Experimental study of the impact of hydrocarbons on the intertidal benthic community: the Mahakam delta (East Kalimantan, Indonesia)

Oil pollution
Toxicity
Dispersant
Benthic community
Indonesia

Pollution par hydrocarbures
Toxicité
Dispersants
Communautés benthiques
Indonésie

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ABSTRACT

Two simulated oil-spills in the Mahakam delta revealed the consequences of this type of pollution on the site's main benthic populations. The results of these trials differentiated between short-term (high toxicity similar to chemical pollution) and long-term effects (similar to organic pollution) of hydrocarbons. Two factors affected the distribution of the main macrofaunal species: intertidal height and degree of pollution. The latter was measured either by the initial quantity of oil spilled or by concentrations measured at each sampling. These trials also showed that dispersants were inefficient and that dredging treatments did not yield positive results. Often no treatment was preferable to these two treatments. A global statistical analysis was generated to define the role of the different environmental variables, both natural or pollution-related, in the spatial distribution of the different species.

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RÉSUMÉ

Étude expérimentale de l'impact des hydrocarbures sur une communauté benthique intertidale du delta de la Mahakam (Kalimantan Est, Indonésie)

Deux déversements de pétrole simulés dans le delta de la Mahakam ont permis de déterminer les conséquences d'une telle pollution sur les principales populations benthiques du site. Les résultats de ces essais ont permis de différencier les effets à court terme des hydrocarbures (toxicité aigüe s'apparentant à une pollution chimique) des effets à long terme (s'apparentant à une pollution organique). Deux facteurs agissent de manière importante sur la répartition des principales espèces de la macrofaune : la hauteur intertidale et le degré de pollution, ce dernier étant mesuré soit par la quantité de pétrole déversée à l'origine, soit par les concentrations mesurées à chaque échantillonnage. Ces essais ont par ailleurs montré que les dispersants étaient peu efficaces et que le traitement par bêchage ne produit pas l'effet favorable escompté. L'absence de traitement reste souvent préférable. Une analyse statistique globale permet de préciser le rôle des différentes variables du milieu qu'elles soient naturelles ou liées à la pollution, dans la répartition spatiale des différentes espèces.

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INTRODUCTION

The experimental study of oil pollution can be approached from three main angles: real case studies (accidents); laboratory experimental studies; and on-site experimental studies. In the first type, the initial site characteristics are rarely known. In addition, studying control zones is often inaccurate, particularly in large-scale accidents, and the results are inconclusive. However, these types of studies do provide a means of examining full-scale effects and a certain number of accidents have yielded a sizeable amount of data. The following oil spills should be mentioned: the Amoco Cadiz spill (Ozouville *et al.*, 1979; Marchand and Caprais, 1979; Levasseur *et al.*, 1979; Gehu and Gehu-Franck, 1979; Vandermeulen and Long, 1980; Vandermeulen *et al.*, 1981; Cabioch *et al.*, 1979; Glémarec and Hussenot, 1979; Glémarec, 1981; Raffin *et al.*, 1981, *etc.*); the Metula supertanker spill in the Magellan straits (Hann, 1977; Veaute, 1982; Guzman and Campodonico, 1980; Straughan, 1981; Baker *et al.*, 1984; Colwell *et al.*, 1978; Gundlach *et al.*, 1982); the Florida barge off Massachusetts, USA (Burns and Teal, 1979; Sanders *et al.*, 1980; Sanders, 1981; Michael *et al.*, 1975) and, in tropical waters, the Zoe Colocotroni spill in Puerto Rico (Nadeau and Bergquist, 1977; Page *et al.*, 1979; Gilfillan *et al.*, 1981); the Saint Peter spill in Equador (Jernelov *et al.*, 1976); the Witwater in Panama (Rutzler and Sterrer, 1970) and the explosion of the Funiwa well 5 off Nigeria (Ekekwe, 1981). Laboratory studies have also yielded numerous results, as shown by the bibliography in Neff and Anderson's book (1981), but these results cannot be extrapolated to actual disasters (Rice *et al.*, 1976; Ramade, 1977; Knap *et al.*, 1983). Halfway between the two methods, on-site experiments make it possible to control a certain number of variables and define reasonable control zones while maintaining the dimension of the natural ecosystem. In mangrove swamps, most experiments in natural environments have focused on plant life (Hoi-Chaw *et al.*, 1984; Getter and Ballou, 1985; Scherrer, 1988; Scherrer *et al.*, 1989; Dutrieux *et al.*, 1990 *a*) and, as we have already demonstrated (Dutrieux, 1989), few trials have been made on the benthic communities.

This report presents results from on-site trials made in the Mahakam delta in Indonesia (*see* Fig. 1) sponsored by an oil company (Total-CFP) working out of this delta. To take advantage of an elaborate experimental design and include uncontrolled environmental variables, we used both analysis of variance and principal component analysis. This made it possible to compare the variable studied (hydrocarbons), indicator variables (macrofauna) and environmental variables.

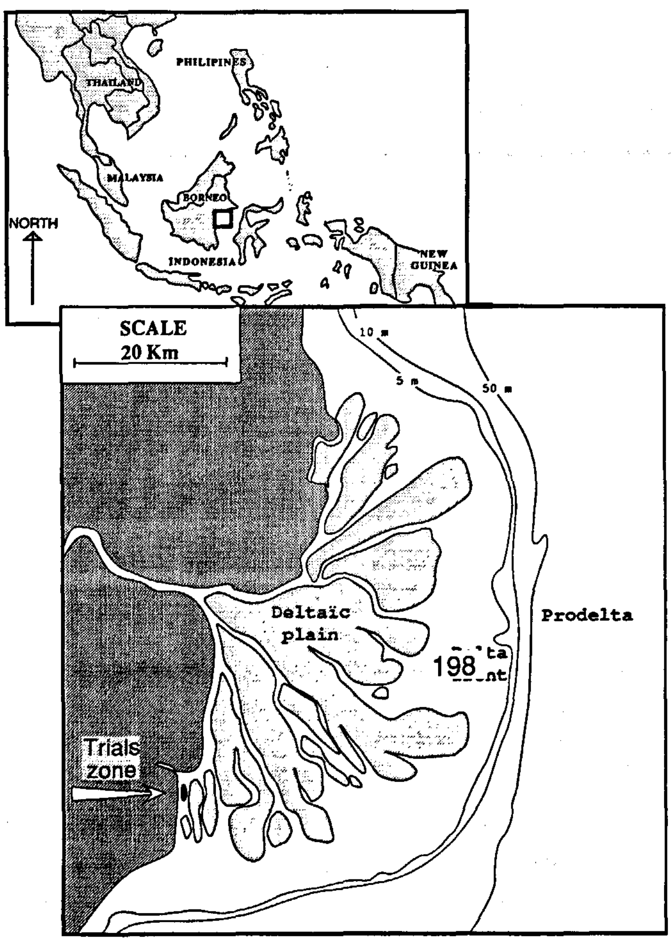


Figure 1

Geographic location of the island where the trials took place in the Mahakam delta.

Localisation de la zone d'étude en Indonésie.

MATERIAL AND METHODS

Goals

The main goal was to describe the response of a benthic community in a mangrove estuary to hydrocarbons. The way communities respond to pollution differs depending on the nature of the pollution, whether chemical contamination or organic enrichment (Harmelin, 1981; Le Gal, 1988). The term hydrocarbon includes a combination of numerous individual compounds (Mc Auliffe, 1976; Rice *et al.*, 1976; Breuel, 1981; Cintrón *et al.*, 1981). All are more or less toxic chemical products essentially composed of organic compounds composed of hydrogen and carbon. One of the goals of this study was to identify the exact nature of this type of pollution by examining its effects on the benthic populations. Another goal was to test the efficiency of different methods of cleaning-up or eliminating hydrocarbons as shown both by the quantity of oil removed as well as the damage to macrofauna.

The site

The experimental trials were performed on an island located in the distributory channel of the southern Mahakam delta (*see* Fig. 1). Since its formation, this island has undergone successive phases of downstream progradation. The recent deposition zones are colonized by a pioneer vegetation of *Sonneratia caseolaris* (Dutrieux *et al.*, 1990 *b*). Figure 2

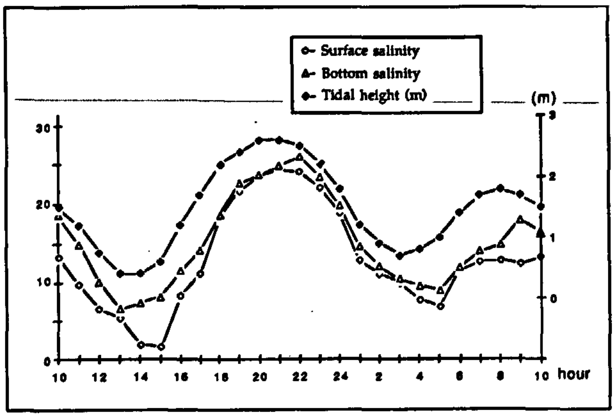


Figure 2

Tidal height (m) and salinity at the surface and at 10 m depth over a 25-hour time period in the trial zone.

Variations de la hauteur intertidale (m) et de la salinité en surface et à 10 m de profondeur en fonction d'un cycle horaire de 25 heures (station située dans le chenal face à la zone des essais).

shows the evolution of salinity over a 25-hour cycle including two tidal cycles for a station located in the channel across from the chosen site. This figure, like other investigations on the delta (Dutrieux, 1991), demonstrates that the salinity of the water at the experimental site varies between 2 to 25 in twelve hours. The stratification phenomenon shown in Figure 2 has been described in detail by Allen *et al.* (1979).

Experimental designs

Two experimental designs were set up at the downstream end of the island on a primarily sandy substrate (Fig. 3).

First sampling design

This initial series of trials tested the feasibility of this type of experiment. Five 100 m^2 parcels (E1 to E5) were placed 10 m from the edge of the Sonneratia. They were sprayed with 2.5 l of Handil crude oil/m^2 on 24 May 1986. Several hours after pollution, 20 l of nitrogen dispersant was spread over E4 and 50 l of a 2nd generation dispersant (hydrocarbon-based dispersant) was spread over E2. Fifteen days after pollution, 50 l of the same dispersant was spread over E3 and the E5 parcel was washed using a high-pressure water hose. No clean-up treatment was used in E1. No hydrocarbons were spread over a zone labelled E0 which acted as a control. Five days after the end of treatments, a sampling of the macrofauna was performed. In October 1986, March 1987 and June 1987 the parcels were resampled. E1 was also sampled in June 1988.

Second sampling design

In May 1987, two rectangular parcels each with a surface area of 90 m^2 (6 x 15 m) were set up below the preceding trials. They were divided into two sections according to intertidal height. Control zones were established between these two parcels. To provide a better distribution of oil, they were individually polluted by hand using a watering can. On 30 May 1987, 2.5 l of oil/ m^2 was spread over parcel A and 6.5 l/m^2 was spread over parcel B. Fifteen days after pollution, four out of eight sub-parcels (A2, A3, B2, B3) were dredged (or tilled) to a depth of 20 cm. This treatment is intended to help biodegrade oil by oxygenating the sediment. Each of the sub-parcels was in turn divided into four parts corresponding to four repetitions of each treatment. This balanced design made it possible to statistically compare data using an analysis of variance (Sokal and Rholf, 1981). In October 1987 and then again in March 1988 and June 1988, the parcels were sampled for macrofauna and environmental variables (hydrocarbons, granulometry, oxygen reduction, intertidal height) were recorded.

Sample analysis

Benthic macrofauna

Sampling was performed at low tide using a 15 cm diameter PVC corer to a depth of 25 cm. Each core was imme-

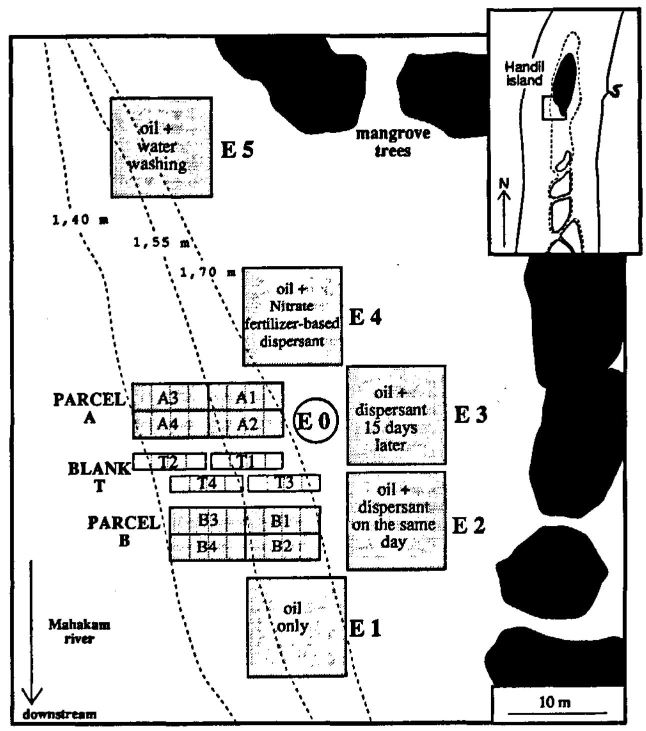


Figure 3

Experimental designs. Parcels E1 to E5 (first trials) were polluted in May 1985. E0 is a blank parcel. Parcels A and B (second trials) were polluted in May 1987. T is a control parcel (controls: T1,..., T4; low pollution: A1,..., A4; high pollution: B1,..., B4; high intertidal level: A1, A2, T1, T2, B1, B2; low intertidal level: A3, A4, T2, T4, B3, B4; dredged parcels: A3, A2, B3, B2; undredged parcel : A1, A4, B1, B4, T1,..., T4).

Dispositifs expérimentaux. Les parcelles E1 à E5 (premiers essais) ont été polluées en mai 1985. E0 est une parcelle témoin. Les parcelles A et B (seconds essais) ont été polluées en mai 1987. T est une parcelle témoin. (parcelles témoin : T1,..., T4; parcelles faiblement polluées : A1,..., A4; parcelles fortement polluées : B1,..., B4; hauts niveaux intertidaux : A1, A2, T1, T2, B1, B2; bas niveaux intertidaux : A3, A4, T2, T4, B3, B4; parcelles bêchées : A3, A2, B3, B2; parcelles non bêchées : A1, A4, B1, B4, T1,..., T4).

Table 1

Comparison of the numbers of one crab and two polychaeta species before pollution and fifteen days after pollution in the experimental design of the first trials.

Comparaison des effectifs d'un crabe et de deux polychètes avant pollution expérimentale et quinze jours après pollution au cours des premiers essais.

SPECIES	TMETHYPOCOELIS		NEREIS		MALACOCEROS	
PARCELS	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
E0A	26	20	73,5	27,5	10,5	8
E0B	12	15	80,3	25,5	5,6	7,5
E1A	20	0	/	0	15,5	0
E1B	22	0	41,5	0	13,5	0
E2A	3	0	39	1	14	0
E2B	15	0	35	0	17	0
E3A	24	0	39	0	3	0
E3B	13	0	109	1	5,5	0
E5A	88	0	5,5	0	9	0
E5B	59	0	53,5	0	5	0
E6A	7	0	93	4	17,5	0
E6B	2	0	78	3	2	0

diately sifted using an 0.8 mm mesh sifter. Three cores corresponding to a surface area of 0.05 m^2 were placed together in the same sack. Samples were then treated with formol and coloured using Bengal Pink on the same day. Sorting was performed under a magnifying glass and the animals were preserved in alcohol. For each sample, individuals were identified, regrouped by species and counted. A prior examination of the population (Dutrieux, 1989) had shown a relatively poor benthic macrofauna (ten species found on the site, most present in small numbers). This led us to use three species with a sufficient number of representatives for statistical analysis. These three species were the crab *Tmethypocoelis ceratophora* (Koebbel, 1897), and the polychaetas *Malacoceros indicus* and *Nereis* sp. The Nereids species is being described at the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER, Centre de Brest) by D. Desbruyères.

Environmental variables

Throughout follow-up, sediment samples were taken to determine the physical and chemical evolution of the soil. Hydrocarbons were extracted from a known quantity of sediment by stirring with carbon tetrachloride (CCl_4). The extract was then analyzed using infrared spectrometry (IR) according to standardized methods (AFNOR, 1979) and the results were expressed in parts per million (ppm). 20 cm-long cores were also taken. They were used for redox potential analysis measured every two centimetres on the core through specially drilled holes (Dutrieux, 1989). These cores then were used for granulometric analysis. The percentages of sixteen categories of fractions smaller than 192 µm were defined using a Cilas 715 granulometer (Dutrieux, 1989).

RESULTS

First trials

Ten days after pollution, sampling of polluted parcels showed that most organisms had completely disappeared (Tab. 1). The number of representatives was also lower in the E0 control parcel after pollution, although it had not received any hydrocarbons. A portion of the dissolved toxic fractions seems to have been transported by interstitial water outside the polluted zone. Although these fractions can rapidly disappear (Oudot, 1984), they were responsible for the complete disappearance of fauna in the polluted parcels. Three months after treatment, *Nereis* recolonized the polluted zones, demonstrating its opportunistic nature. However, "normal" representatives of *Malacoceros* could only be found in controls. *Tmethypocoelis* was found in large numbers on the surface of treated parcels and seemed to be indifferent to a long term pollution of the soil (this species lives on the top of the sediment where it feeds, while oil is found inside the sediment). Two years later, the environment in the E1 parcel treated with hydrocarbons alone could be considered to have recovered (Fig. 4). These trials also showed that dispersants are only effective when rapidly spread after a spill. The nitrogen-based dispersant had a very high residual toxicity for the benthic populations. This effect on growth and death of mangrove shrubs has already been observed both by our team and others (Dutrieux *et al.*, 1990 *a*; Scherrer *et al.*, 1989). The use of this product should therefore be avoided. Treatment using pressurized water initially led to highly negative results, but the environment recovered in the long run since this technique physically eliminates oil. When low amounts of oil are spilled, however, it seems advisable not to undertake any clean-up treatment.

Second trials

Temporal evolution of the three main species

The preliminary trials demonstrated the important role of *Nereis* and *Malococeros* as pollution indicators. The second trials sought to define their behavior by describing their temporal changes.

After treatment, the parcels were completely depopulated, as already shown in the preliminary trials. However, as early as October 1988 (t + 4 months), the number of *Nereis* representatives was close to that in controls and even exceeded them.

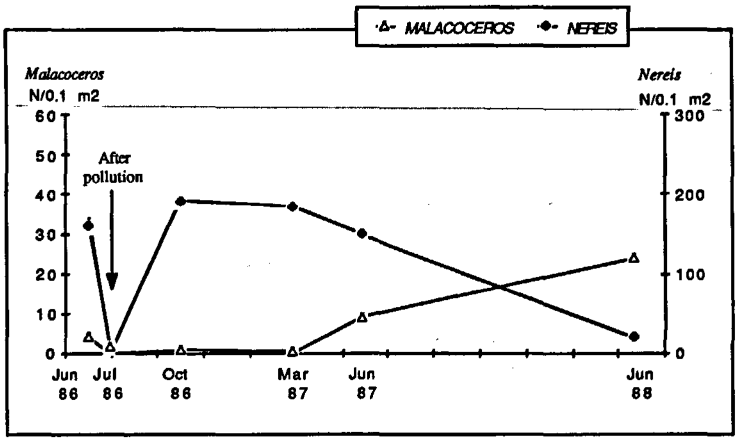


Figure 4

Changes in the number of two species of polychaeta on parcel E6 (polluted, untreated with chemicals), first trials over two years.

Variations des effectifs de deux espèces de polychètes sur la parcelle E6 (polluée, non traitée par des produits chimiques), au cours des premiers essais et durant deux ans.

The analysis of variance (Table 2, October 1987) showed no significant difference between T control parcels, low-polluted A parcels and highly-polluted B parcels (hydrocarbon treatment; P = 0.86). Only dredging treatments helped to increase the number of *Nereis* representatives in the dredged parcels (P = 0.01). In March (t + 8 months) and June (t + 12 months), the number of *Nereis* representatives was systematically higher in oiled parcels than in controls (P < 0.01), as shown in Tables 2 (March 1988) and 2 (June 1988). The intertidal height treatment played an important role. The number of *Nereis* representatives was much higher in low parcels than in high parcels (P < 0.01). However, no effect of the dredging treatment was found (P > 0.4). Figures 5 and 6 show that, one year later, the number of *Nereis* representatives in some treated parcels was close to that found in controls (A3, B1, B2), while other parcels had much higher numbers (B3, B4). Environmental recovery was therefore not yet complete for this species.

The behaviour of *Malacoceros* was completely different from that of *Nereis* and the number of representatives was systematically lower in parcels treated with hydrocarbons than in controls (Fig. 5 and 6), regardless of the time period (October 1987, March and June 1988). The difference was highly significant as shown by Table 2 (P < 0.01). As found for *Nereis*, dredging had a positive short-term influence (P = 0.02 in October). One year later, most parcels were still disturbed. Only a few parcels located at the bottom of the intertidal zone (B1 and B2) appeared to have recovered, which demonstrates the natural cleaning process in water.

The crab *Tmethypocoelis* showed no variations of densities according to spilled oil quantities (including no oil spilled). However, it was particularly sensitive to intertidal height (P < 0.01) and dominated the upper levels, especially in March and June (Tab. 2).

In conclusion, these results confirm those obtained during the preliminary

Table 2

Three-way variance analysis (quantity of spilled hydrocarbons, intertidal height and dredgi-g) in the experimental design of the second trials. A: tables (two-way analysis) include control zones in the comparison which excludes the dredging factor, (the control zones being untreated); B: tables (three-way analysis) exclude control zones and therefore enable us to clarify the effects of hydrocarbon quantity by comparing parcels A and B. Interpretation of F-test and its associated probability value (P), is explained in Green (1979).

Analyse de variance à trois facteurs (quantité d'hydrocarbures déversés, hauteur intertidale et bêchage) pour les seconds essais. Les tableaux A (analyse à deux facteurs) incluent les témoins dans la comparaison ce qui exclue l'utilisation du facteur bêchage, les zones témoins n'ayant pas subi ce traitement. Les tableaux B (analyses à trois facteurs) excluent les zones témoins et permettent donc de préciser les effets de la quantité d'hydrocarbures déversés par la comparaison des parcelles A et B. L'interprétation du test F et de la probabilité (P) qui lui est associée est décrite par Green (1979).

October 87

A

TREATMENTS	OIL SPILLED		INTERTIDAL HEIGHT	
SPECIES	F	P	F	P
Nereis	0,1	0,86	2,4	0,13
Malacoceros	18,9	0,00	4,3	0,04
Tmethypocoelis	0,3	0,77	2,3	0,14

B

TREATMENTS	OIL SPILLED		INTERTIDAL HEIGHT		DREDGING	
SPECIES	F	P	F	P	F	P
Nereis	0,1	0,80	1,2	0,28	7,7	0,01
Malacoceros	6,8	0,02	0,3	0,60	5,9	0,02
Tmethypocoelis	0,4	0,52	3,9	0,06	0,0	0,83

March 88

A

TREATMENTS	OIL SPILLED		INTERTIDAL HEIGHT	
SPECIES	F	P	F	P
Nereis	12,6	0,00	84,7	0,00
Malacoceros	40,8	0,00	0,4	0,54
Tmethypocoelis	1,0	0,36	8,7	0,01

B

TREATMENTS	OIL SPILLED		INTERTIDAL HEIGHT		DREDGING	
SPECIES	F	P	F	P	F	P
Nereis	6,5	0,02	73,9	0,00	0,7	0,41
Malacoceros	0,4	0,50	1,0	0,33	0,1	0,81
Tmethypocoelis	2,7	0,11	10,8	0,00	0,0	/

June 88

A

TREATMENTS	OIL SPILLED		INTERTIDAL HEIGHT	
SPECIES	F	P	F	P
Nereis	25,9	0,00	36,6	0,00
Malacoceros	33,7	0,00	1,3	0,25
Tmethypocoelis	0,0	0,98	13,2	0,00

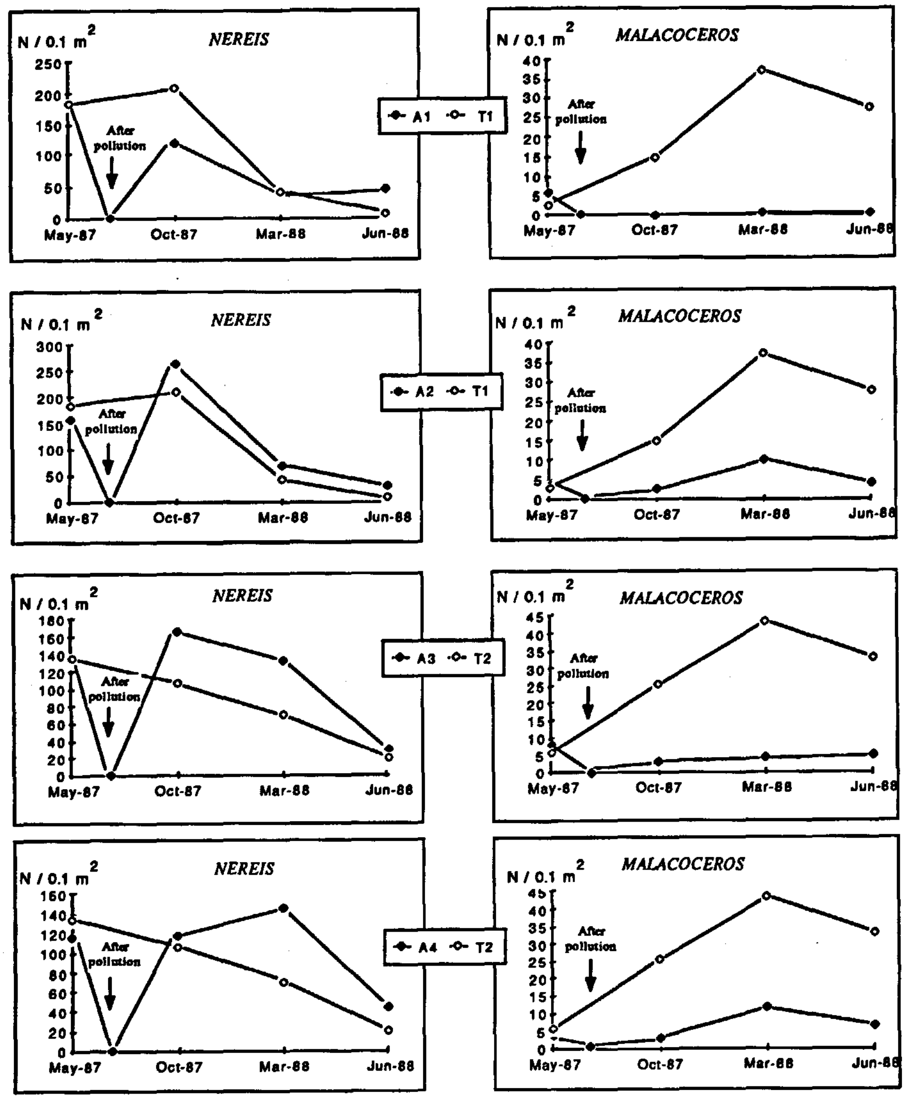
B

TREATMENTS	OIL SPILLED		INTERTIDAL HEIGHT		DREDGING	
SPECIES	F	P	F	P	F	P
Nereis	15,6	0,00	27,6	0,00	0,1	0,81
Malacoceros	2,4	0,13	2,1	0,16	0,0	0,96
Tmethypocoelis	0,0	0,97	8,7	0,01	1,1	0,31

Figure 5

Comparison of numbers of two polychaeta species in polluted parcels and corresponding control zones over one year for the second trials (parcel A, low pollution).

Comparaison des variations des effectifs de deux espèces de polychètes sur les parcelles polluées et sur les parcelles témoin correspondantes pendant un an et au cours des seconds essais (parcelle A, peu polluée).



trials. *Nereis* is favored in polluted zones and its abundance exceed that found in controls. *Malacoceros* is harmed by pollution. After one year, *Nereis* had recovered in some parcels (as shown by a lowering in the number of representatives) though few parcels showed any *Malacoceros* recovery.

Evolution of hydrocarbon concentrations

Figure 7 shows the evolution of hydrocarbon concentrations in controls and parcels. In general, all the curves have a similar shape. Concentrations before treatment in May 1987 were between 0 and 100 ppm, which we attributed to the background. One week after pollution, concentrations were highest (between 2 000 and 4 500 ppm for A and between 4 000 and 12 000 ppm for B), but were lower than the theoretical concentrations which should have been found if all the hydrocarbons spilled had remained in the same place (7 000 ppm for A and 21 000 ppm for B). This means that between 40 and 60 % of the hydrocarbons had been eliminated by dissolving or evaporation in one week. After this period, the overall decrease was very rapid and followed an exponential regression. As of October 1987 (t + 4 months), concentrations were almost all below 1000 ppm. After this, the decrease continued more slowly. In controls which had not been subjected to any direct pollution, a slight contamination was visible in the first months which never exceeded 500 ppm. In addition, the chromatographic analysis of oil in the parcels made it possible to define the amount of biodegradation of residual oil using the method described by Fusey and Oudot (1984). The results, which have been described in detail by Oudot and Dutrieux (1989), are summarized in Table 3 which gives the composition of the different stages of biodegradation. The classic stages of biodegradation (Oudot *et al.*, 1981; Oudot, 1984; Atlas, 1984) were

Table 3

Chromatographic data for the determination of the biodegradation stages in the experimental parcels of the second trials (after Oudot and Dutrieux, 1989).

Données chromatographiques permettant de calculer l'indice de biodégradation pour les seconds essais (after Oudot and Dutrieux, 1989).

Stages	n-alcanes	iso-alcanes	pristane	steranes terpanes	UCM
1	+++	++	+++	+	+
2	++	++	+++	+	+
3	+	+	++	++	++
4	-	+	+	++	++
5	-	-	-	+++	+++

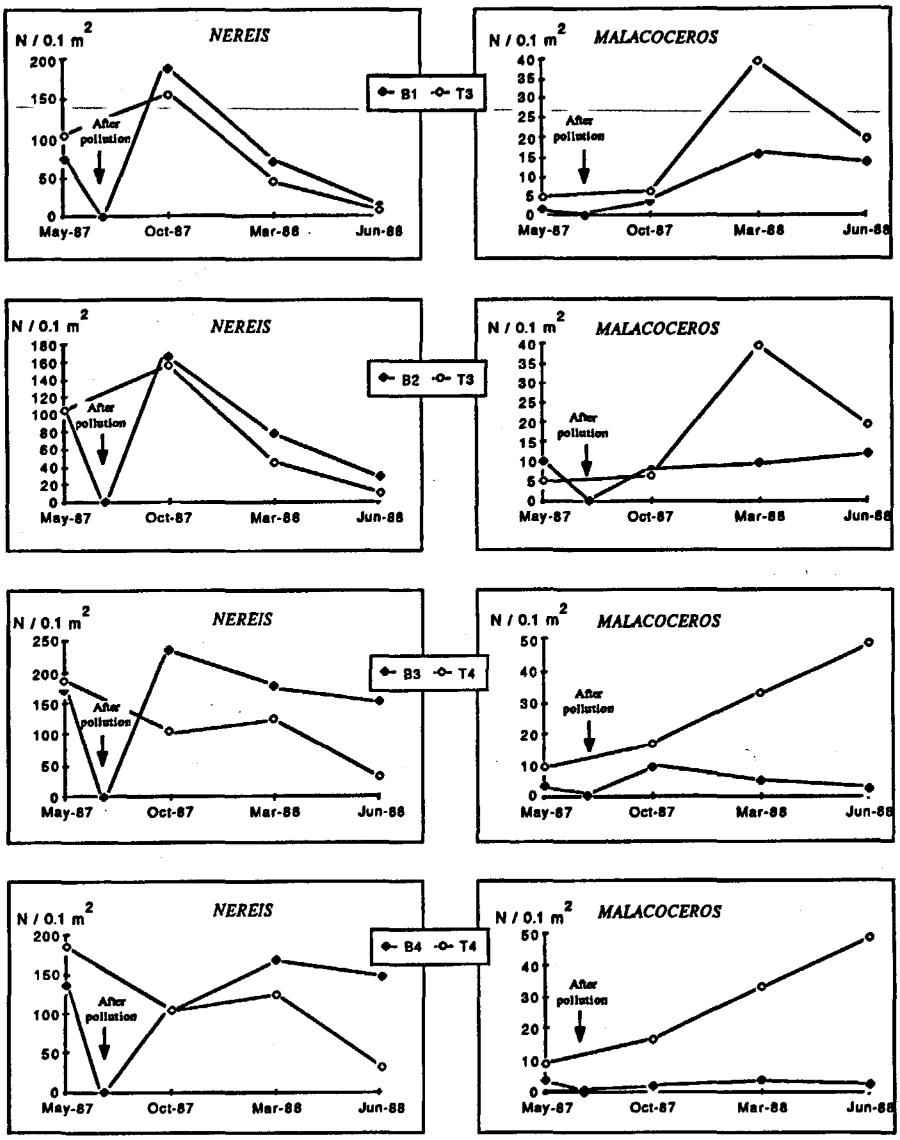


Figure 6

Comparison of numbers of two polychaeta species in polluted parcels and corresponding control zones over one year for the second trials (parcel B, high pollution).

Comparaison des variations des effectifs de deux espèces de polychètes sur les parcelles polluées et sur les parcelles témoin correspondantes pendant un an et au cours des seconds essais (parcelle B, fortement polluée).

found: n-alkanes > iso-alkanes > medium molecular weight polycyclic alkanes and aromatics and unbiodegradable compounds (UCM): heavy polycyclic alkanes and aromatics, steranes-terpanes, resins and asphaltenes.

Redox potential

This variable makes it possible to compare hydrocarbon content and macrofauna. In fact, several authors have shown the high sensitivity of some benthic species to redox values (Leppakoski, 1969, Fenchel and Riedl, 1970; Hily, 1984). In addition, we have already shown the close relationship between hydrocarbon content and redox (Dutrieux, 1989; Dutrieux *et al.*, 1989; Dutrieux *et al.*, 1990 *a*). Figure 8 shows that the profiles measured in each of the experimental parcels can be ranked into several categories from unpolluted profiles to highly polluted profiles. In addition, the effects of dredging are clearly visible and show that this treatment does not yield the intended result of spreading the oil throughout the sediment column, but forces it down deeper which limits aerobic biodegradation (Hambrick *et al.*, 1980). An analysis of all the profiles using a principal component analysis led us to retain the redox values measured at 2 and 10 cm as representative of the whole profile (Dutrieux, 1989).

Granulometry and organic matter

An analysis of all the distributions using a PCA yielded the three representative variables for the distribution among the sixteen measured:

- % of fractions between 6 and 8 µm: silt and clay,
- % of fractions between 64 and 96 µm: fine grained sand,
- % of fractions between 128 and 192 µm: large grained sand.

Figure 9 shows the spatial distribution of these three granulometric fractions. The fraction between 6 and 8 microns has no noticeable spatial structure. However, the fraction between 64 and 96 microns seems to be dominant in the upper layers of the parcels and in a large part of parcel B. The fraction between 128 and 192 microns has a distribution complimentary to the preceding one and is dominant towards the bottom layers of parcel A. No particular spatial structure could be found for organic matter, except for a slight dominance in parcel B.

Global statistical analysis building

The analysis of variance, although a powerful tool, did not allow us to multiply the number of factors without rendering the experimental design overly complicated. The principal

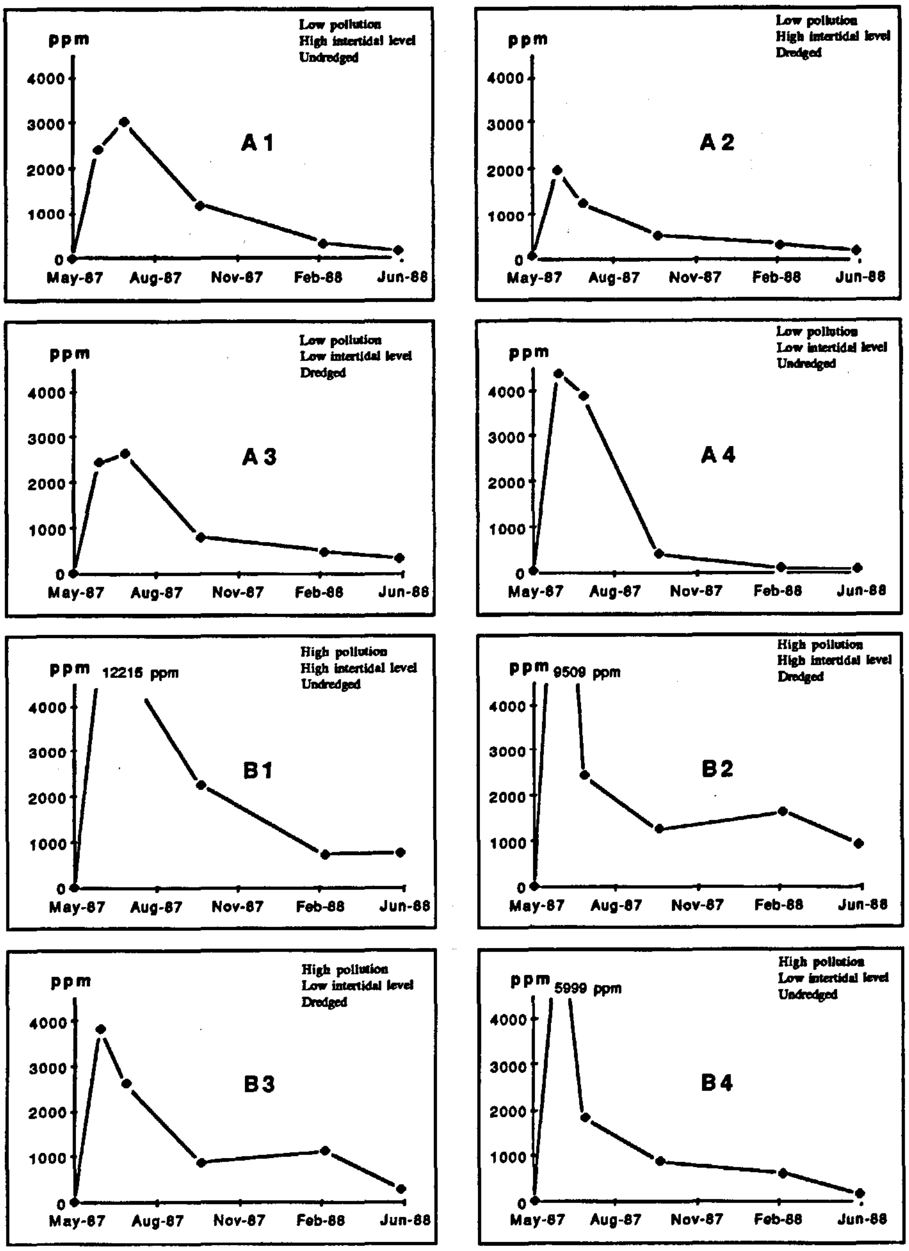


Figure 7

Changes of hydrocarbon concentration in the sediment of the second trial parcels as a function of time.

Variations des concentrations d'hydrocarbure dans le sédiment en fonction du temps au cours des seconds essais.

component analysis (Hotelling, 1933; Legendre and Legendre, 1984) helped solve this problem and create a global statistical analysis including all the previously described variables.

This method centres and reduces variables and thus makes it possible to study very different descriptors simultaneously and to position them in a reduced space, making their interrelationships easily apparent. Table 4 provides a list of the variables used in this analysis.

The result of the PCA for the three sampling periods is shown in Figure 10. In all three cases, the percentage of inertia for the first two axes is approximately 50 %. Only the representation of the descriptors is given as only their correlation interested us. In October, the three species were negatively correlated with the variables characterizing the hydrocarbons. This shows the high toxicity of the product spilled over the community studied. During March and June, a similar situation occurred. *Nereis* was opposite *Malacoceros* on the first axis and *Tmethypocoelis* was opposite the other two species on the second axis.

In March 1988 (t + 8 months), the four variables were closely correlated with *Malacoceros*. Redox at 10 cm and the

Table 4

List of variables used for the PCA analysis.

Liste des variables utilisées pour l'analyse en composantes principales.

Indicator variables	Controlled variables	Uncontrolled environmental variables
Nereis	Hydrocarbons spilled at the outset	Oil content (ppm)
Tmethypocoelis	Intertidal height	Organic matter %
Malacoceros		Granulometric fraction between 64 and 96 μm
		Granulometric fraction between 6 and 8 μm
		Granulometric fraction between 128 and 192 μm
		Redox down to 2 cm
		Redox down to 10 cm
		Biodegradation index

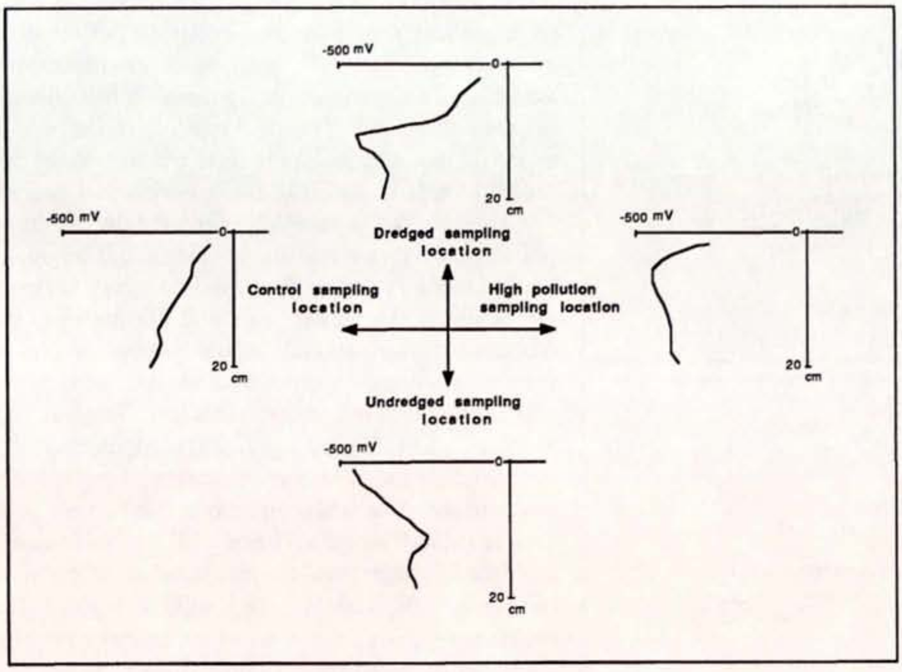


Figure 8

Main types of redox potential profiles characterizing the experimental parcels of the second trials three months after pollution.

Différents types de profils redox caractérisant les parcelles expérimentales des seconds essais trois mois après pollution.

percentage of biodegradation were positively correlated, while the amount of oil initially spilled and measured concentrations were negatively correlated. All these variables strongly contribute to the first axis. *Malacoceros* is thus exclusively linked to variables characterizing pollution by hydrocarbons.

Nereis is opposite *Tmethypocoelis* on both axis 1 and axis 2. The variables influencing these species' distribution are intertidal height and granulometry. *Nereis* is favoured in large-grain sands while *Tmethypocoelis* prefers finer grained sands. *Tmethypocoelis*' contribution to the first axis is low which demonstrates its low sensitivity to pollution. Granulometric fractions between 6 and 8 µm, redox at 2 cm and particularly organic matter do not appear to be important.

In June 1988 (t + 12 months), the situation had changed slightly, primarily due to hydrocarbon changes. *Malacoceros* was still closely correlated with the degree of degradation and the amount of hydrocarbons spilled at the outset. However, Redox at 10 cm and HC concentrations had become independent. Redox at 10 cm was still strongly opposed to HC spilled at the outset. The last two observations should be explained. They demonstrate that, despite an absence of correlation between measured HC and Redox, Redox can reflect the original type of pollution. This means that even if pollution cannot be detected using measurements of hydrocarbon concentration, its effects on the environment can persist, as shown by low redox values. The situation of *Nereis* and *Tmethypocoelis* in terms of intertidal height and granulometry had not changed.

In conclusion, through the PCA we were able to define the role of environmental variables not included in the original design, but which were measured at each sampling, *i. e.* granulometry, organic matter, oxido-reduction potential and actual amounts of hydrocarbons measured at each time period. These variables can significantly influence indicator variables and their role in the analysis of variance is included in the "error", *i. e.* in the unexplained variability in the global statistical analysis based on the experimental design.

DISCUSSION AND CONCLUSION

Experiments in natural environments are rarely undertaken due to the problems existing between experimental designs where all the variables influencing a system are controlled and the uncontrollable nature of variables in a natural ecosystem. The statistical methods we used made it possible to

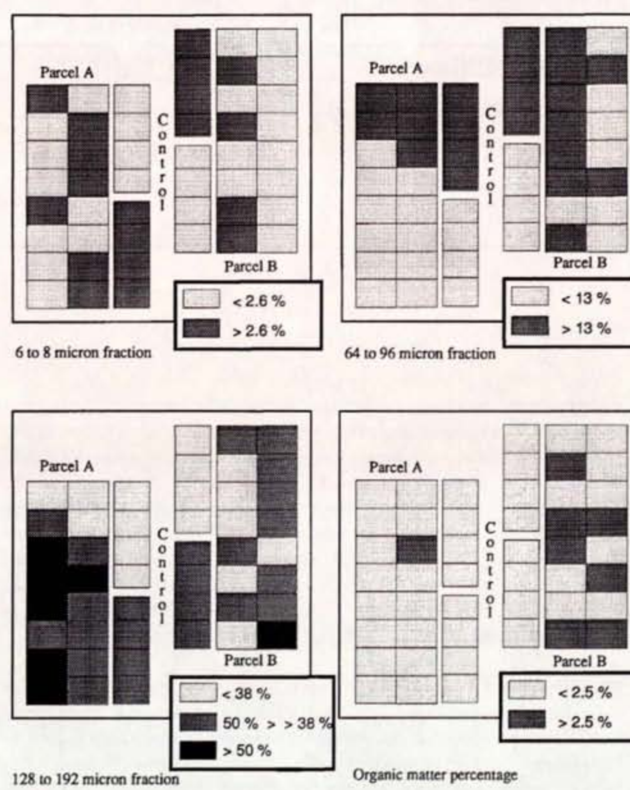


Figure 9

Spatial distribution of some granulometric fractions and of organic matter in the experimental parcels of the second trials.

Distribution spatiale de quelques fractions granulométriques et de la matière organique sur les parcelles expérimentales des seconds essais.

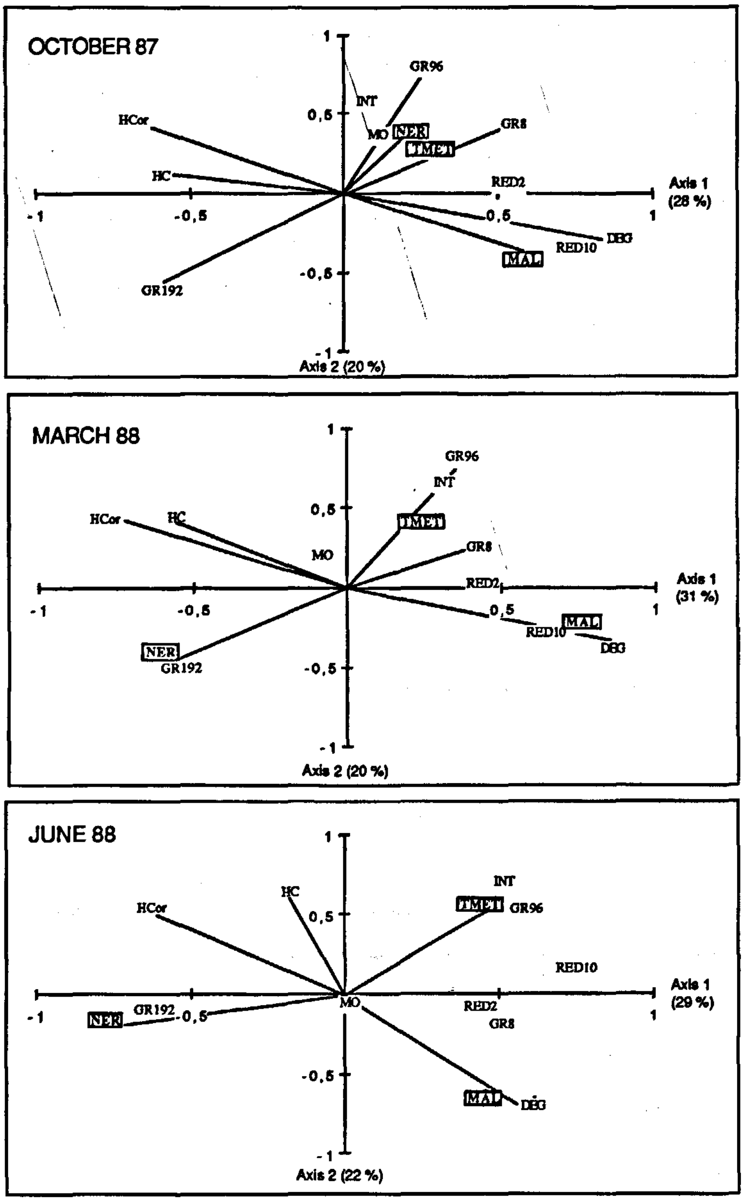


Figure 10

Global statistical analysis generation using principal component analysis of the relationships between the three characteristic species of the benthic macrofauna and the main physico-chemical variables of the environment during the three sampling periods of the second trials. Representation of the descriptors (NER: Nereis*; TMET:* Tmethypocoelis*; MAL:* Malacoceros*; HCor: hydrocarbons spilled at the beginning; HC: measured concentrations; INT: intertidal height; MO: organic matter; GR96: granulometric fraction between 64 and 96 µm; GR8: granulometric fraction between 6 and 8 µm; GR192: granulometric fraction between 128 and 192 µm; RED2: Redox down to 2 cm; RED10: Redox down to 10 cm; DEG: biodegradation index).*

Modélisation à l'aide de l'analyse en composantes principales des relations existant entre les trois espèces caractéristiques de la macrofaune benthique et les principales variables physico-chimiques de l'environnement au cours des trois périodes d'échantillonnage des seconds essais. Représentation des descripteurs (NER : *Nereis* ; TMET : *Tmethypocoelis* ; MAL : *Malacoceros* ; HCor : hydrocarbures déversés à l'origine ; HC : concentrations mesurées ; INT : hauteur intertidale ; MO : matière organique ; GR96 : fraction granulométrique comprise entre 64 et 96 µm ; GR8 : fraction granulométrique comprise entre 6 et 8 µm ; GR192 : fraction granulométrique comprise entre 128 et 192 µm ; RED2 : Redox à 2 cm ; RED10 : Redox à 10 cm ; DEG : indice de biodégradation).

detect a modification in the population structure and to relate this modification directly to pollution by considering several different environmental variables. Two complementary methods helped us to achieve this goal. The analysis of variance is a powerful tool which allowed us to test the role of different factors included in the experimental design. For example, we were able to separate the effects of pollution, the factor studied, from intertidal height, an environmental characteristic, and dredging, a clean-up technique. However, we could not multiply the number of factors studied without making the experiment overly complicated and the analysis of variance was limited to a few essential factors. To solve this problem, we performed a principal component analysis including indicator variables as well as the main environmental variables and those that had not been used in the analysis of variance. This analysis made it possible to compare all the measured environmental factors with the variables we sought to explain. The problem of this analysis was that the relationships could not be tested from a statistical perspective and remained simply descriptive. Finally, ANOVA and PCA are complimentary techniques: one helps statistically test the role of several essential factors while the second helps to define the role of many other additional factors.

Use of both these techniques revealed the effects and nature of oil pollution and the clean-up treatments often used on benthic populations. Understanding these mechanisms leads to an relatively complex concept: *i. e.* the ecosystem's sensitivity. This includes several features. It is possible to define it globally as the capacity to resist a stress factor. Seyle (1956) defined a stress factor as a factor or situation which forces the system to mobilize its resources and spend a greater or lesser amount of energy to maintain homeostasis. Stress is the response of a system to a stress factor. The capacity to resist a stress factor has two aspects. One is represented by the structural variables of the ecosystem, such as richness or specific diversity. In fact, the more rich and diversified an ecosystem, the more it has to lose from a disturbance. The other aspect is represented by functional variables, *i. e.* inertia and resilience. Inertia is resistance by an object or a system to a constraint, while resilience is the way an object or a system changes or returns to its original state after a constraint (Westman, 1978). It can be broken down into several terms: elasticity is the speed at which an environment can recover after a disturbance, amplitude is the maximum degree of damage from which it is still possible to return to the original state, hysteresis is the difference between recovery and degradation stages and malleability measures the difference between the initial state and the stable state established after recovery. These functional variables are very difficult to measure quantitatively, but are very important in impact evaluation (Sherman and Coull, 1980).

The trials performed here easily lent themselves to an analysis requiring a careful study of the ecosystem. The inertia towards hydrocarbons in the studied ecosystem was low, as shown by the complete mortality of macrofauna after pollution. The high toxicity of oil, which is both very strong, yet ephemeral, can explain this feature. We observed the effects of a chemical type of pollution and the use of dispersants often increased the toxic effect and even made it persistant. Elasticity was evaluated over one to two years, which is a shorter time period than that in other studies and could be due to the fact that the delta is a physically controlled environment (Sanders, 1968; Dutrieux, 1989; Dutrieux, 1990 *b*; Dutrieux, 1991) which shelters populations capable of resisting certain types of constraints. Amplitude was maximum as the maximum degree of damage from which the system could recover was the total destruction of the populations. Hysteresis was high. Degradation stages consisted in the sudden disappearance of the population; recovery, however, was gradual and involved several different succession stages. These recovery stages are similar to those previously described by other authors for organic pollution (Bellan, 1976; Pearson and Rosenberg, 1978; Hily, 1984) and show the similarity which exists between these two pollution types (Sanders *et al.*, 1972; Glémarec and Hussenot, 1979). The long term effects of hydrocarbon pollution are thus organic. Finally, malleability was more complex in this type of ecosystem where the environment evolves under the pressure of natural constraints unrelated to stress factors. The stable state at the end of recovery was different from that existing prior to disturbance, but corresponded to a new environmental "standard". The environment in fact evolves during the different recovery phases. In conclusion, elasticity, amplitude and malleability are the parameters which reflect an environment's resistance, while inertia and hysteresis show its sensitivity.

Performing this type of experiment in the natural environment adds a new dimension to the notion of ecotoxicology by studying not only the action of a product on a species taken out of its natural context, but also the action of this product on the ecosystem as a whole.

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