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Impact of the 'Erika' oil spill on the *Tigriopus brevicornis* ecosystem at the Le Croisic headland (France): preliminary observations

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An investigation of the oil spill after the wreck of the tanker 'Erika' on 16 December 1999 showed that the maritime area around the town of Le Croisic (between the Vilaine and Loire river estuaries) was severely contaminated by the resulting black tide. Samples of sediment, oil and water were collected during several missions to the Le Croisic headland and analysed. The toxicity of the oil was tested on two organisms, the harpacticoid copepod *Tigriopus brevicornis* and the alga *Enteromorpha intestinalis*. The tests showed only slight degradation of the oil after three months. In laboratory conditions, the toxicity of the sediment contaminated by the oil was apparent on the copepod after four days of exposure. Seawater in contact with the oil also had an effect on the alga.

INTRODUCTION

Maritime disasters like those involving the 'Torrey Canyon' (1967), 'Amoco Cadiz' (1978) and 'Exxon Valdez' (1989), have provided important information about the biocenotic effects of marine contamination by petroleum products (Smith, 1970; CNEXO, 1978; Laubier, 1991). Seven to ten years are needed to restore the ecosystem after an oil spill, and oceanographic observations have shown that plankton (phytoplankton and zooplankton) are particularly susceptible to contact with hydrocarbons.

Rock-pools visible at low tide in coastal areas constitute natural mesocosms. One type, located among growths of macroalgae, is covered at each high tide, and the period of emersion depends on the distance from the limit of the lowest tide. These pools are of variable size, but can contain several tens of cubic metres of seawater. A second type is found between the upper limit of tidal flats covered with macroalgae and rocky outcrops covered with lichen (*Caloplaca marina*). Pools of this type, which can remain emerged for several days depending on the amplitude of the tide, constitute a special ecosystem at the supralittoral level of rocky areas between zones with lichen (*Xanthoria parietina*, *Caloplaca marina*, *Lichina confinis* or *Lichina pygmae*) and macroalgae (*Pelvetia canaliculata*, *Fucus vesiculosus*). This ecosystem, depending on the slope of the coast, forms an interface between the land and the ocean. Two species are dominant there: the Chlorophyceae alga *Enteromorpha intestinalis* and the harpacticoid copepod *Tigriopus brevicornis*.

This ecosystem is subjected to large variations in the main physico-chemical parameters, particularly salinity and temperature (Davenport, 1997; Crowe, 2000). Water temperature can reach 30°C or more in summer and drop to 1°C in winter. Salinity either increases relative to the mean salinity of the ocean because of evaporation, or decreases because of the runoff of rainwater.

In this extreme environment biodiversity is reduced to only two dominant species: an invertebrate (the copepod) and an enteromorph (the alga).

The harpacticoid copepod lives near the bottom of pools. The females, when fecundated, bear ovigerous sacs containing 30 to 50 embryos. The swimming larva is a nauplius that changes into a copepodite after about 72 h, depending on environmental conditions. As the copepodite stage includes several sub-stages before adult age is reached, the complete cycle from larva to adult takes 21 days. Maximum size is 1 mm, and the life span is four months. The composition of the copepod diet is not clearly known. The experimental studies conducted here, in which the copepod *T. brevicornis* and the alga *E. intestinalis* were exposed to heavy pollution of anthropic origin, provide clear evidence of the effects of a black tide on the natural environment.

MATERIALS AND METHODS

Samples

Samples of sediment, oil and water were obtained on the Le Croisic headland beach on 18 January, 29 March, and 4 May 2000. Sediment was collected close (<15 m) to rock outcrops covered with oil deposits, and oil was scraped from rocks and/or gathered with sediment. Despite the presence of oil on rocks and in pools (iridescence, pellets on the surface or at the bottom), copepod populations and enteromorphs were abundant in January 2000. In May, the beach had been cleaned and appeared to be in good condition.

Chemical analysis

Samples

High-resolution gas chromatography analyses were performed on different samples collected on rocks at the Le Croisic site in January and March 2000, and the chemical composition of these samples was compared with that of oil from the Dunkirk refinery used as a control.

Living enteromorphic alga, free of apparent pollution, and dead, visibly polluted enteromorphic alga were gathered in March 2000 at the same site from pools located near oil-covered rocks. Oil contamination was then studied in both types of alga.

Chromatographic analyses

In this study, oil samples were dissolved in dichloromethane (DCM) and analysed directly by capillary gas chromatography with flame ionization detection (GC-FID). The operating conditions were as follows: HP 4890 chromatograph; Chrompack CP Sil5 column, 50 m×0.32 mm thick; 0.25 μm film; injection with 30 l^{-1} division, injector-detector temperature of 320°C; temperature rise from 80 to 320°C at a rate of 3°C min^{-1}; and helium vector under 1.1 bar. Chromatographic data were processed using software developed in our laboratory.

The analytic protocol for the alga consisted of extraction by exposure to ultrasounds (40 kHz, 30 min) in the presence of DCM. The total lipid extract obtained was passed through a Florisil column, which retained endogenous lipids. The purified extract containing hydrocarbons was analysed by GC-FID after addition of an internal standard (n-1 eicosene).

Biological tests

Tests of environmental quality (seawater and sediment) in the presence or absence of oil were conducted on the copepod *Tigriopus brevicornis* and the alga *Enteromorpha intestinalis*.

On the copepod Tigriopsus brevicornis

The method used to test environmental quality on copepods has been developed during several years in our laboratory on specimens from Le Croisic (Pavillon et al., 2000) in accordance with the norms of ISO/DIS 14669 (1999). The tests involved were performed in climate-controlled conditions at a temperature of 18°C ±1°C and with a L:D cycle.

The oil collected at low tide on 18 January 2000 from rocks close to pools (within about a metre) was a compact, rather rigid brownish paste. Samples were transported to the laboratory on the same day and stored in a refrigerator (4°C ±1°C). When placed in seawater, they sank to the bottom.

Experimental determination of oil toxicity in seawater for the copepod

A preliminary mortality test was performed on *T. brevicornis* using two series of three crystallizing dishes. One series contained 20 ml of filtered (0.45 μm) seawater and about 1 g of oil. The other series (control) contained no oil. Twenty copepods were placed in each dish and maintained in the temperature and lighting conditions noted above. To avoid water evaporation, tinfoil was placed over each dish and pierced with holes to allow air–water exchanges.

Experimental determination of LC_{50} 96 h for the copepod in the presence of variable amounts of oil from slick deposits at the Le Croisic headland

The oil toxicity detected during the preliminary test was studied using natural sediment obtained from the site before the spill. Twenty copepods (ovigerous females) were placed in each dish, and five replications were performed for each concentration. Fifteen grams of sediment were placed at the bottom of a 50 ml crystallizing dish, and 20 ml of seawater were added. The ratio between the height of the water and the thickness of the sediment was 2:1. Oil was added in varying amounts: 0.7, 1.5, 2.5, 3.5, 4.0, and 5.0 g. The mortality rate was checked in each dish after four days.

Determination of the ecotoxicity of Le Croisic sediment before and after oil deposits at the Le Croisic headland

Samples of coarse wet sand containing considerable shell debris were obtained for testing on the beach at the Le Croisic headland. A first sample (around 1 kg) collected on 18 January 2000 contained a mass of oil mixed with sand. A second sample was collected on 5 May 2000 (5.5 months after the wreck). The site had been cleaned and to the naked eye showed no signs of oil. The sand sample used as a control had been collected at the same site in September 1999.

Four series of tests were performed in triplicate for each sediment according to the protocol described above. In each crystallizing dish, 10 g of sediment collected after the black tide were mixed with 5 g of oil.

Toxicity of 'Erika' oil for the alga Enteromorpha intestinalis

One litre of filtered seawater and 1 g of oil collected at the Le Croisic site were bubbled for 24 h in a beaker using an aquarium-type bubbler. To avoid oil deposits on the walls and ejections, the viscous oil was enclosed in 0.5 mm nylon mesh. After 24 h, the oil was removed and the supposedly contaminated seawater was retained for tests.

Ten filaments of the alga *E. intestinalis*, which had been kept in an aquarium after being gathered at the Le Croisic site in an area apparently uncontaminated by the oil slick, were each placed in a crystallizing dish containing either seawater that had been bubbled with oil or clean filtered (0.45 μm) control seawater. All of the dishes were maintained in climate-controlled conditions.

The algal filaments were removed from the dishes at 7, 17, 21 and 28 days. Before extraction, they were placed in the open air on blotting paper to eliminate water. Chlorophyll was then extracted from 10 mg of alga by grinding in a mortar. Five millilitres of acetone 90% were added, and the preparation was centrifuged at 5000 rpm for 5 min.

Quantitative measurement of chlorophyll by colorimetry

A Beckman model 24 spectrophotometer produced an absorption spectrum of the algal pigments which showed two peaks in the visible range: one in the blue at around 430 nm, corresponding to absorption of chlorophylls and carotenoid, and the other in the red at around 663 nm, corresponding to absorption of chlorophylls alone.

RESULTS

Chemical analysis

Oil analyses

Compared to control oil, whose composition is indicated in the chromatogram shown in Figure 1A, practically no change was observed between the oil samples collected in January (Figure 1B) and March (Figure 1C). The slight relative reduction of the smallest fractions was attributable to evaporation and partial dissolution of low-molecular-weight compounds. No trace of

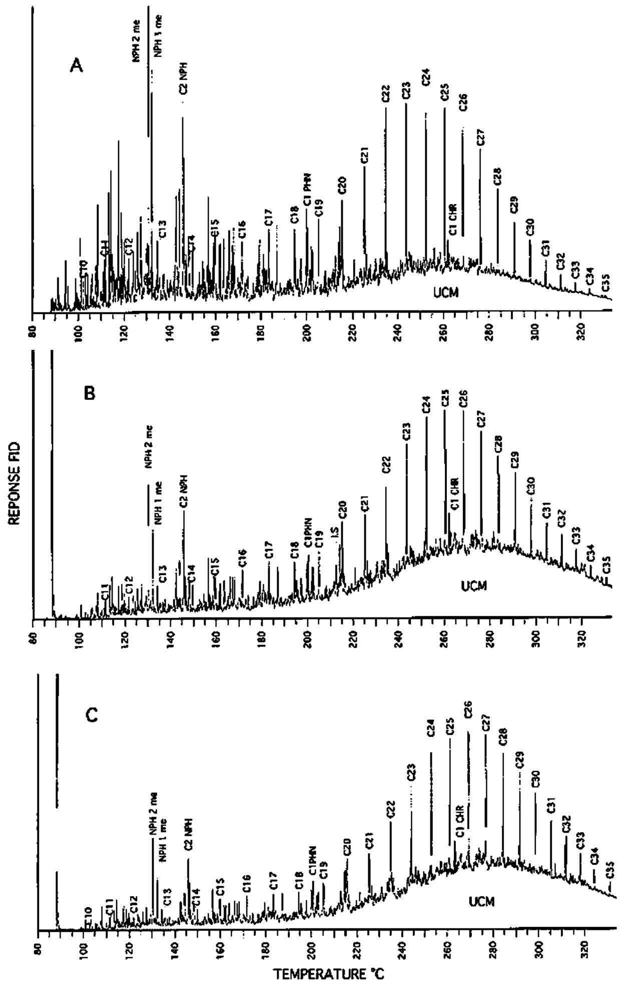

Figure 1. Chromatograms of 'Erika' oil. C10-C35, n-alkanes; NPH, naphthalenes; PHN, phenanthrenes; CHR, chrysenes; UCM, unresolved complex mixed; I.S., internal standard (n-1: eicosene). (A) Oil from the Dunkirk refinery; (B) oil collected at the Le Croisic headland in January 2000; (C) oil collected in March 2000.

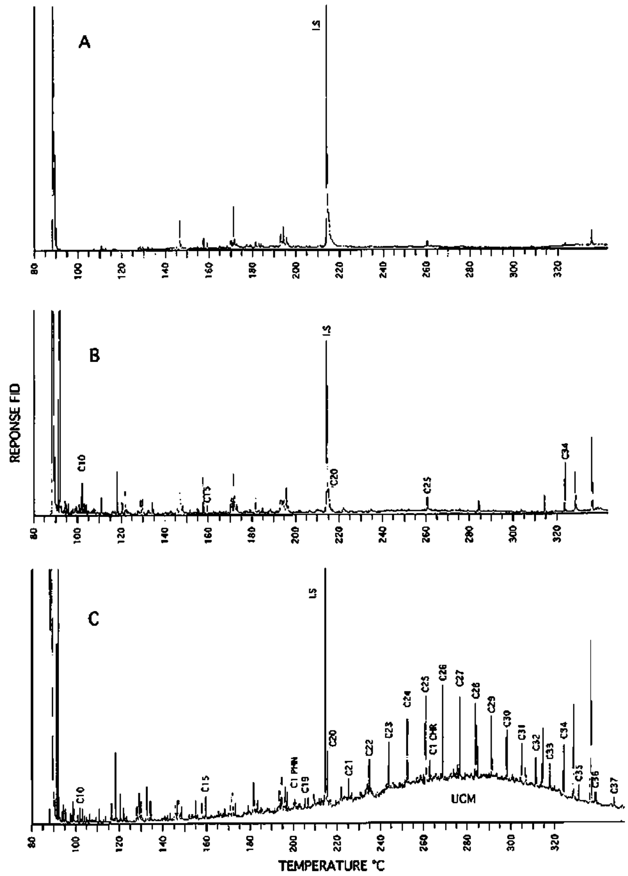

Figure 2. Chromatograms of Enteromorphe algae. (A) Control alga; (B) non-polluted living alga gathered at the Le Croisic headland in March 2000; (C) polluted dead alga gathered in March 2000. Contamination by 'Erika' oil was revealed by the presence of a UCM and characteristic compounds: alkanes, methyl phenanthrenes, and methyl chrysenes.

biodegradation by micro-organisms was apparent after three months. Methylnaphthalenes had not been attacked, nor n-alkanes, despite their sensitivity to biodegradation (Oudot, 2000).

Analyses of enteromorphs

Control alga collected in September 1999 before the disaster (Figure 2A) and stored in a freezer contained no hydrocarbons. The same was true for the living alga gathered in March (Figure 2B). However, the dead alga showed contamination (around 500 ppm) by hydrocarbons from the 'Erika'. No contamination was detected by GC-FID in living alga, even in plants gathered in pools near polluted rocks, but contamination persisted and killed plants in direct contact with oil.

Biological tests

Toxicity of oil in seawater for the copepod

Observations were begun at the third day, and the number of dead copepods was counted. Forty per cent were dead after three days and 60% after four days, whereas 100% of controls were alive (Table 1A).

Experimental determination of LC_{50} 96 h for the copepod in the presence of variable amounts of oil from slick deposits

The highest mortality rate (100%) was observed for the oil concentration of 5.02 g 20 ml^{-1} (Table 1B). The method of Bliss (1935) used to plot the regression line of the dose–effect relation gave an LC_{50} value at 96 h of 3.83 ±2.02 g 20 ml^{-1} of seawater in the experimental conditions described above.

Determination of the ecotoxicity of Le Croisic sediment before and after oil deposits

Significant differences in toxicity (χ^2-tests with 5% risk) were found between contaminated and control sediments (Table 1C). Mortality rates reached 30.6 and 27.8% for the two contaminated sediments (four times the 6.7% rate for control sediment). The difference between the two contaminated sediments was not statistically significant.

*Quantitative measurement of chlorophyll-*a

The results (Table 2) were analysed statistically by the Fisher test for pairwise comparison of means and a Student test. During the first seven days, a sudden drop (up to 50%) in chlorophyll-*a* concentrations was observed in the test and control series, corresponding to the period of adaptation to experimental conditions. At day 14, chlorophyll-*a* concentrations decreased from 512.2 to

Table 1. *Toxicity of 'Erika' oil for the copepod* Tigriopus brevicornis. *(A) in seawater; (B) with different concentrations ranging from 0.7 to 5.0 g 15 g^{-1} of contaminated Le Croisic sediment; (C) in Le Croisic sediment before (control without oil) and after the oil spill (5 g of oil in 10 g of sediment).*

			Mortality rate	
Days	Oil added	Environment	CONTROL	TEST
(A) *Mortality rate of copepods exposed to 'Erika' oil in seawater.*				
0+2	1 g	seawater	0	0
0+3	1 g	seawater	0	40
0+4	1 g	seawater	0	60
(B) *Mortality rate of copepods exposed to 'Erika' oil-contaminated sediment.*				
0+4	0.7g	seawater+sediment	0	21
0+4	1.54	seawater+sediment	0	28
0+4	2.54	seawater+sediment	0	31
0+4	3.53	seawater+sediment	0	42
0+4	4.0	seawater+sediment	0	64
0+4	5.02	seawater+sediment	0	100
(C) *Mortality rate of copepods exposed to Le Croisic sediment before and after the oil spill.*				
0+4	0	seawater+sediment (September 1999)	6.7	
0+4	5 g	seawater+sediment (18 January 2000)		30.6
0+4	5 g	seawater+sediment (29 March 2000)		27.8

Table 2. *Changes in chlorophyll-*a *content of the alga* Enteromorpha intestinalis *placed in seawater that had contained 'Erika' oil (24 h of bubbling).*

Days	0	7	14	21	28
Controls	1079.7	512.2	485.0	755.9	1197.5
	±146.1	±70.9	±93.9	±217.5	±61.7
Tests	1079.7	480.73	383.0	841.1	783.2
	±146.1	±1.27	±63.6	±299.1	±258.9

485.0 μg l^{-1} for control medium and from 480.7 to 383.0 for test medium. From day 14 to 21, the concentrations increased after the alga had adapted to the new conditions, reaching 755.9 μg l^{-1} for oil-contaminated water and 841.5 μg l^{-1} for the control. At day 28, the difference became greater between the control (1197.98 μg l^{-1}) and the contaminated medium (783.2 μg l^{-1}).

The comparison of means test showed no significant differences during the first three weeks of the experiment, whereas differences were significant after 28 days. Necrosis was more extensive on thalli in contaminated water.

DISCUSSION

This appraisal of the effects of the 'Erika' oil slick on the ecosystem of *Tigriopus brevicornis* and *Enteromorpha intestinalis* did not allow explicit ecological assessment after three months in areas partly covered by oil. Chemical analysis indicated that the oil degrades very slowly and will probably be a long-term factor in the ecosystem.

Six months after the disaster, once the beach had been cleaned (manually and mechanically) in February and no oil was visible to the naked eye, the sediment retained all its toxicity for the copepod. Given the nature of the sediment, i.e. biological calcium whose particles had been absorbed or adsorbed on sand grains, it is likely that toxic products were released again from the sand upon contact with water.

Although the results of these tests are significant, it is difficult to determine the real toxicity of the oil for the copepod. First, the tests were conducted in a closed environment favouring the concentration of dissolved products. Secondly, the dry oil collected at Le Croisic, which initially remained at the bottom of the crystallizing dish, rose to the surface after several hours, forming a uniform layer (0.5 mm thick) that prevented any exchange of gases with surrounding air. In these conditions, the death of copepods could have been caused by the dissolved oxygen remaining in the experimental environment or a combined effect of asphyxia and oil toxicity.

Laboratory tests did not clearly indicate the effects of the spill on the natural environment, which will in fact require continuing studies of the ecosystem for a number of years. However, they established that the oil was toxic for the two species considered. The mortality rate increased for the copepod, regardless of the medium tested: seawater in contact with oil for 24 h, or seawater in contact with oil-contaminated sediment. As the *Tigriopus* ecosystem consists of a group of pools largely confined to rocky areas, it can remain isolated from oceanic space for several days, depending on tidal amplitude.

The cleaning operation has not eliminated toxicity from the sediment, which apparently contains microparticles. The mechanical or manual mixing of the sand by machines and tools may have contributed to a greater dissemination of the oil in sediment.

Since the preparation of this communication, successive missions to the Le Croisic headland site in May, September, November and December 2000 determined that populations of the copepod *Tigriopus* and the alga *Enteromorpha* have decreased in pools studied for many years. Although this may be a natural seasonal phenomenon unrelated to the oil spill, no similar situation was observed in previous years.

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