

## Biological effects of the “Erika” oil spill on the common mussel (*Mytilus edulis*)

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**Abstract** – A 3-year survey was made of several biological markers in mussels (*Mytilus edulis*) exposed in situ to the oil that came ashore after the wreck of the “Erika” tanker on the Brittany (France) coast in December 1999. The mussel response was assessed using a set of 7 biomarkers, most of them related to the metabolism of organic contaminants. After a series of validation tests, data was evaluated for only 5 biomarkers: acetylcholinesterase (AChE), glutathione S-transferase (GST), catalase (CAT), malondialdehyde (MDA) and deoxyribonucleic acid (DNA) adducts. No significant reductions in GST or CAT levels were observed, levels of DNA adducts and MDA were high during the 6 months immediately following the accident and levels of AChE were significantly lower during the first year of the survey suggesting a general stress. A simple multivariate graphic method, the integrated biomarker response index, was used to combine 4 of the 5 validated biomarkers and quantify the degree of impact on mussels at different sites. The results show that mussel populations were affected by the oil spill only during the first year after the event.

**Key words:** Oil spill / Polycyclic aromatic hydrocarbon / PAH / Survey / Monitoring / Biomarkers / Mussel / *Mytilus edulis*

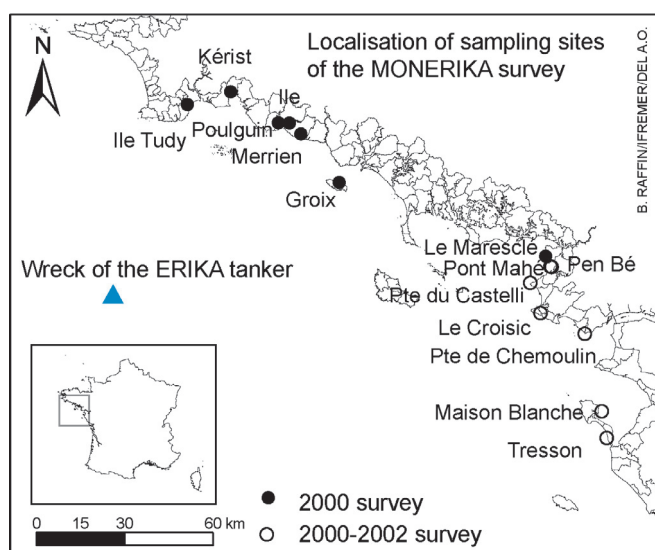
**Résumé** – Suivi des effets biologiques de la marée noire de l’« Erika » sur la moule commune (*Mytilus edulis*). Pendant trois ans, un suivi de plusieurs marqueurs biologiques a été engagé sur des populations de moules (*Mytilus edulis*) exposées au pétrole échoué après le naufrage de l’« Erika » sur les côtes bretonnes en décembre 1999. La réponse biologique du bivalve a été estimée à travers la mesure de 7 biomarqueurs qui, pour la plupart, sont issus des systèmes de métabolisation mis en place lors d’une exposition à ce type de contaminant. Après un exercice rigoureux de validation des données, les valeurs de glutathion S-transférases (GST), catalases (CAT), malondialdéhyde (MDA), adduits à l’ADN et d’activité acétylcholinestérase (AChE) ont été retenues pour l’établissement d’un bilan des effets. Aucune perturbation significative des activités GST et catalase n’a pu être mise en évidence alors que les niveaux d’adduits à l’ADN sont forts dans les premiers mois et que les teneurs en MDA sont élevées pendant les huit mois qui suivent l’accident. Les niveaux d’activité de l’acétylcholinestérase sont plus faibles la première année du suivi. En utilisant une méthode graphique intégrant les réponses multi-marqueurs, une échelle de couleurs a permis de quantifier l’impact biologique en fonction du temps. De manière générale, l’impact biologique du pétrole de l’« Erika » est faible et limité aux huit premiers mois du suivi. Face à ce type de contamination, les adduits à l’ADN et la mesure du niveau de MDA semblent deux marqueurs pertinents et informatifs. La base de données établie au cours de cette étude a valeur de référence.

### 1 Introduction

On the 13th of December 1999, the wreck of the *Erika* oil tanker resulted in the release of 20 000 t of crude oil, including polycyclic aromatic hydrocarbons (PAH), nickel (Ni)

and vanadium (V), into the North Atlantic Ocean close to Brittany (France). The oil slicks drifted for several weeks before coming ashore. Different levels of oil contamination occurred along 400 km of the Atlantic shoreline from Finistère to the mouth of the river Loire (Fig. 1). The northern part of Brittany was the first part of the coast to be affected by oil

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**Fig. 1.** Map of Brittany (France) showing the different sampling sites in 2000 and 2000-2002.

slicks coming ashore (23 December 1999) and the pollution reached the southern part (Pen Bé to Tresson) after the oil had been at sea for 4–5 days.

A survey was started in January 2000 on the potential biological effects of the *Erika* oil on the common mussel (*Mytilus edulis*). Biomarkers were used to detect molecular, cellular or physiological disorders that could be the result of exposure to the oil. The *MONERIKA* programme (Monitoring the effects of the *Erika* oil spill) was designed as a spatial-temporal field monitoring of a set of biomarkers at different contaminated sites over a 3-year period (2000-2002).

The main objectives of the *MONERIKA* programme were:

- to measure the temporal patterns of 7 biomarkers and determine if the *Erika* oil disrupted them,
- to detect any biological changes related to oil exposure at different sites,
- to analyse biological responses in relation to the levels of PAH, nickel and vanadium in mussel tissues, and
- to combine the biological markers to measure the relative biological impact of the oil on mussels at different sites.

Acetylcholinesterase (AChE) activity, benzopyrene hydroxylase (BPH) activity, glutathione S-transferase (GST) activity, catalases (CAT) activity, malondialdehyde (MDA) content, deoxyribonucleic acid (DNA) adducts and multixenobiotic resistance proteins (MXR) content were measured.

All the markers measured in the survey, except AChE, form part of the detoxification system activated in living creatures in response to the hydrophobic nature of contaminants such as PAH. These markers are either directly related to the metabolism of PAH (BPH and GST) or are the result of their metabolic effects especially those related to their effect on the production of reactive forms of oxygen (CAT and MDA are oxidation stress markers) or on DNA adducts.

**Table 1.** List of sites, from north to south, and the maximum observed concentrations of polycyclic aromatic hydrocarbon, PAH, contamination in winter/spring 2000. PAH =  $\Sigma$  13 PAH; in  $\mu\text{g g}^{-1}$  dry weight.

Sites	Reference	Maximum PAH content	Period
Ile Tudy	1	nd*	
Kerist	2	nd*	
Poulguin	3	0.468	January 2000
Ile	4	nd*	
Maresclé	6	0.347	March 2000
Pen Bé	8	1.466	February 2000
Pointe de Castelli	9	1.627	April 2000
Le Croisic	10	1.409	March 2000
Pointe de Chemoulin	11	0.879	October 2000
Maison Blanche	12	1.164	February 2000
Tresson	13	4.745	February 2000

\* Analysis not performed.

## 2 Materials and methods

### 2.1 Sampling

Mussels were chosen as the indicator species because of their local economical importance as well as by for practical reasons related to use of a sampling net. The *MONERIKA* programme was carried out over a 3-year period from January 2000 to December 2002. In 2000, 12 sites in the affected area were sampled, from the south of Finistère to Noirmoutier Island (Fig. 1). In 2001 and 2002, sampling was limited to only 6 sites from the most severely affected areas from the mouth of the river Vilaine to the bay of Bourgneuf (Table 1). 60 mussels were collected for biomarker assays at each of these sites. Gills and digestive glands were immediately removed and pooled samples from 5 individuals were stored in liquid nitrogen. 16 400 mussels were collected during the course of the programme.

During the 3 years of the study, the RNO (French National Observation Network on the quality of coastal environment) sampling network ensured the quality of the sampling at the different sites.

### 2.2 PAH content in mussel tissues

Each month, additional mussel samples were taken for the determination of soft tissue PAH concentrations. The following 13 PAH were measured: fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene.

### 2.4 Data validation and processing

The *MONERIKA* database comprises >40 000 data. The sources of variability due to the analytical methods were rigorously analysed. Only the AChE, GST, CAT and MDA data

### 2.3 Biomarker analytical references (Table 2)

**Table 2.** List of biomarkers.

Biological markers	Abbr.	Organs	Analytical methods	Units
Acetylcholinesterase	AChE	gills	Ellman et al. (1961)	nmol mg <sup>-1</sup> P min <sup>-1</sup>
Benzopyrene hydroxylase	BPH	digestive gland	Akcha et al. (2000)	pmol resorufin mg <sup>-1</sup> P min <sup>-1</sup>
Glutathione S-transferase	GST	gills	Habig et al. (1974)	nmol mg <sup>-1</sup> P min <sup>-1</sup>
Catalase	CAT	digestive gland	Grennwald (1985)	μmol mg <sup>-1</sup> P min <sup>-1</sup>
Malondialdehyde	MDA	digestive gland	Buege and Aust (1978)	nmol mg <sup>-1</sup> P min <sup>-1</sup>
DNA adducts	Add	digestive gland	Genevois et al. (1998)	adducts per 10 <sup>8</sup> nucleotides
Multi-xenobiotic resistance proteins	MXR	gills	Minier et al. (1991)	ng protein

were validated. After finding evidence of analytical errors due to the instability of the control compound, the BPH data were not included in the data set. The multi-xenobiotic resistance proteins (MXR) data were not sufficiently complete and showed great differences that prevented any statistical treatment and ecotoxicological interpretation.

Raw data for each biomarker was plotted against time and evaluated for correlation with the levels of PAH, nickel and vanadium in the mussels. A graphic method for combining all the biomarker data (integrated biomarker response - IBR, Beliaeff and Burgeot 2002) was used to assess the AChE, GST, CAT and MDA data measured in 2000 and 2001 and quantify the impact of the oil spill at each site. Star plots, combining different biomarker values, were used to visualise the biological effects and create an IBR index. So as to use a multi-marker approach, the biomarker data were processed to calculate the IBR index.

## 3 Results

### 3.1 PAH concentration

Before the *Erika* oil spill the chronic contamination of mussels by PAH was estimated to be 0.05 to 0.2 μg g<sup>-1</sup> dry weight. From January to June 2000, the average concentrations in mussels at the exposed sites reached 0.4 to 1 μg g<sup>-1</sup> with high variability between sites (RNO 2002). PAH concentrations decreased slowly from the levels measured in January 2001 and reached baseline levels in April 2001. Maximum PAH contents at the different study sites are given in Table 1. It should be noted that the 13 PAH measured represent only 0.3% of the total amount of the *Erika* oil.

### 3.2 Biomarkers

For technical reasons, it is not feasible to show all the data for each site. Table 3 provides a summary of the AChE, GST, CAT, MDA and DNA adduct data for 2000-2002 and their correlations with tissue contamination data. The sites with the highest responses are identified. Figure 2 provides information on the variability in AChE, GST, CAT and MDA data at the study sites during 2000-2002.

*AChE levels.* The mussel AChE concentrations showed clear seasonal variations every year of the survey with highest levels during the summer. Significantly lower levels were measured during 2000 compared to 2001 and 2002, with a decrease of up to 30%. Variability was greater during the first two years of the survey and levels seemed more stable in 2003. No significant differences were observed between the sites.

There was a negative correlation between AChE activity and PAH levels, but no correlation could be found between AChE and the Ni or V levels. The sites with the lowest AChE levels during the winter and spring 2000 were Ile Tudy, Pointe de Castelli and Pointe de Chemoulin.

*GST levels.* As for AChE, the evolution of GST activity showed seasonal cycles with higher levels in the summer. The highest levels were found from June to October 2001 at Pointe de Castelli and Le Croisic. There was no correlation between GST activity and the levels of PAH, Ni or V contamination. During the six first months following the oil spill, the sites with the highest GST levels were Ile Tudy, Pen Bé and Le Croisic.

*CAT levels.* The highest levels were observed during 2003, while greater variability was recorded during 2000. There was no correlation between the CAT activity and the levels of PAH, Ni or V contamination. The highest CAT levels were measured at the beginning of 2000 at Kerist, Pen Bé and Le Croisic.

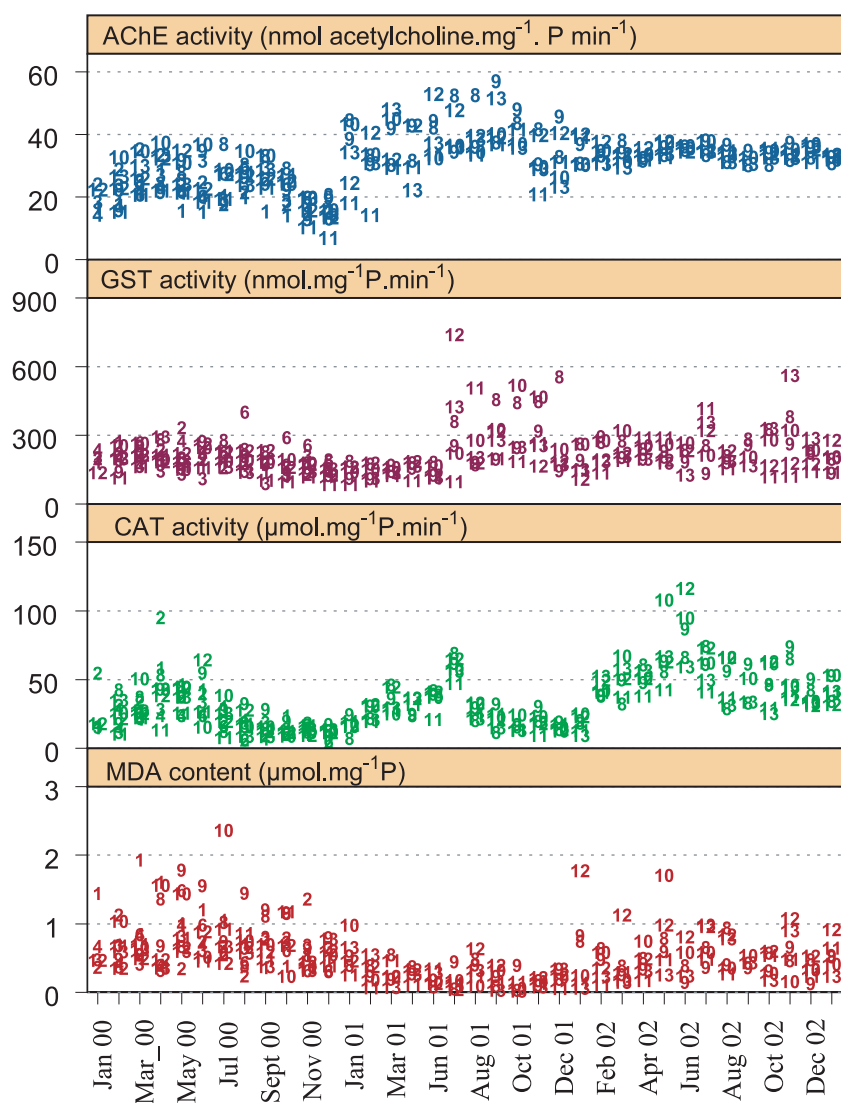
*MDA levels.* MDA levels were high from the beginning of the survey until June. 2000 MDA concentrations were twice as high as in 2001. There was a correlation between the MDA levels and the concentrations of PAH in mussels, but not with the levels of Ni or V. The maximum levels were found at Ile Tudy, Pointe de Castelli and Le Croisic from March to May 2000.

*DNA adducts.* DNA adducts in mussels increased significantly at most sites during the first months of 2000 particularly during January (Fig. 3). The highest values were found at Pointe de Chemoulin, Maison Blanche and Pointe de Castelli but decreased rapidly in February except at Pointe de Castelli. A moderate increase was observed in April at Chemoulin, Tresson and Pointe de Castelli, while summer levels were very low at all sites. Another significant peak was recorded in October and November at Le Croisic, Ile Tudy and Pen Bé. In January, a typical PAH radioactive diagonal zone (Fig. 4) was observed at all stations and some adducts disappeared and others became visible during the following months.

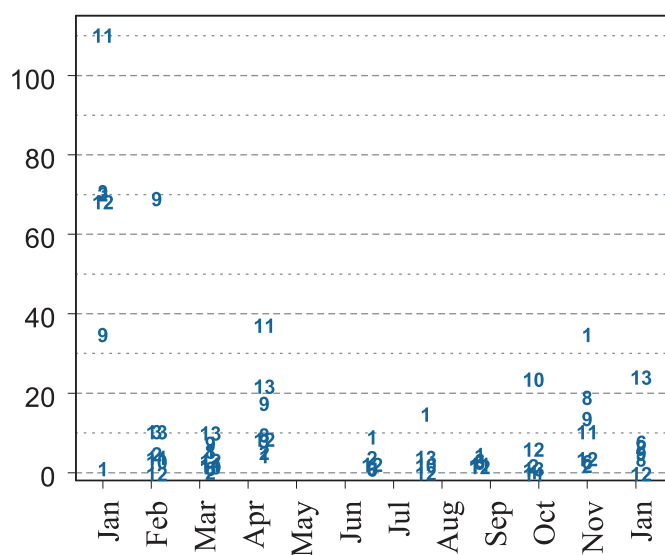
**Table 3.** AChE, GST, CAT, MDA and DNA adduct data for 2000-2002 and their correlations with tissue contamination data. Sites with extreme responses (increase or decrease) in the first months following the oil spill are identified.

	PAH	Ni-V	General comment	Sites with extreme values
<b>Acetylcholinesterase</b> (Physiological index)	Negative correlation	No correlation	Values were lower in 2000 than in 2001 and 2002	Ile Tudy, Pointe de Castelli, Chemoulin
<b>Glutathione-S-transferases</b> (Biotransformation phase II)	No correlation	No correlation	No major change in the seasonal cycles	Ile Tudy, Pen Bé, Le Croisic
<b>Catalases</b> (Lipid peroxydation)	No correlation	No correlation	No major change in the seasonal cycles	Kerist, Pen Bé, Le Croisic
<b>Malondialdehyde</b> (Lipid peroxydation)	Positive correlation	No correlation	Higher levels 2000 than in 2001	Ile Tudy, Pointe de Castelli, Le Croisic
<b>DNA adducts</b> (Genotoxicity)	nd*	nd*	High levels of adducts in February 2000 at 5 sites	Chemoulin, Pointe de Castelli, Tresson

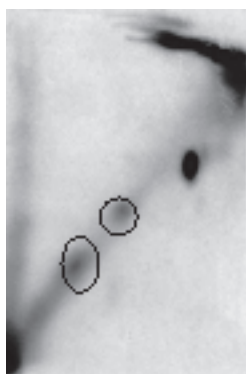
\* analysis not performed.



**Fig. 2.** Levels of AChE ( $\text{nmol mg}^{-1} \text{P min}^{-1}$ ), GST ( $\text{nmol mg}^{-1} \text{P min}^{-1}$ ), CAT ( $\mu\text{mol mg}^{-1} \text{P min}^{-1}$ ) and MDA ( $\text{nmol mg}^{-1} \text{P}$ ) during the 3-year survey (Jan. 2000 – Dec. 2002). All results measured at each site are plotted using the site reference (Table 1).



**Fig. 3.** DNA adducts levels (in adducts per  $10^8$  nucleotides) in mussels from different sites in 2000. All results measured at each site are plotted using the site reference.



**Fig. 4.** The DNA adducts pattern shows two main spots in the diagonal radioactive zone (DRZ) (Maison Blanche in January).

## 4 Discussion

During the *Erika* oil spill, the oil did not cause an immediate and massive acute mortality of the mussel populations probably because the oil was constantly removed from the shore by both the clean-up operation and the tide. Additionally, bivalves can isolate themselves from their environment by closing their shells when faced with hostile conditions. Although mussels are known to be physiologically tolerant of pollution, the possible prolonged effects due to oil exposure can be assessed using biological markers.

Most biological markers used in this study arise from the detoxification mechanisms activated by organisms when exposed to xenobiotics or are the consequences of detoxification. AChE levels were monitored as a general marker of the mussels' physiological status. BPH is a P450-dependent system which catalyses the hydroxylation of benzopyrene (a PAH) in bivalves. BPH is one of the Phase I biotransformation enzymes. The glutathione S-transferase family (GST) belongs to the Phase II detoxification enzymes and play an important role in the detoxification and metabolism of many xenobiotics.

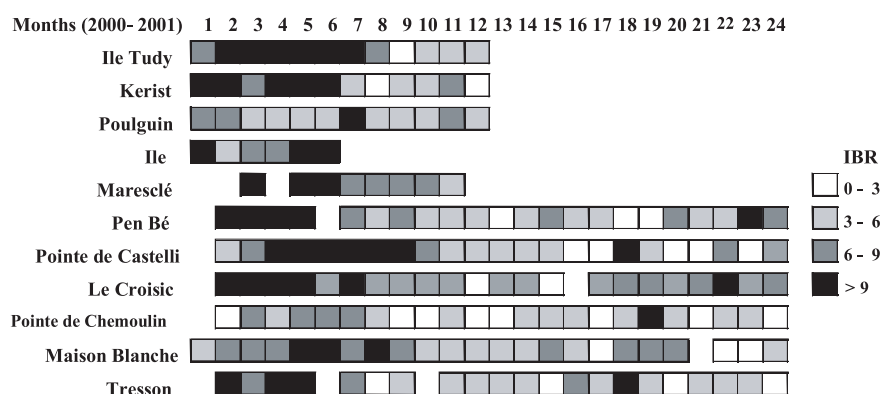
GST catalyses the combination of a wide variety of substances (including PAH) with glutathione to facilitate their solubility and excretion. CAT are powerful antioxidant enzymes acting against various potentially harmful oxidising agents, the reactive oxygen species (ROS, e.g. HO, H<sub>2</sub>O<sub>2</sub>), which are produced as intermediates during the detoxification processes. CAT promote the conversion of ROS to water and molecular oxygen. MDA is a metabolite produced by lipid peroxidation of unsaturated fatty acids resulting from the effects of ROS and a marker for the oxidative degradation of cellular membranes. The metabolites of PAH can combine with DNA, resulting in DNA adducts. This impairment of DNA by genotoxic compounds is thought to be an essential step in chemical carcinogenesis. Furthermore, exposure to PAH can be demonstrated by examination of specific adduct profiles, e.g. the so-called diagonal radioactive zone (DRZ). Multi-xenobiotic resistance proteins (MXR) play a role in the transport of hydrophobic contaminants out of the cells and are part of the Phase III system (transport).

Most enzymatic activities in invertebrate species vary with the environmental temperature. In reality, the levels of enzymatic activity do not directly depend on ambient temperature but on the physiological activity of the organism, which in turn is tightly coupled to water temperature. This point is of great importance because the mussels' main exposure to the *Erika* oil spill of occurred during the winter when biological activity is low.

In mussels, AChE levels are usually lowest in winter-early spring. In winter 2000, the levels of PAH in tissues were high. Although AChE and PAH levels showed a marked negative correlation, it is not possible to determine if the low AChE levels represent true inhibition by oil or just normal low winter activity. Concerning the AChE levels measured in 2000 (Fig. 2), the lower values and their greater variability compared to those measured in 2002 indicate a lower general concentration but it is not possible to clearly identify the cause of this. AChE levels are usually measured to assess the specific inhibitory effects of organophosphorous and carbamate insecticides but Payne et al. (1996) provided evidence that used engine oil contains AChE inhibiting compounds.

GST and CAT data show no significant change from their seasonal patterns. It is possible that this indicates the ability of the mussel to maintain control of its homeostasis. A common feature observed in both AChE and CAT levels was the wide variability recorded during the first year of the survey.

Further information was obtained from the measurements of DNA adducts in the digestive gland. High levels of adducts were observed in the beginning of 2000 at all the exposed sites, with a marked decrease during the summer period. Chromatograms of <sup>32</sup>P-postlabeled DNA from the mussels showed a common pattern at all the sites in January. This pattern is known as the "Diagonal Radioactive Zone" (DRZ) and is caused by complex mixtures of aromatic chemicals. Generally, the DRZs are typical of DNA damage induced by complex mixtures of PAH. The DRZ observed in January at most sites is very similar to the one obtained by Akcha et al. (2000a) after exposing mussels to the benzo(a)pyrene B[a]P congener via their nouriture.



**Fig. 5.** Variation with time of the integrated biomarker response, IBR index, from January 2000 to December 2001 (darker IBRs indicate greater effects).

Some of the PAH compounds measured in this study are known to be genotoxic and carcinogenic and DNA adducts reflect genotoxic effects of xenobiotics. Several studies have been conducted to analyse the capacity of bivalve species to metabolise complex mixtures of PAH. At least two metabolic mechanisms, the diol-epoxide and the radical cation pathways have been implicated as causing DNA damage in Akcha et al. (2000b). The metabolic conversion of oil into genotoxic compounds involves a Phase I enzyme (e.g. cytochrome P450 (CYP) or BPH and Phase II enzymes (e.g. GSTs). In this study, the data on BPH concentrations were discarded because of analytical problems and GST levels showed no significant reaction during the first months of the survey. Michel et al. (1993) and Akcha et al. (2000) demonstrated significant reduction of GST levels in mussels following *in vitro* exposures to B[a]P. However, Fitzpatrick (1997) reported a slight increase in GST concentrations as PAH levels increased in the digestive gland.

No major deviations from the natural seasonal variability of CAT levels were observed and the GST comments apply equally to CAT. Livingstone et al. (1990) reported no increase in mussels CAT levels after exposure to 50 ppb of B[a]P. Other authors have reported that *in vitro* exposure to B[a]P (Akcha et al. 2000) and *in situ* exposure to PAH (Solé et al. 1998) show a positive correlation with CAT concentrations. In Akcha's work, a highly positive correlation also occurred between CAT and the levels of DNA adducts.

MDA shows a markedly high production during the first months following the oil spill. MDA and DNA adducts are end products resulting from the metabolism of PAH by cytochrome P450 monooxygenases, although their metabolic pathways may be different. The activation of the P450 system is only the first step in a cascade of enzymatic defence systems: B[a]P is a P450 inducer and its metabolism causes lipid peroxidation through ROS production (Winzer et al. 2001).

The IBR was found to be strongly related to MDA levels. The index scores were transformed into a colour scale to illustrate the progression of the impact of the oil on mussels at the different sites (Fig. 5). This representation indicates that Ile Tudy, Kerist, Le Croisic and Pointe de Castelli are the most affected sites while the IBR was lower in Poulguin, Maison-Blanche, Tresson and Pointe de Chemoulin.

The evolution of the IBR index over time suggests that the northern sites (Ile Tudy, Kerist and Poulguin) were exposed first and more severely affected.

Local people have described the *Erika* oil spill as a major ecological disaster. Apparently, there is a great contrast between the way the biological impact was perceived and the first observations made by the scientists. No obvious massive deaths were seen in marine invertebrate species. Although thousands of data was collected during this survey, there are only small and fleeting signs of biological impairment in mussels. There are a number of possible explanations for that:

- Were the levels of PAH high enough to elicit a response? In the most highly contaminated areas (with a few exceptions), mean PAH concentrations in mussels were roughly 5–10 times higher ( $0.2\text{--}1.5\ \mu\text{g g}^{-1}$  dry weight) after the oil spill than normal levels ( $0.05\text{--}0.2\ \mu\text{g g}^{-1}$ ). These levels may be thought of as being rather low considering the amount of oil that was released but it must be kept in mind that coastal species were only exposed to the oil for a few hours because of the fast reaction to the spill, the effectiveness of clean-up measures to remove the oil from the rocks and agitation by the waves. Additionally, the capacity of mussels to accumulate PAH is in the range of 4–11 (depending on the congener), which is relatively low. The PAH levels found in the mussels exposed to the *Erika* oil appear lower than those found associated with most large tanker accidents. In the US, 3 years after the “*Exxon Valdez*” oil spill (82 000 t), PAH levels in *Mytilus trossulus* were as high as  $8.1\ \mu\text{g g}^{-1}$  (Carls et al. 2001). In spite of the high PAH levels, Thomas et al. (1999) observed no significant impact on the physiological response of mussels exposed for 3 years to the “*Exxon Valdez*” oil in field conditions. In the UK, 6 months after the “*Sea Empress*” accident (72 000 t),  $22\ \mu\text{g g}^{-1}$  of PAH were found in mussels from the most affected sites in St. Ishmaels (Irish Sea) and levels decreased to  $7.87\ \mu\text{g g}^{-1}$  in 1997 (Widdow et al. 2002).
- Is the mussel a sufficiently sensitive species to be used for biomonitoring? From many ecotoxicological perspectives, vertebrate species are much more relevant biomonitoring mainly because the toxicant uptake and metabolism pathways are better understood (Stegeman et al. 1992). For

example, according to Venier (2001), the levels of DNA adducts in the mussel never reach those detected in vertebrate organisms (fish). On the other hand, the relatively high mobility of fish and difficulties in their capture considerably limit their use. Mussels are a filter-feeding species and can accumulate contaminants (e.g. trace metals, PAH) that reflect contaminant levels at the site of exposure. Coupled with their relatively low sensitivity, mussels should be regarded as good chemical monitors but poor biomonitors. In the current programme, the main reason for choosing mussels was not purely scientifically based. The principle reason was the commercial importance of this species to the region and the concern of mussel farmers regarding the protection of consumers.

The use of biomarkers of sub-lethal effects in environmental monitoring programmes has been widely discussed over the past number of years. Although most of the biomarkers in current use are useful, they do have some important limitations (Lam and Gray 2003). Two major limitations are natural variability and analytical uncertainty and they often overlap. Natural variability can be assessed by seasonal surveys that take into account biological variability. A significant reduction in analytical variability can be achieved through quality assurance (QA) processes but they have not yet been fully developed for all the biomarkers used in marine monitoring (BEQUALM 1998). As a result, biomarkers must be regarded as helping towards a holistic diagnosis, rather than being considered as absolute numerical data, such as a chemical concentration. Biological markers are essential tools in the assessment of the impact of spills on ecosystems.

In terms of recommendations in the event of an accident involving hydrocarbon contamination, this survey confirms the choice of monitoring DNA adducts and MDA as markers for PAH exposure and their effects on mussels. These two markers are end products produced directly by the metabolism rather than enzymes managing metabolism, which are subject to more biological variation. This makes them more significant and reliable indicators of contamination.

Finally, the database from this study contains nearly 40 000 biomarker values combining seasonal variations over three years and is a first in this area where studies are usually short-term. In this respect, the *MONERIKA* database can be considered as a reference source for the levels and variability for a range of biomarkers that most aquatic ecotoxicology laboratories use.

## 5 Conclusion

Most markers measured in this study were enzymes involved in detoxification systems. As biological mechanisms, they are subject to natural variations and for most of them it was not possible to find evidence of disruption to the natural cycle that could be attributed to the toxic effects of the oil. Nevertheless, it is evident that the levels of DNA adducts and MDA were much higher during winter and spring 2000 than during the following years. Moreover, levels of AChE were significantly lower during the first year of the survey suggesting a physiological stress. These results demonstrate a short and

weak effect of the *Erika* oil on mussels exposed to it, resulting in potential genetic damage (DNA adducts) and ROS production (MDA content). The long-term effects on mussel populations require further investigations on reproductive effects and potential physiological or immunological impairments.

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