experimental approach

In press: Marine Environmental Research

DOI information:10.1016/j.marenvres.200912.001

P.Whomersley, M. Huxham, S. Bolam, M. Schratzberger, J. Augley, D. Ridland

The present work describes an experimental test of the effects of different types and intensities of disturbance on infaunal intertidal communities at two different sites with similar suites of species. It tests three null hypotheses, as described below.

- There are no differences in the responses of the same species and trophic groups to the same disturbances at two different but comparable sites. That is, there are no interactions between treatment and site factors for species and trophic group level responses.
- There are no differences in community responses to the same disturbances at two different but comparable sites. That is, there are no interactions between treatment and site factors for community level responses.
- Different types of disturbance produce qualitatively similar community responses, which can be ranked on a single continuum of intensity. Hence there are no community changes that are larger under low, compared with high, intensities of a disturbance

As lead author of this study I was responsible for the experimental design and sampling of the experiment at site one (Creeksea), identification of all biological samples from site one, analysis of all biological data and direction of the study. D. Ridland was responsible for the identification of macrofauna sampled from site two

(Blackness). S, Bolam and J. Augely provided invaluable help during the sampling of both experimental sites.

3.4 Chapter 5.

Differential response of nematode and macrofauna to *in-situ* burial

Published in: The Journal of the Marine Biological Association of the UK (2009) 89(6) 1091-1098.

P. Whomersley, M. Huxham, M. Schratzberger, S. Bolam

The main aims of this chapter are to compare and contrast the effects that two intensities of *in-situ* burial have on the meiofaunal nematode and macrofaunal communities of an intertidal mudflat.

The following null hypotheses were addressed.

- Meiofaunal nematode and macrofaunal communities show the same pattern of response to disturbance by *in-situ* burial
- There are no differences in the effects of different intensities of *in-situ* burial on meiofaunal nematode and macrofaunal communities.

As lead author of this study I was responsible for the experimental design and sampling of the experiment, processing and identification of all nematode samples, the analysis of all biological data and direction of the study. M. Schratzberger provided guidance during the identification of nematodes to species level and S. Bolam provided invaluable help during the sampling of the experimental site.

Chapter two

The use of time-series data in the assessment of macrobenthic community change after the cessation of sewage-sludge disposal in Liverpool Bay (UK)

Published in: Marine Pollution Bulletin (2007) 54 32-41

P.Whomersley, M. Schratzberger, M. Huxham, H. Bates, H. Rees

1. Introduction

Until 1998, sewage-sludge disposal at sea took place at 13 sites around the UK coast (Jones et al., 1997). Sites, which received significant amounts of sewage-sludge, include Garroch Head in the Firth of Clyde (West coast of Scotland), the Lothian disposal grounds (East coast of Scotland), the Tyne and Thames disposal grounds (East coast of England), and the Liverpool Bay disposal ground (Northwest coast of England) (Rees et al., 1990, Rees, E.I.S 1993).

Liverpool Bay has been used as a site for sewage-sludge disposal for over 100 years and has been studied for at least the past 40 years (e.g. Best 1972, Norton et al., 1982, 1984, Rees and Walker 1984, 1991, Rees et al., 990, 1992b, Rees, E.I.S. 1991, 1993, Rowlatt et al., 1991, Leah et al., 1993, Rowlatt and Ridgeway 1997, Widdows et al., 2002). The annual amounts of sludge disposed of at the Liverpool Bay disposal site increased from 0.5 million tonnes per annum in 1900 to around 2 million tonnes per annum in 1995 (Data from disposal returns). Sewage sludge disposed of post 1980 was predominantly anaerobically digested primary and secondary sludge originating from Manchester, Salford, Warrington and Runcorn (Norton et al., 1984). Other anthropogenic inputs into this area include the disposal of dredged material, agricultural run-off and discharges from rivers, estuaries and coastal outflows (Taylor and Parker 1993). The disposal site dimensions were changed in 1994 due to the construction and position of an oil platform, increasing the size of the site by 0.8 square nautical miles. The Bay also provides important services, such as commercial fisheries for fish and shellfish including sole, cod and whiting, and acts as a spawning and nursery ground for both sole and plaice. Other uses include recreation and navigation to and from the port of Liverpool (Norton et al., 1984, Taylor and Parker 1993).

Statutory control of sewage-sludge disposal at sea in the UK dates back to 1974 with the passing of the Dumping at Sea Act (DASA). This was replaced in 1985 by the passing of the Food and Environment Protection Act (FEPA). At the same time, two groups of experts representing various regulatory agencies were established to provide a national oversight of associated monitoring activities: the Marine Pollution Monitoring Management Group (MPMMG, now the Marine Environment Monitoring Group, MEMG) and the Coordinating Group on the Monitoring of Sewage-Sludge Disposal (CGMSD, now the Group Co-ordinating Seabed Disturbance Monitoring, GCSDM). During the period of disposal in Liverpool Bay, large-scale grid surveys were carried out to assess the impacts of sewage sludge on the receiving environment (e.g. Rowlatt et al., 1991, Norton et al., 1984, Rees and Walker 1984). After the cessation of this activity in 1998, a limited amount of follow up surveys continued in order to monitor any long-term changes at the disposal site.

The present dataset was produced from an independent 'check monitoring' programme carried out by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) on behalf of the regulator, the Department for Environment, Food and Rural Affairs (Defra) (Rowlatt et al., 1991). Its principal purpose was to provide an annual quality check against the outcome of more extensive monitoring by the licensee (North West Water plc). The macrofauna and sediments for later determination of a suite of environmental variables were collected at stations adjacent to and distant from the disposal site over 13 years, spanning a pre- (1990-1998) and post- (1999-2003) cessation period. Time-series data sets of this length are relatively rare (Wolfe et al., 1987, Hawkins et al., 2002, Hardman-Mountford et al., 2006) and not only allow an evaluation of the near-field effects of the disposal of sewage sludge on the resident macrobenthic biota and their habitat, but also provide an opportunity to examine community responses after the cessation of disposal. The data thus provide an opportunity to follow potential community recovery, in a 'real world', large-scale disturbance experiment. The null-hypotheses addressed in this study were:

- Macrobenthic communities at the reference and disposal-site stations do not differ in terms of univariate (density, species richness) and multivariate (community structure) attributes.
- The cessation of sewage-sludge disposal has no effect on macrobenthic communities, or the abundance of indicator species at the disposal site.

2. Material and methods

2.1. Collection and processing of macrofauna samples

Macrofauna and sediment sub-samples for determination of particle size and organic carbon/nitrogen content were collected once in September per year between 1990 and 2003 (excluding 1995) from one station near to the eastern edge of the disposal ground (M10: 53° 26.4 N, 3° 49.4 W) and one distant station to the west (M10X: 53° 31.3 N 3° 31.2 W) (Figure 2.1). At each sampling time three or four samples were taken at each site using a 0.1m^2 Day grab. Both stations corresponded with locations on a larger grid sampled annually on behalf of the licensee (Rees and Walker 1984). Surface- and bottom-water residual flows within Liverpool Bay are predominantly in a landward direction. (Ramster and Hill 1973), thereby dispersing sewage sludge disposed of within the licensed area in an easterly direction over M10. This direction of flow also limits the scope for any impact at the reference station M10X. All macrofauna samples were washed over a 1000 µm mesh sieve and the retained fauna preserved in 3 % buffered formaldehyde solution. All specimens were identified where possible' to species level.

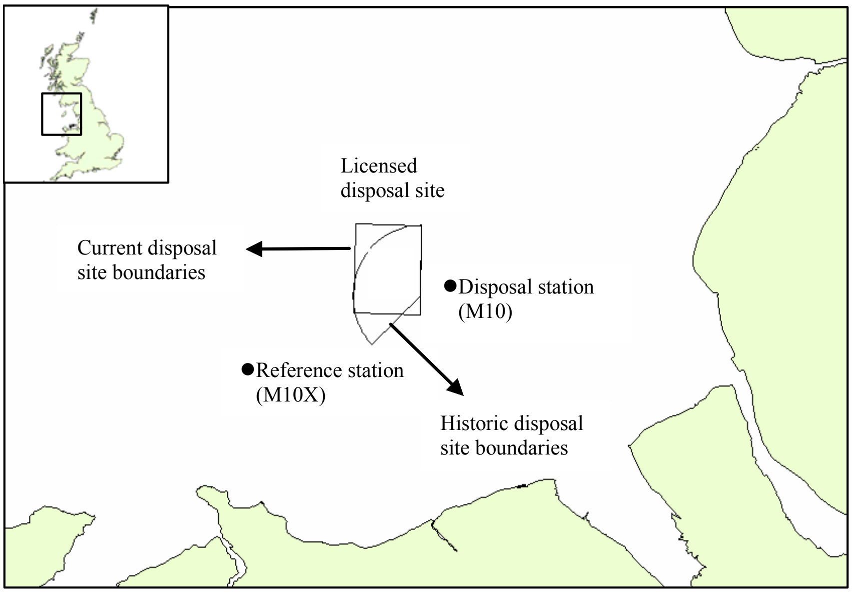


Figure 2.1. Location of Liverpool Bay sewage-sludge disposal site and position of sampling stations.

2.2. Collection and processing of sediment samples

Sediment sub-samples were collected using a 3cm diameter syringe core to a depth of 5cm from each Day grab and then frozen. Before processing each sample was allowed to defrost for several hours. Samples were wet-sieved at 63µm. The sediment fraction

>63μm was oven dried at ~90°C for 24 hours and then sieved for 10 minutes on a sieve shaker on a stack of Endecotts^{tm} stainless steel test sieves (63mm- 63μm at $^{1}/_{2}$ Phi intervals). The <63μm sediment fraction was frozen and then freeze-dried using an Edwards super modulyo freeze drier^{tm}. A sub-sample of the <63μm freeze dried fraction was analysed on a Malvern Mastersizer 2000 laser diffraction analyser^{tm}. Both dry-sieve and laser diffraction results were combined to give the full particle size distribution. The sorting coefficient, median particle size and % silt/clay content were calculated from these results. A further sub-sample from each sampling date was processed as above and organic carbon and nitrogen content determined using an elemental analyser (Leeman CE440 Analyser^{tm}).

2.3. Univariate data analyses

The total number of individuals and species were counted for each sample. Aggregation files were compiled for each site to facilitate the analysis of the benthic macrofauna using indices of taxonomic diversity (the average taxonomic distance apart of every pair of individuals in the sample) and taxonomic distinctness (the expected taxonomic distance apart of any two individuals, belonging to different species, chosen at random from the sample) described by Warwick and Clarke (1994). Pielou's evenness, $J = H / \text{Log}(S)$ was calculated to examine the variability in number of individuals per species between sites (Pielou 1966)

Homogeneity of variance was determined using Bartlett's and Cochran's tests. Two-way Analysis of Variance (ANOVA) was used to assess the significance of the factors 'site' (i.e. reference and disposal site stations averaged over time) and 'disposal group vs. cessation group' (i.e. samples collected during the time of sewage-sludge disposal (1990-1998) vs. samples collected after the cessation of sewage-sludge disposal

(1999-2003) on (a) univariate community attributes abundance (N) and species number (S) and (b) abundances of dominant species. The latter included those identified from the literature (Rees, E.I.S and Walker 1984, 1991) as possible indicators of disturbance (e.g., *Lagis koreni* and *Ampharete lindstroemi*). Samples taken at the same station in the same year were random factors nested within the fixed factors ‘site’ and ‘disposal vs. cessation’. This analysis is similar to a classic BACI (Before After Control Impact) design (Underwood, 1994), although the current study reverses the normal sequence of pristine to impacted, by looking for recovery after impact. In common with the usual BACI approach, this analysis focuses on interactions between the two main factors as a way of detecting the effects of a ‘press’ disturbance (Underwood, 1994).

The relationships between environmental variables and univariate indices were assessed using correlation analysis. All univariate analyses were performed using MINITAB version 13.0 (Minitab Statistical Software 2000).

2.4. Multivariate data analyses

To complement interpretations of the data based on univariate measures, a suite of multivariate techniques was applied to double square-root transformed species abundance data which reduces the contribution to resultsof dominant species. All multivariate analyses were performed using PRIMER version 6.0 (Clarke and Warwick 1994). Non-metric Multi-Dimensional Scaling (MDS) ordinations derived from Bray-Curtis similarity matrices, were carried out to assess differences in the structure of macrofauna communities. Two-way crossed Analysis of Similarities (ANOSIM) was carried out to assess the significance of the factors ‘site’ and ‘disposal vs. cessation’ on macrofauna community structure. In addition to the two-way analysis, one-way ANOSIMs were conducted as exploratory analyses. These were performed to allow comparison of the magnitude of differences between ‘disposal vs. cessation’ at the two stations. The similarity percentages (SIMPER) procedure was utilised to identify the main species contributing to the observed patterns. In order to assess whether the cessation of sewage sludge had a significant effect on macrofauna communities, Spearman rank correlations were calculated between similarity matrices derived from the reference samples and those derived from the disposal-site samples. This allowed the investigation of directional changes (ie sereation) in macrofauna communities over time at both stations, with a significant correlation indicating comparable temporal trends at the reference and disposal-site station. The relationships between macrofauna community structure and environmental variables were assessed by maximising the Spearman rank correlation between environmental and biotic similarity matrices. This resulted in the identification of environmental parameters that best explained macrofaunal community patterns.

3. Results

3.1. Relationship between environmental variables and macrofauna communities

Mean values for sediment characteristics at both stations between 1990 and 2003 are listed in Table 2.1. Sediments at the reference and disposal-site station differed little in terms of median particle diameter and sorting coefficient. Though significant differences in % silt/clay content were observed. (Table 2.2).

Table 2.1. Means (± S.E) for environmental variables at the reference and disposal site stations between 1994 and 2003.

Year	% Silt/Clay				Sorting Coefficient				Median Particle size (Phi)				% Organic carbon	% Nitrogen
	Disp	SE	Ref	SE	Disp	SE	Ref	SE	Disp	SE	Ref	SE	Disp	Disp
1994	2.83	1.30	0.32	0.56	1.52	0.24	0.9	0.35	0.85	0.10	1.46	0.14	3.04	0.35
1996	2.32	1.24	0.98	0.74	1.62	0.44	0.9	0.16	1.04	0.08	1.57	0.21	2.78	0.29
1997	6.10	2.04	1.16	0.74	1.78	0.19	1.36	0.15	0.99	0.11	1.64	0.08	2.63	0.29
1998	6.45	6.00	0.43	0.24	1.57	0.69	1.13	0.49	1.11	0.21	1.46	0.29	2.83	0.33
1999	3.35	2.31	0.58	0.34	1.42	0.41	0.86	0.37	1.01	0.06	1.44	0.25	2.38	0.28
2000	6.85	1.73	1.50	1.50	2.01	0.05	1.08	0.28	1.02	0.08	1.28	0.29	2.26	0.23
2001	4.41	2.43	##	##	1.63	0.32	##	##	1.02	0.11	##	##	2.06	0.25
2002	23.0	2.20	0.42	0.09	2.17	0.80	0.87	0.80	2.47	1.20	1.16	0.27	2.18	0.26
2003	1.62	0.81	0.40	0.21	1.03	0.16	0.93	0.20	1.14	0.04	1.45	0.11	1.92	0.23

No data available Disp = Disposal station Ref = Reference station

Table 2.2. Results from the nested two-way analysis of variance (ANOVA) of environmental data.

Factor	Median (Phi)		% Silt / Clay		Sorting coefficient	
	F	p	F	p	F	p
Site	1.92	0.18	4.7	0.04	2.51	0.12
Disposal vs. Cessation	0.01	0.91	0.77	0.39	0.22	0.64
Site * Disposal vs. Cessation	2.87	0.10	0.36	0.56	4.92	0.03
Year (Site Disposal vs. Cessation)	0.81	0.67	0.96	0.51	2.32	0.02

Significant interaction (P = 0.03) between site and disposal vs. cessation for sorting coefficient indicated that the stations behaved differently over time. Differences in median particle diameter and sorting coefficient at both sites were also found to be non-significant when disposal and post-disposal years were compared. Analysis of the % silt/clay content from both sites showed no significant differences over time and between disposal and post-disposal years. There was, however, a significant difference between stations. Further analyses using Tukey's multi-comparison tests revealed significant differences between stations for 1994 (p = 0.037) and 1997 (p = 0.017). None of the post-disposal years were found to be significantly different. Several significant correlations between environmental variables and univariate community attributes were revealed for the reference site: total abundance was positively correlated with % silt/clay content (r = 0.797, p = 0.018) and sorting coefficient (r = 0.790, p = 0.001), while species number was related to sorting coefficient (r = 0.678 p = 0.045). In contrast, no significant relationship with % silt/clay content emerged at the disposal site.

The small amounts of the silt/clay fraction in samples found at the reference station were insufficient for analyses of % organic carbon and nitrogen content, and therefore data were only available for the disposal site. Both showed a reduction over time. However, C:N ratios remained between 8-10:1 which is within the normal range for marine sediments (Degens and Mopper 1976). There were significant correlations between % organic carbon content and species number (r = 0.9, p = 0.006), abundance (r = 0.876, p = 0.010) and evenness (r = -0.876, p = 0.020). and, similarly, between % nitrogen and species number (r = 0.952 p = 0.001) and abundance (r = 0.857 p = 0.014).

Multivariate analysis of the data from the disposal-site station using the BIOENV procedure (Clarke and Warwick 1994) showed that a combination of % organic carbon and sorting coefficient best explained macrofauna community structure (R = 0.789).

3.2. Macrobenthic infauna

A total of 91 benthic samples were processed and analysed, resulting in the identification of over 400 macrofaunal species. Abundance and number of species were significantly higher at the disposal station compared with the reference station (Figure 2.2a and b and Table 2.3). In contrast, changes over time and interaction terms were not significant at $p < 0.05$. No significant differences between stations were observed when the “disposal group” (1990-1998) and the “cessation group” (1999-2003) were compared (Table 2.3). However evidence for a proportionately greater reduction in densities and a rather more even spread of individuals among taxa post-cessation was observed at the disposal-site station (Figure 2.3).

Table 2.3. Results from the nested two-way analysis of variance (ANOVA) of univariate indices.

Factor	Log N		S		Δ*	
	F	p	F	p	F	p
Site	183.50	<0.01	264.06	<0.01	3.26	0.08
Disposal vs. Cessation	1.41	0.25	2.93	0.10	0.49	0.49
Site * Disposal vs. Cessation	0.83	0.37	2.31	0.14	0.02	0.88
Year (Site Disposal vs. Cessation)	1.26	0.24	1.24	0.24	1.63	0.07

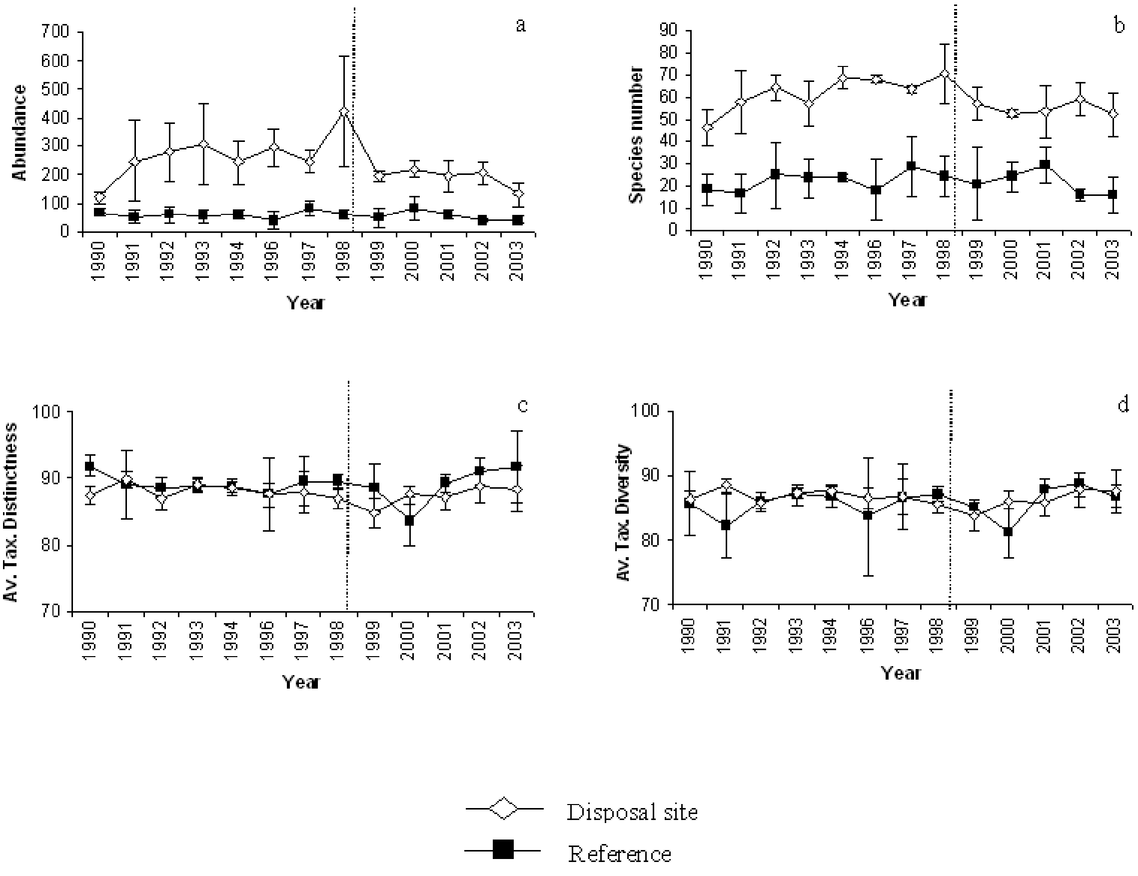


Figure 2.2. a to d. Mean (± S.E) abundance, species number, taxonomic distinctness and taxonomic diversity of macrofauna assemblages collected at the reference and disposal site stations. (Hashed line indicates cessation of sewage sludge disposal at sea.)

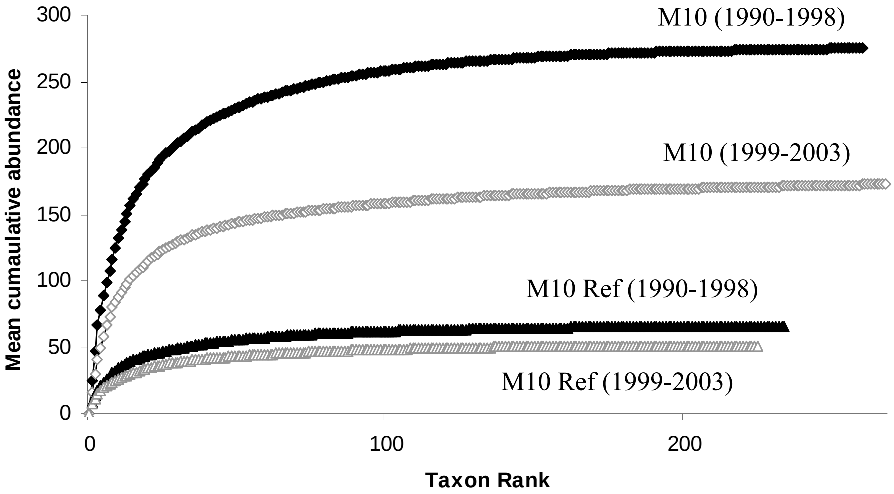
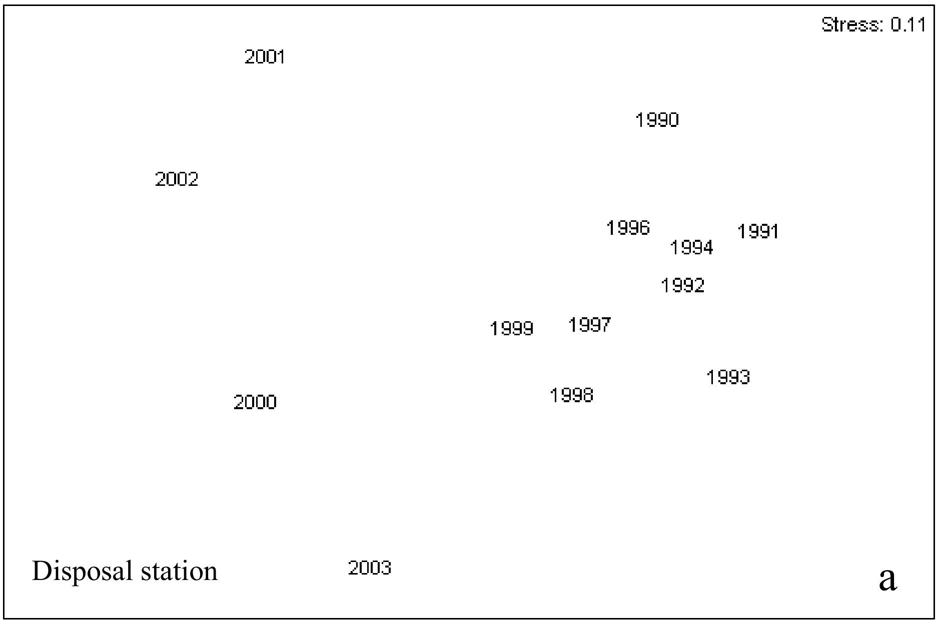


Figure 2.3. Mean cumulative species abundance at the disposal and reference station pre- (1990-1998) and post- (1999-2003) cessation of sewage disposal.

Measures of taxonomic diversity (Δ) and taxonomic distinctness (Δ*) gave similar values for the disposal and reference site stations with 82-88 and 85-92 respectively (Figure 2.2c and d). Differences in taxonomic distinctness were not significant at $p<0.05$ and there was no evidence of a significant difference over time or between “disposal group” and “cessation group” (Table 2.3). Formal significance testing of the taxonomic diversity index was precluded by the non-normal distribution of the data.

Notable changes were observed in community structure over the 13-year time series (Figure 2.4a-b). A clear difference was observed between the disposal and reference station in terms of temporal development of macrobenthic communities. The disposal site station appeared less variable during disposal years (1990-1998), and became more variable post-disposal (2000-2003, Figure 2.4a). This is in contrast to the reference station, where variability remained similar over time (Figure 2.4b).



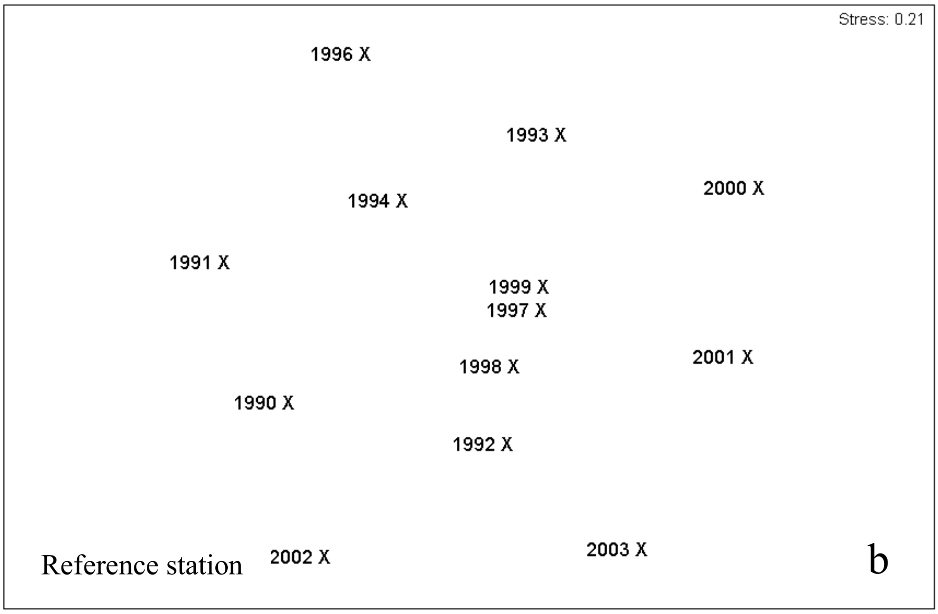


Figure 2.4. a to b. Non-parametric multi-dimensional scaling (MDS) ordination based on double square-root transformed mean abundance of macrofauna species at the reference and disposal site stations between 1990 and 2003. X denotes reference site. Stress value <0.05 = excellent representation, <0.1 = Good ordination, <0.2 = Useful 2 dimensional picture, > 0.3 = Points close to being arbitrarily placed.

Results from the two-way crossed ANOSIM confirmed that both ‘site’ and ‘year’ significantly affected observed community patterns. However, differences between sampling stations were greater ($R = 0.87$ $p = 0.001$) than differences over time ($R = 0.34$ $p = 0.001$). This is corroborates results obtained from the univariate analyses. Comparisons between years of disposal (i.e. ‘disposal group’) and post-disposal (i.e. ‘cessation group’) within sampling stations using one-way ANOSIM gave R values of 0.54 ($p < 0.01$) for the disposal site station and 0.14 ($p < 0.01$) for the reference station. Therefore, there were significant changes at both stations, but the magnitude of these changes was greater at the disposal site.

Results from the correlation analyses suggested that the cessation of sewage-sludge disposal did indeed have a notable effect on macrofauna communities. The comparison of similarity matrixes derived from the biotic time-series data at the reference and the disposal site station resulted in a non-significant R-value of 0.11 ($p = 0.25$), indicating that there was no common time pattern at the two stations. The two similarity matrices were subsequently related to a model, describing simple time trends. A resultant R value of zero would imply that changes in the community have no definite direction. R values greater than zero, in contrast, imply a directional change over time. Our analysis yielded R values of 0.62 ($p = 0.01$) for the disposal site station and 0.20 ($p = 0.05$) for the reference station, suggesting a comparatively greater change at the former compared to the latter over time and in particular after the cessation of sewage-sludge disposal.

SIMPER analysis identified *Lagis koreni*, *Scalibregma inflatum*, *Urothoe marina* and *Mysella bidentata* as species which consistently contributed highly to the dissimilarity between macrofaunal samples collected at the two stations. Over a 13-year period, a reduction in the number of *L. koreni* and increases in *S. inflatum*, *M. bidentata* and U. marina post-disposal (Figure 2.5) were observed. Since disposal ceased in 1998, dominant species at the disposal site station included the amphipod *U. marina*, the polychaetes *S. inflatum* and *Ampharete lindstroemi* and the bivalve *M. bidentata*. Significant interaction terms in the 2-way ANOVA for *S. inflatum*, *L. koreni* and *U. marina* (Table 2.4.) revealed that changes in abundances at the reference and disposal site station differed significantly.

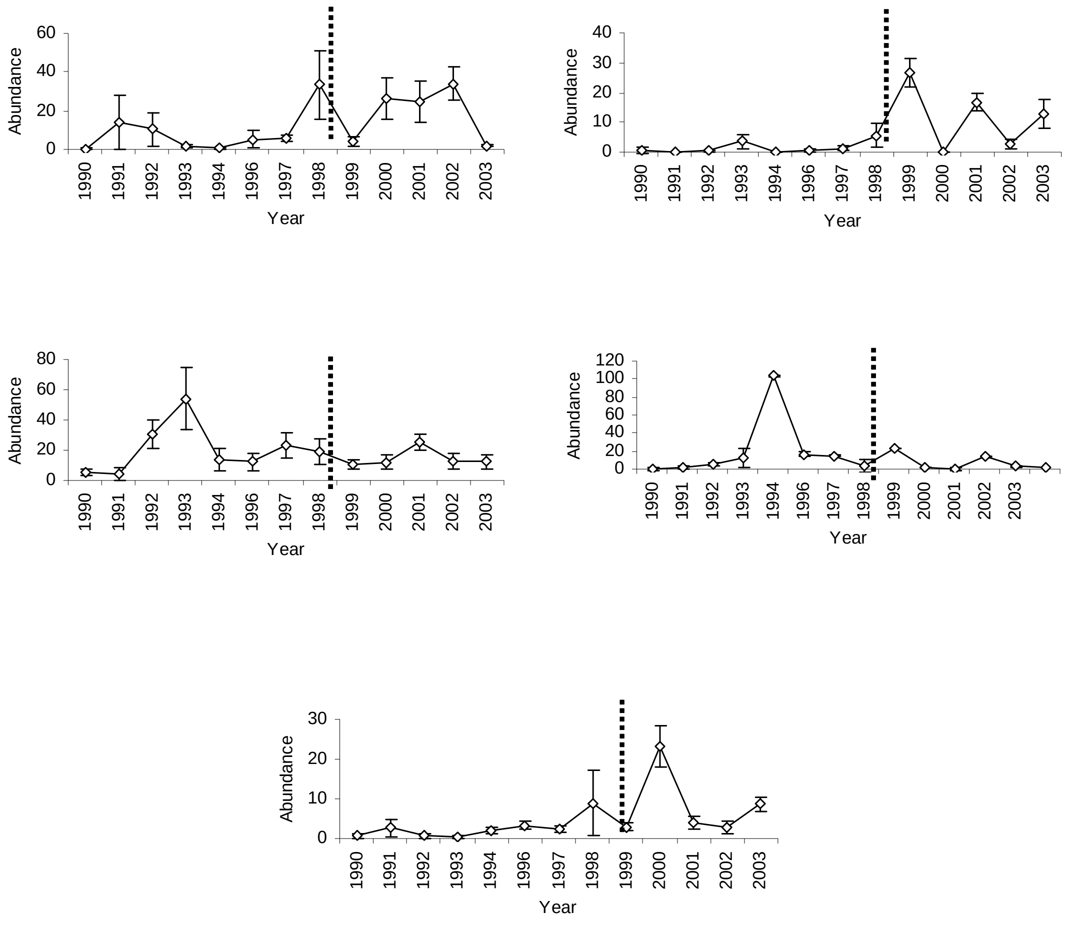


Figure 2.5. Mean (± S.E) abundance of discriminating species at the Liverpool Bay disposal site between 1990 and 2003. Dashed line indicates cessation of sewage-sludge disposal.

Table 2.4. Results from the nested two-way analysis of variance (ANOVA) of discriminating species.

Factor	*A. lindstroemi*		*L. koreni*		*S. inflatum*		*M. bidentata*		*U. marina*	
	F	p	F	p	F	p	F	p	F	p
Site	46.51	<0.01	10.59	<0.01	21.29	0.01	33.54	<0.01	66.76	<0.01
Disposal vs. Cessation	1.22	0.27	4.92	0.03	5.77	0.02	2.94	0.09	42.13	<0.01
Site * Disposal vs. Cessation	1.80	0.18	5.55	0.02	5.35	0.02	2.94	0.09	41.83	<0.01
Year (Site Disposal vs. Cessation)	2.41	<0.01	4.10	<0.01	1.90	0.02	1.69	0.05	6.55	<0.01

4. Discussion

4.1. Methodological considerations

Due to the long history of sewage-sludge disposal in Liverpool Bay, it is difficult to assess the extent of any recovery of the disposal site to date, as no pre-disposal data are available. Equally, as a result of the spatial heterogeneity of marine benthic environments, especially in inner Liverpool Bay, the macrofauna sampled at the reference station cannot fully represent undisturbed communities in the vicinity of Liverpool Bay disposal site. The present work relies on the analysis of data collected during an annual check-monitoring program. As such, it has the benefit of dealing with long-term data taken from a large-scale disturbance, characteristics not usually found in controlled small-scale experimental studies. However it suffers from a lack of replication; the main objective was to conduct annual ‘check monitoring’ alongside spatially extensive surveys of the area carried out by the licensee (e.g., Rees and Walker 1984, Rolwatt et al., 1991). Thus, due to the low level of spatial replication in the present time-series, site-specific factors may confound some of the results.

Although the main effects of sewage-sludge disposal on the benthic fauna are generally manifested through organic enrichment of the sediment (Rees 1993), the effects of trace contaminants, especially heavy metals, must also be considered.

Concentrations of a range of trace metals for the period 1979-1991 showed no significant trends over time (Rowlatt and Ridgeway 1997), though elevated levels of copper, mercury, zinc and lead were observed within the disposal site (Norton et al., 1984). The analysis of intertidal mussels (*Mytilus edulis*) collected from Liverpool Bay and other locations within the Irish Sea also demonstrated that trace metals had not accumulated to levels that could cause a significant effect and that tissue concentrations were all considerably below the recorded 'no observed effect thresholds' (Widdows et al., 2002). The absence of any recent trend towards increasing concentrations of trace metals in sediments and mussels may be explained by the reduction of inputs into Liverpool Bay through improved regulatory control over sewage and other industrial discharges (Chris Vivian, Cefas, pers. comm., Leah et al., 1993). However, at present the occurrence of subtle effects on the macrofauna community cannot entirely be discounted at the disposal site itself.

4.2. Macrofauna community structure

Both uni- and multi-variate approaches showed significant community differences between reference and disposal stations but only multivariate analyses identified significant community changes at the disposal site stations following the cessation of sewage sludge disposal. Relative species abundance plots showed a decrease in density and dominance at the disposal site after cessation, which may be attributed to reduced carbon inputs post-disposal. Similar changes were not observed at the reference station, and hence the two null-hypotheses are rejected.

The cessation of disposal appeared to induce a greater degree of inter-annual change as the community recovered. Analysis of total abundance and species number showed the disposal-site station to be more species-rich than the reference station which may, in part, be attributed to disposal activities. However, as this difference was sustained after the cessation of disposal, it is probable that it also reflects natural variations in the habitat at the two stations (Rees and Walker 1991). These relatively subtle changes which could be attributed to sewage-sludge disposal may be contrasted with those observed at Garroch Head (W Scotland), a sediment accumulating site where macrofauna densities increased by two orders of magnitude due to the proliferation of small opportunistic deposit-feeding organisms (Pearson 1987, Pearson and Coates 1997).

Taxonomic diversity and taxonomic distinctness indices changed little post-disposal. This may be due to (a) that this may be due to the lack of species totally lost from the community during disposal and (b) that the main changes observed were dominance shifts amongst the species present. In these circumstances, the indices therefore appear to lack sensitivity. Although dominance shifts within the disposal-site community were observed, the macrofaunal community remained stable over time, showing none of the expected characteristics of reduced species diversity and high dominance.

Macro-infaunal soft bottom communities vary in their resilience to disturbance, with species recovery rates ranging from months (Huxham et al., 2000) to years (Johnson and Frid 1995). Communities are thought to recover in three progressive steps in response to an improving habitat: species dependent increases in abundance, increasing species diversity and a switch in the dominant organisms from pollution-tolerant, opportunistic species to pollution- sensitive species (Pearson and Rosenberg 1978, Borja et al., 2000). Multivariate analyses of our data revealed such shifts after the disturbance (i.e. sewage-sludge disposal) ceased. A comparison between the disposal site station and reference station revealed a comparatively greater community change at the former post-cessation (1999-2003). Univariate community attributes remained relatively stable over time but dominance within the community changed annually. This accords with the observation of Pearson and Rosenberg (1978) that species dominance within a recovering community is in constant flux.

During disposal years (1990-1998) macrofaunal communities at the reference station were more variable over time than communities at the disposal site station. There was also greater variability in the macrofauna communities between years after disposal ceased in 1998. Work carried out by Warwick and Clarke (1993), using a variety of biological studies, demonstrated that variability between replicate samples increased as the level of perturbation increased. It is interesting to note that community variability between years was lower during sewage-sludge disposal and increased one year after the perturbation ceased. This suggests that disturbance seems to have acted to dampen, rather than enhance, variability, possibly by allowing a relatively stable dominance by tolerant species to become established. Disturbance-tolerant species which dominated at the disposal site during disposal years (1990-1998), included the tube-building deposit-feeding polychaetes *L. koreni* and *A. lindstroemi*. Members of both families can reach high population densities (Rouse and Pleijel 2001) and are capable of colonising disturbed habitats (Rees et al., 1992b, Heath 2004).

After disposal ceased, the abundance of disturbance-tolerant species decreased and other, less tolerant species became more numerous. After 1998, dominant species in the vicinity of the disposal site included *U. marina, S. inflatum*, and *M. bidentata*. Alteration in community structure post-disturbance may reflect successional changes driven by facilitation. An example of this is the dominance of *S. inflatum* followed by an increase in the number of *M. bidentata*. *Scalibregma inflatum* has been described as a transitory species with a slower colonisation rate than pioneer community species (Rosenberg 1972). It is thought to be a detritivore and an active burrower, forming galleries down to a depth of 60 cm (Fauchald and Jumars 1979). *Mysella bidentata* is known to associate with other burrowing organisms such as the brittle star *Amphiura filiformis* and the sipunculid *Golfingia elongata* (Ockelmann and Muus 1978). Studies by Ockelmann and Muus (1978) found increased numbers of *M. bidentata* in association with the oxidised layers of *A. filiformis* burrows. Since burrow-constructing organisms are classed as one of the major functional groups in biotic habitat transformation (Reise 2002), it is possible that the galleries created by the burrowing of *S. inflatum* facilitated the colonisation of the sediment by *M. bidentata*. Significant interactions between 'site' and 'disposal group vs. cessation group' were found for the abundances of *L. koreni, U .marina* and *S. inflatum*. Hence these species could be useful indicators of disturbance at sites similar to the current one.

The present results are in agreement with a spatially extensive 4-year study of Liverpool Bay carried out at by Norton et al., (1984). Analysis of species distribution patterns at over 40 sampling stations also found the disposal site to be dominated by *L. koreni* with sites to the north and west of the disposal site dominated by *S. inflatum*. Several authors hypothesised that the increased dominance of *L. koreni* within the disposal site may be related to an increased input of organic material and fine sediment fractions from the disposal of sewage sludge (Norton et al., 1984, Rees and Walker 1984). The analysis of this long-term data set showed that the disposal site contained a significantly greater proportion of silt/clay than the reference site. Macrofauna community structure was highly correlated with the organic carbon content in the sediment, suggesting that increased levels of fine sediment fractions and increased levels of organic carbon may be a determining factor in structuring benthic communities at sewage-sludge disposal sites. Furthermore, the negative correlation between carbon content and evenness also highlights the tendency of organically enriched environments to be dominated by high numbers of pioneer species (Rees et al., 1992a).

This study has demonstrated the value of extensive time-series data in the assessment of macrobenthic community change during and after the cessation of sewage-sludge disposal in Liverpool Bay. Analysis of the data revealed significant though relatively subtle community changes, with a strong inference that the cessation of disposal was indeed responsible for these changes. This is the case even when, as here, impact and reference stations are faunistically different for other reasons. In this ‘real world’ experiment, multivariate measures performed well as did focusing on key discriminating species. These subtle faunistic changes at the Liverpool Bay disposal site indicate that the near-field effects of the disposal of sewage sludge were small and therefore could be considered environmentally acceptable

Chapter three

Biological indicators of disturbance at a dredged material disposal site in Liverpool Bay, UK: an assessment using time-series data

Published in: ICES Journal of Marine Science (2008) 65(8) 1414-1420

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1. Introduction

The development of reliable indicators of disturbance is essential due to the increasing utilisation and exploitation of the marine environment and the associated need for more effective regulation of activities both singly and in combination (Rogers and Greenaway 2005). Major drivers for indicator development include commitments to achieving international ecosystem targets set within OSPAR (The Biological Diversity and Ecosystems Strategy) and the EU Water Framework directive.

Scientists have a wide range of analytical tools available to measure both physical and biological shifts within marine ecosystems (Washington 1984, Elliot 1994, Danilov and Ekelund 2001, Quintino et al., 2006). Difficulties arise when trying to identify and evaluate the most reliable and informative metrics to use for a given situation (Salas et al., 2006). Some of the tools available for assessing the health of biological communities include primary and derived univariate indices, number of individuals (N) species number (S), species richness (Margalef, d), species diversity (Shannon-Wiener, H') and taxonomic distinctness and diversity (Clarke and Warwick 2001). Multivariate techniques include multi-dimensional scaling (MDS), similarity percentage (SIMPER) and the RELATE procedure (Clarke and Gorley 2006). In addition, there are biological metrics which take into account the pollution tolerances and ecological strategies displayed by individual benthic faunal species e.g. the Azti Marine Biological Index (AMBI) (Borja et. al., 2003) and the Infaunal Trophic Index (ITI) (Word 1979, Maurer et al*.,* 1999).

In an indicator context these tools can be used in several ways including: monitoring tools to assess natural variation within a biological community (Marine Environment Monitoring Group 2004, Schratzberger et al., 2004), assessing the effectiveness of management practice (Whomersley et al., 2007) or monitoring communities continually impacted by anthropogenic activities such as dredge material disposal at sea (Rees et al., 1992, Rees et al., 2006).

Appropriate sample designs accompanied by a good understanding of the recent history of human activities of interest are a pre-requisite for effective indicator application. For the initial evaluation of possible indicators (or 'metrics'), the potential for confounding natural and anthropogenic-induced variation must first be discounted, typically through the selection of adequate reference sites. In the case of dredged material disposal, impacts may be caused by the physical act of burial (amount deposited), a change in sediment type (type of sediment being deposited), the presence of contaminated material e.g. heavy metals, or a combination of all these factors. Such considerations are clearly important for the selection of metric(s) that will be effective in discerning the impacts of the disturbance being studied. Many selection criteria have already been suggested and used to evaluate metric performance in varying circumstances (ICES 2001, Defra, 2004, EEA 2005, Sneddon et al., 2006). In the current study, criteria selected were representative of those identified by a variety of national and international organisations e.g. EEA, Defra and ICES. They included scientific validity, correlation to manageable human activities, ease of communication, relevance to decision making, sensitivity and ability to show spatial and temporal trends, and cost effectiveness.

The aim of this study was to apply these recommendations in the evaluation of a suite of potential metrics using a large data set derived from the monitoring of a major dredge disposal site. The data used came from annual (1996-2003) macro-invertebrate infaunal surveys of Liverpool Bay dredged disposal site.

The main questions that were addressed are:

- What measurable environmental impacts associated with dredged material disposal can be linked with/correlated to a faunal response?
- Should an individual metric or a suite of metrics be employed when assessing dredged material disposal in Liverpool Bay?
- What are the wider lessons for indicator application?

2. Material and methods

2.1 Site description

The Liverpool Bay 'Site Z' disposal site on the west coast of the UK, was first licensed in 1982 following closure of a nearby site due to shoaling. In 1996 the new site was extended to the west, also due to shoaling in the centre of the licensed area (Figure 3.1). From 1996-2003 this site received fifteen million wet tonnes of dredged material, an average of two million wet tonnes per annum. Material disposed of largely originated from maintenance dredging of docks or navigational channels in the Mersey Estuary and its approaches (Somerfield et al., 1995). The location is shallow (10 m) and is exposed to wave action principally from westerly to northerly winds

(Rees et al., 1992, Somerfield et al., 1995) with residual bottom currents flowing in a predominantly landward (eastward) direction (Ramster 1973).

2.2 Metric assessment

A macro infaunal dataset was produced from samples (4 replicates from each station) collected between 1996-2003 from one station within the disposal ground and two near field reference stations (Figure 3.1).

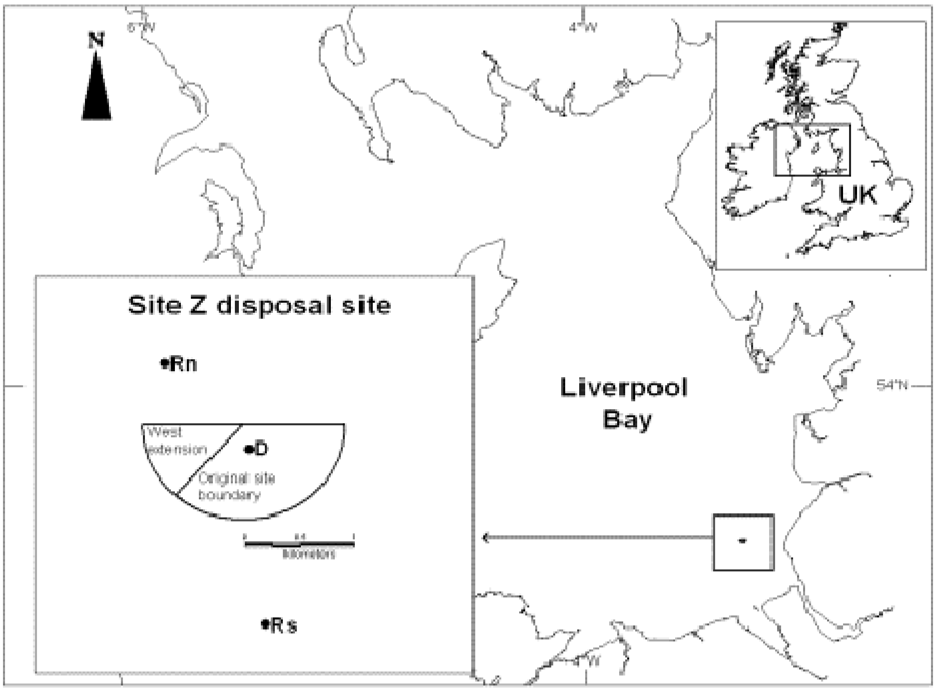


Figure 3.1. Site Z disposal site and temporal sampling stations.

A range of metrics including both primary and complex derived biological indices (Table 3.1) were calculated for each sample.

Table 3.1. Performance of metrics from the disposal site when correlated (Anderson Darling test) with (a) same- year disposal quantities and (b) with values lagged by one year.

Metric	Normally distributed data	Correlation (a)	Correlation (b)
Abundance	✓	X	X
Species number	✓	X	✓
Species richness	✓	X	✓
Species diversity	X	X	X
Taxonomic diversity	✓	X	X
Taxonomic distinctness	✓	X	X
Av. Taxonomic distinctness	X	X	X
AMBI coefficient	✓	X	X
AMBI % Sensitive species	✓	X	X
AMBI % Tolerant species	✓	X	X
ITI Score	✓	X	X
ITI % Detrital feeders	✓	X	X
ITI % Deposit feeders	✓	X	X

Normality of data was tested using the Anderson Darling test. Criteria for judging the utility of the metrics were selected to represent those identified by a variety of national and international organisations, e.g. EEA, Defra and ICES, and were divided into two groups:

Group 1.

A Scientifically valid

B Tightly linked to manageable human activity

Group 2.

C Communicable to non-scientists and other users

D Easily and accurately measured

E Cost effective

F Show spatial and temporal trends

If the metric was not scientifically valid (i.e. scientifically relevant to the objectives of the study) or not tightly linked to the disturbance in question then no further analysis or assessment of the metric was carried out. Metrics that passed the group one criteria were then evaluated using criteria from group two. Scores (1 = Very poor, 2 = Poor, 3 = Good, 4 = Very good, 5 = Excellent) were subjectively allocated in consultation with other experienced benthic ecologists and found to be comparable with a previous study which also assessed the merits of several commonly used univariate, multivariate and biological metrics (Suzanne Ware pers com). The highest scoring metric(s) were then used to evaluate the effects of dredged material disposal at site Z in Liverpool Bay. The subjective nature of this approach is accepted. The initial list of indicators used is not exhaustive and was produced using expert judgement and existing literature (Aubry and Elliot 2006), the choice of the criteria used may be biased towards certain activities and management objectives (Rice and Rochet 2005) and the method of scoring the indicators e.g. based on expert judgement will be sensitive to the experiences of those involved (Rochet and Rice 2005). However, the increasing need for advice on the implications of human activities within the marine environment, combined with the large array of potential indicators and the restrictions on resources available for regulators, demands that alternative methodologies of developing and assessing indicator performance are explored.

2.3. Univariate analysis

Due to the absence of any significant difference in contaminant levels and sediment type at the three sites (data not shown) it was concluded that the best pressure indicator of disturbance was the amount of material disposed per annum.

To assess if the chosen metrics were tightly linked to dredged material disposal within Liverpool Bay, Pearsons product moment correlation was calculated between each indicator metric and quantities of material deposited (annual amounts) in the same year and with a one-year time lag e.g. 1996 disposal quantities with 1997 metric data. A General Linear Model ‘site year sample (station) station*year’ was then constructed to evaluate differences between years and stations. Metrics that had displayed significant ($P<0.05$) correlations (positive or negative) with disposal quantities were then tested; significant interaction terms between years and stations were of particular interest, since they implied different temporal trajectories at different stations. In addition, Tukey's multiple comparison test was used to investigate differences between sites within years. 'Treatment' / 'Reference' ratios were also calculated using a pairwise comparison of annual measures *[(Treatment/Reference) – 1] X 100* to investigate the degree of community change and synchrony at stations within and outside the disposal area. A mean cumulative species abundance plot was constructed to investigate species dominance within each site.

2.4 Multivariate analysis

In order to assess whether dredged material disposal had a significant effect on macrofauna community structure, Spearman rank correlations were calculated between similarity matrices derived from the reference samples and those derived from the station within the disposal site. This allowed the investigation of directional changes (i.e. seriation) in macrofauna communities over time at the three stations, with a significant correlation indicating comparable temporal trends at the disposal site station and reference stations.

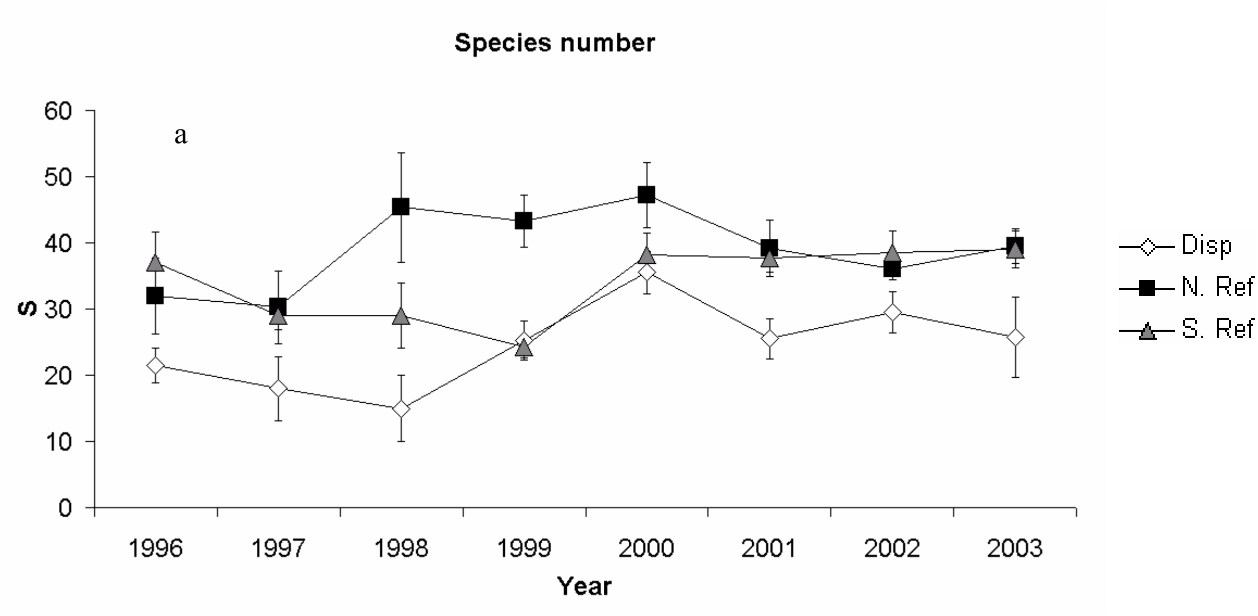
Non-metric Multi-Dimensional Scaling (MDS) ordinations derived from Bray-Curtis similarity matrices were carried out to display differences in the structure of macrofauna communities. Two-way Analysis of Similarities (ANOSIM) was performed to assess differences in macrofauna community structure between stations and over time. The similarity percentages (SIMPER) procedure was utilised to identify the main species contributing to the observed community patterns. The

BVSTEP procedure was also employed to ascertain if the same groups of species from the different stations correlated with the quantity of material disposed of each year. All multivariate analyses were performed on double square-root transformed species abundance data using PRIMER version 6.1.5 (Clarke and Gorley 2006).

3. Results

3.1 Univariate analyses

There were no significant differences between mean values of sediment characteristics (median particle size, percent silt/clay and the content of several metals) when stations within the disposal and reference sites were compared. Species number (r = -0.831 p = 0.021) and species richness (r = -0.824 p = 0.023) were the only metrics to correlate significantly with lag one year (year – 1) amounts of dredged material deposited. No such relationships were found using metrics derived from either of the reference stations data (Table 3.2).



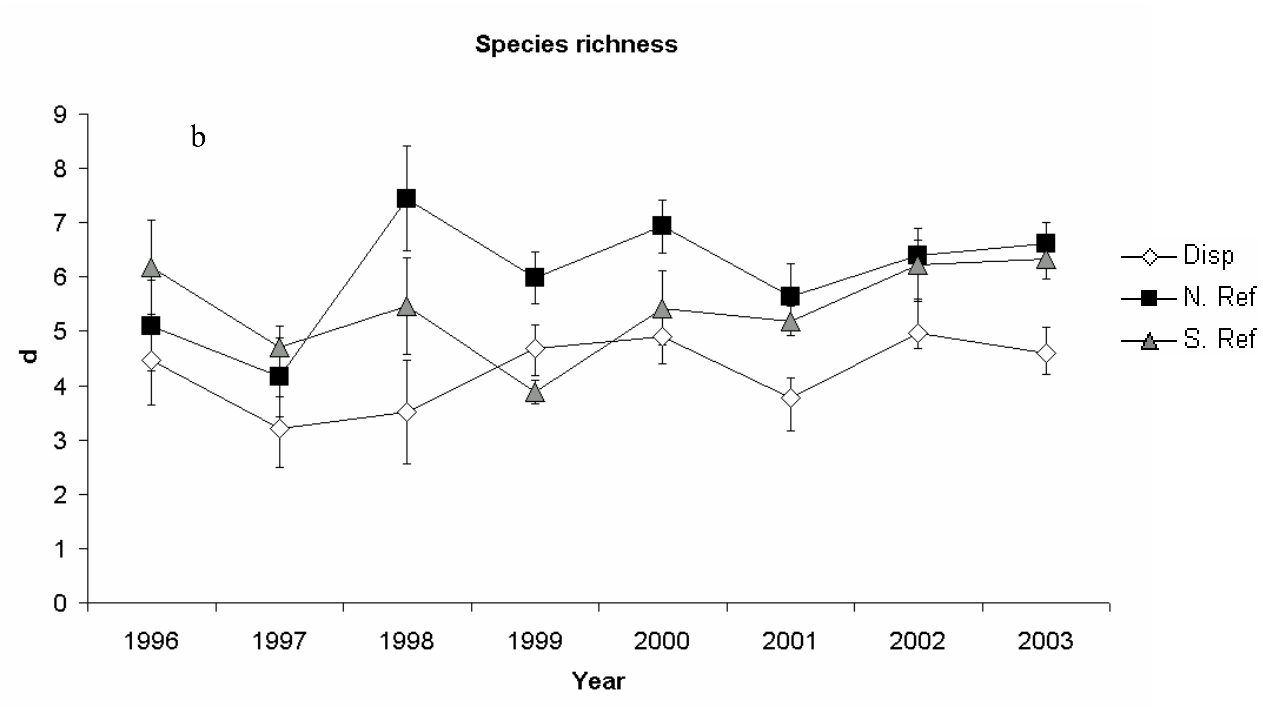


Figure 3.2a and b. Species number (S) and species richness (d) plots for each site from 1999-2003. (Mean + / - 95%CI).

Throughout the time-series number of species and species richness (Margalef 'd') tended to be higher at the reference stations than the station within the disposal site (Figure 3.2). The primary and derived univariate indices of species number and richness both scored highly when assessed using the group 2 criteria with mean scores of 4 (very good) (Table 3.2). Therefore to ensure the metrics used to further assess the effects of dredged material disposal at Liverpool Bay met the majority of the selection criteria both species number and species richness were selected.

Table 3.2. Metric scoring matrix.

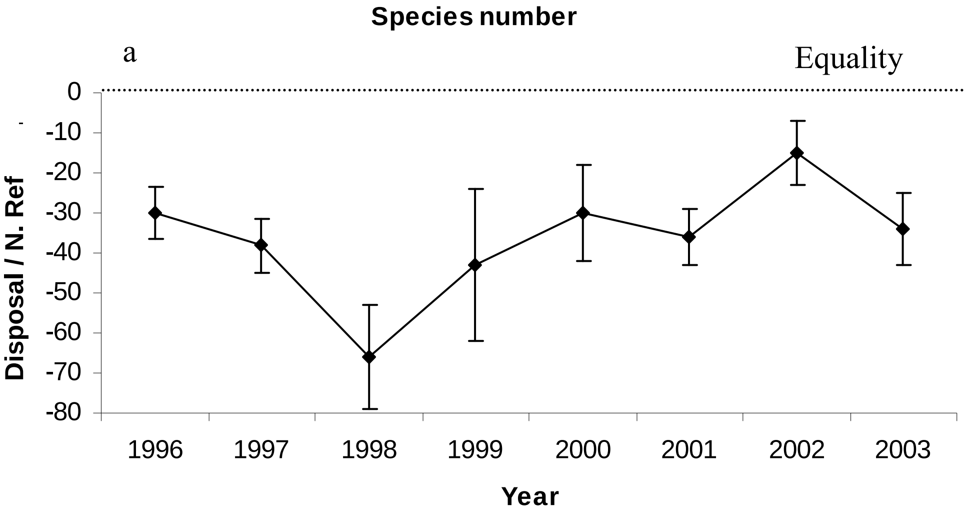
	Criteria						
Indices	A	B	C	D	E	F	Total Score
Ratio **[(Treatment/Reference)-1]*100**	**5**	**4**	**5**	**4**	**3**	**5**	26
Species number	**5**	**4**	**4**	**4**	**3**	**4**	24
Species richness	**5**	**4**	**3**	**3**	**3**	**4**	22
Multivariate (MDS)	**5**	**1**	**2**	**3**	**3**	**1**	15

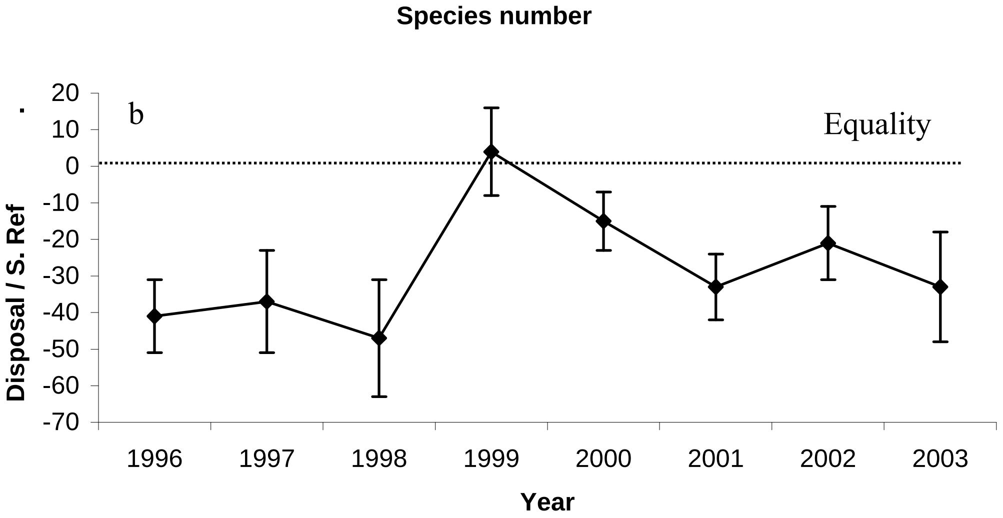
Results from General Linear Model ‘station year rep (station) station*year’ revealed significant differences in values of the metrics species number (S) and species richness (d) between the reference and disposal site stations (F = 97.42, p= <0.01 and F = 88.94, p = <0.01) and over time (F 13.68, p = <0.01 and F = 11.37, p = <0.01). Significant interaction between stations were also identified for species number (F = 5.74, p = <0.001) and species richness (F = 5.08, p = <0.001) indicating that the 3 stations behaved differently over time. Tukey’s multiple comparisons test revealed consistent significant differences between disposal site and reference stations within years for both species number and richness (Table 3.3 and 3.4).

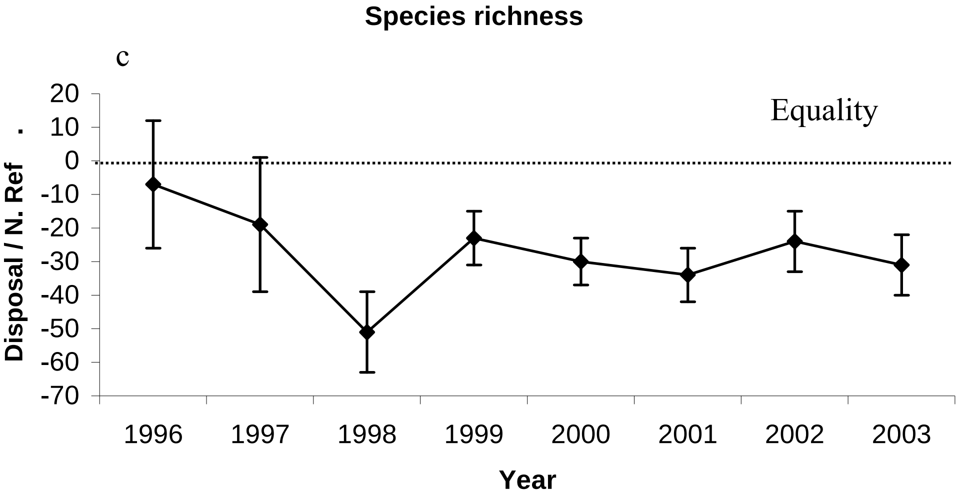
Table 3.3. Results from Tukey’s multiple comparison test and ANOSIM comparing species number (S) and species richness (d) between reference and disposal site. stations within years.

Year	Stations	*S*		*d*		ANOSIM	
		Test statistic	Significance	Test statistic	Significance	Test statistic	Significance
1996	Rn / D	-3.50	n.s.	-2.43	n.s.	1	0.03
	Rs / D	-5.38	<0.01	-5.01	<0.01	1	0.03
	Rn / Rs	-3.50	n.s.	2.58	n.s.	0.78	0.03
1997	Rn / D	-4.22	<0.01	3.90	0.04	0.82	0.03
	Rs / D	-3.77	n.s.	3.77	0.05	0.79	0.03
	Rn / Rs	-0.45	n.s.	0.14	n.s.	0.94	0.03
1998	Rn / D	-9.01	<0.01	-8.60	<0.01	1	n.s.
	Rs / D	-4.04	0.02	-6.29	<0.01	1	0.02
	Rn / Rs	-4.98	<0.01	-4.77	<0.01	1	<0.01
1999	Rn / D	-6.58	<0.01	-8.51	<0.01	1	<0.01
	Rs / D	-0.27	n.s.	-4.13	0.02	0.91	n.s.
	Rn / Rs	-0.28	<0.01	0.62	n.s.	0.67	n.s.
2000	Rn / D	-4.13	0.02	-4.00	0.03	1	n.s.
	Rs / D	-1.08	n.s.	4.37	<0.01	0.98	n.s.
	Rn / Rs	-3.05	n.s.	-2.86	n.s.	0.88	n.s.
2001	Rn / D	-4.98	<0.01	4.96	<0.01	1	0.03
	Rs / D	-4.40	<0.01	4.24	0.01	1	0.03
	Rn / Rs	0.54	n.s.	0.72	n.s.	1	0.03
2002	Rn / D	-2.15	n.s.	-3.64	n.s.	1	0.03
	Rs / D	-0.36	n.s.	2.95	n.s.	1	0.03
	Rn / Rs	0.99	n.s.	0.75	n.s.	0.25	n.s.
2003	Rn / D	-4.93	<0.01	-4.81	<0.01	0.55	0.03
	Rs / D	-4.75	<0.01	-4.39	<0.01	0.49	0.03
	Rn / Rs	0.18	n.s.	-0.41	n.s.	0.19	n.s.

Treatment / reference ratios remained below equality (zero) for the majority of the time-series indicating values of both species number and richness were lower within the disposal site, except for the year 1999 (S. Ref / Disp) when values for species number and richness both rose above the equality value (Figure 3.3b and d).







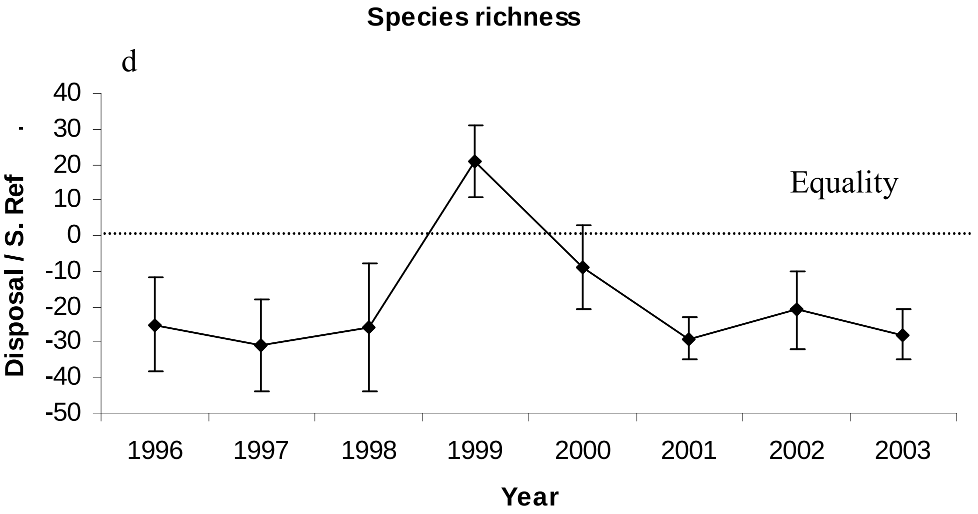


Figure 3.3a-d.'Treatment'/'Reference' ratios of species number and species richness, dashed line represents equality between the reference and disposal site stations (+ / - 95% CI).

Although species number and richness indices were higher at both reference stations for the duration of the time-series, a more even spread of individuals at the disposal site was observed (Figure 3.4).

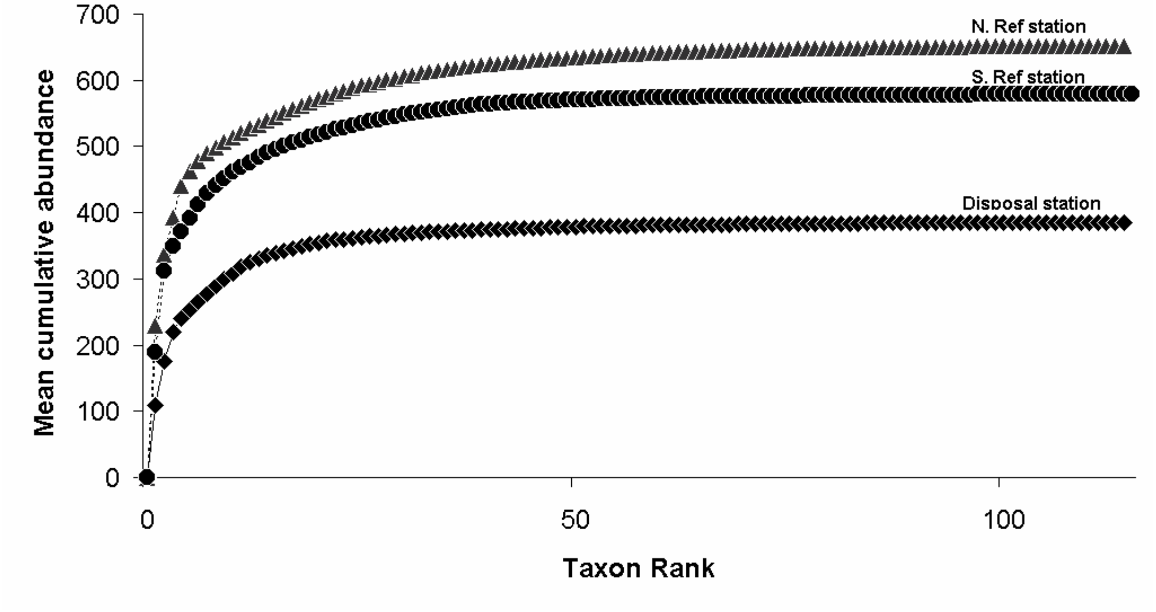


Figure 3.4. Mean cumulative species abundance at the disposal and reference sites from 1996-2003.

Densities of *Lagis koreni* the dominant species at all three stations, showed large fluctuations over time. These fluctuations appear to be inversely related to a reduction in disposal quantity (Figure 3.5). However no significant correlations were identified.

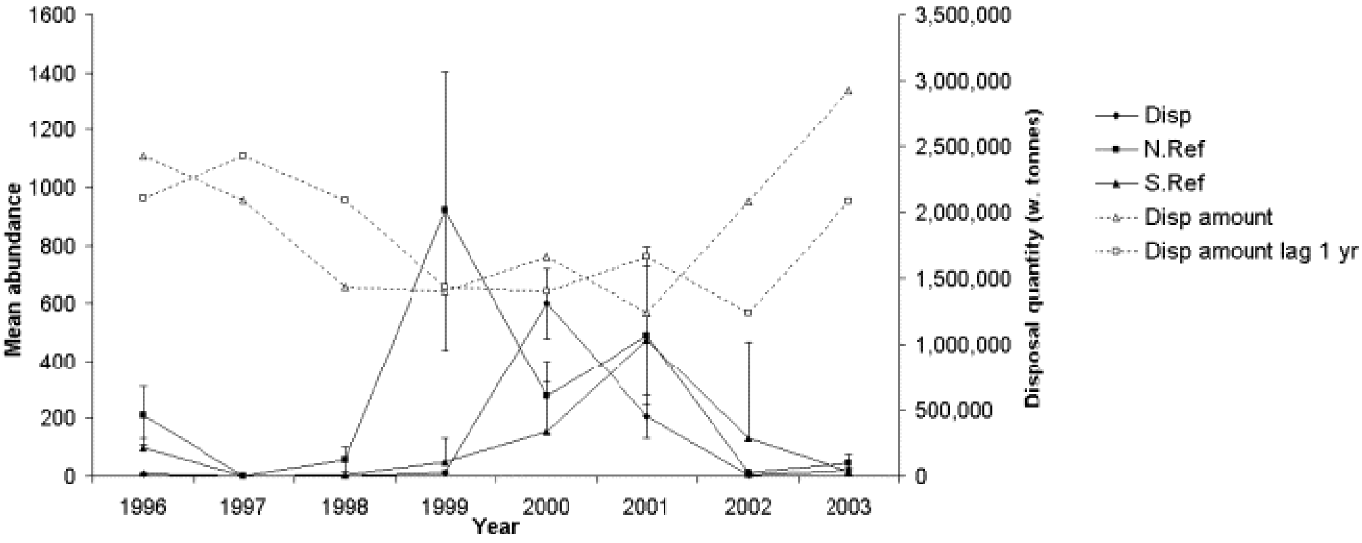


Figure 3.5. Fluctuations in the abundance of *Lagis koreni* at reference and disposal sites stations from 1996-2003 (Mean + /- 95% CI).

3.2. Multivariate analyses

Comparison of Bray-Curtis similarity matrices derived from the biotic time-series data at the disposal and reference stations (RELATE procedure) resulted in significant R-values of N Ref / Disp 0.546 (p = < 0.01), S Ref / Disp 0.486 (p= <0.01) and N Ref / S Ref 0.607 (p = < 0.01) indicating that there were common time patterns between all sites as illustrated in Figure 3.6.

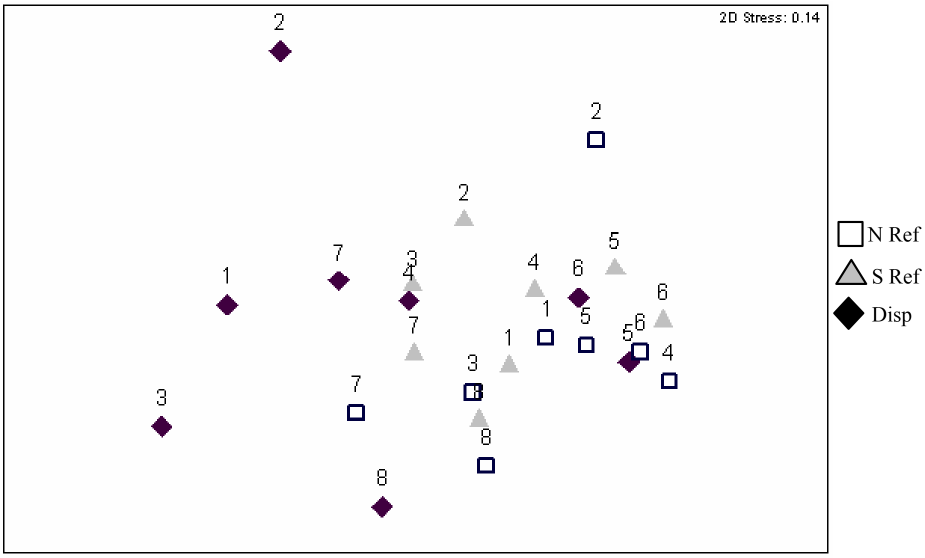


Figure 3.6. Multi-dimensional scaling plots of averaged biotic data. Numbers represent consecutive years. Stress value <0.05 = excellent representation, <0.1 = Good ordination, <0.2 = Useful 2 dimensional picture, > 0.3 = Points close to being arbitrarily placed.

Results from the two-way ANOSIM with replication revealed significant differences in community structure between disposal site stations and reference stations and also between the two reference stations (R = 0.788, p = < 0.01). Further analysis using one-way ANOSIM also showed significant differences between sites within years. (Table 3.3).

Similarity percentage (SIMPER) analyses showed the species that contributed highly to the dissimilarity between stations were the polychaete worm *Lagis koreni* and the bivalve mollusc *Spisula subtruncata.* The main dissimilarities (17%) between stations were in the abundances of these two species with higher average abundances being found at the disposal site station. Results from the BVSTEP routine identified different groups of species that correlated with the quantity of material disposed of each year. Within the disposal site the group that correlated most strongly with the quantity disposed of ($r = 0.909$) contained mainly predatory polychaete species from the families Glyceridea, Nephytidae and deposit-feeding species from the family Ampharetidae. Within the reference sites (N Ref $r = 0.898$, S Ref $r = 0.895$) species groups contained mainly ophuiroids, amphiurids and bivalve molluscs.

4. Discussion

The criteria in group one are necessary pre-requisites for the use of any potential metric; any initial selection must involve an informed decision on whether the measurement is likely to be relevant in any given situation. In the current case, this involved looking for evidence of some sensitivity to impact. The criteria in group two facilitate the weighting of metrics ensuring that the chosen metric is fit for purpose e.g. providing environmental managers with accurate, cost effective, easily communicable results and outcomes. The method analysed here raises important questions, including how do we ensure that observed changes in metric values are part of a cause-effect relationship and therefore tightly linked to the manageable anthropogenic activity in question? Potential impacts of dredged material disposal on the receiving environment are dependent on the nature and quantity of the material deposited (Bolam et al., 2006, Bolam and Rees 2003, Rees et al., 1992, 1994, 2006). These impacts include burial, changes in sediment type and organic enrichment. Since none of the stations differed significantly in sediment type, trace metal and organic content, the amounts deposited per annum were considered the best pressure indicator to use in the assessment of the effects of dredged material disposal at site Z dredged disposal site.

Only two (species number and species richness) out of thirteen potential metrics passed the group one criteria. This was due to the lack of any correlation between the remaining metrics and amounts of material deposited in the same year as the metric data and also when the quantities data were lagged by 1 year. This is not surprising as some of the metrics chosen e.g. the biotic indices (AMBI, ITI) were primarily derived to assess impacts of organic enrichment. However the absence of a biological response e.g. an organic enrichment indicator, may in itself have value in determining the actual cause-effect relationship at site Z dredged disposal site. To avoid the possibility of misinterpreting results, metrics which were not tightly linked to the disturbance were discounted from any further analysis.

Both univariate (general linear model with species number and richness as responses) and multivariate (ANOSIM test) analyses showed persistent significant differences between all three sites. However, when multivariate community data were analysed using the RELATE routine no significant differences in the macrofaunal communities over time were observed. The fact that communities at the three stations remained significantly different but exhibited common time patterns over an eight year period shows that factors other than dredge disposal impacts, such as natural community variation (Hall et al., 1994) and climate change (Rees et al., 2006), must have contributed to observed community variation. This illustrates the central challenge in interpreting long term data sets: to discriminate between low amplitude, low frequency drivers such as those associated with climate change and high frequency point source impacts including fishing, aggregate extraction and dredged material disposal.

Species number and richness were found to be lower within the disposal site station when compared with the reference site stations. SIMPER analysis showed that *Lagis koreni* and *Spisula subtruncata* were the dominant species at all three stations. *Lagis koreni* has been identified previously as a possible indicator of disturbance within Liverpool Bay dredged disposal ground, and is thought to dominate in such disturbed areas due to its opportunistic life cycle which enables it to colonise recently deposited material, (Rowlatt et al., 1990, Rees and Rowlatt 1994). However, despite *L. koreni* being found in large numbers at the disposal site station it was not as abundant as in both reference site stations. This may be an example of how disturbance may increase evenness within benthic communities by preventing the community from reaching a climax represented by a few well-adapted dominant species (Paine 1974, Connell 1978).

The identification of metrics capable of detecting and quantifying the effects of dredging disposal (or any other point source impact) generally relies on the comparison of reference and impacted stations. In this study the method of producing treatment/reference ratios [(Treatment/Reference)-1]*100 indicated the differences between ‘reference’ and ‘treatment’ communities in an easily communicable output thus permitting changes in primary metrics to be summarised and communicated to environmental managers in an unambiguous way. Using this approach it may be possible to set action levels to help guide managerial decisions relating to the future use of the site. This approach may help to set action levels to guide managerial decisions relating to the future use of the site. The setting of such action levels (environmental quality standards) has been applied previously to other marine disposal activities (Ministry of Agriculture, Fisheries and Food 1993, Rees et al., 2006), based on an acceptable deviation from reference values of primary and derived metrics.Of interest when assessing licensed impacts is the sphere of influence of the activity. Any deterioration in the benthic community within a licensed area, although interesting, would be considered to be acceptable under the licensing agreement. However, if the activity began to affect areas outside the licensed boundaries then action would need to taken (Food and Environment protection act 1985). Using this method it would be possible to set action levels based on the distance from equality of the relevant reference and treatment sites. A reduction in the magnitude of the difference could be an indication that communities were being affected at the reference sites and further assessments would be necessary. A number of management decisions would arise in the application of such action levels, in particular the choice of the critical threshold that would precipitate action. It is likely that such action levels would be site specific and would need to be adapted over time e.g. in response to systematic changes in disposal practices.

Similar work to that described here, for disposal sites in other locations and with other types of anthropogenic disturbance, would be helpful in refining the use of indicators of disturbance. Only metrics that are actually linked to the disturbance in question should be used at well defined sites such as this, where the aim is to monitor the extent of known disturbance, rather than to detect it. The univariate indices, species number and species richness should currently be used as front line indicators to monitor future biological effects of dredged material disposal on macrofaunal communities within the site Z disposal ground and at the far-field reference stations.

Within the disposal ground there were significant negative correlations with disposal quantities for the previous year (lag 1 year relationship). Therefore a trend towards increasing correlation between amounts deposited and species richness and number at the far-field sites could be used as an indicator of increasing disturbance due to the activity of dredged material disposal.

Chapter four

Response of intertidal macrofauna to multiple disturbance types and intensities. An experimental approach

In press: Marine Environmental Research **DOI:10.1016/j.marenvres.200912.001**

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1. Introduction

In ecological science, disturbance can be defined as “any discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability or the physical environment” (Pickett and White 1985). This definition highlights the complexity of factors and processes that can be affected by disturbance, and is broad enough to imply that disturbance is ubiquitous in ecology. Marine benthic communities are subject to a variety of abiotic and biotic disturbances and their interactions (Posey 1990). These include both natural (e.g. waves, currents and storms) and anthropogenic events (e.g. fishing, offshore disposal and construction). Such events are often dominant structuring forces within benthic communities (Pearson and Rosenberg 1978, Thistle 1983, Hall 1994, Cowie et al., 2000, Huxham et al., 2000, Bolam et al., 2002a) and despite the large range of potential causes they can all in principle be characterised by their frequency, duration, size and intensity (Bengtsson 2002).

For the purpose of this study, disturbance will be defined as an‘externally imposed destructive force’ (Huxham et al., 2000). Numerous studies have assessed the impacts of various types and intensities of disturbance on benthic communities (Pearson and Rosenburg 1978, Heip 1980, Rees 1982, Zajac and Whitlach 1982, Coull and Chandler 1992, Hall 1994, Austen et al., 1998, Schratzberger and Warwick 1999, Cowie et. al., 2000, Huxham et al., 2000, McCabe and Goatelli 2000). These have established that benthic communities show a wide range of responses to disturbance, and that these responses can vary depending on the frequency and intensity of disturbance. However, uncertainty remains within the literature and in practical applications over the epistemological status of the term ‘disturbance’. As defined above, and as used in predictive models such as the Intermediate Disturbance Hypothesis and diagnostic tools such as abundance biomass curves, ‘disturbance’ is a general concept that can have many causes but which manifests at the community level in predictable ways. However, there is evidence that different types of disturbance may have very different effects on communities and also that disturbance effects are community-specific (Table 4.1). Thus regarding ‘disturbance’ as a single category, which can be applied to different events ranked according to intensity along a single continuum, may be misleading, as may the use of models and tools based on this assumption.

Table 4.1. The effects of different disturbance types on benthic macrofaunal communities.

Disturbance type	**Effects**	**References**
Oil	Localised toxicity, burial, organic enrichment, suffocation, immobilisation, varying species and phylum (Mollusca, Echindamata) sensitivity, reduced species number and diversity, increased occurrence and dominance of opportunistic short lived species, changes in trophic structure.	Addy et al., 1984 Glemarec 1986 Kingston 1992 Daan et al., 1994 Westerland et al., 2001 Nititik and Robinson 2003
Dredge material relocation	Localised effects of isolated disposals, burial, smothering, chemical contamination (Hg, Cd, Cu, Zn, Pb and organotins), changes in sedimentology, increased levels of organic carbon, reduction in abundance, species richness, and diversity, increased dominance of tolerant and opportunistic species (*Lagis koreni and Abra alba)*	Norton et al., 1984 Rowlatt et al., 1990 Rees et al., 1992a Reed 2000a Reed 2000b Stronkhorst et al., 2003 Cruz-Motta and Collins 2004 Bolam et al., 2006 Curtis et al., 2006a Curtis et al., 2006b
Sewage sludge disposal	Localised impacts, immunological effects on flatfish populations, enhancement of a range of taxa local to the area, increases in Ph, organic carbon and heavy metal (Cd, Cu, Zn, Pb) levels, rising of the redox potential discontinuity layer, changes in sediment grain size, increased occurrence and dominance of opportunistic short lived species (*Capitella capitata*). Changes in trophic structure from surface deposit feeders and predators to a predominantly sub surface deposit feeding community. Increased total biomass.	Eleftheriou et al., 1982 Rowlatt et al., 1991 Rees et al., 1992b Rees and Rowlatt 1995 Moore 2003 Kress et al., 2004 Elias et al., 2005 Rees et al., 2005 Whomersley et al., 2006
Fishing	Changes in sedimentology and roughness, frequency of sediment plumes, changes in trophic structure, increased scavengers (*Asterias rubens*), removal of target species, death / injury of large long lived benthic species (*Glycymerus glycymerus*) and bioturbating species (*Echinocardium cordatum*) reduction in biomass, abundance, species number and diversity	Hall 1994 Dayton 1995 Macdonald et al., 1996 Kaiser and Spencer 1996 Brown and Wilson 1997 Jennings and Kaiser 1998 Hansson et al., 2000 Lindergarth et al., 2000 Jennings et al., 2001 Kaiser et al., 2001 De Biasi 2004

Under most conditions of anthropogenic impact, different types of disturbances (such as toxic pollution and organic enrichment) occur simultaneously, thus making distinctions between their effects difficult. It is possible that, if considered separately, these different types of disturbance will produce qualitatively different outcomes. The potential effects of the same disturbance on different species raise similar problems. If the effects of disturbance are emergent properties of communities, then they should not rely on the presence of a single or few particularly sensitive species. But this may not be true when dealing with keystone species i.e. species that have a disproportionately large effect on any aspect of ecosystem function (Paine 1974, Menge 1994, Davic 2003). Since the keystone role can be context, rather than species, specific, the removal of the same species from two apparently similar communities can have dramatically different effects. Similar disturbances in similar communities may also cause different outcomes through chance effects, such as different assembly or disassembly trajectories caused by different abundances of important species (Lindsay et al., 2006). For example, the order in which species are lost from a community might affect both abiotic and biotic interactions, which are known to structure benthic communities (Ostfield and LoGiudice 2003).

Under most field conditions, differences between species are confounded by differences between community types, and hence detecting species-specific effects can be difficult. The present work describes an experimental test of the effects of different types and intensities of disturbance on infaunal intertidal communities at two different sites with similar suites of species. It tests three null hypotheses, as described below.

- There are no differences in the responses of the same species and trophic groups to the same disturbances at two different but comparable sites. That is, there are no interactions between treatment and site factors for species and trophic group level responses.

- There are no differences in community responses to the same disturbances at two different but comparable sites. That is, there are no interactions between treatment and site factors for community level responses.

- Different types of disturbance produce qualitatively similar community responses, which can be ranked on a single continuum of intensity. Hence there are no community changes that are larger under low, compared with high, intensities of a disturbance

2. Material and methods

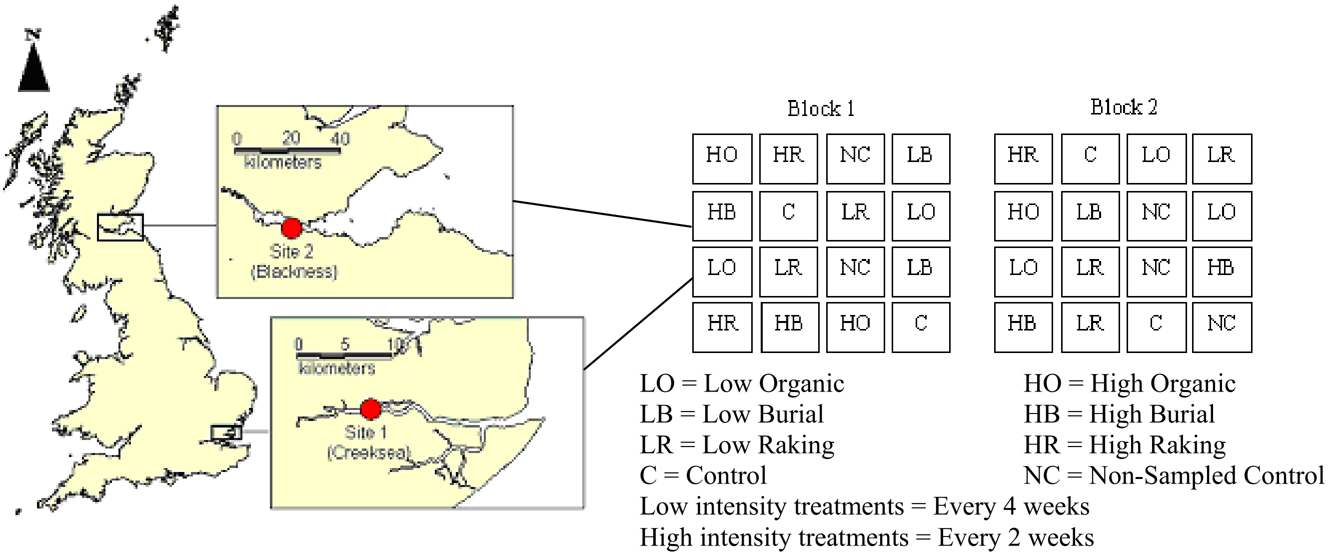
2.1 Study sites

2.1.1 Site 1. Creeksea

The Crouch Estuary is a sea inlet (Figure 4.1) which is dominated by tidal ebb and flow of high-salinity waters. The experiment was carried out on mud flats mid way along the estuary (51°38.20N, 00°42.80E) that are sheltered from the main prevailing wind and tide. The sediments are fine (90% silt/clay) with 12% organic content (loss on ignition) (own analysis). The macroinfaunal community is dominated by tubificid oligochaetes, polychaetes from the family cirratulidae and the gastropod mollusc *Hydrobia ulvae* (Bolam et al., 2004).

2.1.2 Site 2. Blackness

The intertidal mud flat is situated east of the village of Blackness in the lower Forth Estuary, Scotland (56°00.00N, 3°30.00W) and is approximately 650 m from MHWS to MLWS (Figure 4.1). This area is classed as a relatively unpolluted muddy site with a silt/clay fraction of 29% and organic contents of around 6.4% (loss on ignition) (Mark Huxham pers com). The macroinfaunal community is dominated by tubificid oligochaetes, polychaetes from the family spionidae, the gastropod mollusc *Hydrobia ulvae* and the bivalve mollusc *Macoma balthica* (Huxham et al., 2000, Bolam and Fernandes 2002b).



Block 1			
HO	HR	NC	LB
HB	C	LR	LO
LO	LR	NC	LB
HR	HB	HO	C

Block 2			
HR	C	LO	LR
HO	LB	NC	LO
LO	LR	NC	HB
HB	LR	C	NC

LO = Low Organic
LB = Low Burial
LR = Low Raking
C = Control
HO = High Organic
HB = High Burial
HR = High Raking
NC = Non-Sampled Control
Low intensity treatments = Every 4 weeks
High intensity treatments = Every 2 weeks

Figure 4.1. Locations of experimental sites and experimental setup. Site one Creeksea, Crouch Estuary, Essex. Site two Blackness, Forth Estuary, Edinburgh

2.2 Experimental design

At each site, eight treatments were randomly allocated to 32 1m^2 plots, divided between two blocks with two replicates of each treatment per block (Figure 4.1). Disturbance treatments consisted of two intensities each of organic enrichment, burial and raking. The low intensity treatments were applied every four weeks and the high intensity treatments applied every two weeks. Two control treatments were set up. Sampled control plots had samples taken at each sampling time, whilst non-sample controls were sampled only at the end of the experiment, to allow for the detection of any effects of sampling *per se* on community structure. The experiment was initiated at both sites in October 2003. Samples were taken at 1, 3, 6 and 9 months at Creeksea; the experiment was then terminated here after ten months, two months earlier than planned due to unusually high densities of seasonal weed growth. Samples were collected and analysed from Blackness after 1 month and at the termination of the experiment at 12 months; logistical constraints prevented using samples at intermediate times here.

2.2.1 Organic enrichment

900g (to ensure full coverage of each plot) of FINASCO A120 food grade powdered *Ascophyllum nodosum* (macroalgae) was applied to each organic enrichment plot by carefully sprinkling it onto the surface of the mud. This powder has a maximum particle size of 120μm and contains 31.5% organic carbon and 0.9% nitrogen. This product was chosen as it is a natural, non-toxic substance derived from a species present at the sites, and has been used previously in other organic enrichment experiments (Schratzberger and Warwick 1998, Bolam et al., 2004).

2.2.2 Burial

Anoxic mud was spread evenly to a depth of 4cm on top of each treatment plot. The mud was taken from areas adjacent to the plots, and was obtained by scraping off the surface oxic layer and digging up the underlying mud. This was done to avoid transferring macrofauna on to the plots.

2.2.3 Raking

Each plot was raked twice to a depth of 4cm using a common garden rake. Each raking treatment was applied in a direction perpendicular to the previous application.

2.3 Sampling and processing of samples

2.3.1 Macrofauna

Macrofauna core samples were collected at 1, 3, 6 and 9 months at site one and 1 month and 12 months from site two using an 8cm diameter core inserted to a depth of 10 cm. The samples were preserved in 4% buffered formaldehyde solution. Samples were washed over a 500 μm sieve and all retained organisms identified where possible to species level.

2.4 Data analyses

2.4.1 Univariate analyses

Number of individuals (N), species number (S), species richness (Margalef, d) and species diversity (Shannon Wiener, H') were calculated for each sample. Species were split into five different trophic groups (Table 4.2) and the percentage contribution of each feeding type to the community was calculated. The abundance of species that were common to both sites and contributed >10% to the total number of individuals from each of the experimental sites were also included as factors in the univariate analyses. All data were checked for heteroscedasticity and normality and transformed (log (x+1)), 4th route or arcsine) as appropriate. Differences between the factors site, treatment type and block at the conclusion of the experiment were then assessed using a general linear model (GLM), with blocks nested within sites. Significant site × treatment interactions were analysed further using two-way Analysis of Variance (ANOVA) with treatment type and block as factors.

Table 4.2. Trophic groups allocated by species.

Feeding Type	**Site 1**	**Site 2**
Sub-surface deposit	*Tubificoides benedii, Tubificoides pseudogastre, Capitella capitata Aphelochaeta 'A'*	*Paranais litoralis, Tubificoides benedii, Tubificoides pseudogastre, Capitella capitata, Notomastus sp. Terebellida sp. Aphelochaeta 'A'*
Surface deposit	*Pygospio elegans, Streblospio benedicti, Manayunkia sp, Abra tenuis, Macoma balthica, Scrobicularia plana, Cerastoderma edulis*	*Aricidea catherinae, Pygospio elegans, Streblospio benedicti, Cossura longicirratta, Scoloplos armiger, Magelona sp, Polydora sp., Chaetozone setose, Manayunkia sp, Macoma balthica, Cerastoderma edulis, Corophium volutator*
Grazer	*Hydrobia ulvae, Limopontia depressa*	*Hydrobia ulvae, Limopontia depressa*
Omnivore	*Nereis diversicolor, Talitroides dorrieni, Leptocythere castanae, Carcinus maenas*	*Nereis diversicolor, Leptocythere castanae, Carcinus maenas*
Predator	*Nemertean sp, Tubellaria sp, Nephyts hombergii, Eteone flava, Parapionosyllis minuta.*	*Nemertean sp, Nephyts hombergii, Eteone flava, Phyllodoce mucosa, Gylcera sp., Sigalionid sp.*

Feeding types identified from Fachald and Jumars 1979, Hayward and Ryland (volume 1 and 2) 1990, Graham 1988, Thompson 1988, Ingle 1996, Rouse and Pleijel 2001.

2.4.2 Multivariate analyses

Data matrices were created using averaged abundance data from the four replicates collected at each sampling date for each site. Bray-Curtis similarity was then calculated using forth root transformed data, and from these Multi-dimensional scaling (MDS) plots were created to enable the visualisation of any community differences between treatments and also differences over time. R values (treatment vs. control) generated by one way Analysis of Similarity (ANOSIM) were used to rank the treatments in order of effect (distance from control). The similarity percentages routine (SIMPER) was utilised to assess the contribution of individual species to the dissimilarity between experimental plots. Principal Components Analysis (PCA) was carried out to assess the contribution of different trophic groups to observed multivariate patterns. Analyses were performed using Minitab 14 and PRIMER v6 software. (Minitab Statistical Software 2000, Clarke and Gorley 2006).

3. Results

Forty six species were identified, twenty six at site one (Creeksea) and thirty five at site two (Blackness). Of these, fifty eight percent of site one species and forty three percent of site two species were common to both sites. Only two species (*T. benedii* and *H. ulvae*) contributed > 10% to the total number of individuals at each site throughout the experiment.

3.1 Univariate analyses

The General Linear Model (GLM) Site Block (Site) Treatment Treatment*Site was used to assess treatment effects within blocks and site and between sites at the conclusion of both experiments (Table 4.3). At the conclusion of both experiments, no significant differences between control and non-sampled controls were observed; hence sampling *per se* did not have a detectable impact on the communities. Significant (P = <0.05) site and treatment effects were detected in all measures (with the exception of a site effect for H').

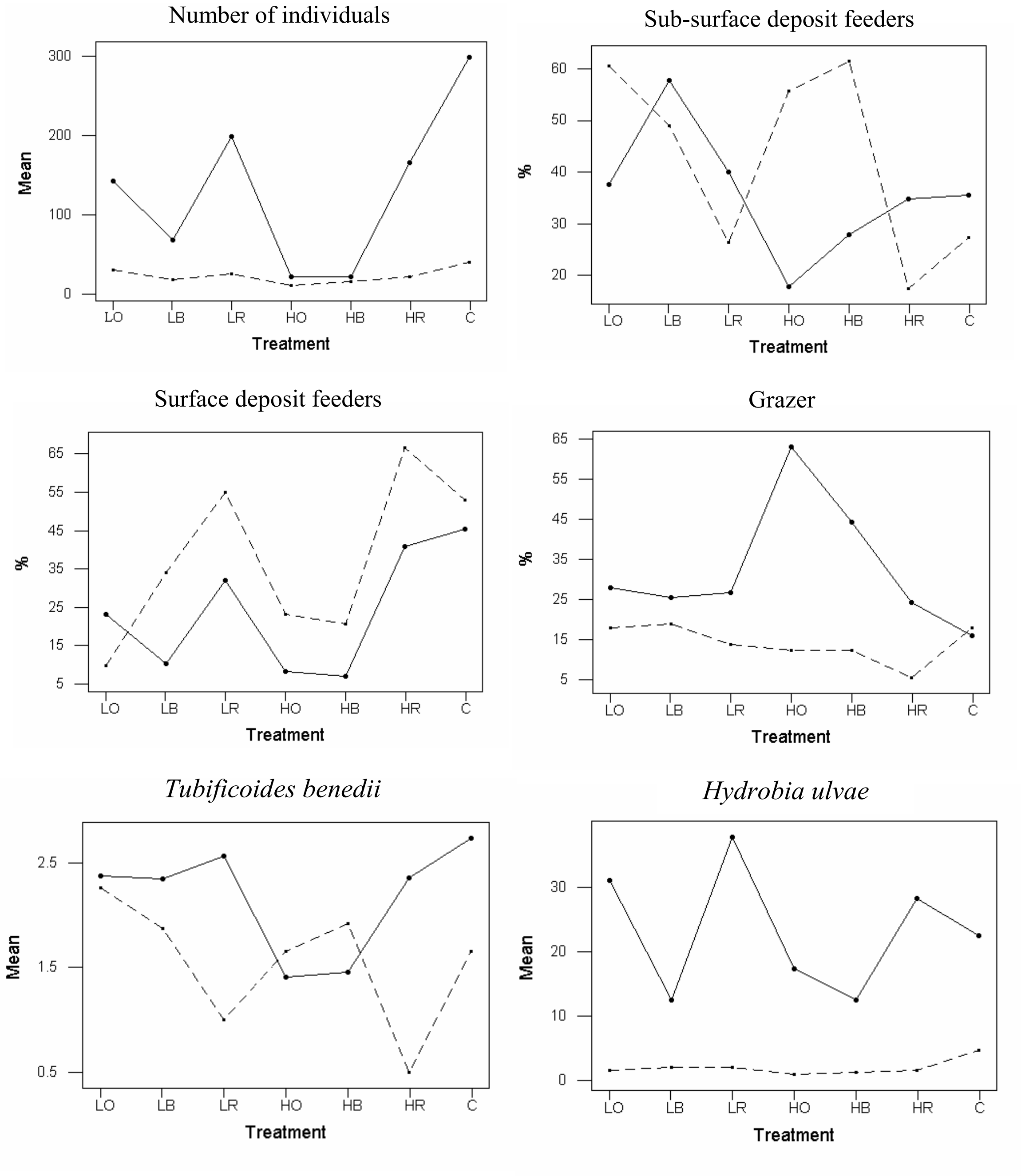
Table 4.3. Results from GLM showing significant treatment effects and site* treatment interactions (significance < 0.05). SSD = Sub surface deposit feeders, SD = Surface deposit feeders, G = Grazers, P = predators, O = Omnivores.

Measure	Site				Block(Site)				Treatment				Treatment*Site			
	DF	Adj SS	F	p	DF	Adj SS	F	p	DF	Adj SS	F	p	DF	Adj SS	F	p
N	1	160821	125.05	**<0.001**	2	609	0.24	0.79	6	145911	18.91	**<0.001**	6	102551	13.29	**<0.001**
S	1	18.286	6.69	**0.013**	2	0.714	0.13	0.878	6	196.71	12	**<0.001**	6	18.714	1.14	0.356
d	1	1.6186	11.49	**0.002**	2	0.0805	0.29	0.735	6	5.4297	6.43	**<0.001**	6	0.5242	0.62	0.713
H	1	616.2	2.229	0.138	2	0.04871	0.31	0.735	6	0.86	10.94	**<0.001**	6	0.89707	1.9	0.104
SSD	1	2562.2	4.6	**0.038**	2	560.5	2.09	0.137	6	4802.1	5.97	**<0.001**	6	6813.2	8.47	**<0.001**
SD	1	2562.2	19.61	**<0.001**	2	5.1	0.02	0.981	6	14530.1	18.53	**<0.001**	6	2207.4	2.82	**0.022**
G	1	4758	44.09	**<0.001**	2	253.4	1.17	0.319	6	2832.9	4.38	**0.002**	6	3812	5.89	**<0.001**
P	1	237.81	5.12	**0.029**	2	33.51	0.36	0.699	6	725.06	2.6	**0.032**	6	75.97	0.27	0.946
O	1	2567.23	66.32	**<0.001**	2	55.3	0.71	0.496	6	1488.13	6.41	**<0.001**	6	343.89	1.48	0.21
H. ulvae	1	6237.16	100.73	**<0.001**	2	170.64	1.37	0.265	6	1167.46	3.14	**0.013**	6	1122.46	3.02	**0.016**
T. benedii	1	16193.5	36.32	**<0.001**	2	554	0.62	0.542	6	16404.9	6.13	**<0.001**	6	20390.2	7.62	**<0.001**

Significant interaction terms between site and treatment were identified for number of individuals (N), the trophic groups sub-surface deposit feeders (SSD), surface deposit feeders (SD) and grazers (G), and for abundances of the species *H. ulvae* and *T. benedii* (Figure 4.2). Further within site analysis of these variables using two-way ANOVA gave significant treatment effects for N (F = 17.24, p = <0.001), SSD (F = 6.89, p=0.01), SD (F = 8.20, p = 0.001), G (F = 15.35, p = <0.001), *T. benedii* (F = 25.03, p = <0.001) and *H. ulvae* (F = 3.39, p = 0.028) at site one and SSD (F = 6.39, p = 0.002) and SD (F = 10.46, p = <0.001) at site two.

3.2 Trophic group analyses

The functional groups sub-surface deposit feeders, surface deposit feeders, grazers, omnivores and predators were represented at both sites (Table 4.2), and at both sites the sub-surface deposit feeding and surface deposit feeding trophic groups contained the most species (ten sub-surface deposit and three surface deposit feeding species at Creeksea (site 1) and sixteen sub-surface deposit and seven surface deposit feeding species at Blackness (site 2). The raking treatments had the smallest effects on trophic group distributions at both sites, although effects on individual species (*Aphelochaeta 'A'* and *A. tenuis*) were observed at Creeksea (Figure 4.3a). A reduction in the contribution of sub-surface deposit feeders (particularly *T. benedii*) and surface deposit feeders (particularly *S. benedicti*) in both the high organic and burial treatments were observed at Creeksea (Figure 4.3a). Grazers increased in proportional importance under burial and organic enrichment treatments at Creeksea, a result driven largely by *H. ulvae* and reflected in the significant treatment effect on this species at this site. In contrast, at Blackness (Figure 4.3b) the proportional contribution of sub-surface deposit feeders increased in organic enrichment and burial treatments. This reflected an increase in the abundance of *T. benedii* under low organic enrichment, and a reduction in the surface deposit feeding organisms (*S. benedicti* and *M. balthica*) in the high burial and organic treatments. Grazers showed little response to treatments at this site, despite the presence of *H. ulvae* as the dominant grazer here.



Number of individuals

Sub-surface deposit feeders

Surface deposit feeders

Grazer

Tubificoides benedii

Hydrobia ulvae

Figure 4.2. Significant interactions between site one and site two. LO = Low organics, LB = Low burial, LR = Low raking, HO = High organics, HB = High burial, HR = High Raking, C = Control.

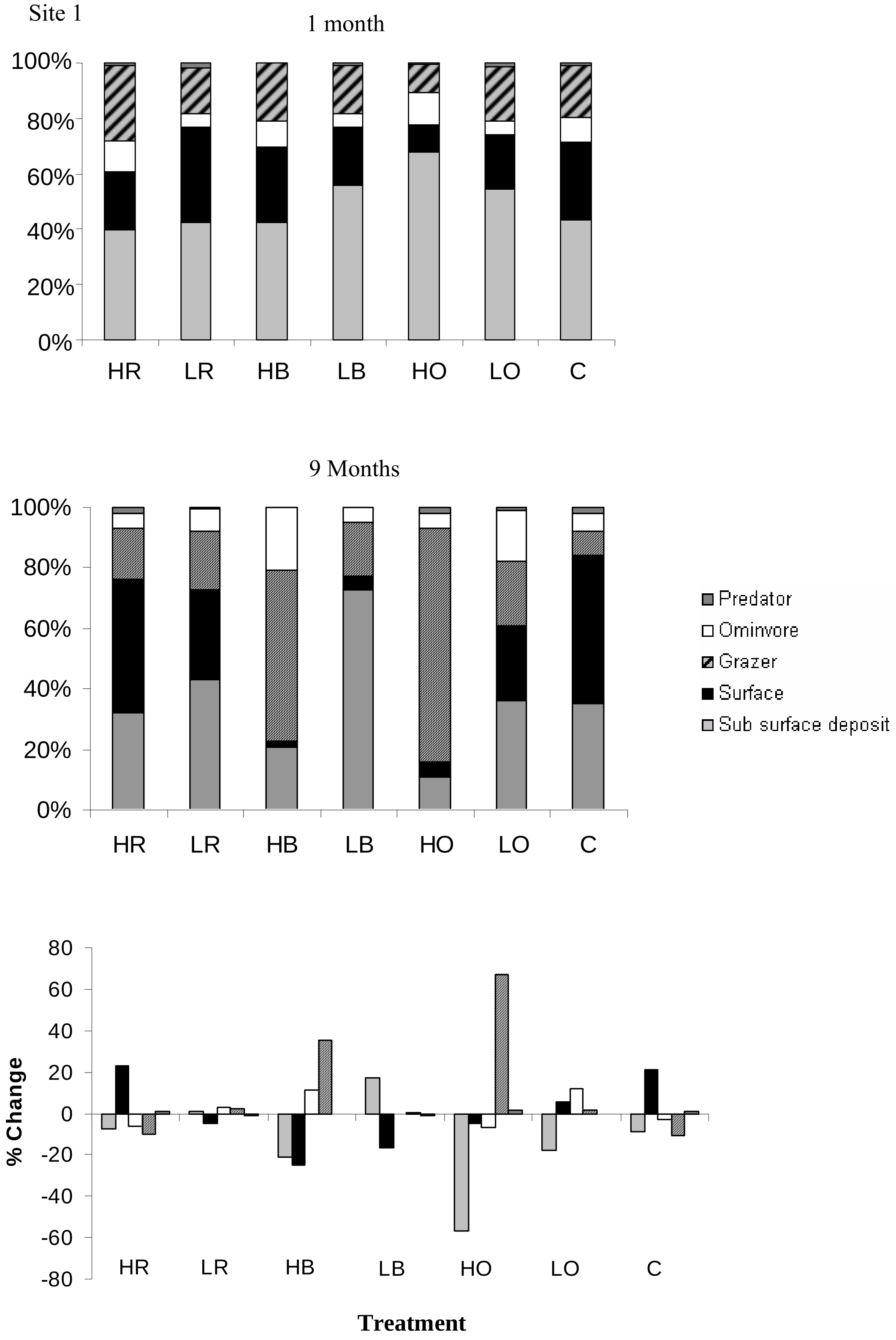


Figure 4.3a. Percentage contribution and change over time of feeding groups at site 1 (Creeksea) 1 and 9 months. LO = Low organics, LB = Low burial, LR = Low raking, HO = High organics, HB = High burial, HR = High Raking, C = Control.

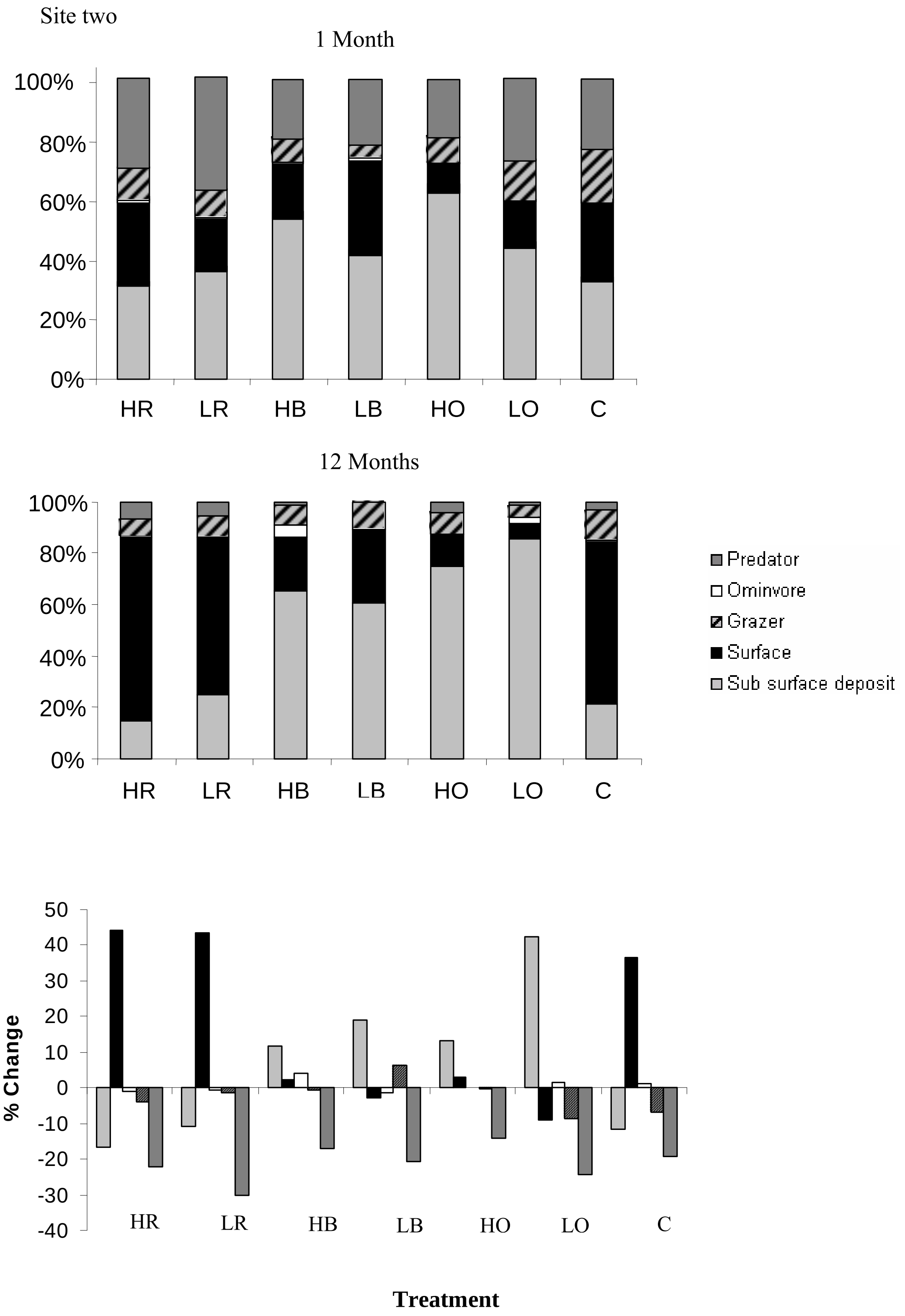


Figure 4.3b. Percentage contribution and change over time of feeding groups at site 2 (Blackness) 1 and 12 months. LO = Low organics, LB = Low burial, LR = Low raking, HO = High organics, HB = High burial, HR = High Raking, C = Control.

3.3 Multivariate analyses

Treatments were ranked at both sites using the R statistic generated from a one way ANOSIM test; the higher the R value (0-1) the greater the differences between treatment and control. Whilst both high organic enrichment and low burial treatments scored the same rank at each site, the other treatments differed in rank score between sites (Table 4.4).

Table 4.4. Results from one way ANOSIM. 9 month treatments vs. 9 month controls (Site one) and 12 month treatments vs. 12 month controls (Site two). Treatments ranked using the test statistic (R). 1 = high, 6 = low. LO = Low organics, LB = Low burial, LR = Low raking, HO = High organics, HB = High burial, HR = High Raking, C = Control.

Treatment	Rank		R Statistic		Significance	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
HO	1	1	1.000	0.792	0.029	0.029
LO	4	2	0.844	0.729	0.029	0.029
HB	1	4	1.000	0.396	0.029	0.029
LB	3	3	0.990	0.646	0.029	0.029
HR	6	5	0.410	0.208	0.057	0.500
LR	5	6	0.630	-0.031	0.029	0.170

The high and low intensity organic enrichment treatments were more dissimilar to the control than any of the other treatments at Blackness. In contrast, the high organic and high burial treatments were ranked joint first at Creeksea, with low organic enrichment ranked only fourth here. Interestingly, the low burial treatment was more dissimilar to the control samples than the high intensity burial treatment at Blackness, and the low raking treatment caused a greater difference than the high raking treatment at Creeksea. Species most responsible for the dissimilarities between treatments and controls at each site were the polychaete worms *Streblospio benedicti* (Spionidae) and *Aphlochaeta 'A'* (Cirratulidae), the bivalve mollusc *Abra tenuis* (Tellinacea) and the oligochaete worm *Tubificoides benedii* (Tubificidae) at Creeksea and the polychaete worms *Pygospio elegans* and *Streblospio benedicti* (Spionids), the bivalve mollusc *Macoma balthica* (Tellinacea) and the oligochaete worm *Tubificoides benedii* (Tubificidae) at Blackness (Figure 4.4).

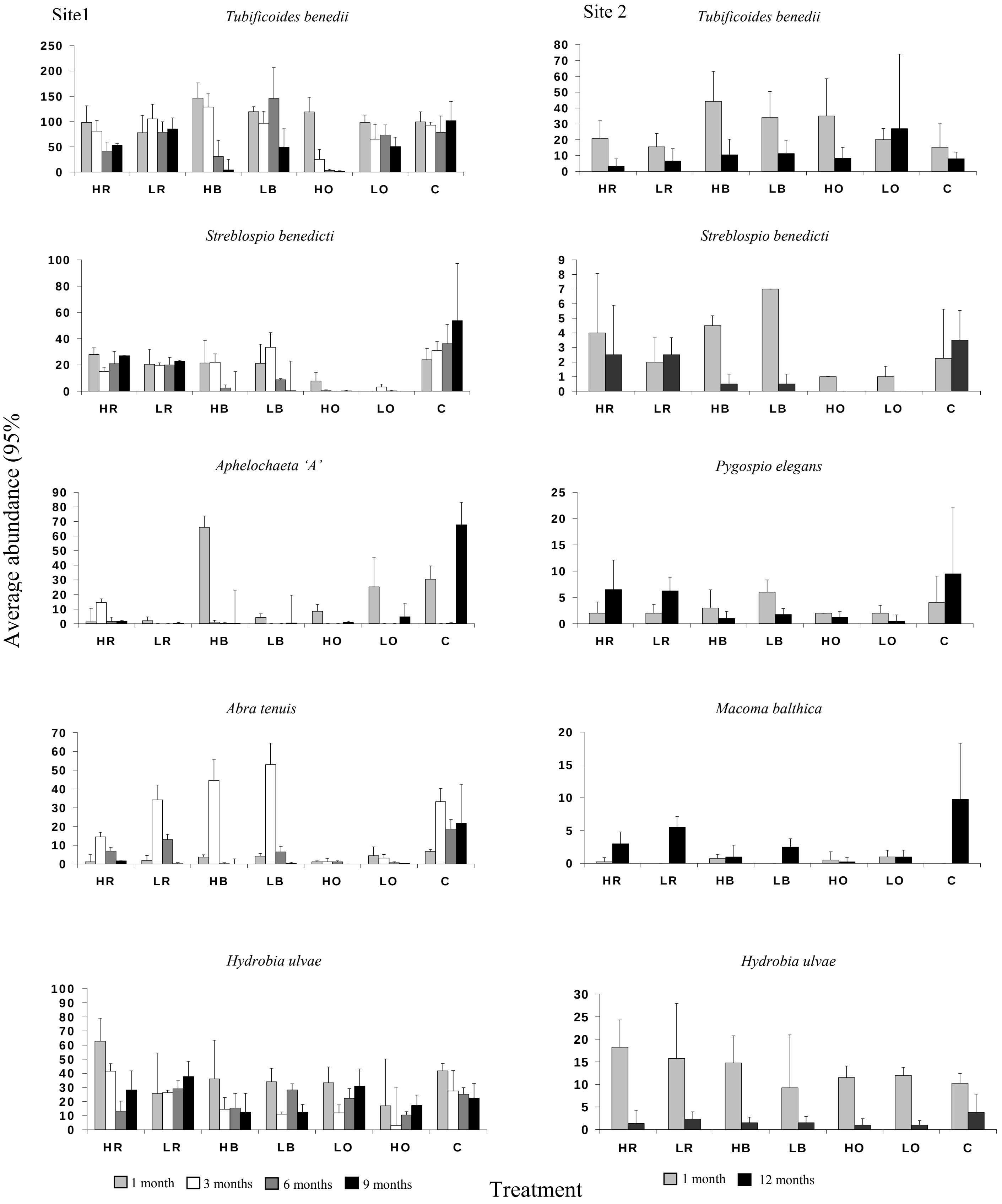


Figure 4.4. Average abundance of species that consistently contributed to the dissimilarity between sites. LO = Low organics, LB = Low burial, LR = Low raking, HO = High organics, HB = High burial, HR = High Raking, C = Control.

At Creeksea numbers of *T. benedii* increased in both burial treatments until the third month (high burial) and sixth month (low burial). In the high organic treatment their abundance increased initially (one month) then decreased thereafter. Numbers of *S. benedicti* were reduced in all treatments. *Aphelochaeta 'A'* was severely affected by the raking treatments, interestingly more so by the low intensity treatment. Within the high burial treatment numbers of *Aphelochaeta 'A'* increased initially in month one then dropped in the following months. The bivalve *A. tenuis* was particularly affected by both the organic enrichment treatments, but increased abundances were observed in both burial treatments. At Blackness increased numbers of *T. benedii* and *S. benedicti* were found in both burial treatments after one month. Increased numbers of *T. benedii* were also observed in the low organic treatment after twelve months. The low and high intensity raking treatments appeared to have little effect on *P. elegans, T. benedii, S. benedicti* or *M. balthica.* The multi-dimensional scaling plots showed clear differences between treatments at both sites, and site-specific responses to treatments (Figure 4.5).

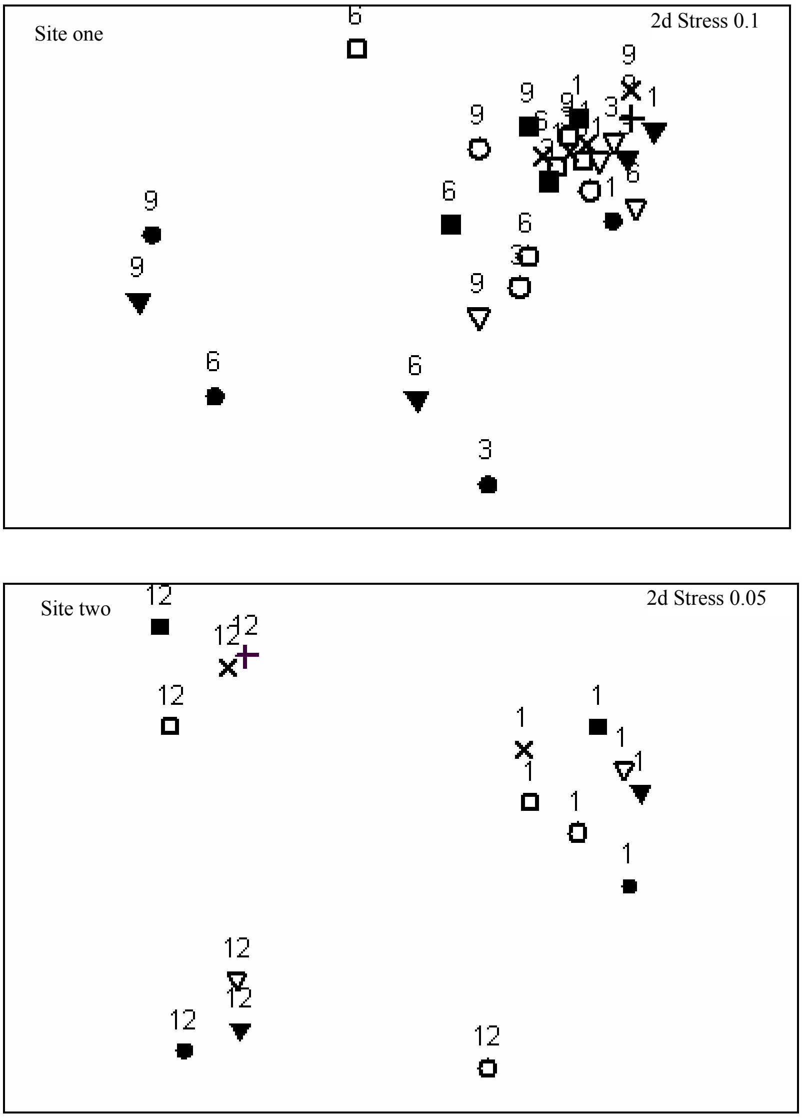


Figure 4.5. MDS plot of averaged square root transformed community data from site one (1 to 9 months) and site two (1 month and 12 months). LO =○ Low organics, LB = ▽ Low burial, LR =☐ Low raking, HO = ● High organics, HB = ▼ High burial, HR =■ High Raking, C = X Control, NSC = + Non sampled control

Organic enrichment and burial treatments from both experiments produced the greatest changes in community structure when compared with the control community. PCA analysis on trophic groups showed that numbers of individuals within the sub-surface deposit feeders, surface deposits feeders and grazing trophic groups were found to be the main contributing factors to the multivariate patterns found at both experimental sites. (Figure 4.6a /b).

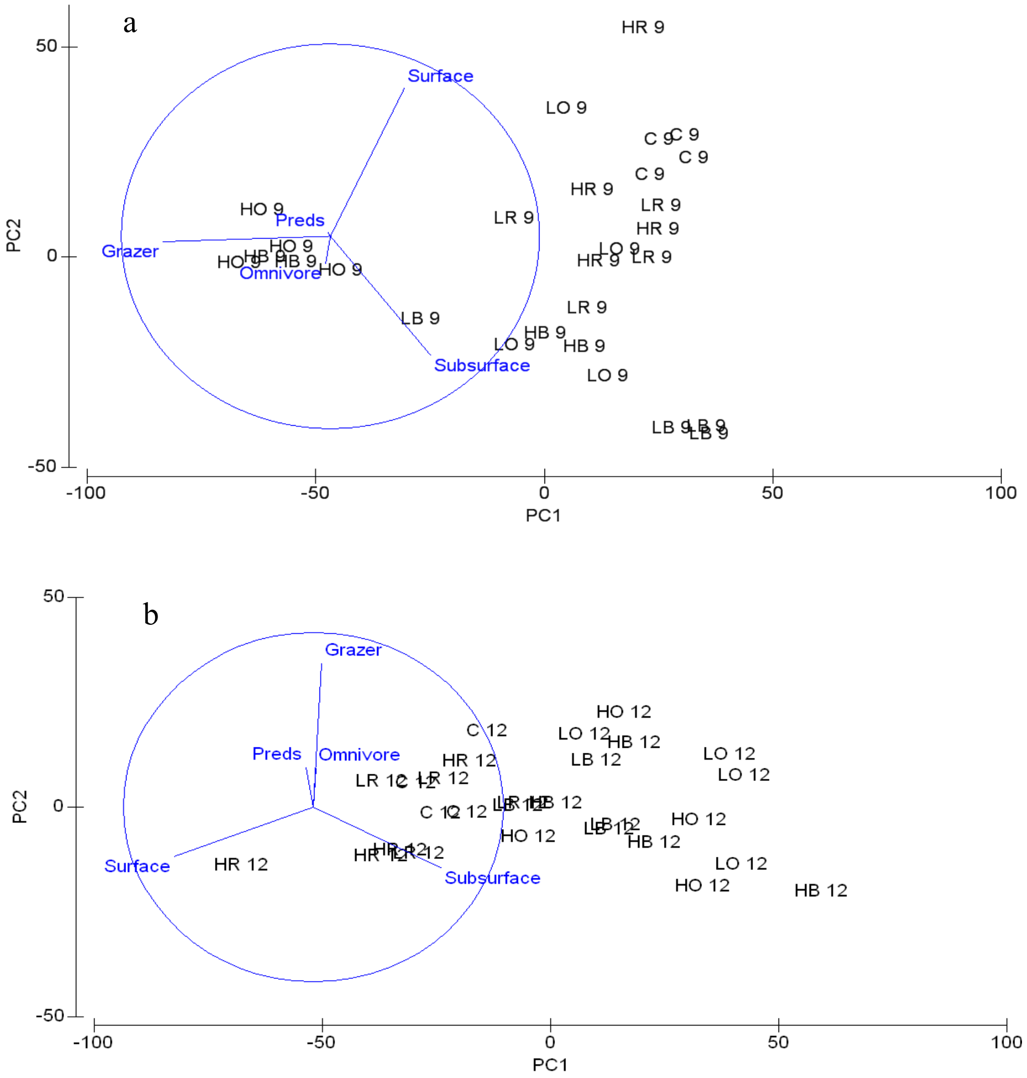


Figure 4.6a-b. Principal Components Analysis (PCA) of feeding types at the conclusion of each experiment. Vector plot indicates variable most responsible for the orientation of points LO = Low organics, LB = Low burial, LR = Low raking, HO = High organics, HB = High burial, HR = High Raking, C = Control.

Principal components (PC) 1 and PC2 accounted for 94.3% and 90.3% of variability at Creeksea and Blackness respectively. At Creeksea Eigenvector values indicted that sub-surface deposit feeders (0.481) defined the low burial treatments, surface deposit feeders (0.770) the high raking treatment (PC2) and grazers (-0.802) the high burial and high organic treatments (PC1). While at Blackness sub-surface deposit feeders (0.675) characterised treatments high organic, low organic and high burial with surface deposit feeders (-0.735) defining the high raking and low raking treatments (PC1).

4. Discussion

The same type and frequency of disturbance, applied at two different sites, produced different responses at the community level. Communities exposed to the high burial, low burial, high organic and low organic enrichment treatments differed significantly from the control assemblages at both experimental sites. In contrast, the raking treatments had an effect at Creeksea only. The magnitude of community change, relative to controls, was different between sites, as were the rankings of different disturbance types. Whilst the raking treatments caused the smallest community changes at both sites, burial had a larger relative effect at Creeksea, and organic enrichment caused greater changes at Blackness. Hence the first null hypothesis of no site × treatment interaction was rejected.

The effects of the same disturbance type at the different experimental sites were also different at the trophic group and species level. Trophic group responses at Creeksea were dominated by proportional changes in grazer numbers (PC1, Figure 4.6a) whilst at Blackness surface and sub-surface deposit feeders were most important (PC1, Figure 4.6b). Most disturbance treatments had greater impacts on total abundance at Creeksea compared with Blackness (Figure 4.2), although this was a species-specific effect. For example, *T. benedii* showed mean reductions in every disturbance treatment, compared with controls, at Creeksea, but mean increases in three treatments at Blackness. In contrast, *H. ulvae* abundance was reduced in all disturbance treatments at Blackness, but showed increases at Creeksea. Hence the second null hypothesis of no site × treatment interactions at the species and trophic group levels is also rejected.

The effects of disturbance, of whatever kind, may be considered as pushing communities along a single continuum from an undisturbed to a severely disturbed state (Rhoads and Boyer 1982, Rhoads and Germano 1986). In models assuming this pattern, knowledge of the intensity of any novel disturbance, combined with the original disturbance regime experienced by a community (i.e. its ‘starting point’), should be sufficient to predict final community characteristics. The current results do not conform to such a linear interpretation. At both sites, the intensity of treatments did not always predict the degree of disturbance, and the anomalous treatment differed between sites. Low intensity raking (Creeksea) and burial (Blackness) had larger effects than the relevant high intensity treatments. Hence the third null hypothesis of linear response is also rejected.

At Creeksea the high burial treatment was ranked equal first with the high organic treatment. In contrast, this treatment was ranked only fourth at Blackness with the low organic treatment having a larger relative effect. These differences reflect the physical and biological conditions at the two experimental sites (Huxham et al., 2000, Bolam and Fernandes 2002b, Bolam et al., 2004). Blackness appears more dynamic than Creeksea, with a relatively large change between the start and end of the experiment in control treatments, whilst Creeksea has higher ambient carbon levels than Blackness. Due to the development of communities within natural disturbance regimes the physical disturbances (burial and raking) had less relative effect on the Blackness community than on the relatively sheltered Creeksea community, whilst organic enrichment had a greater relative impact at Blackness. Blackness generally showed smaller absolute responses to all treatments (as demonstrated by the smaller R values generated in the ANOSIM tests) reflecting the higher natural resilience to physical disturbance of communities there. However, there were exceptions to this pattern. For example, *H. ulvae* was generally more resilient at Creeksea than at Blackness, and showed a 68% relative increase in mean abundance in the low raking treatment compared with the control at Creeksea.

Communities that are frequently disturbed by sediment movement or naturally rich in organic material would be expected to contain species capable of surviving in such environments, and may therefore show greater resilience in the face of further physical disturbance or enrichment. Our results support this idea for Blackness and physical disturbance. In contrast, the effects of organic enrichment at Creeksea are more complex. Although the relative importance of the enrichment treatments is lower here, the absolute effect is higher than at Blackness (Table 4.4). The dominant species *T. benedii,* which is known to be highly tolerant of enrichment (Mendez 2002), suffered significant reductions in abundance in the high organic treatment at Creeksea (and all treatments showed some reductions here, in contrast to Blackness). Hence high ambient carbon at Creeksea does not necessarily enhance community or species' resilience in the face of further organic enrichment. Instead, it is likely a threshold was passed here beyond which most species and individuals cannot survive. Such an effect was not noticed for *H. ulvae*, perhaps because its epibenthic niche allows it to avoid severe anoxia.

The most influential general theory relating disturbance to community structure is the Intermediate Disturbance Hypothesis (Grime 1973, Connell 1978). Previous work (Mackey and Currie 2001) including intertidal benthic communities at Blackness

(Huxham et al., 2000), has generally failed to find supporting evidence for this model, possibly because these communities experience levels of natural disturbance high enough to obviate competitive effects. The current work is mostly consistent with this, with all types and intensities of disturbance lowering diversity and abundance in comparison with controls. However, there was limited evidence of enhanced numbers of some species, such as a (non-significant) increase in *T. benedii* at Blackness under the low organic treatment, and a significant increase of *H. ulvae* at Creeksea under low raking. The Pearson and Rosenberg (1978) model of gradual replacement of large bodied sensitive species with smaller bodied opportunist ones under increasing intensity of disturbance provides a better picture of the responses that was recorded. Although this was originally developed to describe community response to organic enrichment, it has also been proposed (with modification) as a general model of benthic succession under other types of disturbance (Rhoads and Boyer 1982, Rhoads and Germano 1986, Bolam and Rees 2003) However, the non-linearity of our results, with lower intensity treatments having bigger impacts in some cases than high intensity ones, demonstrates the complex responses encountered when assessing effects of disturbance at the community level. Although models based on a single successional continuum, such as that described by Pearson and Rosenberg (1978), may allow accurate general predictions, they cannot accommodate site-specific contingencies that result in changes in the usual ordering of disturbance effects.

Allocating species to feeding types and assessing changes in their percentage contribution allows the effects of disturbance to be examined at a functional level. This approach can simplify complex food webs, and allow the comparison of similar communities that contain different species but the same functional groups (Gaston et al., 1998). Such comparisons may lead to a better understanding of community dynamics, and to predictive tools of wide potential relevance. However the trophic groups used in the current work showed quite different responses at the two sites. Whilst Creeksea saw a proportional expansion in grazers in the high impact treatments, sub-surface deposit feeders showed the largest proportional response at Blackness. In the present study, trophic groups and species were strongly correlated, since the dominant species in these two groups (*T. benedii* and *H. ulvae* respectively) were the same at both sites. This overlap further emphasises the importance of site-specific factors in determining their disparate responses; disturbance treatments were identical, as were the niches of the dominant species involved. Despite the greater between-site variation in species in the surface deposit feeders, the response of this trophic group was more consistent, with reductions in proportional abundance under high disturbance at both sites. This perhaps reflects the higher relative sensitivity of most species within this niche to disturbance; they are generally less well adapted to anoxia than sub-surface deposit feeders (Mucha and Costa 1999), and many (including *S. benedicti, P. elegans, Aphelochaeta sp.* and *C. volutator)* construct burrows considered to be sensitive to physical disruption. (Wilson 1981, Flach 1992, Brown and Wilson 1997).

Under most field conditions multiple sources of disturbance, both natural and anthropogenic, are confounded. For example, whilst the physical disturbance regime often provides a good explanation for broad trends in subtidal community structure (e.g. Hall 1994), the level of physical disturbance may be correlated with anoxia and hypoxia, grain size, organic content and other variables. The current work demonstrates that different types of disturbances may produce different types of response. Regarding these multiple sources of disturbance as examples of the same phenomenon differing only in intensity, may be a useful approximation when covariance is high, but could prove misleading if the effects differ (such as relative enhancement of one species or trophic group under organic enrichment, but reduction under burial). Similarly, considering different sites as representing different points along the same linear disturbance continuum is an over-simplification. The current work demonstrates that this is not simply because of differences in species or trophic groups at different places. Even when the species or trophic group is the same, its response to disturbance may depend on site-specific factors such as the history of prior disturbance and the inherent ecological plasticity exhibited by many benthic species (Davic 2003). Whilst current models perform well in predicting benthic responses to gross disturbance, detecting subtler effects requires a recognition that community response may depend on the site, the species and the sources of disturbance.

Chapter five

Differential response of nematode and macrofauna to *in-situ* burial

Published in: Journal of the Marine Biological Association of the UK (2009) 89(6) 1091-1098

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1. Introduction

Historically, most indicator work has focused on the macro-benthic infauna (defined as organisms retained on a 500µm mesh sieve (Warwick 1986)). Until recently the importance of small zoobenthic organisms within the marine environment has been underestimated. Although their biomass may be insignificant compared to larger invertebrates, the have been shown to contribute disproportionately to benthic production (Platt and Warwick 1980, Kuipers et al., 1981, Raffaelli and Hawkins 1996). This complex "Small Food Web", consisting of bacteria, microfauna, meiofauna, temporary meiofauna and small macrofauna is characterised by the small size of individuals (< 500µm), a high turnover rate, and relatively short life spans. Kuipers et al., (1981) showed that in intertidal environments these groups contribute to the production of food for juvenile stages of commercially viable carnivores such as shrimp, crab and fish.

Macrofaunal communities are regularly utilised in impact studies. (See Table 4.1 chapter 4.) However, very few studies are carried out utilising additional data from nematode communities (Warwick et al., 1990, Somerfield et al., 2006). A literature search using the search engine Scopus (www.Scopus.com) covering the last twenty years, using the key words 'Macrofauna' AND 'Disturbance' then 'Meiofauna' AND 'Disturbance' and finally 'Macrofauna AND Meiofauna' AND 'Disturbance', gave 210, 115 and 36 hits respectively. The topics covered were very similar and included physical disturbance, organic enrichment, effects of fishing gear (dredges and trawls), mariculture and the assessment and monitoring of anthropogenic activities.

Nematode and macrofauna communities may behave differently under the same disturbance regime, increasing their utility as disturbance indicators when used together. The short life cycle, fast recolonisation rates and all year round reproduction of nematodes is thought to affect their response to disturbance events (Warwick and Buchanan 1971, Schwinghammer et al., 1986, Alves et al., 2003, Lampadariou 2005), hence meiofaunal communities might be expected to show higher resilience in the face of disturbance than macrofaunal communities. For example, Bolam et al., (2006) found that, when benthic communities were buried during the beneficial recharge of a mudflat, the disturbed nematode communities recovered rapidly and univariate community metrics were comparable to those of reference communities with respect to spatial and temporal patterns. In contrast, macrofauna community indices were found to be significantly lower when compared with reference sites. Sherman and Coull (1980) found that, after sediment was disturbed creating anoxic conditions, meiofauna communities recovered within one tidal cycle, much faster than any reported results from macrofaunal studies. However, other studies investigating disturbance events using meiofauna communities have shown that responses can be similar to those displayed by some macrofauna (Coull and Chandler 1992, Austen and Widdicombe 2006). Observed effects of various types and intensities of disturbance on nematode communities have included increased evenness, initial increases in abundance, reduced species richness and changes in community assemblage (Moore et al., 1987, Sandulli and Giudici 1989, Schratzberger et al., 2002a Schratzberger et al., 2002b, Lampadariou 2005, Dye 2006). Nematode species-specific responses to burial have also been documented (Schratzberger 2000). These have been attributed primarily to the variable ability of nematode species to migrate upwards through the deposited material.

Meio- and macrofaunal assemblages do not exist in isolation and therefore are part of an interacting system (Zobrist and Coull 1992, Tita et al., 2000). Our understanding of benthic systems and how they behave in response to disturbance events may therefore be improved if a more holistic ecosystem approach to disturbance impact studies was taken, considering more than a single faunal group (Warwick et al., 2005).

This chapter differs from previous studies (Austen et al., 1989, Austen and Widdecombe 2006) in that this data was derived from a controlled experiment set up in the field and not from data collected from mesocosm experiments (lack of natural conditions) or field data (little control and no accuarate quantification of the impact being studies) The main aims of this chapter are to compare and contrast the effects that two intensities of *in-situ* burial have on the meiofaunal nematode and macrofaunal communities of an intertidal mudflat.

The following null hypotheses were addressed.

- Meiofaunal nematode and macrofaunal communities show the same pattern of response to disturbance by *in-situ* burial

- There are no differences in the effects of different intensities of *in-situ* burial on meiofaunal nematode and macrofaunal communities.

2. Materials and methods

2.1. Study sites

The Crouch Estuary is a sea inlet which is dominated by tidal ebb and flow of high-salinity waters. A field manipulation experiment was carried out on mud flats mid-way along the estuary (51°38.20N, 00°42.80E) that are sheltered from the main prevailing wind and tide. The sediments are fine (90% silt/clay) with 12% organic content (loss on ignition). The macroinfaunal community is dominated by tubificid oligochaetes, polychaetes from the family cirratulidae and the gastropod mollusc *Hydrobia ulvae* (Bolam et al., 2004). Dominant meiofaunal nematode species include the chromadorids *Sabatieria punctata*, *Molgalaimus demani*, *Pytcholaimellus ponticus Metachromadora vivipara* and *M. remanei*, *Chromadora macrolaima*, *Cyatholaimus gracilis* and the monhysterids *Terschellingia longicaudata* and *Terschellingia communis*.

2.2 Experimental design.

The experiment involved a replicated random block design and was initiated in October 2003 (Figure 5.1). Samples were taken at one, three, six and nine months post set-up; the experiment was then terminated after ten months, two months earlier than planned, due to unusually high densities of seasonal algal growth. Non-sampled control, control, high intensity and low intensity burial treatments were randomly allocated to twenty four 1 m^2 plots, divided between three blocks, with two replicates of each treatment per block. These treatments represented a subset of the experimental treatments described in Chapter four. Control plots were sampled at the same time as the treatment plots, whilst non-sampled controls were only sampled at the end of the experiment, to allow for the detection of any effects of sampling *per se* on community structure. Due to time constraints only one replicate from each of the blocks (two burial intensities and two control treatments) were processed.

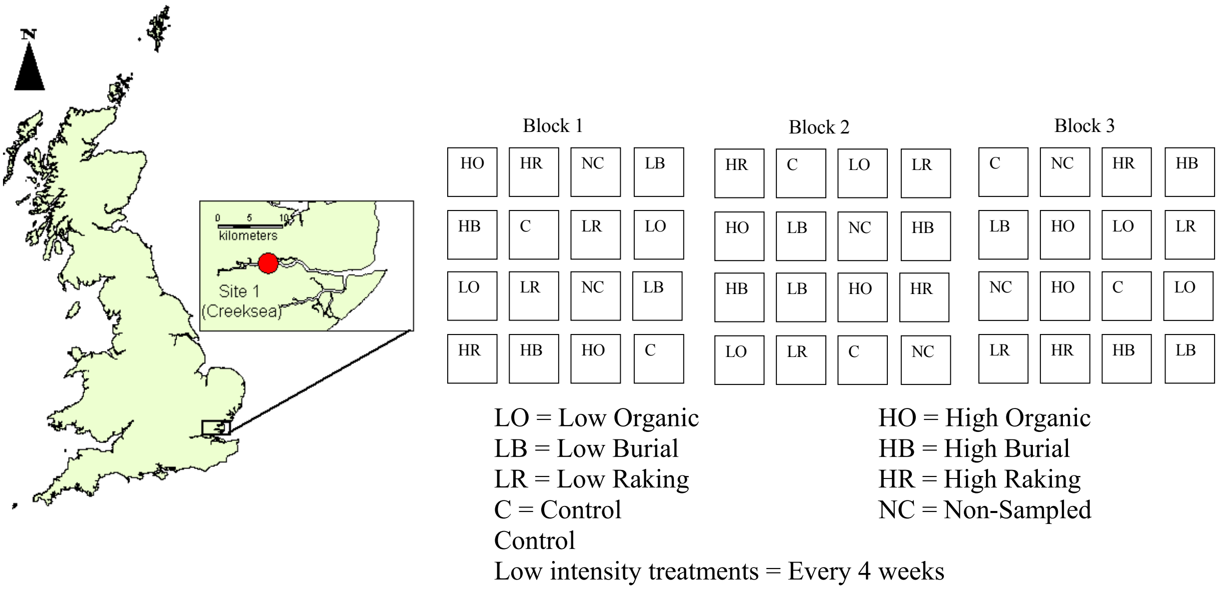


Figure 5.1. Locations of experimental site and experimental setup. Site one Creeksea, Crouch Estuary, Essex.

2.3 Disturbance

In-situ burial was achieved by spreading 4cm of anoxic mud on top of each treatment plot. The mud was taken from areas adjacent to the plots, and was obtained by scraping off the surface oxic layer and digging up the underlying anoxic mud. This was done to avoid transferring macrofauna on to the plots. The high intensity treatments were applied every two weeks and the low intensity treatment every four weeks.

2.4 Sampling and processing of samples

2.4.1 Macrofauna

Samples were collected one, three, six and nine months after set-up using an 8cm diameter core inserted to a depth of 10 cm. The samples were preserved in 4%

buffered formaldehyde solution. Samples were washed over a 500 μm mesh sieve and all retained organisms identified, where possible, to species level.

2.4.2 Meiofauna

Samples were collected one, three, six and nine months after set-up using a 5cm diameter core inserted to a depth of 5 cm. The samples were preserved in 4% buffered formaldehyde solution. Samples were washed over a 63 μm sieve and extracted using Ludox (McIntyre and Warwick 1984; Somerfield and Warwick 1996). As nematode abundances were high, 5% sub-samples were taken. The sub-samples were then evaporated slowly in anhydrous glycerol and mounted on semi-permanent slides for identification and counting.

2.5 Data processing

2.5.1 Univariate

Number of individuals (N), species number (S), species richness (Margalef, d) and species diversity (Shannon Wiener, H') were calculated for each nematode and macrofauna sample. All data were checked for heteroscedasticity and normality and transformed where necessary. Differences in the response of the two faunal communities over time were assessed using repeated measures General Linear Models with within-subject factor of time (1, 3, 6 and 9 months) and between-subject factor faunal type. Separate analyses were performed for each treatment (high burial, low burial and control) in order to test for significant interactions between meiofaunal nematodes and macrofauna. The responses of the two types of community to the treatments were analysed in separate repeated measures analyses with time and treatment as factors; these were supplemented with one-way Analysis of Variance

(ANOVA) within times where significant interactions were found. Repeated measures analysis was carried out using SPSS version 14 (SPSS UK).

2.5.2 Multivariate

Bray-Curtis similarity matrices were calculated for both faunal types using square-root transformed data and, from these, Multi-dimensional scaling (MDS) ordinations were produced to display any community differences between treatments over time. R-values (treatment vs. control) generated by one way Analysis of Similarity (ANOSIM) were used to rank the treatments in order of effect (distance from control).

Supplementing the illustration of fauna-specific temporal patterns in MDS ordination plots, the RELATE permutations test was used to determine the temporal inter-relationships between faunal matrices based on univariate indices of meiofaunal nematode and macrofauna communities. This test compares the relationships between independently derived similarity matrices (based on Bray-Curtis similarity) from matched sample data, therefore allowing the significance of the relationship of temporal patterns in the two types of community to be assessed. A Spearman rank correlation of $\rho = 1$ implies a perfect match between assemblages whereas a Spearman rank correlation of $\rho = 0$ implies no match (Clarke et al., 2006). The Similarity Percentages (SIMPER) routine was carried out to assess the contribution of individual species to the dissimilarity between treatments and controls (Clarke and Warwick 1994, Clarke and Gorley 2006).

3. Results

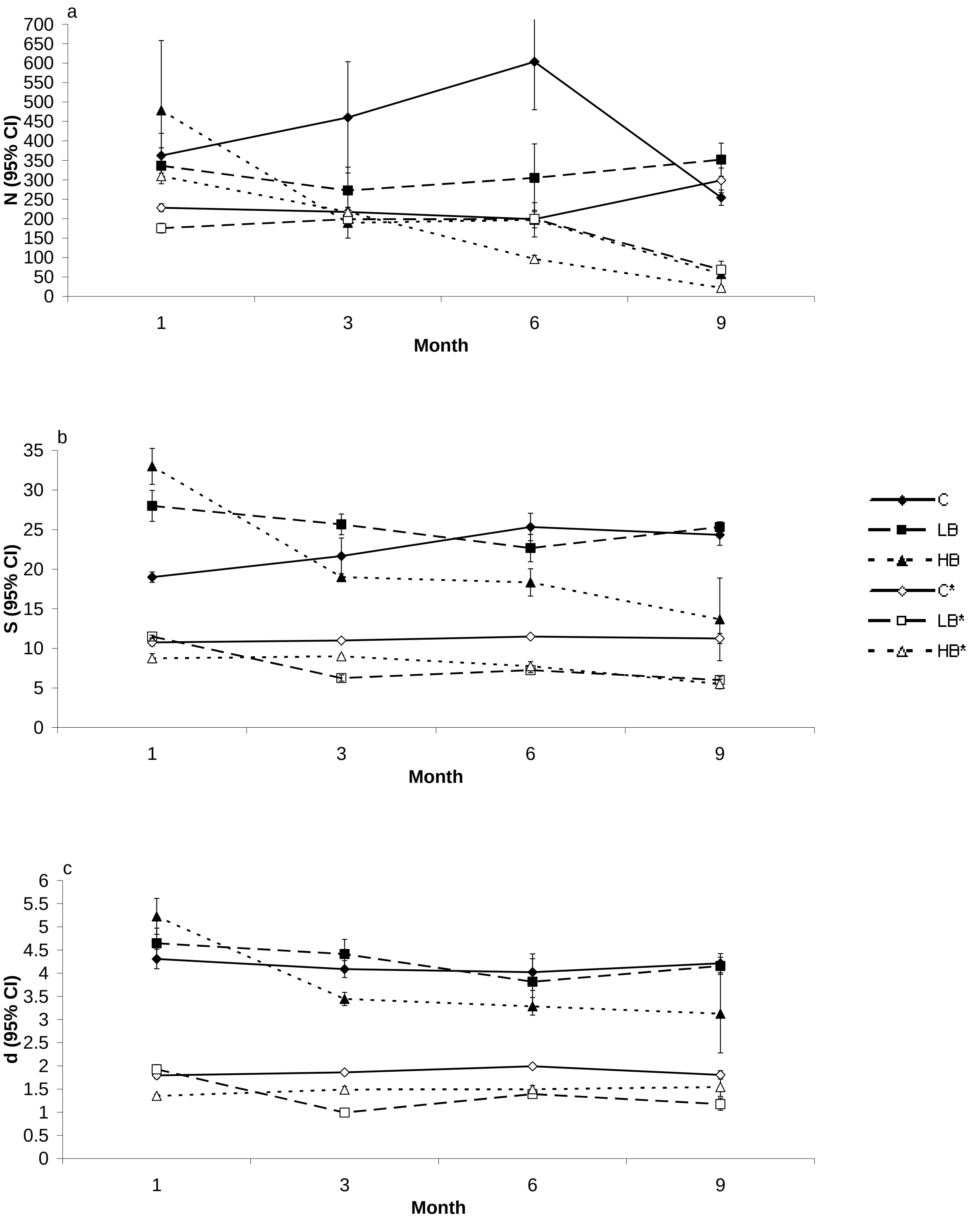
Forty-six meiofaunal nematode and twenty six macrofaunal species were identified during this study. At the end of the experiment at Creeksea, no significant differences between control and non-sampled controls were observed; hence sampling *per se* had no impact on the communities.

3.1 Physical observations

Mounds were formed at the sites of the high and low burial treatments, though these never exceeded the height of the lowest tide. Anoxic black patches of mud were observed at the sites of the high organic treatments and recesses observed at the sites of the high raking treatments.

3.2 Univariate analyses

Figures 5.2 a-d illustrate observed spatio-temporal patterns in the univariate measures for meiofaunal nematode and macrofaunal communities. Clear differences in community behaviour over time and in response to disturbance treatments were observed. Increases in nematode abundances, species number, richness and diversity in response to disturbance were revealed in month one of the experiment. This was not apparent in results from the macrofaunal data, where there was a reduction in S, d and H'. In the case of species richness and diversity, the low intensity burial appeared to have a greater effect over time on the macrofaunal community than high intensity burial. This was not observed in the meiofaunal data. In month six, nematode abundance increased significantly in the control only and this was primarily attributed to seasonal fluctuations in *Chromadora macrolaima* and *Molgalaimus demani* densities.


a
N (95% CI)
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600
550
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S (95% CI)
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d (95% CI)
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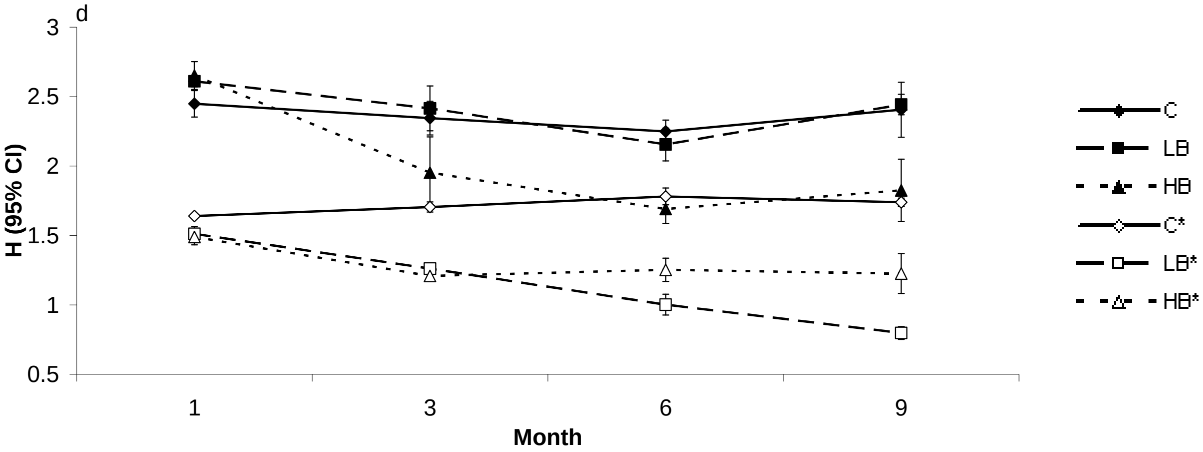


Figure 5.2. a-d. Mean (± 95% C.I.) nematode and macrofauna* community indices over time. Open symbols = macrofauna, shaded symbols = meiofaunal nematodes. C = control, LB = low burial, HB = high burial.

All assumptions of sphericity (equality of the variances of the differences between levels of the repeated measures factor) were passed for all Repeated Measures General Linear Model analyses. Due to multiple testing and therefore an increased risk of false positive results, an adjusted alpha value (Bonferroni correction) of p = 0.0042 was used. Repeated Measures analyses with within-subject factors of time (1, 3, 6 and 9 months) and the between-subject factor of faunal type (meiofaunal nematodes and macrofauna) revealed several significant interactions (Table 5.1). Interestingly, these interactions included control treatments.

Table 5.1. Significant interactions between meiofaunal nematodes and macrofauna (p = 0.0042) results from the Repeated Measures General Linear Model.

Indices	Treatment	DF	MS	F	p
Abundance (N)	Control	5	61204.9	12.33	<0.001
Species number (S)	High burial	5	94.01	30.11	<0.001
Species richness (d)	High burial	5	1.923	15.160	<0.001
Species diversity (H’)	Control	5	0.248	18.166	<0.001
	Low burial	5	0.262	8.996	0.001
	High burial	5	0.337	0.328	0.001

Assessing differences between treatments within faunal type, results from repeated measures ANOVA revealed significant interactions between time and treatment for all univariate measures for both macrofauna and meiofauna.

Results from additional one-way ANOVAs (Table 5.2 and 5.3) indicated when community responses differed during the experiment. In month one, the high intensity burial treatment caused significant increases in nematode species number, richness and diversity. These values then declined as the experiment progressed. Due to a smaller number of individuals and number of species recorded per sample, only subtle changes in the macrofauna community were apparent. At different stages during the experiment all indices identified significant differences between both treatments. However, at the end of the experiment after nine months, only nematode abundance and macrofauna species diversity were sensitive enough to discriminate between the three experimental treatments. Analysis of the macrofaunal data revealed differences between the control and the two burial treatments. The magnitude of these differences changed during the experiment with the greatest differences occurring in month three.

Table 5.2. Results from one-way ANOVAs carried out to assess significant differences between treatments at each time point within the meiofaunal nematode community. Letters a, b, c denote significant differences between treatments.

Metric	**Month**	**DF**	**MS**	**F**	**p**	**Post hoc**		
						C	**LB**	**HB**
Abundance (N)	1	2	7290	1.77	0.249	a	a	a
	3	2	57837	8.67	0.017	a	ab	b
	6	2	133122	20.62	0.002	a	b	b
	9	2	67261	78.74	<0.001	a	b	c
Species number (S)	1	2	36.11	14.77	0.005	a	b	c
	3	2	46.78	26.31	0.001	a	b	a
	6	2	52.11	22.33	0.002	a	a	b
	9	2	125.44	16.36	0.004	a	a	b
Species richness (d)	1	2	0.6469	8.24	0.019	a	ab	b
	3	2	0.7305	18.28	0.003	a	a	b
	6	2	0.433	3.8	0.086	a	a	a
	9	2	1.121	5.4	0.046	a	a	a
Species diversity (H')	1	2	0.03485	6.39	0.033	a	ab	b
	3	2	0.1864	6.70	0.03	a	a	a
	6	2	0.26839	32.69	0.001	a	a	b
	9	2	0.3589	14.55	0.005	a	a	b

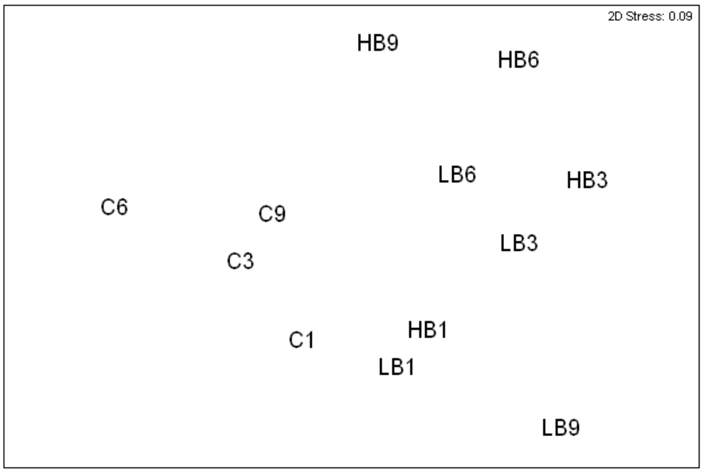
Table 5.3. Results from one-way ANOVAs carried out to assess significant differences between treatments over time within the macrofaunal community. Letters a, b, c denote significant differences between treatments.

Metric	**Month**	**DF**	**MS**	**F**	**p**	**Post hoc**		
						C	**LB**	**HB**
Abundance (N)	1	2	8294	11.33	0.003	a	b	c
	3	2	456	0.40	0.684	a	a	a
	6	2	14043	6.70	0.017	a	a	b
	9	2	87569	28.72	<0.001	a	b	b
Species number (S)	1	2	8.08	5.02	0.034	a	a	b
	3	2	22.750	30.33	<0.001	a	b	c
	6	2	16.583	22.96	<0.001	a	b	b
	9	2	40.58	14.18	0.002	a	b	b
Species richness (d)	1	2	0.3618	7.14	0.012	a	a	b
	3	2	0.7550	33.44	<0.001	a	b	c
	6	2	0.4106	8.02	0.010	a	b	b
	9	2	0.8885	12.75	0.002	a	b	b
Species diversity (H')	1	2	0.0263	1.49	0.276	a	b	b
	3	2	0.29725	67.67	<0.001	a	b	b
	6	2	0.6330	13.21	0.002	a	b	c
	9	2	0.8885	12.75	0.002	a	b	c

3.3 Multivariate analyses

Non-metric multi-dimensional scaling (MDS) ordinations (Figure 5.3) revealed different spatial trajectories for the two faunal types. In both ordinations, the two burial treatments behaved differently when compared to the control. In the ordination of meiofaunal nematode communities, samples within treatments are more variable than those of macrofauna. In both ordinations, the high intensity burial treatment has caused greatest community variation.

Meiofauna



Macrofauna

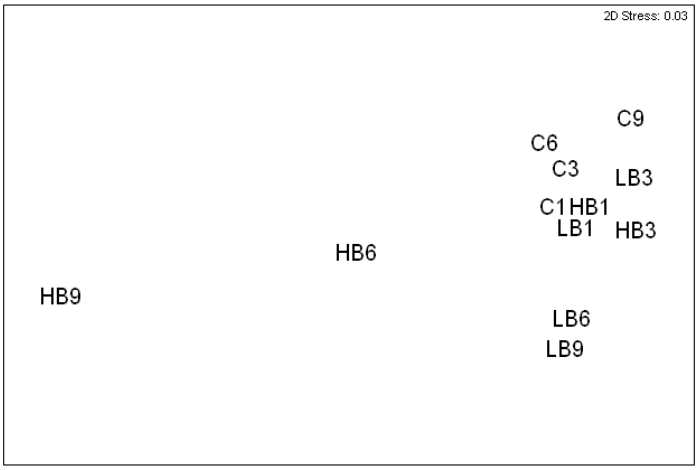


Figure 5.3. Multidimensional scaling ordinations of square-root transformed averaged community data displaying community differences between treatments over time. LO = Low Organics, LB = Low Burial, LR = Low Raking, HO = High Organics, HB = High Burial, HR = High Raking, C = Control. Numbers correspond to month the sample was taken.

In order to quantify the magnitude of treatment effects, treatments at 9 months were ranked using the R statistic generated from the one-way ANOSIM test; the higher the R-value (0-1) the greater the differences between treatment and control. For both faunal types high burial was ranked highest with R-values of 1. Both the low burial treatments were also found to differ greatly from the control values with R-values of 0.963 (meiofaunal nematodes) and 0.990 (macrofauna). Nematode species primarily responsible for the dissimilarity between treatments and controls included *Sabatieria punctata, Molgalaimus demani Pytcholaimellus ponticus*, *Chromadora macrolaima*, *Spilophorella paradoxa, Calyptronema maxweberi, Cobbia trefusiaeformis* and *Dichromadora cephalata.* For macrofauna, discriminating species were *Tubificoides benedii*, *Hydrobia ulvae*, *Streblospio benedicti* and *Aphelochaeta 'A'*.

To assess temporal inter-relationships between faunal matrices based on univariate indices of nematode and macrofauna communities the RELATE procedure was carried out. Non-significant test statistics of $\rho = 0.011$, 0.084, 0.078 and 0.019 for N, S, d and H', respectively, showed there were no common temporal patterns. Results from a comparison of similarity matrices created from community data also showed there were no common temporal patterns. ($\rho = 0.376$). This confirms the observation made from the MDS ordinations in Figure 5.3.

4. Discussion

Nematode and macrofaunal communities responded differently over a period of nine months when exposed to high and low frequencies of *in-situ* burial. Within one month following disturbance, burial treatments had caused significant increases in the species number, richness and diversity of the meiofaunal nematode community, whilst values for macrofauna were significantly reduced. Only abundance of macrofauna responded positively, as a result of a temporary increase in the numbers of opportunists such as *Tubificoides benedii* and *Aphelochaeta 'A'* 'The initial increase in nematode species richness is congruent with the intermediate disturbance hypothesis which describes a predictable unimodal relationship between the intensity and frequency of disturbance and the species richness of a perturbed community (Grime 1973, Connell 1978). Although such a response has been reported from a wide range of communities (Mackey and Currie 2001), it is not the usual pattern for inter-tidal macrofauna (Huxham et al., 2000). Hence this difference implies that macro- and meiofaunal nematode communities may have fundamentally different constraints and drivers (Warwick et al., 1990, Dial and Roughgarden 1998, Austen and Widdicombe 2006); in particular competition (Svensson et al., 2007) may be a more important structuring force for meiofauna.

Macrofauna species richness and diversity were more affected by low than high burial frequencies. This is in contrast to the meiofaunal nematode communities where the high burial treatments consistently had the greatest effects. It is possible that the macrobenthic community benefited from high frequency burial due to increased stability of the sediments and a greater surface area as a result of the three dimensional shape of the mounds of sediment which formed (Bingham pers com). For example, at the end of the experiment, extensive crab burrows (*Carcinus maenas*) were found in the sides of the mounds and increased numbers of *Hydrobia ulvae* were observed on the upper surfaces.

Overall macrofauna were found to be more sensitive to disturbance than meiofaunal nematodes. This was apparent from the number of significant contrasts between control and burial treatments and also between the two treatment intensities for meiofauna (22) and for macrofauna (35). Significant interactions between the two control communities were also observed. These interactions appeared to be driven by the seasonal pulses of nematode individuals entering the community. However the fact that a single meiofaunal taxon (nematodes) has been compared to several macrofaunal taxa (polychaetes, oligogchaetes, mollusca and crustacea) must be considered. It is possible that had more meiofauna taxa e.g. copepoda been included in the analysis, sensitivity of meiofauna may have been greater than that of macrofauna (Moore and Bett 1989).

The magnitude of effects caused by burial on the meiofaunal nematode and macrofaunal changed throughout the experiment. Within the macrofaunal community, number of individuals (month one) and species number (month three) showed significant differences among all treatments. However, at the end of the experiment, only species diversity was sensitive enough to distinguish between the two disturbance types and the control community. After one month significant differences in species number, species richness and species diversity were observed between the nematode communities disturbed by burial and those in the control. However, after nine months only number of individuals was significantly different between all three treatments. These observations are important when deciding which fraction of the infauna and univariate measure to use during experimental and monitoring studies. The frequency of sampling is also an important factor which needs much consideration. The correct sampling frequency will ensure that no changes in community structure are overlooked and then masked by natural variability in the community attributes being monitored.

Generally, small body size enables meiofauna to utilise a greater variety of niches and thus achieve considerable diversity at both the species and the functional group level compared to macrofauna (Warwick 1981). Meiofaunal generation times are much shorter than those of macrofauna (Somerfield et al., 1995, Bongers and Ferris 1999, Schratzberger et al., 2000). Meiofaunal nematode species also exhibit continuous reproduction and direct development within the sediment rather than seasonal reproduction and pelagic larval stages exhibited by many macrofaunal species (Warwick 1981, Gunter 1992). Continuous reproduction results in the constant presence of individuals, facilitating the recovery of disturbed areas. This renders meiofauna less vulnerable than macrofauna to disturbance events during the recruitment period, which could destroy the population until the next recruitment. This means that potentially, and as demonstrated here, meiofauna are more responsive than macrofauna to the early stages of disturbance. The fact that macrofauna were found to be generally more sensitive to burial than meiofaunal nematodes (Austen et al., 1989, Warwick et al., 1990, Gee et al., 1992, Austen and Widdicombe 2006, Somerfield et al., 2006) may also be explained by the different life history characteristics of nematodes. In previous studies these characteristics have been shown capable of dampening the longer-term effects of disturbance events (Warwick and Buchanan 1971, Schwinghammer et al., 1986, Alves et al., 2003, Lampadariou 2005).

Species variability was greater in the meiofaunal nematode community. Changes in the species composition of the macrofaunal community involved a ‘set’ number of species, with no new species entering the community. At the end of the experiment the dominant macrofaunal species were still present, although reduced in number. The dominant meiofaunal species (*S. punctata, M. demani and P. ponticus*) were also still present in all treatments although their abundances were also reduced and the order of dominance changed when compared with the control treatments. In contrast to the macrofauna, new species entered the nematode communities disturbed by burial. Species not observed in the control treatments included *S. paradoxa* and *C. maxweberi* in the low burial and *C. trefusiaeformis* in the high burial treatment. *D. cephalata* was found to be present in all the control samples but by the end of the experiment had disappeared from both burial treatments. These species were also shown to contribute highly to the dissimilarity between control and disturbance treatments. Therefore, their presence or absence could be used as an indication of a disturbed nematode community.

Results from RELATE analyses (combined with the significant interactions in the repeated measures analyses) showed that meiofaunal nematode and macrofaunal community attributes (abundance, species number, richness and diversity) did not follow the same trajectory. The current work is congruent with previous studies, suggesting that meiofaunal nematodes and macrofauna respond differently to the same type and frequency of disturbance. Several studies (Warwick et al., 1990, Pranovi et al., 2004, Austen and Widdicombe 2006, Bolam et al., 2006) found that macrofauna communities revealed clear signs of disturbance while nematode communities were apparently undisturbed by physical perturbation. This may be due to either meiofaunal communities being structured by other environmental factors, shorter adaptation times to changes in the physical environment or because they are simply more resilient to this form of disturbance.

The mechanisms of diversity maintenance are thought to be different for meiofauna and macrofauna. Diversity in nematodes is thought to be maintained through specialised feeding behaviour and food partitioning while macrofauna diversity is thought to be controlled by the spatial partitioning of the habitat (Whitlach 1980, Warwick et al., 1981, Austen and Warwick 1995). Nematodes are generally found in the surface layers of intertidal muds where the habitat is very homogenous. Therefore, high diversities of nematodes are thought to be maintained by the portioning of resources which is facilitated by the different feeding mechanisms found within the nematode taxa. (Weiser 1979). This was apparent from the increased utilisation of the high burial treatment mounds by the macrofaunal species *C. maenas* and *H. ulvae*. The different mechanisms of diversity maintenance and possible interactions between meiofauna and macrofaunal communities (Zobrist and Coull 1992, Tita 2000, Olafsson 2003) may further explain why different responses to the same disturbance were observed.

Whilst attributes of meiofaunal nematode communities were more sensitive to the initial impacts of disturbance, indices derived from macrofaunal data were more responsive to burial throughout the whole duration of the experiment. The shorter generation time and all year round reproduction of most nematode species may provide an early indication of disturbance (Schratzberger et al., 2000). Conversely the longevity of some macrofaunal species may result in changes in community structure taking longer to appear (Somerfield et al., 2006). Community-specific responses and sensitivities of meiofauna and macrofauna to the physical disturbance associated with *in-situ* burial highlights the importance of using both faunal types in the assessment of the effects of seabed disturbance in the marine environment (Heip 1992, Somerfield et al., 1995, 2006).

Chapter six

General discussion and conclusions

The main aims of this study were to assess the effects that different intensities and types of disturbance can have on benthic meiofaunal nematode and macrofaunal communities. This was achieved through the analysis of macrofaunal time series data from 2 different disturbance events occurring in the same locality and a field experiment, which investigated and compared the effects of several kinds of disturbance at two similar sites and on two faunal groups. Throughout, various analytical techniques were utilised and assessed as tools capable of indicating changes within benthic communities.

Chapters 2 and 3 of this PhD utilise data from actual disposal sites. The data in both cases has arisen from historic annual surveys that were primarily designed as check monitoring schemes under the Food and Environment Protection Act (FEPA). Therefore, they may not be considered as being optimally designed to detect disturbances (Underwood 1994). However, due to finite resources, time and the rarity and value of such time series data sets it was considered acceptable to proceed with the analyses carried out within this thesis. In both studies indices that were used were selected to be comparable to existing studies and include those cited in recent European Directives e.g. Water Frame Work Directive.

The controlled field experiment was conducted over a 10 – 12 month period therefore accounting for seasonal variability. The study was designed to investigate the effects of different intensities and disturbance types on macrofaunal communities at two locations in the UK. The disturbance types were chosen to mimic those commonly found within the marine environment. The methods used to assess the effects were again chosen to be comparable to past studies and test existing conceptual models designed to describe and predict the effects of disturbance on macrofauna community structure and dynamics. A comparison of the response of macrofauna and meiofaunal nematodes to *in-situ* burial was also carried out. Methodologies used were those that could be applied to both faunal groups. Other indices designed predominantly for macrofauna (AMBI) and nematodes (Maturity Index) were not included in the analysis as direct comparisons would not have been possible.

At the sewage sludge disposal site (Chapter 2) and the dredged material disposal site (Chapter 3) in Liverpool Bay both uni- and multivariate approaches showed significant community differences between reference and disposal stations. At the sewage sludge disposal site, relative species abundance plots showed a decrease in density and dominance within the disposal site after cessation, which may be attributed to reduced carbon inputs post-disposal. The cessation of disposal appeared to induce a greater degree of inter-annual change as the community recovered. At the dredged material disposal site, species number and richness were found to be lower within the disposal site when compared with the reference site. This differs from the sewage sludge disposal site where during sewage disposal species numbers were found to be higher within the disposal site, highlighting the fact that different disturbance types within relative close proximity to each other can have different effects on the receiving communities. Changes within communities were also found to be disturbance dependent. Community variability at the sewage sludge disposal site was less during the disposal years than after the perturbation ceased. This suggests that the disturbance seems to have acted to dampen, rather than enhance, variability, possibly by allowing the establishment of a relatively stable community dominated by disturbance tolerant species. This is in contrast to the dredged material disposal site where the greatest between year variability was observed throughout disposal activity. At both sites in Liverpool Bay, treatment and reference sites were found to exhibit common time patterns showing that factors other than anthropogenic disturbance may be responsible for some of the observed community variation. This illustrates the central challenge in interpreting the effects of disturbance: to discriminate between low amplitude, low frequency drivers such as those associated with climate change and high frequency point source impacts such as mans' activities.

During the work carried out to assess the effects of dredged material disposal within Liverpool Bay (Chapter 3), a methodology to define which metric best shows the effects of the disturbance being studied was further developed (Ware et al., In Press) and trialled. Several commonly used indices, including examples relying on univariate measures of taxon and functional diversity and on multivariate analyses, were assessed using two groups of criteria. The criteria which were selected were representative of those identified by a variety of national and international organisations (ICES 2001, Defra, 2004, EEA 2005, Sneddon et al., 2006) and included scientific validity and being tightly linked to manageable human activity (group 1), easily communicable to non-scientists and other users, easily and accurately measured, and cost effective (group 2). It became apparent that during this assessment very few of the metrics were actually correlated with the perturbation being studied, i.e. few were tightly linked to manageable human activity. This method raised important questions, including:

- How do we ensure that observed changes in metric values are part of a cause-effect relationship?

- How do we identify / choose which of the potential impacts of the disturbance in question to use as a pressure indicator?

To try and address some of our earlier observations and conclusions in a more controlled environment an experimental study was carried out to determine the effects that different types and intensities of disturbance have on infaunal intertidal communities. The experiment was carried out at two different locations, which contained several common species (Chapter 4). The main aims were to ascertain if species-level responses were site and disturbance dependent, and to determine if there were any differences in community responses to the same disturbances at the two localities.

Significant differences between how the macrofaunal communities at the same sites behaved towards different disturbance types were observed. This is in accord with observations from the two studies carried out in Liverpool Bay, which also demonstrated that different disturbance types i.e. sewage sludge disposal and dredged material disposal caused different community and species responses. A comparison between the two experimental sites revealed that, in some cases, species found at both locations responded differently to the same disturbance. Hence species' responses to disturbance may be dependent on both the type and location of the disturbance. The response of communities to disturbance is thought to depend on site-specific factors such as the history of prior disturbance (Dernie et al., 2003), the natural environmental regime (Huxham et al., 2000, Bolam and Fernandes 2002b, Bolam et al., 2004), biological interactions (Posey 1990) and the inherent ecological plasticity exhibited by many benthic species (Davic 2003). All these factors must be considered when attempting to identify and use indicator species or community based indices for the assessment of perturbations within the marine environment.

Intensity of disturbance was also found to be an important factor which should be considered when assessing and comparing the effects of disturbances. The effects of a disturbance are sometimes portrayed as pushing communities along a continuum from an undisturbed to a severely disturbed state. However, the non-linearity of our results, with lower intensity treatments having greater impacts in some cases than high intensity ones, demonstrates the complex responses that may be encountered when assessing effects of disturbance at the community level. Although models based on a successional continuum, such as that described by Pearson and Rosenberg (1978), may allow accurate general predictions, they cannot accommodate site-specific contingencies that result in changes in the usual ordering of disturbance effects.

Several methods of assessing the effects of disturbance on macrofaunal communities were used during our field experiment. These included the allocation of macrofaunal species into feeding types and the assessment of changes in their percentage contribution. This method allowed the effects of disturbance to be examined at a functional level. Although the same trophic groups were present at both sites, and although all groups contained overlapping suites of species, they showed quite different responses at the two sites. Compartmentalizing species in this way can simplify complex food webs and allow the comparison of similar communities that contain different species but the same functional groups (Gaston et al., 1998). Since responses to disturbance should relate to the niche occupied by a species, the use of functional groups offers the prospect of identifying general predictions on the effects of disturbance. For example, predators (Pimm, 2002) might be expected to show greater sensitivity. However, the experimental results reported here show site-specific responses for functional as well as taxonomic categories.

Macrofaunal assemblages do not exist in isolation they are part of a complex food web which also contains bacteria, microfauna and meiofauna (Platt and Warwick 1980, Kuipers et al., 1981, Raffaelli and Hawkins 1996, Olafsson 2003). From this complex food web, meiofaunal nematode communities are also regularly utilised in the assessment of athropogenic disturbances in the marine environment. However, very few studies have been carried out using both meiofauna and macrofauna together (Warwick et al., 1990, Somerfield et al., 2006). Our results, which compared the response of the meiofaunal nematode and macrofaunal communities showed that the communities responded differently when perturbed by the same disturbance.

Overall, macrofauna were found to be more sensitive to disturbance than meiofauna, though meiofauna were found to be more responsive to the initial impacts of disturbance (Schratzberger et al., 2000). Previous work comparing meiofauna and macrofauna as indicator groups has shown that in some cases meiofaunal nematodes and macrofauna behave differently to some disturbance events e.g. studies examining the effects of organic enrichment found similar responses (Coull and Chandler 1992, Austen and Widdicombe 2006). Nematodes were found to be more sensitive to the effects of dredge disposal (Somerfield et al., 1995) and macrofauna more sensitive to sewage pollution (Austen et al., 1989) and burial (Bolam et al., 2006). Our work is consistent with this in that, the number of significant contrasts between control and burial treatments and also between the two treatment intensities was higher for macrofauna (35) than for meiofauna (22). The fact that macrofauna were found to be generally more sensitive to burial than meiofauna may be explained by the different life history characteristics of nematodes which, in previous studies, have been shown to be capable of dampening the effects of disturbance events (Warwick and Buchanan 1971, Schwinghammer et al., 1986, Alves et al., 2003, Lampadariou 2005). The shorter generation times and all year round reproduction displayed by nematodes may allow early indications of disturbance at the community level to be more readily detected. Conversely, the longevity of some macrofaunal species may result in changes in community structure taking longer to materialise resulting in occasional impacts being overlooked. However, the longevity of macrofauna species may also result in impacts being detectable for a longer period of time. Community-specific responses and sensitivities of meiofauna and macrofauna to the physical disturbance associated with *in situ* burial highlights the importance of using both faunal types in the assessment of the effects of seabed disturbance in the marine environment (Heip 1992, Somerfield et al., 1995, 2006).

A better understanding of how different disturbances affect benthic ecosystems and of how best to measure and monitor these effects will provide accurate ecologically based information to licensing officials and policy makers responsible for preparing and enforcing effective legislation. The ability to detect and manage anthropogenic disturbances in the marine environment is more important than ever, given increasing pressure from a range of sources and the growing awareness of the sensitivity of some marine habitats. It is clear that no simple method exists for detecting disturbance which is applicable to all sites and situations. Hence, careful consideration, informed by ecological knowledge of sites and species, need to be given to each case.

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