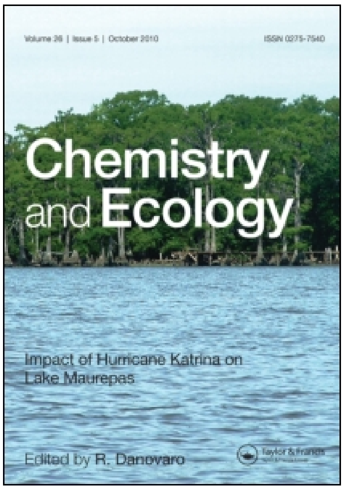

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Use of Ordination Techniques to Follow Community Succession from Oil Impact to Recovery in the Field

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USE OF ORDINATION TECHNIQUES TO FOLLOW COMMUNITY SUCCESSION FROM OIL IMPACT TO RECOVERY IN THE FIELD

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INTRODUCTION

Much effort has been directed toward elucidating pollution effects on marine benthic communities (Pearson and Rosenberg, 1978; Sanders *et al.*, 1980; NAS 1985). Less effort has been directed at methods of data analysis which will identify distribution patterns and relationships between communities. Traditional community structure summary parameters such as species richness and various indices of diversity utilize only part of the information contained in a data set and are not very useful in elucidating relationships between communities of animals.

A variety of multivariate analytical techniques has been developed to analyze complex species by station data sets, primarily by terrestrial plant ecologists (Harris, 1975; Orloci, 1978; Whittaker, 1978 a and b). Some of these techniques have been applied to data from unpolluted marine benthic communities (Sanders, 1958; Hughes and Thomas, 1971; Stephanson *et al.*, 1972; Haedrich *et al.*, 1980; Poore and Obley, 1980; Field *et al.*, 1982; Weston, 1988).

Multivariate methods have seen less use in marine pollution studies (Sanders *et al.*, 1980; Reid *et al.*, 1982; Warwick, 1986; Gray *et al.*, 1988; Heip *et al.*, 1988; Underwood and Peterson, 1988; Warwick, *et al.*, 1988; Zenetos and Papathanassiou, 1989). With the exception of the Sanders *et al.* (1980) work, those situations which have been investigated using multivariate techniques have examined the distribution of steady state pollutant effects over space.

Communities subjected to an acute impact such as an oil spill undergo dynamic changes in structure. Species richness and diversity are reduced while the number of individuals/unit area increases; normally occurring species are replaced by opportunists. Over time, the community undergoes a succession to an unimpacted state. Because community structure is a dynamic process, it is important to consider that succession to recovery occurs within the context of normal temporal and spatial variations in community structure (Buchanan, *et al.*, 1978; Eagle, 1975; Glemarec, 1979; Princz *et al.*, 1983; Rachor and Gerlach, 1978; Rachor *et al.*, 1982). Multivariate analysis of benthic infaunal data taken over space and time can yield valuable data about the time course of succession and about those species most important in defining succession. Multivariate techniques can also be used to assess similarity between impacted locations and unimpacted reference locations.

This paper presents an application of Principal Component Analysis (PCA) to benthic community structure data arising from a field experiment in which similar intertidal sedimentary environments and their associated infauna were exposed to equal amounts of untreated crude oil and chemically dispersed crude oil and to no treatment.

EXPERIMENTAL DESIGN

The object of the experiment was to compare the consequences of dispersing or not dispersing a small slick of crude oil near shore. The scenario on which the experimental design is based envisages a moderate (11233 l/hectare) spill of crude oil into the nearshore environment which is dispersed near shore to avoid damage to low energy depositional environments.

The experiment was carried out in the upper part of Long Cove, Searsport, Maine, USA. Figure 1 shows the location of Long Cove and the locations of the three test areas. Long Cove is a shallow body of water which is well flushed by tidal action. Long Cove is exposed to low levels of sewage pollution from the Penobscot River Plume. During periods of SW winds, wind-driven currents enter Long Cove along the West shore of Kidder Point and leave along the western shore of the cove.

The test areas were as comparable as possible. They were laid out so that in SW wind conditions the reference area is "upstream" of the undispersed oil area, which is "upstream" of the dispersed oil area. Within each test area, 2 sampling stations were established. Each of these stations consisted of a 5 × 5 m grid having 25 possible sampling locations. The upper station was just below the boulder/cobble beach at approximately mid tide level. The lower stations were just above mean low water. The upper part of the beach in Long Cove consists of boulders and cobble. The interstices of this area are filled with poorly sorted sediments containing a significant proportion of silt-clay size particles (Mayo *et al.*, 1978). The lower portion of the intertidal zone consists of a broad, gently sloping flat. The sediments become finer toward the lower water line.

Oil releases (6 bbl; 1136 l) were carried out over two 60 × 100 meter test areas. Light crude oil (Murban) was chosen because it would contain a wide variety of the compounds found in those oils that are likely candidates for dispersion.

The untreated oil was not pre-treated to simulate weathering. It was released several hundred feet offshore during a falling tide and was carried to the beach by the wind. It was stranded on the untreated oil test area during a time when the water table was falling to result in the maximal penetration of oil into the sediments. Following two tidal cycles of exposure, oil remaining on the surface of the sediment was physically removed. To enable detection of possible cross contamination between dispersed oil and untreated oil a unique hydrocarbon pattern was produced by spiking. The dispersed oil was spiked with 1.5 × normal concentration of nC-18; the untreated oil was spiked with 2 × normal amount of nC-16. Following the spills no cross contamination was detected.

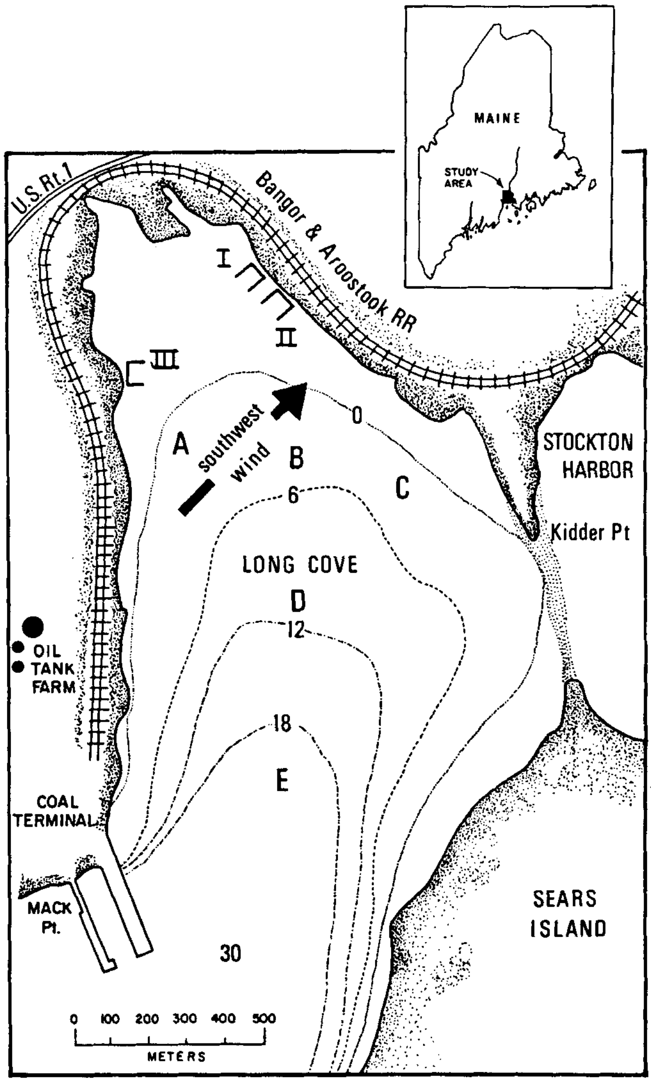

Figure 1 Location map showing Long Cove and the location of the experimental areas. Area I was exposed to untreated oil, Area II was the reference area; Area III was exposed to chemically dispersed oil.

The dispersed oil was pre-mixed with 10% v/v of a widely available glycol ether based dispersant. This dispersant was chosen because it is highly effective; it has been used in previous experiments (McAuliffe, *et al.*, 1981; Cretney *et al.*, 1981; Buckley *et al.*, 1981). Dispersed oil was released into the water over two intertidal sampling stations within the test area. The oil was released during the hour preceding high water. The use of two small boats with mixing gates provided maximal exposure of the intertidal benthos to the resulting cloud of dispersed oil.

A third test area (reference) received no treatment at all.

MATERIALS AND METHODS

Materials and methods for the data presented here have been previously reported in Page *et al.*, 1984; Gilfillan *et al.*, 1983; Page *et al.*, 1983. In the interest of brevity, they will not be repeated here except to say that five replicate 0.018 m^2 core samples were taken to a depth of 15 cm at each station in each of the areas on 12 dates from July 1980 to August 1982. The spills took place on 21 August 1981. Each of the replicate core samples was sieved through a 0.5 mm screen. Those organisms retained on the screen were identified to species as far as possible. Data were reported as abundances of each species within each replicate sample.

Programs to carry out PCA and associated file-handling tasks were written in a compiled 16-bit version of BASIC (TML Systems, Jacksonville, FL). These programs run on an Apple IIGS. The PCA program was checked by comparison examples given in Pielou (1984). It is a normal analysis carried out on the Q matrix. Source code for these programs and a sample data set are available from the authors. PCA was carried out on a species × station data matrix in which the mean abundance of each species was transformed to a standard normal deviate. This transformation was chosen to minimize the effects of the differing mean abundances of the various species on the analysis (Pielou, 1984).

RESULTS

PCA was carried out on a 36 station (12 sampling dates at each of three test areas) × 44 species data matrix. The most important axis (axis 1) contained 15.8% of the total variance in the data set; examination of the eigenvector for axis 1 showed that axis 1 was defined in terms of the abundance of *Heteromastus filiformis, Streblospio benedicti, Macoma balthica* and *Etone heteropoda, i.e.* these species had the largest weighting values. The next most important axis (axis 2) contained 9.7% of the total variance and was defined in terms of the abundance of *Mya arenaria* spat and Harpacticoid copepods.

Clearly, the signal produced by the oil spill was not large. The reason for this is that the amount of oil reaching shore was not large. The total volume of oil released was 1136 l. As much as half may not have been incorporated into beach sediments and was lost through evaporation and clean up efforts. The majority of the oil stranded over a 1200 m^2 area at the top of the beach; the maximum possible oil dose was 0.9 l/m^2; it is more likely that the actual dose was 0.4 l/m^2. Sediment hydrocarbon chemistry was done concurrently with infaunal sampling at all sites. No incorporation of petroleum hydrocarbons into sediments was detected in the reference area or the area exposed to chemically dispersed oil (Page *et al.*, 1983; 1984). Petroleum hydrocarbons were incorporated into sediments in the area exposed to untreated oil (Page *et al.*, 1983; 1984). Sediment hydrocarbon content increased from 131 ppm total petroleum hydrocarbons (TPH) to 259 ppm TPH. The kinetics of biodegradation and weathering of the sediment bound oil approximate a first order decay process with a half life of 7 weeks (Gilfillan *et al.*, 1986). Changes in sediment hydrocarbon concentration are lagged by the observed changes in the infaunal community, because of the reproductive delays involved in the community response. The results indicate that the transfer of organic carbon from petroleum to infaunal communities *via* heterotrophic microorganisms is probably of major importance in producing the observed changes in community structure. Once the oil had been removed from the environment, the community recovered.

Oil incorporated into sediments eliminated no species from the infaunal community; no new species invaded the oiled area (Gilfillan *et al.*, 1983). There were, however, some dramatic changes in the abundance of several species already present in the community. Three species of opportunistic (Grassle and Grassle, 1974) polychaetes increased in population density following the oil spill. *Streblospio benedictii* increased in density from 500–1000/m^2 to 10–12,000/m^2; *Heteromastus filiformis* increased in density from 2–3000/m^2 to 11–14000/m^2 following the spill; *Capitella capitata* increased in density from 500–1000/m^2 to 1700–2200/m^2 following the spill and then declined to baseline densities.

Other detritivores also increased in population density following the spill. The polychaete *Etone heteropoda* increased from 500–1000/m^2 to 3–4000/m^2; the bivalve *Macoma balthica* increased from 800–3000/m^2 to 3–8000/m^2. Spat of *Mya arenaria* were settling at the time of the spill. Initially their population density was reduced, but after three weeks their population density began an increase to very high levels. Sediments in the area exposed to untreated oil were very slightly coarser than those in the other two areas. Population densities of *M.* arenaria spat were always higher there than in the other areas.

It is clear that the succession to an unimpacted state began near the midpoint of the succession defined by Pearson and Rosenberg (1978), where both opportunists and species typical of a biologically accommodated community are present. As all of the species which increased in density following exposure to untreated oil are detritivores, a major part of the effect of the oil was probably caused by a temporary increase in biomass of hydrocarbon-degrading bacteria which they utilized as food.

Figure 2 shows a plot of ordination scores (axis 1 *v* axis 2) for all areas and stations. In this plot, the centroid formed by samples from the reference area, the area exposed to chemically dispersed oil and the pre-spill samples from the area exposed to untreated oil is readily apparent. Outliers represent the post spill samples from the area exposed to crude oil.

Figure 3 shows ordination scores for Axes 1 and 2 plotted against each other for the 12 samples from the reference area. The pattern shown in Figure 2, a centroid of points, is typical of a situation where variability is low. No trends are apparent.

Figure 4 shows a similar plot of ordination scores obtained for the 12 samples from the area exposed to chemically dispersed oil. As with the samples from the reference area variability is low. The points from the dispersed oil site form a centroid that merges with that formed by the reference area points.

Figure 5 shows a similar plot of ordination scores obtained for the 12 samples from the area exposed to untreated oil. Here there is a definite trend. The post spill samples in 1981: 8/26, 9/3, 9/22 and 10/20 are progressively further displaced from the centroid along axis 1. Two of the three 1982 samples (6/82 and 8/82) have rejoined the centroid formed by pre-spill samples.

Figure 6 shows the same plot shown in Figure 5 with a minimum spanning tree projected onto it. A minimum spanning tree is created when points on an

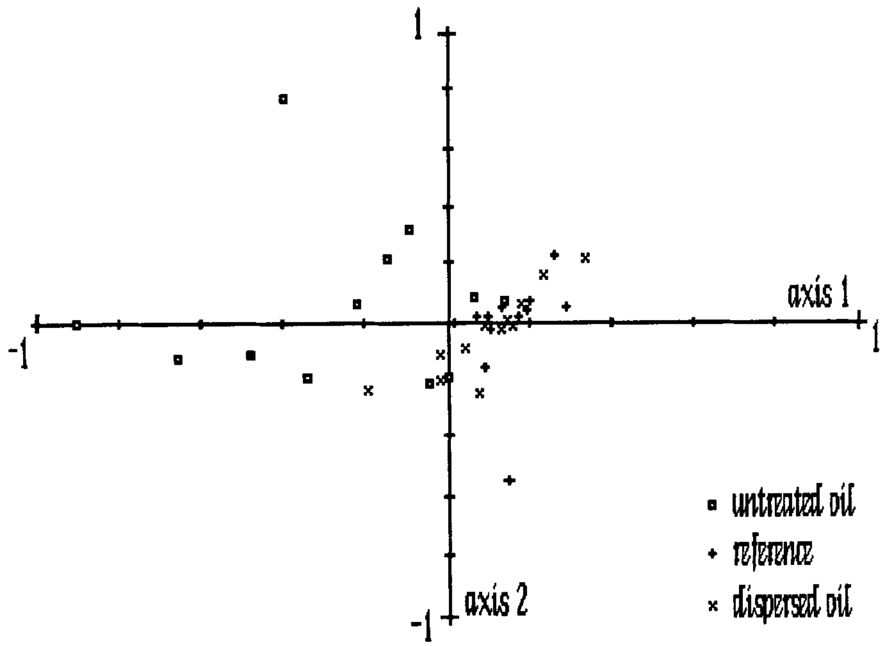

Figure 2 Plot of ordination scores for axis 1 plotted against ordination scores for axis 2 showing data for all areas.

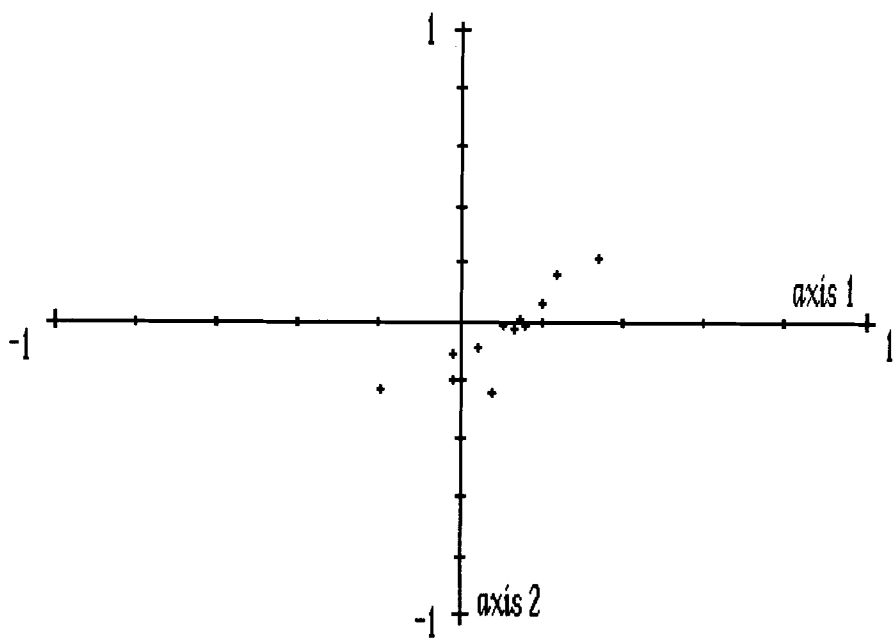

Figure 3 Plot of ordination scores for axis 1 plotted against ordination scores for axis 2 showing data for the reference area. An outlier (6/81) is identified.

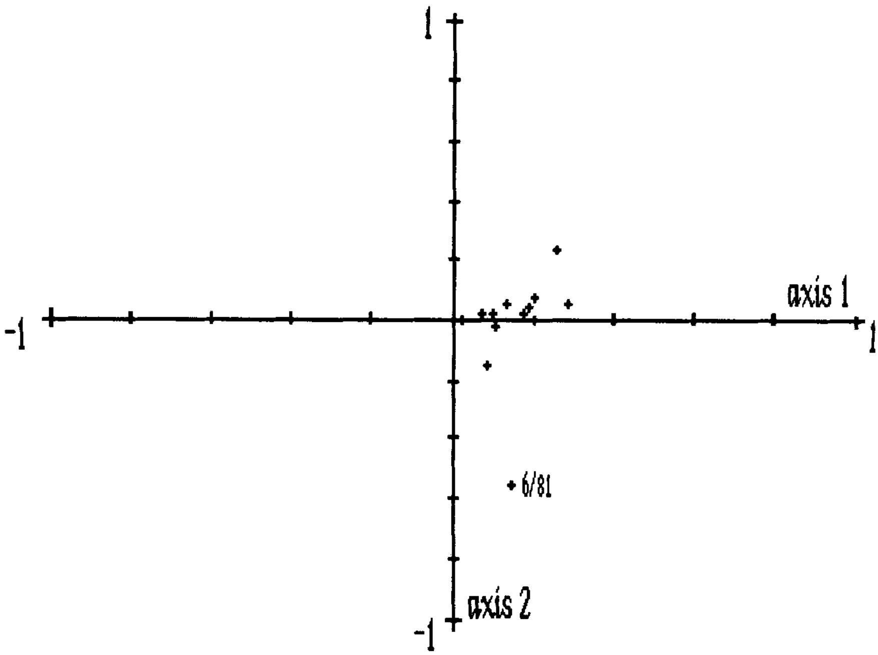

Figure 4 Plot of ordination scores for axis 1 plotted against ordination scores for axis 2 showing data for the area receiving chemically dispersed oil.

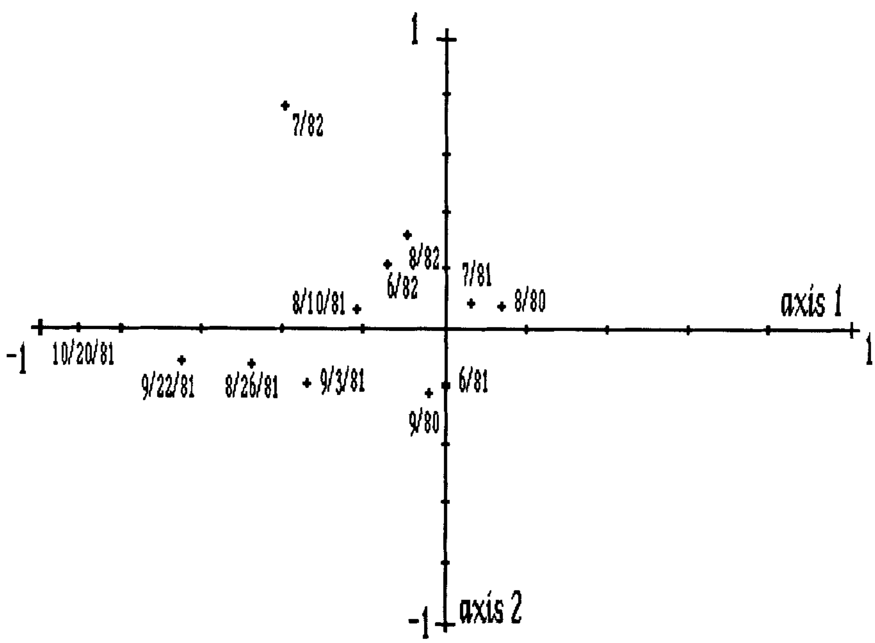

Figure 5 Plot of ordination scores for axis 1 plotted against ordination scores for axis 2 showing data for the area receiving untreated oil. Sampling dates are shown next to each point.

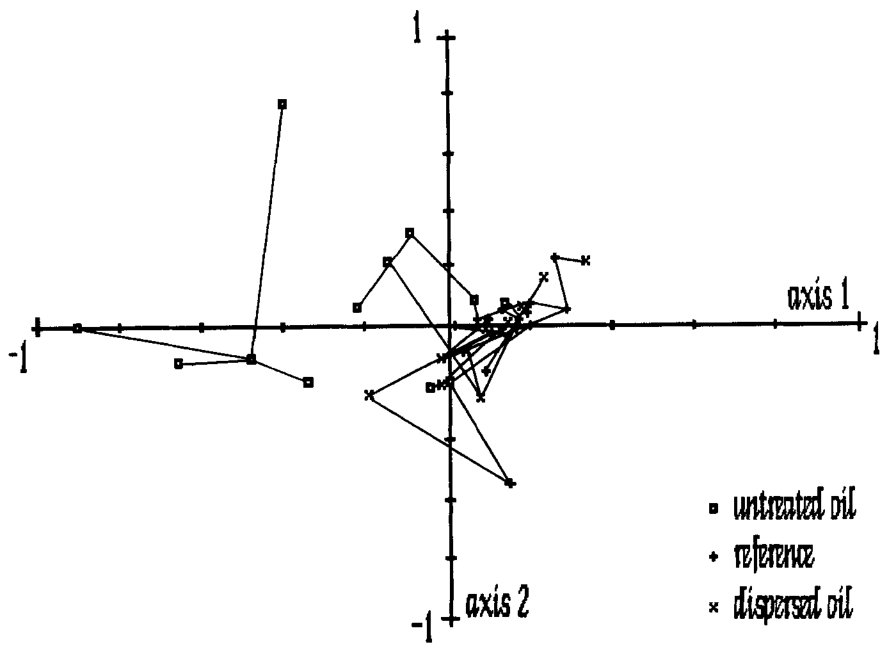

Figure 6 Plot of ordination scores for axis 1 plotted against ordination scores for axis 2 showing data for all areas. A minimum spanning tree has been projected onto the plot.

ordination plot are joined to those points representing samples to which they are most similar. Similarity is determined by calculation of a similarity matrix. In this instance Ruzicka's similarity index was calculated (Goodall, 1978). It is clear from the data shown in Figure 6 that there are two families of samples. There is the unperturbed group which is formed by samples from the reference area, the area exposed to chemically dispersed oil and the pre-spill samples from the area exposed to untreated oil. The perturbed group is formed by post-spill 1981 samples from the area exposed to untreated oil and the 7/82 sample from the same area.

DISCUSSION

When oil is incorporated into fine-grained sediments, it is useful to consider that oil is a toxic material which is also a rich source of organic carbon. Ecological effects of spilled oil are a function of the amount of oil reaching an environment and its toxicity. The toxicity of oil is largely resident in the 1, 2 and 3 ring polynuclear aromatic hydrocarbons (PAH) (Anderson *et al.,* 1987). If large amounts of toxic oil are incorporated into sediments, all species may be eliminated. Once the toxicity of the oil is reduced through weathering processes, a succession from a physically controlled, simple community, to an unimpacted biologically accommodated, diverse community will occur (Sanders, 1969). Pearson and Rosenberg (1978) have described successions associated with organic loading.

Similar successions have been described following oil spills (Gilfillan *et al.*, 1983; Glemarec and Hussenot, 1981; Sanders *et al.*, 1980). Initially the environment is invaded by opportunistic species which may become extremely abundant. Over time, as both the amount and toxicity of the remaining oil are reduced, opportunists are replaced by species typical of biologically accommodated communities which are better competitors. In time, the community will return to an unimpacted state. However, the unimpacted state may not be that state in which the community was immediately prior to the oil spill.

It is useful to consider that community structure is the result of a complex process affected by changes in both physical and biotic boundary conditions, which result in annual and inter-annual variability in community structure (Buchanan *et al.*, 1978; Eagle, 1975; Glemarec, 1979; Princz *et al.*, 1983; Rachor and Gerlach, 1978; Rachor *et al.*, 1982). Therefore, the often frequent lack of data on the community structure prior to an oil spill may not be a serious handicap. The appropriate reference for determining recovery from oil spill impacts is when the community has returned to the state that it would have been in, had the oil spill not occurred. If proper reference locations are sampled, recovery to an unimpacted state can be gauged by comparison with them.

Multivariate methods use all of the information in a data set. They are preferred for analyzing large station × species data sets. (Green, 1980; Pielou, 1984). A wide variety of multivariate methods has been proposed for use on environmental data (Green, 1980; Field *et al.*, 1982; Hill and Gauch, 1980; Hill 1973; Kruskal and Wish, 1978; Pielou, 1984). Ordination by Principal Component Analysis (PCA; Pielou, 1984; Seber, 1984) is easily performed and is effective in extracting distribution patterns and relationships from large species × station data sets. A drawback to PCA is that non-linearity in the data structure can cause distortions in results (Gauch and Whittaker, 1972; Gauch 1982). However, linear data structures are frequently found in nature (van der Maarl, 1980) and problems arising from non-linearity in the data set can be minimized by analyzing data from similar environments. Data reported in this paper are from the upper stations. At this level the sediments in all three test areas were similar; they were muddy sands. Projection of minimum spanning trees on to ordination plots will detect any distortions which may exist (Gower and Ross, 1969).

Results of PCA showed that 25.5% of the variance in the data set was associated with the two most important axes fitted by PCA. A plot of ordination scores for axis 1 *v* ordination scores for axis 2 using data for all stations and dates is shown in Figure 2. Development of oil impact on community structure is clearly visible as the post-spill points for the area exposed to untreated oil move further away from the centroid as time passes after the oil spill. Those species important in defining axis 1 are *Heteromastus filiformis, Streblospio benedicti, Macoma balthica* and *Etone heteropoda.* These are the species most important in defining the impact of the untreated oil and in defining recovery to an unimpacted state.

Axis 2 is defined in terms of harpacticoid copepods and *Mya arenaria* spat. Inspection of the data set reveals no clear relationship between oil exposure and population density of harpacticoid copepods. *Mya arenaria* spat are always much more abundant in the area exposed to untreated oil. There is a very strong seasonal component in abundance of *M. arenaria* spat. The major events along axis 2 are associated with high densities of *M. arenaria* spat (6/10/81 in the reference area; 7/82 in the untreated oil area). Information contained in axis 2 appears to relate to natural annual and interannual variability.

A plot of ordination scores for axis 1 *v* ordination scores for axis 2 using data for the reference area is shown in Figure 3. Apart from one outlier (6/10/81), these points form a centroid which shows no persistent trends in community structure.

A plot of ordination scores for axis 1 *v* ordination scores for axis 2 using data for the area exposed to chemically dispersed oil is shown in Figure 4. These points also form a centroid indicating no persistent trends in community structure. No impacts on community structure were associated with exposure to chemically dispersed oil.

A plot of ordination scores for axis 1 *v* ordination scores for axis 2, using data for the area exposed to untreated oil, is shown in Figure 5. Here the development of oil spill impact is shown as an excursion to the left side of axis 1. Points for 6/82 and 8/82 have joined the centroid of points formed by the pre-spill samples from this area which also corresponds to the centroid of points from the reference area and the area exposed to chemically dispersed oil (Figure 2).

Figure 6 shows the same plot as Figure 2 except that a minimum spanning tree has been projected on to it. It is clear that there are two families of points. Points representing unimpacted samples form one group. Points representing impacted samples form another. The impacted group contains all the post-spill samples from the area exposed to untreated oil from 1981 and the July 1982 sample. The July 1982 sample contained 5 times as many *Mya arenaria* as the samples from other areas. Population densities of species associated with Axis 1 were not very different from those in other areas. Thus it appears that infaunal communities in the area exposed to untreated oil had recovered by the end of the summer of 1982, and that this recovery can be shown by the fact that ordination scores are similar to those for unimpacted samples, and that these samples are more similar to unimpacted than to impacted samples.

The multivariate methods used in this study utilize all the information contained in the data set; they provide a quantitative way to visualize the impacts of the untreated oil on community structure. They also provide a way to identify those species which are most important in defining the succession to an unimpacted community. In addition, they provide an objective way to define the end of the succession that is not dependent on having information about pre-spill community structure.

SUMMARY AND CONCLUSIONS

Ordination techniques utilize a major portion of the information contained in the data set to describe relationships between samples across space or time. Principal component analysis was applied to infaunal community structure data collected at a number of locations prior to and following a series of experimental oil spills in which intertidal infaunal communities were exposed to untreated crude oil, chemically dispersed crude oil and no treatment.

Ordination results are expressed with respect to axes fitted to the original data set. Examination of the eigenvectors associated with each axis will reveal which species are most important in defining it. The most important axis in the data set examined was defined in terms of two species of opportunistic polychaete worms and two species of detritivores typical of unimpacted communities. The next most important axis was defined in terms of *Mya arenaria* spat and a group of copepod species. Together these axes accounted for 25.6% of the variance in the data set.

When coordinates for the two most important axes were plotted against each other, those stations receiving untreated oil showed dramatic changes followed by succession to an unimpacted state. After 12 months, ordination scores for the impacted stations were similar to those for pre-spill samples from the same locations and to control samples taken at the same time. Ordination scores for the area exposed to chemically dispersed oil were similar to those for the reference area.

Ordination techniques offer a quantitative way to follow recovery of communities from oil spill or other impacts. Ordination techniques may also provide an objective way, in the absence of pre-spill data, to determine when the recovery succession has gone as far as it can, i.e. when ordination scores for successive samples from a location are similar to each other and are similar to scores for unimpacted reference stations.

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