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Impact of the *Aegean* Sea oil spill on the subtidal fine sand macrobenthic community of the Ares-Betanzos Ria (Northwest Spain)

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Abstract

Two sites located in the sublittoral fine-sand macrobenthic community of the Ares-Betanzos Ria were sampled over four years (December 1992–November 1996) in the wake of the *Aegean Sea* oil spill. This sampling revealed that the petroleum had affected the structure and abundance of this community, as well as the number of taxa present. In this context, the results of the biotic index and the biotic coefficient were insufficient; however, study of the synthetic parameters, particularly through multivariate analysis, showed that the community went through three successive and distinct phases over time. A short period of high mortality in some species, especially amphipods, was followed by a period of low abundance that lasted until the spring of 1995. A period of recovery began in the second half of 1995 and continued through to the end of 1996, when the survey ended. The community showed a gradual evolution back towards the conditions observed immediately after the spill, when abundance of the more resistant species was still high. Despite this similarity, the last period exhibits a new structure, clearly separate from the two previous periods. This study provides information about the short-term effects of the *Aegean Sea* oil spill on the fine sand bottoms of the sites

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surveyed in the Ares-Betanzos Ria. This information could also serve as a baseline for identifying the effects of a more recent accident, the Prestige oil spill, in which similar communities in other Galician rias were polluted in 2002–2003.

Keywords: Marine macrobenthos; Sublittoral community; Oil spill; Monitoring; Bio-indicators, NW Spain

1. Introduction

The headlands of Brittany (France) and Galicia (Spain) have been particularly affected by oil spills due to tanker wrecks. The area of A Coruña (NW Spain), adjacent to a refinery, has experienced three major oil spills in the last 30 years. The first accident happened on 12 May 1976, when the super tanker *Urquiola*, carrying 107,000 tons of Persian Gulf crude, struck bottom while passing through the entrance channel into the A Coruña harbour (between 15 and 30 m). A total of 100,000 tons of oil were lost, most of which burned, but an estimated 25–30 thousand tons washed ashore, affecting approximately 215 km of coastline (Anonymous, 1976; Gundlach & Hayes, 1977; Stein, Gundlach, & Hayes, 1977). Sixteen years later, on 3 December 1992, the Greek tanker *Aegean Sea* released 79,000 tons of ''brent blend'' crude oil in a similar accident occurring just outside the A Coruña harbour (Martín Bauza, 1996). Prevailing winds pushed the tanker firmly onto the rocky coast, where it ran aground a few meters from A Coruña city, leaking and burning for five days. The severe weather conditions and the natural dispersive quality of this oil caused it to spread rapidly along 200 km of coast. In both cases, the heaviest oil contamination occurred in the three rias closest to the sites where the tankers ran aground: A Coruña, Ares-Betanzos and Ferrol.

On 13 November 2002, a third accident occurred 28 nautical miles from Cape Finisterre, when the *Prestige* began to list due to a developing crack on its starboard side. The ship, then transporting 77,000 tons of fuel oil M-100 (fuel oil number 6 in English terminology), drifted to a distance of less than 5 nautical miles off the Spanish coastline. There, with the assistance of two tugs, it was finally turned about and headed back out to sea. On 19 November, the tanker broke in two and sank off the Galician coast, 130 nautical miles from Cape Finisterre. During that seven-day period, more than 10,000 tons of oil is estimated to have leaked out to pollute the Spanish coast. Presently, oil continues to escape from the sunken hull of the Prestige, whose stern lies at a bathyal depth of 3500 m.

According to Lee and Page (1997), anywhere from 1% to 13% of the oil may penetrate shallow subtidal sediments (less than 10 m in depth) after large spills, depending on the type of hydrocarbon and sediment type. The biological effects of oil spills are determined mainly by the hydrodynamics at the site and the chemical composition and concentration of hydrocarbons that reach the subtidal sediments, with the aromatic hydrocarbons being more toxic than the aliphatic hydrocarbons (Dauvin, 1987, 1998, 2000). Kingston (1992) holds that benthic community structure can clearly be altered by hydrocarbon concentrations higher than 50 ppm in the sediment. Research into the effects of hydrodynamic gradients on contamination has shown that benthic communities exposed to strong tidal currents (coarse sand and pebbly substrates) were less affected by hydrocarbon contamination than the ones located in low tidal current areas (medium and fine sand, mud), such as those touched by the Amoco Cadiz oil spill off the Brittany coast (Dauvin, 1987).

In the *Aegean Sea* spill, most of the crude oil burned, while another fraction was lost through evaporation following the tanker's grounding and subsequent explosions. It is difficult to determine what will happen to the remaining oil. One reasonable hypothesis is that large quantities of oil will be washed ashore, with possible contamination of the subtidal sediments near the site of the wreck in the Artabro Gulf and its three tributary rias (Martín Bauza, 1996).

Marine pollution management is based on monitoring various physico-chemical and biological parameters to detect changes in the environment. Macrobenthic communities provide the ideal context for such monitoring because their basic properties are useful for assessing marine pollution: (i) their lifespan is relatively long and stable; (ii) they exhibit moderately fast responses to stress; (iii) they are predominantly sessile or slow-moving organisms, vulnerable to the effects of sediment contamination; and (iv) they assimilate the effects of pollutants over time (Dauer, 1993; Gray, Clarke, Warwick, & Hobbs, 1990; Kingston, 1992). The effects of oil pollution on shallow subtidal macrobenthic communities (<30 m depth) have been widely reported in the literature for several significant spills (Sanders et al., 1980; Cabioch, Dauvin, & Gentil, 1978; Bonsdorff, 1981; Dauvin, 1982; Elmgren, Hansson, Larsson, Sundelin, & Boehm, 1983; Bilyard, 1987; Gray et al., 1990; Kingston, Dixon, Hamilton, & Moore, 1995; Parra & López-Jamar, 1997; Jewett, Dean, Smith, & Blanchard, 1999; Hawkins et al., 2002). Reported responses of infauna after an oil spill include very high initial mortalities in species sensitive to hydrocarbons, such as crustaceans and especially amphipods, and their subsequent disappearance. This process is followed, 1–3 years after the spill, by a significant increase in the abundance of opportunistic species, such as polychaetes, which proliferate due to increased organic matter (Pearson & Rosenberg, 1978; Sanders et al., 1980; Glémarec and Hussenot, 1982; Gray and Pearson, 1982). This initial impact has been correlated with the numbers of sensitive species in natural conditions, and data indicate that, after an oil spill, the re-colonisation of affected species in shallow subtidal areas generally takes more than 10 years (Gomez Gesteira & Dauvin, 2000).

Different methods have been used to monitor benthic communities affected by natural and anthropogenic stress. These methods can be divided into three main groups, according to approach. The first, based on the Pearson and Rosenberg model (1978), describes changes in benthic communities using basic quantitative parameters [species richness (S), abundance (A), and biomass (B)]. The most common technique is to track changes in parameters S, A and B, or in community structure indices (e.g. a diversity index), over space and time. However, the relationship between diversity and human-induced disturbances is quite complex, and a single diversity index is often not sufficient to indicate the degree of alteration in a benthic community. Gray and Mirza (1979) and Gray and Pearson (1982) have suggested using graphic techniques to identify changes in community structure along a pollution gradient. These techniques suggest that under the influence of a pollution-induced perturbance, the distribution of the number of individuals in each species is different from the distribution of species biomass (Warwick, 1986).

The second monitoring approach uses a single species, or group of species, to characterize the degree of community alteration. Such bio-indicator models have been widely used in freshwater quality control (Woodiwiss, 1964; Cairns, Douglas, Busey, & Chaney, 1968), and attempts have been made to define similar appropriate indicator species in benthic marine environments. Starting in the late 1970s and early 1980s, a variety of pollution indices have been used to describe the structure and spatio-temporal changes of marine benthic communities (Bellan, 1980; Bellan, 1984; Glémarec & Hily, 1981; Raffaelli & Mason, 1981; Washington, 1984; Lechapt, Bellan, & Retière, 1993; Grall & Glémarec, 1997a, 1997b; Borja, Franco, & Pérez, 2000; Blanchard, Feder, & Shaw, 2003). These studies highlight the usefulness of grouping macrobenthic species according to their susceptibility, resistance or proliferation with respect to a pollution gradient.

The third approach to monitoring is to use multivariate, classification and ordination analyses in conjunction with one of the methods noted above. Such analyses, developed over the past two decades, are employed because of their usefulness in demonstrating changes in community patterns (Field, Clarke, & Warwick, 1982; Gray et al., 1988; Gray et al., 1990; Warwick, Platt, Clarke, Agard, & Gobin, 1990; Rakocinski et al., 1997; Blanchard, Feder, & Shaw, 2002).

The objective of this paper is to identify the short-term effects (1992–1996) of the *Aegean Sea* oil spill on the macrobenthic sandy community of the Ares-Betanzos Ria. We employ a variety of the methods described above to assess the effects of oil pollution on macrobenthic communities.

2. Materials and methods

2.1. Study area

According to Evans and Prego (2003), rias are incised valleys whose estuarine zones shift with climatic changes. For both hydrographic and sedimentological reasons, only the inner zones of Galician rias can be considered estuaries, which confines the main estuarine processes to this relatively small sector of brackish water.

The Ares-Betanzos Ria has a double estuarine system (Nonn, 1966; Asensio-Amor & Grajal-Blanco, 1981) covering a surface of 73 km^2. Two inner channels, influenced by estuarine processes, come together in an inner confluence zone that connects freely to the open sea opposite the ria's mouth. Depths range from 2 to 43 m. Sediments are distributed in three principal zones. The *inner* zone, which as noted above can be considered as two estuaries, has a depth of <10 m, and is dominated by muddy sediments with high silt and clay content. Muddy areas exist in both channels of this zone due to deposition (Mora et al., 1996). The *central* zone, whose depth ranges between 10 and 30 m, is dominated by medium to fine sands, and the *mouth*, the deepest and most exposed zone of the ria, is dominated by gravel and coarse sand with a notable shelly input (Sánchez-Mata, Glémarec, & Mora, 1999).

Sampling sites for the survey initiated three days after the *Aegean Sea* oil spill (December 1992) replicated those of a previous year-long study (1988–1989) of this ria's macrobenthic communities, in which monthly samples were taken from five sites in two distinct areas from August 1988 to July 1989. Two sites were located in the inner zone of the ria in a muddy *Abra alba* community. The three remaining sites were located in the sandy central zone of the ria, in an ecotone between the *Abra alba* and *Venus gallina* communities (Garmendia, 1997; Gomez Gesteira, 2001). [The main characteristics of the study area and sampling design are described in detail in Gomez Gesteira and Dauvin, 2000].

Two of the sites presented in this paper (Fig. 1), X (43°23.90′N; 8°14.15′W) and Z (43°23.85′N; 8°15.75′W), are located in the sandy central zone of the ria (10–15 m in depth). Because the dynamics of the third sandy site were so similar to site X (The Pearson correlation coefficient between densities was both large [$r = 0.76$] and significant [$P < 0.01$]), it was excluded from this study in order to simplify the interpretation of the results. The two muddy inner-zone sites of the Ares-Betanzos Ria were not included in this study because they were less affected by the oil spill (Gomez Gesteira, 2001); in fact, they were almost completely free of petroleum hydrocarbons after the first year (Pastor, Sanchez, Porte, & Albaigés, 2001).

2.2. Oil spill

Measurements of the hydrocarbons in the water column during the first year after the spill were carried out in 37 sites corresponding to the Artabro Gulf and the nearest continental shelf (González, Schultze, Escánez, & Cerqueira, 1997). Sites located

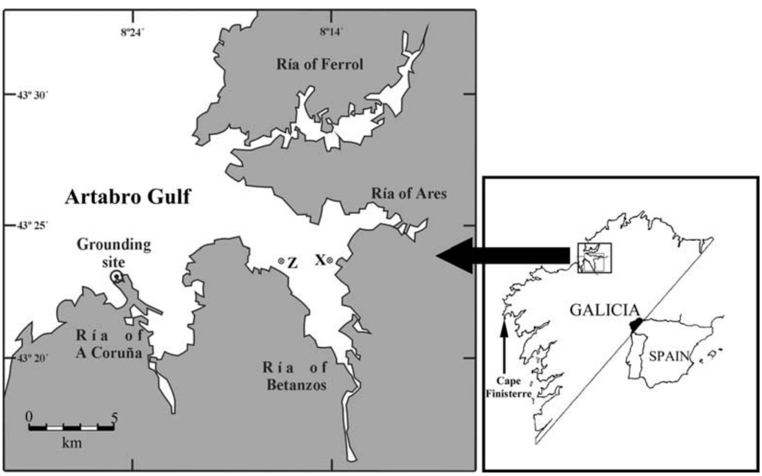


Fig. 1. Map showing the investigated area in the Artabro Gulf, NW Spain. Location of the sampling sites studied in the Ares and Betanzos Ria in relation to the grounding site of the *Aegean Sea*.

in the sandy central zone of the Ares-Betanzos Ria showed high concentrations of aromatic hydrocarbons in the bottom water in February 1993 (mean 79.1 $\mu g\, l^{-1}$ and maximum 257$\mu g\, l^{-1}$). Their sharp reduction in May 1993 (mean 3.2 $\mu g\, l^{-1}$ maximum 6 $\mu g\, l^{-1}$) was followed by a slight increase in September 1993 (mean 18.8 $\mu g\, l^{-1}$ maximum 63 $\mu g\, l^{-1}$), probably due to the physical disturbance of the sediment during beach cleaning operations, including dredging and dumping, which occurred in this area.

Two years after the wreck, the concentrations of hydrocarbons were measured in 39 sites throughout the ria seabed. The total polycyclic aromatic hydrocarbons (PAHs) content, analysed by HPLC-UV, indicated a moderately contaminated environment (Neira et al., 1997). The most polluted sites were located at the mouth of the ria (10,174 ng g^{-1} PAHs), especially on the south shore of the central zone (34,228 ng g^{-1} PAHs). These concentrations clearly exceeded the 'Effects Range Low' (ERL) sediment toxicity of 4000 ng g^{-1} per total resolved PAHs, proposed by Long and Morgan as a measure of oil's potential toxic effect (see Lee & Page, 1997). In contrast, in the innermost zone of the ria, levels of petroleum hydrocarbons remained very low (Neira et al., 1997), and oil subsurface layers were only detected in March 1993 (Sánchez-Mata, 1996). In addition, sediment samples collected in April, July, September and November of 1993 at two sites situated in the estuarine areas reflected low hydrocarbon levels (Pastor et al., 2001), although higher concentrations of hydrocarbons were found at a site in the same central zone of the ria, where PAHs exceeded ERL on all four occasions.

2.3. Sampling

Based on a variety of criteria (Cain & Castro, 1959; Boudouresque & Belsher, 1979; Martín, Ballesteros, Gili, & Palacín, 1993), calculations of the minimum required sampling surface were performed at sites X and Z. The calculations demonstrated that three fauna samples were sufficient to determine the number of species present in the community as well as their abundance and diversity. Thus, every month from August 1988 to July 1989, three samples were taken at each site. Biological samples were collected with a 1/127 m^2 box corer, which penetrated approximately 16 cm into the sediment. Samples were sieved immediately through a 1 mm screen, and preserved in 6% neutralized formaldehyde. Using a PVC corer tube, a sediment subsample was taken from the grab for sediment analysis, which included particle-size determination. Bottom water samples were also taken in order to obtain information about salinity, temperature, pH and oxygen concentration (Gomez Gesteira, 2001).

The sites originally sampled were re-sampled after the oil spill using identical methods. However, due to the reduction in species numbers and abundances subsequent to a polluting event, a higher minimum sampling surface was required. Thus, six samples were taken at each site from December 1992 to November 1996, instead of the three taken originally. The sampling period varied from year to year and season to season: monthly from December 1992 to December 1993; three times (March, June and September) in 1994; on eight occasions in 1995 (January, April, June, July, August, September, October and, November), and once a season in 1996 (January, April, July and November). Samples were taken on 28 occasions during the 4-year post-spill survey.

2.4. Laboratory analysis

Sorting was facilitated by staining the fauna with rose Bengal. The organisms were separated from the remaining sediment and counted; polychaetes, molluscs, crustaceans, and echinoderms were classified to the lowest possible taxonomic level (generally species), while other taxa were only classified to the level of major groups (Gomez Gesteira, 2001). The organic content of the dry sediment, estimated as the loss of weight after burning, was calculated by weighing the ashes. Sediment grain size was determined by the standard mechanic wet-sieved procedure (Buchanan, 1984). Size classes <62 μm were estimated by pipette, using the Robinson method.

2.5. Data analysis

Descriptive statistics were used in order to describe the evolution of the macrobenthic community at each site before and after the pollution: richness (total species numbers per date) and total density (ind m^2). To quantify the temporal variability of these parameters, a variance estimate was obtained using a method based on successive differences, described in Scheaffer, Mendenhall, and Ott (1996). Also, different calculations were made to assess the qualitative and quantitative changes in the structure of both sites studied, following the oil spill. Diversity was estimated using the Shannon diversity index (H', $\log_2$ based, Shannon & Weaver, 1963). The Biotic Index (Biotic Coefficient, BC: Borja et al., 2000) was used to detect temporal changes in the quantitative structure of the macrobenthic fauna. Based on previous models by Glémarec (1986) and Hily (1984), this index applies Salen-Picard's logic (1983), concerning the four progressive steps in stressed environments, to the classification of soft-bottom fauna, resulting notably in the classification of soft-bottom macrofauna into five groups according to their sensitivity to an increasing stress gradient. These groups have been summarized by Grall and Glémarec (1997a): Group I (species very sensitive to organic enrichment), Group II (species indifferent to enrichment), Group III (species tolerant to excess organic matter enrichment), Group IV (second-order opportunistic species) and Group V (first-order opportunistic species).

A single formula based upon the percentages of abundance for each ecological group produces a continuous BC, with values ranging from 0 to 6, rising to 7 when the sediment is azoic. BC validation of the five ecological groups was done using Borja's list (2000) of more than 900 taxa from various studies of systems affected by human disturbances. Species not assigned to a group, and those not found on the list published by Borja et al. (2000), represented only 4.20% of the total abundances at site X and 5.46% at site Z. These species were not included in the results. Additionally, as suggested by Eaton (2001), two variables – total taxon numbers (TT) and amphipod and caridean shrimp taxon numbers (A + C) – were used as evidence of the spill's impact. The latter taxa are known to be very sensitive to oil pollution (Gomez Gesteira & Dauvin, 2000).

In the same way that the nematode/copepod ratio has been used to track temporal changes in meiobenthos communities (Raffaelli & Mason, 1981; Warwick, 1981; Lambshead, 1984; Montagna & Harper, 1996; Ansari & Ingole, 2002), the changes in soft-bottom macrobenthic communities can be shown via the relationship between opportunistic polychaete abundance and amphipod abundance, expressed as Log_{10} (polychaetes/amphipods + 1) (Gomez Gesteira & Dauvin, 2000). Crustaceans, particularly amphipods, have been chosen as indicator species in several studies because they are highly sensitive to toxicants, particularly oil and its aromatic components (Dauvin, 1987, 1998; Ho, Mckinney, Khun, Pelletier, & Burgess, 1997; Lee & Page, 1997). Opportunistic polychaete species were selected for observation in this study because of their ability to proliferate following an increase in organic matter. Typically, these species are members of the following families (Bellan, 1984; Glémarec & Hily, 1981; Lechapt et al., 1993; López-Jamar, 1981; Wass, 1967):

- Capitellidae [*Capitella capitata* (Fabricius, 1780), *Mediomastus fragilis* (Rasmussen, 1973), *Heteromastus filiformis* (de Claparède, 1864)] and other unidentified Capitellidae;
- Cirratulidae [*Chaetozone setosa* (Malmgren, 1867), *Cirriformia tentaculata* (Montagu, 1808), *Aphelochaeta filiformis* (Keferstein, 1862), *Aphelochaeta marioni* (de Saint-Joseph, 1894)] and other unidentified Cirratulidae;
- Spionidae [*Polydora flava* (de Claparède, 1870) and *Malacoceros fuliginosus* (de Claparède, 1868),
- Eunicidae [*Nematonereis unicornis* (Grube, 1840)], and
- Spiochaetopteridae [*Spiochaepterus costarum* (de Claparède, 1868)].

The mean values for density and taxon numbers at the two sites were compared using paired T tests. In addition, Pearson correlation coefficients were calculated for the same descriptive statistics in order to compare the sites' temporal patterns.

Species density values after the spill were analysed at both sites, using multivariate analysis. First, the matrix of abundance data was double square root transformed to reduce the relative importance of the most abundant species. A hierarchical classification technique using UPGMA (Unweighted Pair Group Average Linkage) was performed, followed by non-metric multi-dimensional scaling (MDS) ordination. Both of these multivariate methods were performed on similarity matrices calculated using the Bray Curtis index. Principal component analysis (PCA) was also used on double square-root transformed data matrices. To assess the potential relationships between the biotic parameters and the PCA, Spearman's correlation coefficients were calculated.

To provide a visual representation of the differences between pre-spill and post-spill conditions, MDS ordination at each site was also applied to the square-root transformed species density data of both periods combined.

3. Results

The dominant sediment grain sizes at both the X and Y sites included medium, fine and very fine sand. The fine sediment fraction had a particle size of less than 62 μm (silt and clay) and reached mean values of 16.7% (±SD 4.3%) and 15.2% (±SD 8.5%) at sites X and Z, respectively. The sediment's organic matter content was similar at both sites: 1.51% (±SD0.25%) at site X and 1.70% (±SD 0.24%) at site Z.

3.1. General macrofaunal patterns

Site X. At site X, the number of species collected after the spill was 162 (Table 1A); polychaetes were dominant, accounting for more than 40% of the total number of species. Most of the species (60%) were rare (found less than six times in 28 sampling dates). The total number of individuals found over the 4-year period was 6.138. Polychaetes formed the largest percentage of the total number of individuals, followed by molluscs, crustaceans and other groups. The polychaetes – *Paradoneis armata* Glémarec, 1966 with a total abundance of 11.4%, *Spio decorata* Bobretzky, 1871 with 4.8%, *Lanice conchilega* (Pallas, 1766) with 4.3%, and *Notomastus latericeus* Sars, 1850 with 3.2% – dominated, and showed peaks of abundance from the spring-summer 1995 until the end of the survey. A similar pattern of abundance was found for certain other relatively abundant species, such as *Mediomastus fragilis*, *Diplocirrus glaucus* (Malmgren, 1867) and *Ampharete acutifrons* (Malmgren, 1866). On the other hand, *Hyalinoecia bilineata* Baird, 1870, *Glycera tridactyla*

Table 1
Total number of taxon and total number of individuals at the two sites (A) from December 1992 to November 1996 and (B) from August 1988 to July 1989.

Sites	X				Z			
	Total number of species	%	Total number of individuals	%	Total number of species	%	Total number of individuals	%
1992–1996								
Polychaetes	69	42.6	3331	54.3	60	39.5	2409	39.5
Molluscs	39	24.1	1198	19.5	30	19.7	885	24.6
Crustaceans	33	20.4	323	5.3	44	28.9	252	5.3
Others	21	12.9	1286	20.9	18	11.8	1160	28.9
Total	162		6138		152		4706	
1988–1989								
Polychaetes	49	40.2	1347	57.5	38	38	1073	57.1
Molluscs	34	27.9	512	21.9	21	21	358	19.1
Crustaceans	25	20.5	143	6.1	25	25	161	8.6
Others	14	11.4	340	14.5	16	16	287	15.2
Total								

Schmarda, 1861, *Lumbrineris gracilis* (Ehlers, 1868) and *Nephthys hombergii* de Savigny, 1818 were relatively constant through the study, though low in abundance. No proliferation was observed at this site.

Molluscs, mainly represented by *Chamelea gallina* (L., 1758) (6.4% of total abundance) and *Fabulina fabula* (Gmelin, 1791) (1.8% of total abundance), showed more erratic fluctuations. However, an increase in this group was observed over the last year of the study due to the high recruitment of the bivalve species *Thracia phaseolina* (Lamarck, 1791) (3.8% of total abundance) and *Mysella bidentata* (Montagu, 1803) (1.82% of total abundance). Crustaceans, especially amphipods, were particularly affected after the spill, resulting in the total disappearance of several species from December 1992 to January 1993 to the beginning of 1995. The most abundant species, such as *Ampelisca brevicornis* (Costa, 1853) (1.2% of total abundance) and *Pariambus typicus* (Kröyer, 1845) (1.1% of total abundance), were present only after April 1995. Other highly abundant species included the cnidarian *Edwarsia claparedii* (Panceri, 1869) (8.9% of total abundance), which maintained a high number of individuals throughout the four years, and the nemertean *Tubulanus polymorphus* Reiner, 1804 (5.6% of total abundance), which was especially abundant from spring 1995 to the end of the survey in 1996.

The total number of species and individuals encountered at site X during the pre-spill survey was 122 and 2343, respectively. Polychaetes dominated in number of species and individuals following by the molluscs; with approximately 25% of the number of species, Crustaceans formed only 6% of the total abundance, while others groups formed 11% of the number of species and 14% of the total abundance (Table 1B).

Site Z. At site Z, the total number of species collected from December 1992 to November 1996 was 152 species (Table 1A), the majority of them belonging to the polychaetes (40%). Rare species (found less than six times in 28 sampling dates) accounted for 61.8% of the total number of species. During this survey, 4706 individuals were collected at site Z. Polychaetes, the most representative group in terms of abundance, formed 50.6% of the total number of individuals. The cnidarian *Edwardsia claparedii* (9.7% of total abundance) dominated, maintaining a higher number of individuals throughout the survey, followed by the polychaetes spionidae *Spiophanes bombyx* (de Claparède, 1870) (7.5% of total abundance), also characterized by constant high abundances, and *Spio decorata* (4.5% of total abundance), whose temporal variations were more erratic. The bivalves *Fabulina fabula* (7.1% of total abundance) and *Chamelea gallina* (4.5% of total abundance), and the Phoronida *Phoronis muelleri* Selys-Longchamps, 1903 (4.9% of total abundance) had stable, high populations throughout the survey. Other abundant species included the polychaetes *Magelona mirabilis* (Johnston, 1865), *Hyalinoecia bilineata*, *Diplocirrus glaucus*, *Notomastus latericeus* and *Nephthys hombergii*.

Over the four-year survey, only ten species occupied the first rank as the most abundant for each sampling date at site Z. Crustaceans showed very low abundance; they were dominated by *Ampelisca brevicornis* (0.7% of total abundance), *Iphinoe trispinosa* (Goodsir, 1843) (0.6% of total abundance) and *Hippomedon denticulatus* (Bate, 1857) (0.6% of total abundance). Amphipoda were mainly absent from the site from December 1992 to January 1993 to the spring 1995. The sea urchin *Echinocardium cordatum* (Pennant, 1777) was collected only after August 1995.

The total number of species and individuals found at site Z over the 12-month pre-spill sampling period was 100 and 2343, respectively. As at site X, polychaetes dominated in number of species and individuals, followed by the molluscs; others groups, for example the Crustaceans, formed less than 10% of the total abundance (Table 1B).

3.2. Temporal changes in the macrofauna

At both sites, temporal changes in the total taxon numbers (TT) (Fig. 2B) roughly followed the changes in density. Paired *T* tests showed differences between the mean values of the taxon numbers for the two sites ($t = 2.60$; d.f. = 27; $P < 0.01$), and a

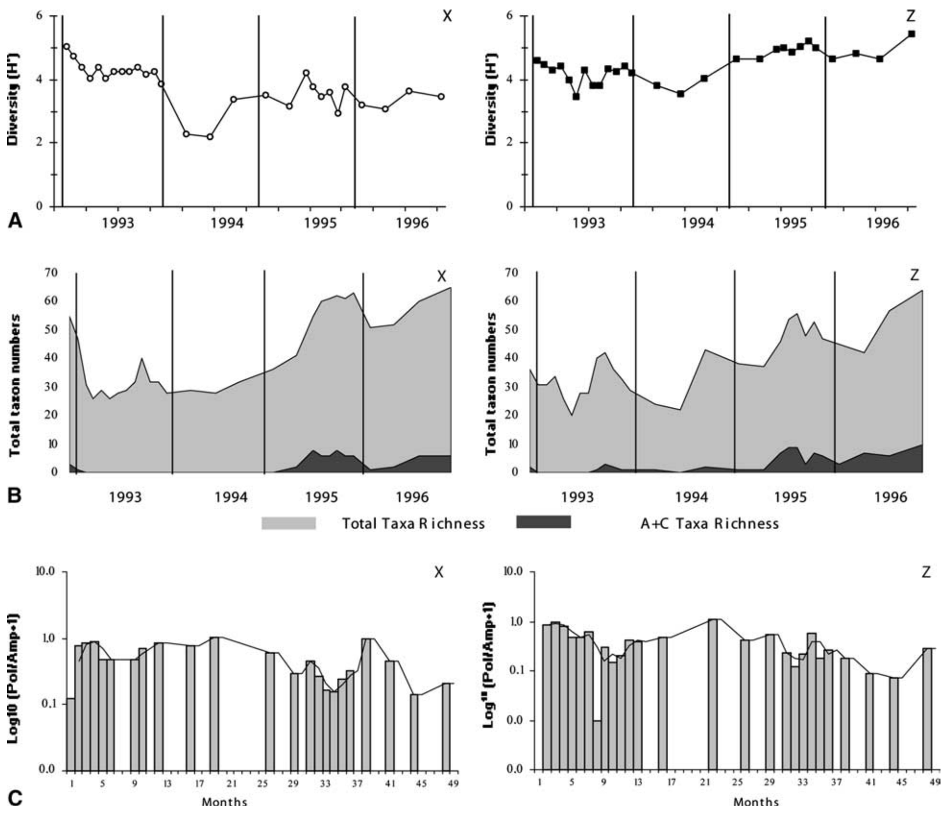


Fig. 2. Temporal changes at both sites X and Z after the spill. (A) Total diversity (H′). (B) Amphipod and caridean shrimp taxa richness (A + C) and total taxa richness. (C) Ratio between the opportunistic polychaete abundance and the amphipod abundance +1, log 10 scale for the ratio (each value is indicated by a bar the line for each site is the mobile mean). *T* in months: from t1 (December 1992) to t48 (November 1996).

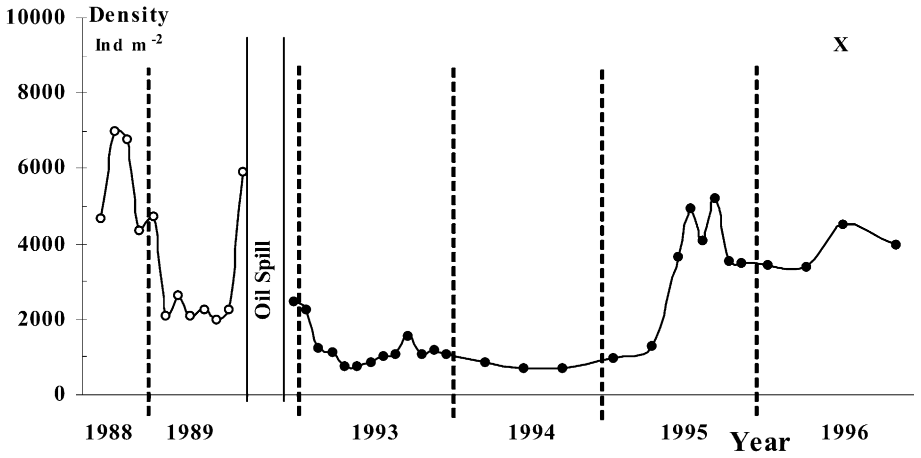
Pearson correlation indicated a strong correlation between the two sites ($r = 0.858$; $P < 0.01$). TT values for site X reveal: (1) a considerable decrease from December 1992 to March 1993, (2) persistently low values until the spring 1995, and (3) an increase during the summer 1995, with high values remaining until the end of the survey. Values at site X averaged 42.71 ± 1.07 species, ranging between 22 species in June 1994 and 64 species in November 1996. Site Z showed a similar trend, though a resilient seasonality was observed from 1992 to 1996: maximums occurred at the end of summer or the beginning of autumn (maximum of 65 in November 1996), and minimums were seen at the end of winter or the beginning of spring (minimum of 16 in March and May 1993), with an overall tendency towards an increase from spring 1995 to the end of the survey in November 1996. The mean TT at this site (*Z*) was 38.92 ± 1.27 species. Though only three samples were considered for every sampling date from August 1988 to July 1989, the mean TT at site X showed higher pre-spill values (47.08) than those after the spill. At site Z, pre-spill TT values, which averaged 37.67, were of the same order of magnitude as post-spill values.

The temporal changes in density at each site revealed two slightly different patterns after the oil pollution. A paired *T* test showed significant differences between the mean values ($t = 2.05$; d.f. = 27; $P < 0.01$). The Pearson correlation coefficient between the two sites was poor ($r = 0.494$, $P < 0.05$).

Site X. At site X, the mean total density after the spill was 2193 ind m^2 ± 132.90 (the estimation error was calculated using the variance estimate based on successive differences). Quantitative changes in this period could be separated into 4 periods (Fig. 3). During the first period just after the *Aegean* Sea wreck (December 1992–May 1993), densities decreased. Densities in the second period (spring-summer 1993 until spring-summer 1995) were persistently low, with values ranging between 710 and 1560 ind m^{-2}. The third period began in spring 1995, and was characterised by an increase that peaked during the summer of 1995 (>5000 ind m^{-2}). This increase could be attributed to two factors: (1) the influence of certain species that accounted for a large portion of total density values, such as *Paradoneis armata*, *Spio decorata*, *Lanice conchilega*, *Notomastus latericeus*, *Mediomastus fragilis*, *Ampharete acutifrons*, *Diplocirrus glaucus*, *Tubulanus polymorphus* and *Thracia phaseolina*, and (2) the re-colonisation of crustaceans, especially amphipods. The last period, from summer 1995 to the end of the survey in 1996, produced persistently high densities (≅ 4000 ind m^{-2}). During this period, a seasonal change was observed, with a minimum in winter and a maximum in summer.

The pre-spill sampling period at site X averaged a total density of 3905 ind m^2, ranging from 6980 ind m^2 in September 1992 to 2000 ind m^2 in May 1989. The seasonal pattern was characterised by a peak during late summer - early autumn 1988 and July 1989, a sharp decrease through late autumn and winter, and lower value in late winter and spring.

Site Z. At site Z, total macroinfaunal density averaged 1681 ind m^2 ± 133.28 over the 4-year period surveyed, ranging from 610 ind m^2 in May 1993 to 3190 ind m^2 in July 1996. Densities show a consistent evolution of the temporal pattern (Fig. 3). Maximums were reached at the end of summer or at the beginning of autumn, and minimums appeared at the end of winter or at the beginning of spring, due to



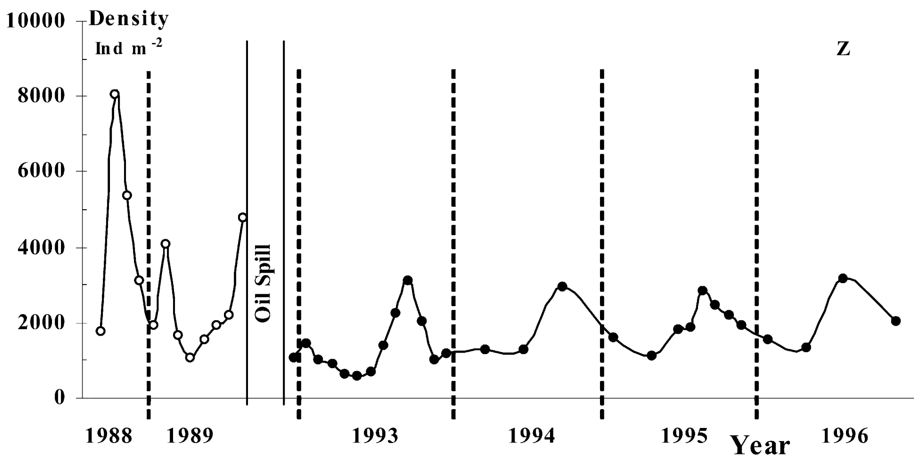


Fig. 3. Changes in density from June 1988 to August 1989 and from December 1992 to November 1996.

the seasonal cycles of the dominant species, especially polychaetes. These species included *Edwardsia claparedii*, *Fabulina fabula*, *Spio decorata*, *Chamelea gallina*, *Magelona mirabilis*, *Pherusa monilifera* delle Chiaje, 1841; *Ampharete acutifrons* and *Phoronis muelleri* with peak densities in September 1993; *Spiophanes bombyx*, *Fabulina fabula*, *Mediomastus fragilis*, *Hyalinoecia bilineata*, *Glycera tridactyla* and *Phoronis muelleri* with peak densities in September 1994; *Edwardsia claparedii*, *Phoronis muelleri* and *Tubulanus polymorphus* with peak densities in August 1995; and *Spiophanes bombyx*, *Phoronis muelleri*, *Spio decorata*, *Owenia fusiformis*, *Ampelisca brevicornis*, *Echinocardium cordatum* and *Ampelisca spinimana* (Chevreux, 1900) with peak densities in July 1996.

The mean density for the pre-spill sampling period at site Z was 3132 ind m^2, ranging from 8080 ind m^2 in September 1992 to 1100 ind m^2 in May 1989. There was a general seasonal pattern in total macroinfaunal density. Thus, values were maximal in September and October 1988 and in July 1989, followed by a remarkable decrease during autumn 1988 and low values in late winter and spring, interrupted by a brief rise in January.

3.3. Temporal changes in the synthetic parameters

3.3.1. Shannon diversity

The Shannon diversity index (H′) (Fig. 2A) averaged 3.77 ($\delta = 0.67$) at X site and 4.45 ($\delta = 0.50$) at Z site during the 4-year observation after the spill. At both sites, a similar pattern was found from December 1992 to December 1993, with high values always >3 and a slight overall decrease. From December 1993 to June 1994, the pattern was again similar for both sites, although values for H′ were consistently lower at site X than at site Z. This pattern was characterized by a decrease, followed by more or less regular increases from that point to the end of 1995, and flat values throughout 1996. Although both sites showed regular increases in diversity over the last two years of the survey, H′ values were always higher for site Z: a maximum value of 5.4 was reached at site Z in November 1996, whereas at site X, H′ reached 4.0 in June 1995, and then fluctuated between 3.0 and 4.0 until the end of the survey. For the sampling period August 1988–July 1989, the mean diversity was 4.77 ($\delta = 0.25$) at site X, higher than that observed after the spill. Nevertheless, at Z site, diversity attained similar values for both periods (4.44; $\delta = 0.32$).

3.3.2. Changes of faunal ratios

After the spill, a few individuals of the A + C group survived at site X and were noted during the first two months of the survey (December 1992–January 1993) (Fig. 2B). However, this group was totally absent from the contaminated sediments for a long period (February 1993 - April 1995). At site Z, some caridean shrimp were observed during the autumn of 1993 and 1994, indicating an initial recovery of this group, but no amphipods were observed prior to the beginning of 1995. At both sites, high increases and seasonal changes in A + C group abundance occurred from spring 1995 to the end of the survey in November 1996. Before the spill, crustaceans, particularly amphipods, had been a permanent presence, with this group accounting for approximately 10% of the mean TT at both sites during 1988–1989 survey.

The polychaete/amphipod ratio (Fig. 2C) showed very slight changes at both sites from the beginning of the spill in December 1992–January 1995 (ratio $\cong 1$). Then, the ratio decreased overall from April 1995 to November 1996, reflecting amphipod recovery. However, this ratio showed strong temporal variability from one month to another, related to natural changes in the abundance of the polychaete selected, such as *Spio decorata* or *Spiochaetopterus costarum*.

3.3.3. Changes of ecological groups

Fig. 4 shows the percentage of the five ecological groups (I–V) present each month at both sites X and Z, and the changes in the biotic coefficient (BC) over the life of

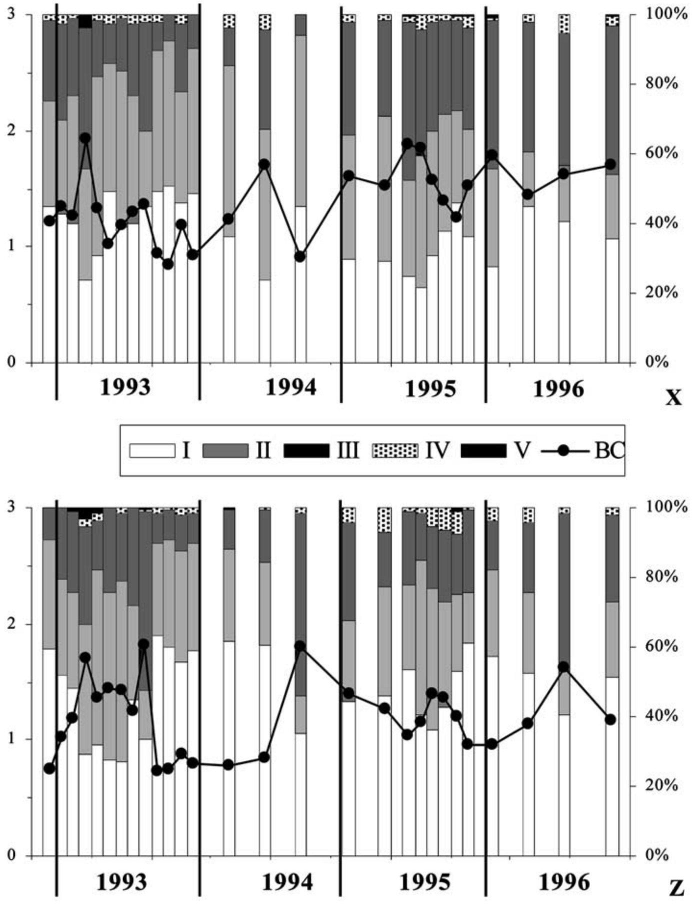


Fig. 4. Evolution of the percentage of ecological groups (I–V) and the Biotic Coefficient (BC) for the stations X and Z.

the survey (1992–1996). BC values ranged from 0.85 to 1.92 at site X and from 0.73 to 1.82 at site Z. According to the bio-indicator model proposed by Borja et al. (2000), both sites appeared to be slightly polluted (BC = 0.2–1.2 means an impoverished benthic community; BC = 1.2–3.3 means an unbalanced benthic community). Temporal changes in the BC did not reveal any clear pattern. Low BC values appeared to be the result of the persistent dominance throughout the survey of species from group I (sensitive to pollution) (ie. *Chamelea gallina*, *Fabulina fabula*, *Diplocirrus glaucus* or *Phoronis muelleri* at both sites and *Thracia phaseolina* or *Ampelisca* genus only at site X) and species from group II (indifferent) (ie. *Edwardsia claparedii*, *Tubulanus polymorphus*, *Nephthys hombergii*, *Glycera tridactyla* or *Lanice conchilega* also at both sites). The total of these last two groups represented a mean value of 72.21% at site X and 76.35% at site Z.

3.3.4. Cluster analysis

Fig. 5 shows a cluster analysis of the data. At site X, two main groups were recognized with a level of dissimilarity of 50%:

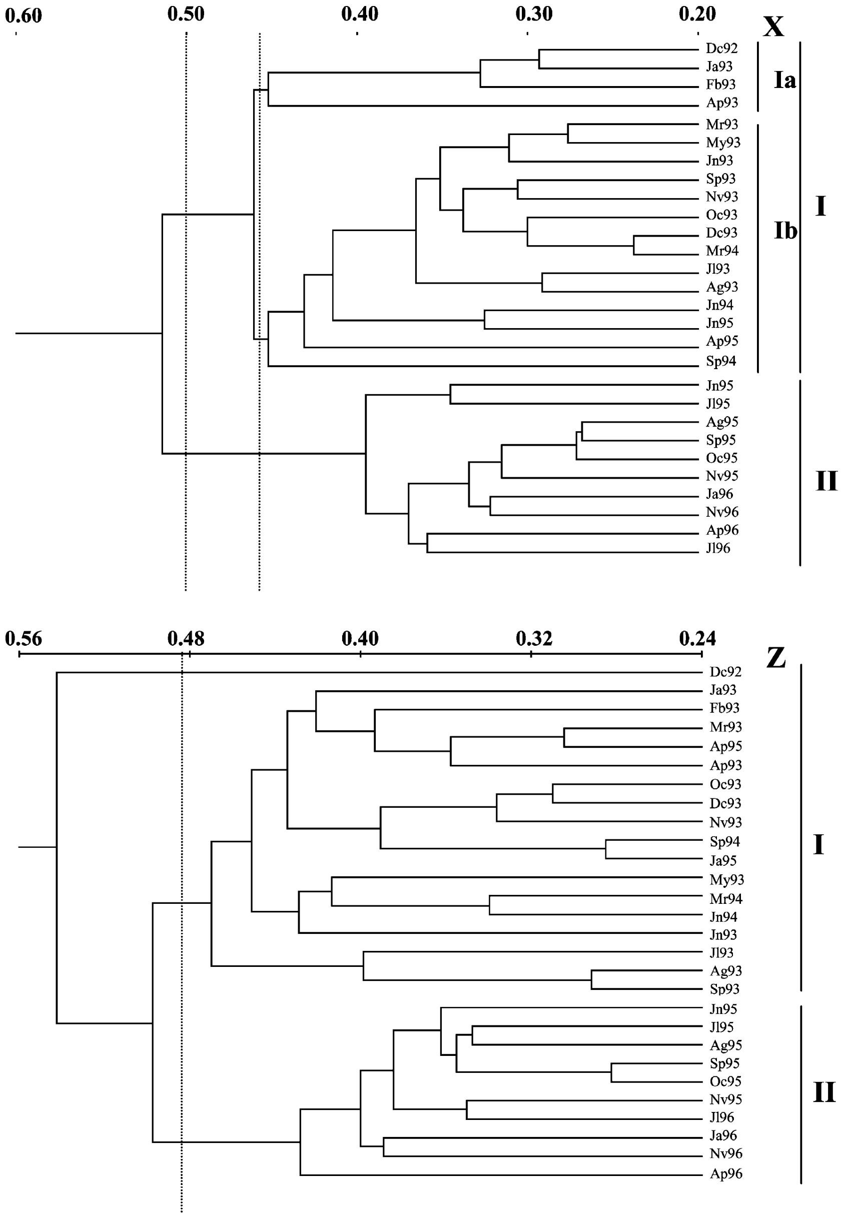


Fig. 5. Cluster analysis showing classification of the 28 dates for sites X and Z.

- group I was subdivided into sub-group Ia, comprising samples from the four months immediately following the spill (Dc92, Ja93, Fb93 and Ap93), and sub-group Ib, composed of the samples taken at the end of 1993, those from 1994, plus Ja95 and Ap95;
- group II included the last ten dates of the study (June 1995 to November 1996).

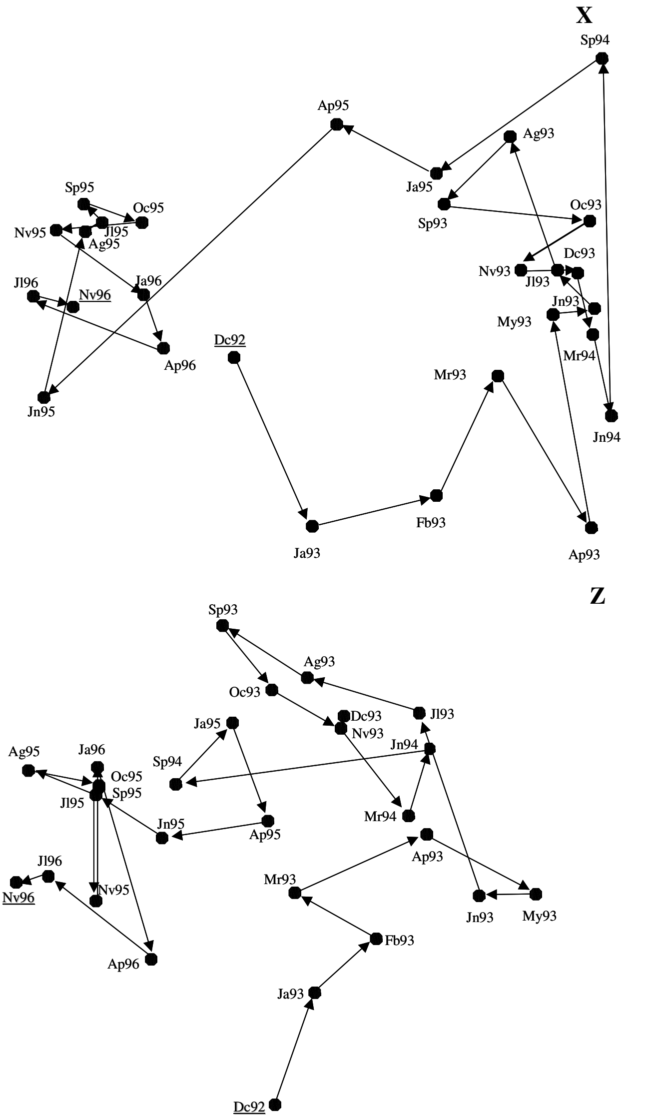


Fig. 6. Multi-dimensional scaling ordination performed on both sites X and Z, at each dates between December 1992 and November 1996. The points are linked in successive time. Stress values for the two-dimensional ordinations are: $X = 0.195$, $Z = 0.209$.

At Z site, December 1992 was isolated in the dendrogram, and two main groups were recognized with a level of dissimilarity of 49%:

- group I included all dates from January 1993 to April of 1995, and
- group II included the remaining dates, from June 1995 to November of 1996.

Thus, there was a similar temporal pattern with two main periods at both sites, even though seasonal changes remained dominant at site Z throughout the four-year survey. The recovery of the most oil-sensitive species, especially amphipods, was obvious at both sites by the summer of 1995.

3.3.5. MDS ordination

Two-dimensional MDS ordinations show acceptable stress levels ($0.25 > S > 0.1$) (Kruskal, 1964) at sites X and Z, indicating that there was an acceptable representation of the similarities in community structure. The MDS configuration in Fig. 6 shows a comparable circular cycle shape at both sites, with samples collected in the first period of the study occupying positions in the ordination space close to those from the last year. This trend was especially clear at site X, where three main periods could be identified from cluster analysis: December 1992–April 1993, May 1993–April 1995, and June 1995–November 1996. Segregation of samples at site Z was more complicated, although both an initial and a terminal period could be differentiated from the rest of the survey. PCA revealed a pattern similar to those of the MDS.

The results of MDS with the mixed pre-spill and post-spill data are shown in Fig. 7. Four groups are identified for site X. All samples surveyed during 1988–1989 period are located to the right on the plot, in two separate groups whose samples correspond to the high abundance months in the lower right (August to December 1988 and July 1989), and the low abundance months in the upper right (January to June 1989). Two periods were identified from the post-spill samples: one from May 1993 to January 1995 and one from April 1995 to November 1996. Samples from the five months immediately after the spill (December 1992–April 1993) are not part of any group. MDS separates site Z into three periods. To the right of the plot is a first period, corresponding to the samples collected before the spill (August 1988 to July 1989). In contrast, all the samples from the post-spill period, from December 1992 to November 1996, are located to the left of the plot, with two groups corresponding respectively to the beginning of the monitoring period (lower group) and to the end of the monitoring period (upper group).

4. Discussion

4.1. Global effects of oil spills on benthic communities

According to the literature (Glémarec & Hussenot, 1982; Elmgren et al., 1983; Dauvin, 1998, 2000; Hawkins et al., 2002), the standard response of shallow subtidal

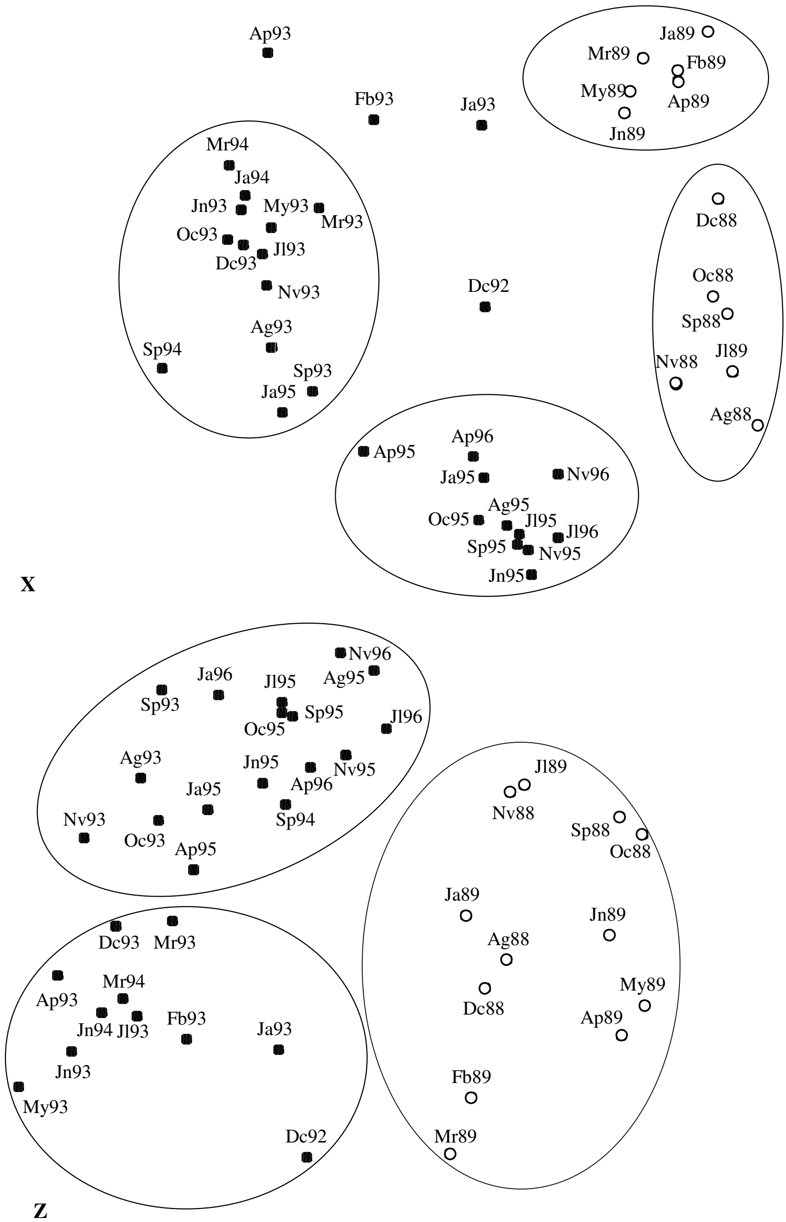


Fig. 7. Results of multivariate analyses for density data. Hollow circles correspond to the period before the oil spill (June 1988-August 1989) and solid circles correspond to the period after the oil spill (December 1992–November1996). Month groups are denoted in each figure. Stress values for the two-dimensional ordinations are: $X = 0.190$, $Z = 0.210$.

benthic communities affected by an oil spill includes four main phases: (1) a period of rapid mortality in the species sensitive to the presence of oil; (2) a period with low numbers of species and abundance; (3) a period of increasing abundance in opportunistic taxa, such as polychaetes and oligochaetes; and (4) a period of rapidly decreasing abundance in opportunistic species concurrent with a re-colonisation of the area by the sensitive species destroyed during the initial period of stress.

During the first few months after an oil-spill, high concentrations of polynuclear aromatic hydrocarbons (PAHs) provoke an initial toxic response, killing the more sensitive species, especially amphipods, (Cabioch et al., 1978; Sanders et al., 1980; Elmgren et al., 1983; Gundlach et al., 1983; Dauvin, 1987; Widbom & Oviatt, 1994; Ho et al., 1997). Amphipods have been used in a variety of toxicity test methods (Swartz, Deben, Sercu, & Lamberson, 1982; Ho et al., 1997), and several authors have observed high amphipod mortalities after an oil spill (e.g. Elmgren, Hansson, Larsson, & Sundelin, 1977; Sanders et al., 1980; Elmgren et al., 1983; Dauvin, 1987). Due to their weak capacity for dispersion and their low fecundity, these amphipods exhibit a low colonisation rate (>10 years) after a spill (Dauvin, 1987). However, decreasing hydrocarbon concentrations (intermediate levels) provoke faunal responses similar to those resulting from organic pollution (Glémarec & Hussenot, 1982). The increased availability of the remaining hydrocarbons results in increased numbers of hydrocarbon-degrading microbes, which provide food for opportunistic fauna (e.g. certain species of nematodes and polychaetes). In addition, hydrocarbon degradation reduces oxygen levels in sediments, enabling some opportunistic species to become dominant (Gilfillan, Page, & Foster, 1986; Spies, Hardin, & Toal, 1988). Similar effects have been observed in the benthos around the North Sea platform (Davies et al., 1984; Kingston, 1992) and at an oil terminal in Port Valdez, Alaska, (Blanchard et al., 2003), where various spatial patterns of faunal responses point to the consequences of toxic effects and organic enrichment. Detailed study of oil-seep communities (Steichen, Holbrook, & Osenberg, 1996; Spies & Davies, 1979; Spies & Des-Marais, 1983) has shown that while high concentrations of petroleum hydrocarbons initially reduce infaunal densities due to toxic effects, intermediate hydrocarbon concentrations stimulate infaunal densities due to organic enrichment.

Nevertheless, Blanchard et al. (2002) observed that the effects of marine pollution are often difficult to determine because the natural variability of environmental factors may mask responses to contamination (Pearson & Rosenberg, 1978; Gray and Mirza, 1979; McManus & Pauly, 1990). Observations made after the *Exxon Valdez* oil spill (Jewett et al., 1996) pointed out the need to be aware of natural population changes in an area after an oil spill, in order to avoid attributing all changes to the effects of hydrocarbons. Jewett et al. (1999), for example, found that periods of natural hypoxia-anoxia were mixed with anthropogenic pollution in one area affected by the spill. Moreover, macrobenthic sampling designs can only identify temporal or spatial disturbances at discrete intervals whereas the effects of stress are a continuum (Blanchard et al., 2002).

Unlike amphipods, polychaetes are species resistant to high levels of hydrocarbons in sediment (Dauvin, 2000; Blanchard et al., 2003) and demonstrate increased abundances after an oil spill. Still, no major proliferation of opportunistic polychaetes was observed at the two sites surveyed, as was also the case in the deep benthic environment of Prince William Sound after the *Exxon Valdez* oil spill (Feder & Blanchard, 1998), and in shallow depths after the *Braer* oil spill (Kingston et al., 1995) and the 1978 *Amoco Cadiz* oil spill in the Bay of Morlaix (Brittany, France) (Dauvin, 1998, 2000). In the case of the *Aegean Sea* oil spill, however, a large increase in *Diplocirrus glaucus* abundance was observed in September 1995 at a third site (not included in this study), also located in the sandy central zone of the Ares-Betanos Ria (Gomez Gesteira, 2001). In addition, at a site located in the muddy inner part of the same ria, an increase in abundance of *Chaetozone setosa* was recorded in March 1994 (Gomez Gesteira, 2001). A similar response was also reported for a series of subtidal sites (~10 m depth) after the *Florida* oil spill in Buzzards Bay, Massachusetts (Sanders, 1978; Sanders et al., 1980), where the effects of the spill occurred approximately one year later, resulting in a recruitment of the opportunistic capitellid *Mediomastus ambiseta*. As Kingston et al. (1995) has suggested, the response of the benthic communities may not be immediate, given a low toxicity of the polluting oil or an initial sub-acute impact.

4.2. Main features of the Aegean oil spill on the benthic communities in the Ares-Betanzos Ria

The Biotic Coefficient did not detect temporal changes at either of the two sites examined in this study. This evolution was not associated with changes in density and total taxon number or disappearances of most sensitive species due to the oil pollution. For this reason, Hily (1984) and Grall and Glémarec (1997) have stated that while heavy pollution is relatively easy to detect using biotic indices, such indices do not appear suitable for intermediate levels of pollution due to their lack of sensitivity. Depending on their sensitivity to pollution stress, models based on the recognition of ecological groups are also of limited use. Although a number of opportunistic species (Pearson & Rosenberg, 1978) and species more sensitive to pollution (Elmgren et al., 1983; Bellan-Santini, 1980; Dauvin, 1987) seem to exhibit universal features, little is known about the lifestyle of several macrobenthic species, and thus assigning them to one group or another is not an easy task.

In terms of abundance, each site in this study showed a different response to the *Aegean Sea* spill. Site X underwent three successive periods of change. Period 1 corresponds to a short time lapse just after the spill (≅ 3 months), and is characterized by the reduction and disappearance of sensitive species (A + C) and a considerable decrease in total abundance. Period 2, which spans the two years up until the spring 1995, exhibits persistently low abundances values, while period 3, which runs from the summer of 1995 to the end of the survey, displays important increases in taxon numbers and abundance resulting from the successful recruitment of species that were rare during period 2. Site Z, on the other hand, showed a pattern of regular seasonal variations, and the spill seems to have had less of an effect on the macrobenthic community here than at site X. Low numbers of amphipods + shrimp were noted at this site prior to summer 1995; however, since the abundance of these species was naturally low at this location, the effects of the spill appear to have been moderate, and total abundance changes reflect mainly the seasonal variation of polychaete abundance. In the study carried out monthly from August 1988 to July 1989, crustaceans, particularly amphipods, were the third zoological group in terms of density and taxon numbers. Nevertheless, under unpolluted conditions, they were permanently present at both sites, which was not the case for this study.

Mean density at both sites showed a reduction in December 1992 as compared to pre-spill data. Total density revealed a clear change after the spill (Fig. 3). At site X, the spill seems to have had serious effects, with low densities from the beginning of the post-spill survey in December to spring 1995 and the absence of seasonal cycles, except for the last year of the survey when the total abundance again reached values similar to those of the pre-spill observations. No change in the general trend in annual cycles was apparent at site Z throughout the four year study, but a clear decrease in total density was registered, reaching values lower than those observed during the pre-spill cycle (June 1988–August 1989).

Because no major proliferation of opportunist polychaetes was observed after the spill, the polychaete/amphipod ratio was particularly influenced by the recovery of amphipods from April 1995. Some of the polychaetes selected as opportunist are dominant species under normal conditions at these sites, and the temporal change observed is reflected in the strong variability of the polychaete/amphipod ratio values throughout the post-spill survey.

The MDS ordination of the X and Z sites reveals changes in quantitative community structure, represented by a circular time-course with a partial reversion towards the beginning of the sampling period, when abundance began to decrease. Nevertheless, the last period indicates a new structure corresponding to the recovery of taxon richness and abundance. Both the initial and final periods can be differentiated from the intervening sampling periods. At site Z, sample segregation was less marked because of the dominant seasonal changes in community composition there. This would tend to confirm Clarke and Warwick (1998) and Warwick and Clarke (1993), who have discussed changes in benthic assemblages, and consider that these assemblages respond to two distinct timescales for environmental change: a major medium-term change resulting from a pollution incident and a series of short-term seasonal changes.

At both sites, the multivariate analysis of the mixed pre- and post-spill periods reveals differences in the assemblage characteristics between pre-spill and post-spill data. The mixed MDS showed that no reversion of the community to the pre-spill conditions occurred; observations from the 4-year survey never matched those of the 1988–1989 survey, indicating a post-spill change in community composition.

Though there is no information on the persistence of hydrocarbons in the sediment at the study sites, it is apparent, particularly at site X, that during the spring-summer of 1995, successful recruitment occurred for the first time after the spill. Presumably a non-toxic benthic environment permitted successful colonisation of the more sensitive species. Indirect data obtained from the analysis of mussel tissue collected 11 months after the oil spill in an area near site X showed some evidence of oil pollution from the *Aegean Sea* spill. However, two years later, in October 1995, there was no evidence of oil pollution in the area (Porte, Biosca, Pastor, Solé, & Albaiges, 2000). The delay of colonisation in the sandy-bottom of the ria was relatively short in comparison with other sites, such as the fine sand Pierre Noire community in the Bay of Morlaix where colonisation of *Ampelisca* was only successful four years after the 1978 *Amoco Cadiz* oil spill (Dauvin, 1987, 1998).

5. Conclusions

The effects of a particular oil spill depend upon many factors, so each pollution incident must be examined in its own context. According to Lee and Page (1997), it is not the total amount of oil spilled which is important for evaluating the biological effects of a spill, but the types and concentrations of hydrocarbons in the subtidal sediments. The most toxic components of oil, the aromatic hydrocarbons, are evacuated rapidly through evaporation when oil is spilled. In the case of the crude oil carried by the *Aegean Sea* (a light Bren type coming from the North Sea), the residue (68% of the original oil) without volatile compounds was 28% mono- and di-aromatics and 4% polyaromatics. In samples taken 12 months (Pastor et al., 2001) and 20 months (Neira et al., 1997) after the spill, PAH concentrations recorded in the sediment in the central zone of the Ares-Betanzos Ria exceeded the minimum level required for causing toxic effects (ERL). This zone of the ria contained not only petrogenic hydrocarbons, but also pyrolytic aromatic hydrocarbons, the latter probably coming from the tanker fire.

The *Aegean Sea* oil spill took place just off A Coruña harbour, where the severe weather conditions drove the oil towards the north and east (Martín Bauza, 1996; Porte et al., 2000) flowing into the Artabro Gulf ria. To produce high concentrations of hydrocarbons in the subtidal region, two conditions must be met: (1) large amounts of oil must be trapped in a semi-enclosed estuary or bay (Lee & Page, 1997; Glémarec and Hussenot, 1982), and (2) elevated concentrations of particulate, or detrital, matter with densities greater than seawater must be present to absorb the oil and sink to the sedimentary layer (Boehm, Judith, Barak, Fiest, & Elskus, 1982). The Ares-Betanzos Ria provide favourable conditions for such oil sedimentation. The ria contains large quantities of gravel and coarse sand, and the double estuarine system is sheltered from direct oceanic influence, except at the mouth. Fine sand, silt and clay bottoms respectively characterize the middle and inner sections of the mouth (Garmendia, 1997). The discharge of several rivers, laden with suspended solids, arrives in the estuarine areas of the ria, providing favourable conditions for the sedimentation of oiled particles.

In addition, the residual estuarine circulation (Prego & Fraga, 1992; Taboada et al., 1998) is typical of a normal positive estuary with a mixture of partially stratified waters. Several vortices form because its circulation pattern drags and suspends the fine sediment into the water column (Sánchez-Mata et al., 1999). The depth of the ria, except near the external sector, ranges from 10 and 25 m, and no reduction in the polluting effects of hydrocarbons was detected at these shallow depths (See Feder & Blanchard, 1998).

Since the initial impact of a spill on benthic communities can only be determined in relation to the sensitive species present in natural conditions (Dauvin, 2000), it is necessary to have recent faunal observations prior to a pollution event in order to assess the initial and successive results of the stress (López-Jamar & Mejuto, 1985). Given these circumstances, it will be difficult to anticipate the impact of the *Prestige* oil spill on the deep subtidal benthic community. However, the data from this study could be useful in the event of future spills in the Galician rias, especially in the Artabro Gulf, as it provides the most recent status of the subtidal macrobenthos in this area.

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