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

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Abstract

Sewage sludge was disposed of in Liverpool Bay for over 100 years. Annual amounts increased from 0.5 million tonnes per annum in 1900 to approximately 2 million tonnes per annum by 1995. Macrofauna and a suite of environmental variables were collected at a station adjacent to, and a reference station distant from, the disposal site over 13 years, spanning a pre- (1990–1998) and post- (1999–2003) cessation period. Univariate and multivariate analyses of the time-series data showed significant community differences between reference and disposal site stations and multivariate analyses revealed station-specific community development post-disposal. Temporal variability of communities collected at the disposal station post-cessation was higher than during years of disposal, when temporally stable dominance patterns of disturbance-tolerant species had established. Alterations of community structure post-disturbance reflected successional changes possibly driven by facilitation. 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.

Keywords: Time-series; Macrofauna; Community structure; Liverpool Bay; Sewage-sludge disposal

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, 1993a,b).

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 and Rowlatt, 1982; Norton et al., 1984; Rees and Walker, 1984, 1991; Rees et al., 1990, 1992b; Rees, 1991, 1993a,b; 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. Sewage sludge disposed of post-1980 was predominantly anaerobically digested primary and secondary sludge originating from Manchester, Salford, Warrington and Runcorn. Total solids were approximately 3% of the totals disposed of each year. Trace metals and organic compounds present within the sludge included Mercury, Cadmium, Chromium, Nickel, Lead and hydrocarbons. Other anthropogenic inputs into this area include the disposal of dredged material, agricultural run-off and discharges from rivers, estuaries and

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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 by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) on behalf of the regulator, the Department for Environment, Food and Rural Affairs (Defra) (see 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:

H_0: Macrobenthic communities at the reference and disposal-site stations do not differ in terms of univariate (density, species richness) and multivariate (community structure) attributes.

H_0: 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 later determination of particle size and organic carbon/nitrogen content were collected once 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) (Fig. 1). At each sampling time three or four samples were taken at each site using a 0.1 m^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 (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 sieve and the retained fauna preserved in 3% buffered formaldehyde solution. All specimens were identified where possible to species level.

2.2. Collection and processing of sediment samples

Sediment sub-samples were collected using a 3 cm diameter syringe core to a depth of 5 cm from each Day grab and then frozen. Before processing each sample was allowed to defrost for several hours. Samples were wet-sieved at 63 μm. The sediment fraction >63 μm was oven dried at ~90 °C for 24 h and then dry-sieved for 10 min on a stack of Endecotts™ stainless steel test sieves (63 mm–63 μm at 1/2 Phi intervals). The <63 μm sediment

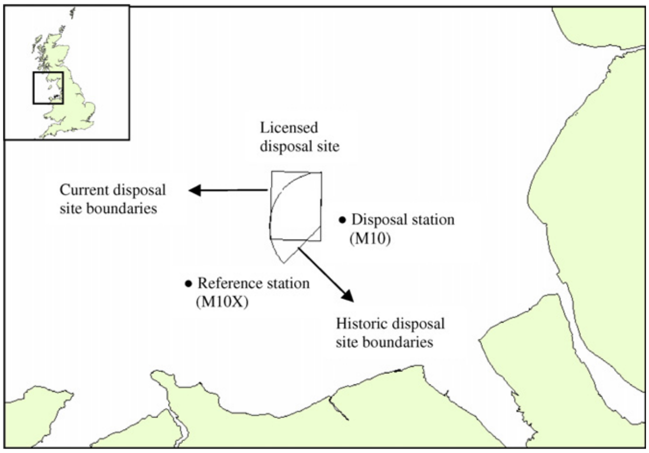

Fig. 1. Location of Liverpool Bay sewage-sludge disposal site and position of sampling stations.

fraction was frozen and then freeze-dried using an Edwards super modulyo freeze drier™. A sub-sample of the <63 μm freeze dried fraction was analysed on a Malvern Mastersizer 2000 laser diffraction analyser™. Both dry-sieve and laser diffraction results were combined to give the full particle size distribution. The sorting coefficient, median particle size and percentage 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™).

2.3. Univariate data analyses

The total number of individuals (abundance) and species (species number) were calculated for each sample. Aggregation files were compiled for each site to facilitate the analysis of the benthic macrofauna using indices of taxonomic diversity and distinctness described by Warwick and Clarke (1995). These indices consider the path length between each species within the classification tree and so are measures of the phylogenetic relatedness of individuals within the assemblage. Pielou's evenness 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 and (b) abundances of dominant species. The latter included those identified from the literature 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. 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 visualise differences in the structure of macrofauna communities. Following the procedure applied to univariate data, 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 macro- fauna 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 (i.e., 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 1. Sediments at the reference and disposal-site station differed little in terms of median particle diameter and sorting coefficient (Table 2). 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 stations were also found to be non-significant when disposal and post-disposal years were compared. Analysis of the percentage silt/clay content from both stations 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 HSD multi-comparison tests revealed significant differences between stations for 1994 ($p = 0.037$) and 1997 ($p = 0.017$). None of the post-disposal years was found to be significantly different. Several significant correlations between environmental variables and univariate community attributes were revealed for the reference station: total abundance was positively correlated with percentage silt/clay content ($r = 0.797$, $p = 0.018$) and

Table 1
Means (±SE) for environmental variables at the reference and disposal site stations between 1994 and 2003

Year	Percentage silt/clay				Sorting coefficient				Median particle size (Phi)				Percentage organic carbon	Percentage 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
1995	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
Results from the nested two-way analysis of variance (ANOVA) of environmental data

Factor	Median (Phi)		Percentage 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

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 percentage 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 percentage 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 percentage 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 percentage 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 percentage 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 (Fig. 2a and b and Table 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 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 (Fig. 3).

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 (Fig. 2c and d). Spatial 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 3). Formal significance testing of the taxonomic diversity index was precluded by the non-normal distribution of the data.

We observed notable changes in community structure over the 13-year time series (Fig. 4a and 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, Fig. 4a). This is in contrast to the reference station, where variability remained similar over time (Fig. 4b).

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 commensurate with results obtained from the univariate

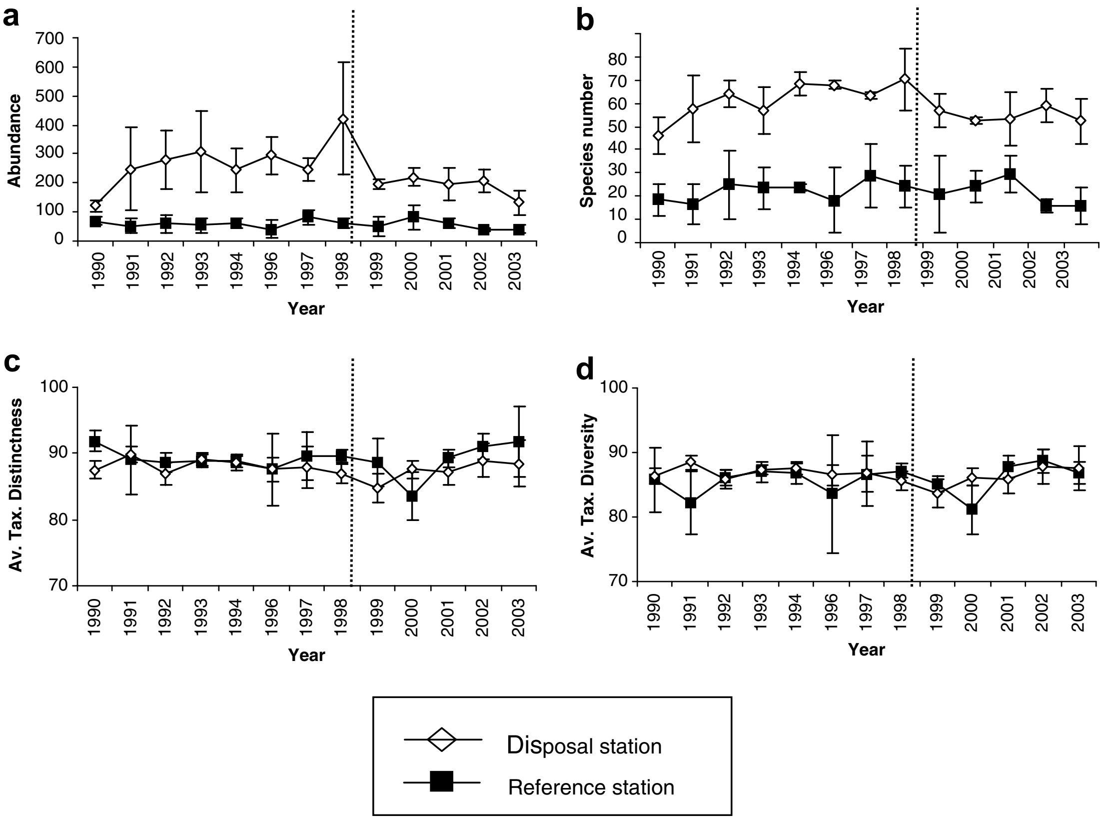

Fig. 2. (a)–(d) Mean (±SE) abundance, species number, taxonomic distinctness and taxonomic diversity of macrofauna assemblages collected at the reference and disposal-site station between 1990 and 2003. Dashed line indicates cessation of sewage sludge disposal.

Table 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

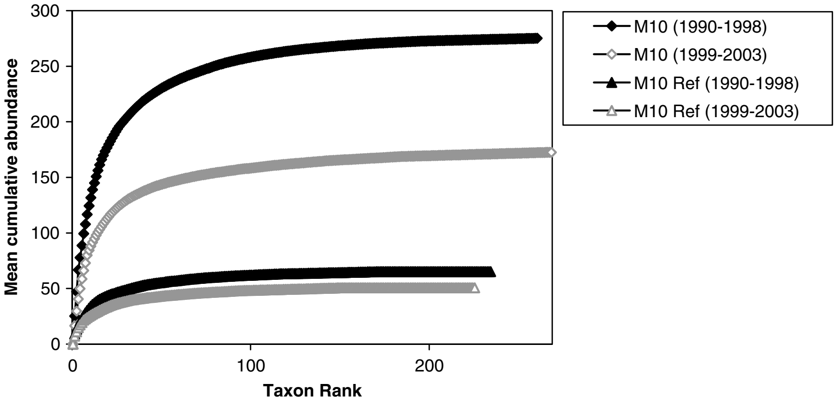

Fig. 3. Mean cumulative species abundance at the disposal and reference station pre- (1990–1998) and post- (1999–2003) cessation of sewage disposal.

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

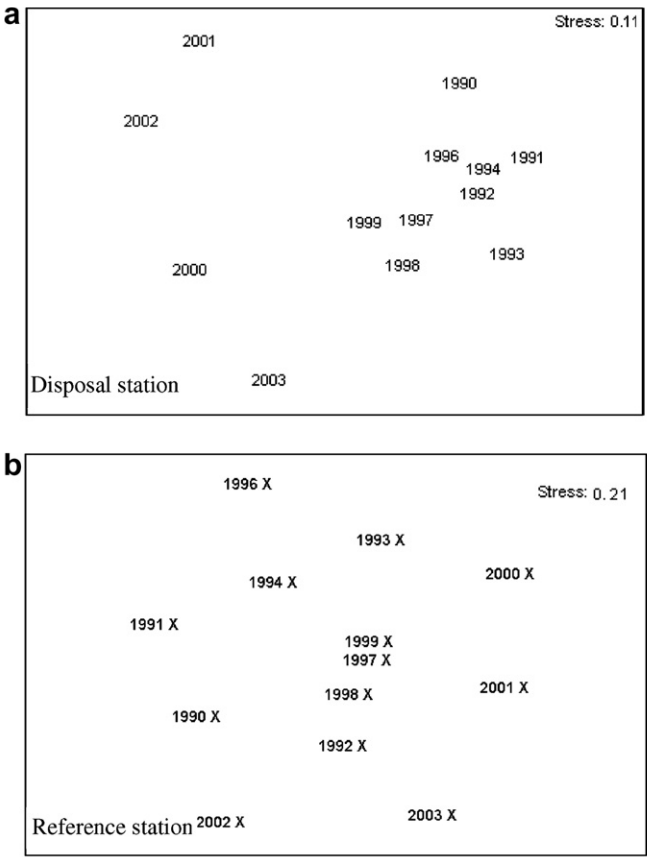

Fig. 4. (a)–(b) Non-parametric multi-dimensional scaling (MDS) ordination based on double square-root transformed mean abundance of macrofauna species at the reference and disposal site stations between 1990 and 2003. X denotes reference site.

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 (Fig. 5a–e) were observed. Since disposal ceased in 1998, dominant species at the disposal site station included the amphipod *U. marina*, the polychaetes *S. inflatum* and *A. lindstroemi* and the bivalve *M. bidentata*. Significant interaction terms in the 2-way ANOVA for *S. inflatum*, *L. koreni* and *U. marina* (Table 4) revealed that changes in abundances at the reference and disposal site station differed significantly.

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 the monitoring of a 'real world' experiment. 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; Rowlatt et al., 1991). Thus, due to the low level of spatial replication in the present time-series, site-specific factors may confound some of our results.

Although the main effects of sewage-sludge disposal on the benthic fauna are generally manifested through organic enrichment of the sediment (Rees, 1993a,b), 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 (C. Vivian, Cefas, Pers. Comm.; Leah et al., 1993). However, at present we cannot entirely discount possible subtle effects on the macrofauna community at the disposal site itself.

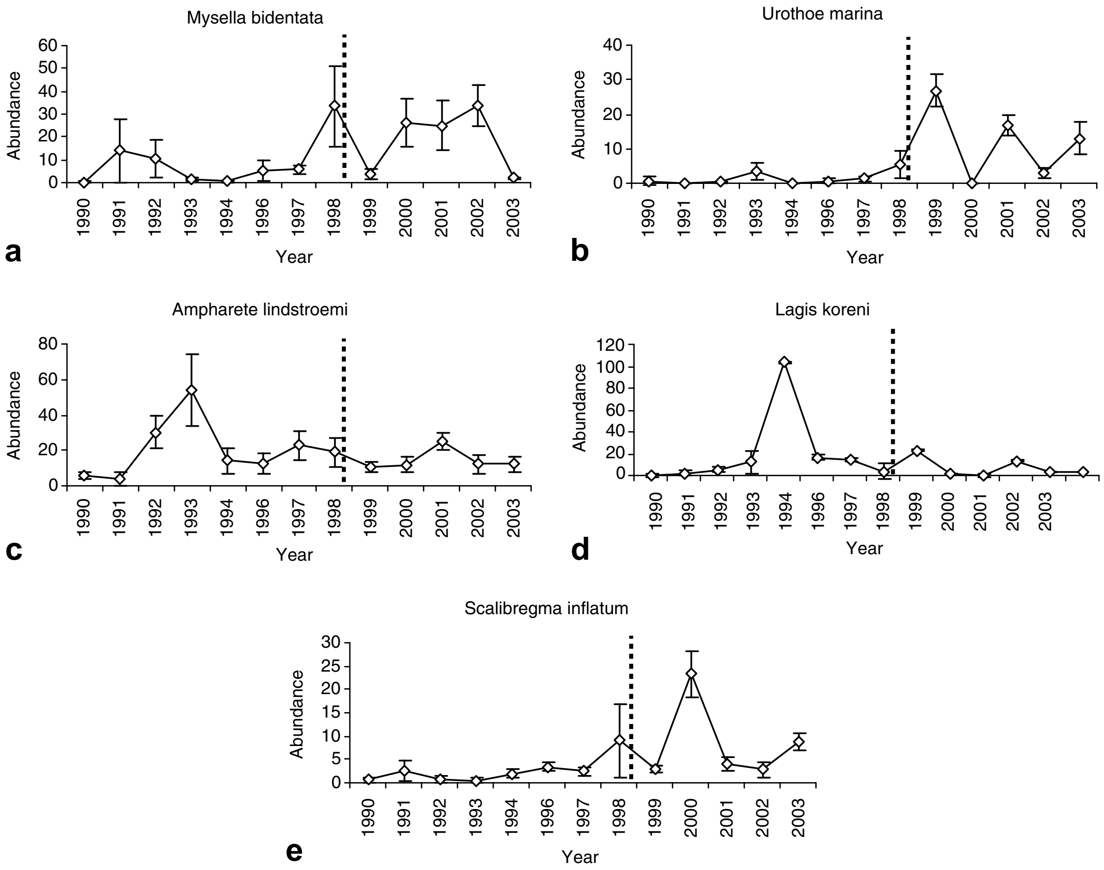

Fig. 5. Mean (±SE) abundance of discriminating species at the Liverpool Bay disposal site between 1990 and 2003. Dashed line indicates cessation of sewage-sludge disposal.

Table 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	0.00	10.59	0.00	21.29	0.01	33.54	0.00	66.76	0.00
Disposal vs. cessation	1.22	0.27	4.92	0.03	5.77	0.02	2.94	0.09	42.13	0.00
Site * disposal vs. cessation	1.80	0.18	5.55	0.02	5.35	0.02	2.94	0.09	41.83	0.00
Year (site disposal vs. cessation)	2.41	0.00	4.10	0.00	1.90	0.02	1.69	0.05	6.55	0.00

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), an 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. We therefore postulate (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 we found dominance shifts within the disposal-site community, evenness was stable over time, showing none of the expected characteristics of reduced species diversity and high dominance.

Macroinfaunal 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: increasing 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. Though in the present study we are comparing time-series data 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 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*. *S. 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). *M. 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 the area 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 our 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.

This contribution forms part of an ongoing assessment of the effect of sewage-sludge disposal and its aftermath around the UK coastline, being conducted under the auspices of the UK Group Co-ordinating Seabed Disturbance Monitoring. Further publications on work at Liverpool Bay disposal site (and elsewhere) will address in greater detail the spatial dimension for any observed changes.

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