Accumulation and depuration of hydrocarbons in the Mangrove oyster *Crassostrea rhizophorae*

Accumulation et purification des hydrocarbures chez l'huître de palétuviers Crassostrea rhizophorae

CARLOS E. DEL CASTILLO, JORGE E. CORREDOR, JULIO M. MORELL
Department of Marine Science, University of Puerto Rico
Mayaguez, P.R. 00680

Abstract

Adult specimens of *Crassostrea rhizophorae* were exposed to diesel oil dispersed in seawater. The experiment consisted of a 10 days contamination period and 15 days depuration period. Oyster samples were extracted, and analysed by gas chromatography and UV fluorescence spectroscopy. The concentrations of n-alkanes, branched alkanes, unresolved complex mixture (UCM) and aromatic hydrocarbons were determined. The oysters accumulated the hydrocarbons rapidly, reaching a maximum concentration of 387.5 µg/g (wet weight). After exposure to uncontaminated seawater, the oysters were able to reduce their hydrocarbon load to 5.6 µg/g. The data indicates that *C. rhizophorae* is capable of preferential accumulation and depuration of hydrocarbons. Apparently, the oysters are capable of modifying the straight chain alkanes and branched alkanes to yield a mixture of components eluting as UCM .

Keywords: accumulation, contamination, depuration, fuel, hydrocarbons, oyster, pollution.

Résumé

Des individus adultes de *Crassostrea rhizophorae* ont été exposés à une pollution d'hydrocarbures. La source de contamination utilisée était composée de gazole dispersé dans de l'eau de mer. L'expérience comportait une période de contamination de 10 jours et une période de purification de 15 jours. Des échantillons d'huître ont été extraits, puis dosés par chromatographie gazeuse et spectroscopie à fluorescence UV. Les concentrations en N-alcanes, alcanes ramifiés, en UCM (mélange complexe non résolu) et en hydrocarbures aromatiques ont été analysées. Les huîtres accumulent rapidement les hydrocarbures, atteignant des teneurs maximales de 387,5 µg/g (poids humide). Après remise en eau de mer non contaminée, les huîtres s'avèrent capables de relarguer les hydrocarbures pour descendre à des concentrations de 5,6 µg/g. Les données montrent que *C. rhizophorae* est capable d'accumulation préférentielle et de relargage des hydrocarbures. Il semble que les huîtres parviennent à modifier les alcanes non ramifiés et ramifiés pour produire un mélange de composants s'éluant sous forme de UCM.

Mots-clés : accumulation, contamination, purification, gazole, hydrocarbures, huître, pollution.

INTRODUCTION

The accumulation and depuration rates of hydrocarbons in tissues of several species of bivalves from temperate climates has been extensively documented (Lee *et al.* 1972; Ehrart, 1972; Fossato and Siviero, 1974; DiSalvo *et al.* 1975; Fossato, 1975; Fossato and Canzonier, 1976; Boehm and Quinn, 1977;

Farrington *et al.* 1982; Nasci and Fossato, 1982; Pruell *et al.* 1986; Cocchieri *et al.* 1990 and others). However, little work has been done with bivalves from tropical and subtropical environments.

Ballow *et al.* (1987) found that organisms growing at the intertidal zone are the most affected during oil spills. As *Crassostrea rhizophorae* grows in this zone, it constitutes a useful organism for studies on accumulation and depuration of hydrocarbons. Since *C. rhizophorae* is found in tropical environments, a high degradation rates of hydrocarbons, through their own metabolism or by associated bacteria, might be expected. Moreover, possible degradation of hydrocarbons by a related species *C. virginica* and other molluscs is documented (Stegeman and Teal 1973 and Livingstone 1985).

In this work, we studied the uptake and depuration of hydrocarbons in *C. rhizophorae*. We used diesel oil as source of hydrocarbons because it is the most likely form of petrogenic pollution in the oysters' habitat in Puerto Rico.

Method

Mangrove oysters were collected in a mangrove area at Boquerón Bay on the west coast of Puerto Rico. The animals were placed in a 210 x 120 x 15 cm fiberglass tray. Natural seawater was pumped through an open system at a rate of 650 ml/min. The oysters were allowed to acclimatise for seven days.

The diesel fuel was analysed by gas chromatography and UV fluorescence spectroscopy. The contaminant was introduced in the system following the method suggested by Anderson *et al.* (1974). A diesel-in-water dispersion (DWD) was obtained by vigorously shaking one part of diesel oil in 1000 parts of seawater (256 motions per minute during 15 minutes). The dispersion was allowed to rest for 30 minutes. The aqueous fraction was mixed with seawater through a funnel, and distributed over the oysters at a rate of 20 ml/min. through a perforated PVC pipe. The oysters were exposed to the pollutants for 10 days. Samples were taken before contamination and then every five days. After the 10 days contamination period, the oysters were removed to clean the tray and replaced to begin the depuration period. The oysters were sampled every five days for consecutive 15 days. This experiment was duplicated.

The analysis of samples was performed following the CARIPOL method (CARIPOL IOCARIBE, 1984). For each sampling, two groups of oysters, with an approximate wet weight of 10 grams each (approximately 20 oysters) were selected. Tissues were dissected using a stainless steel knife rinsed with hexane, rinsed with pre-extracted distilled water, weighed and placed in 500 ml round bottom flasks. A solution of NaOH 5N was added to digest the tissues. The flasks were kept closed for 18 hours. After digestion, samples were extracted three times with 20 ml of pentane. The extracts were dried under vacuum at 40°C. The dried samples were diluted in 5 ml of hexane, and then cleaned and fractionated by passing through chromatographic columns containing silica and alumina. Aliphatic hydrocarbons were eluted with hexane and polynuclear aromatic hydrocarbons (PAHs) were eluted with a hexane/methylene chloride (8:2) solution. The extracts were dried under a current of nitrogen and diluted in

hexane for analysis. Aliphatic hydrocarbons were analysed by gas chromatography, using a Hewlett-Packard 5890-A instrument. Table I shows the operational conditions of the instrument. The concentration of resolved hydrocarbons was determined with reference to a standard of nonadecane. The area of the unresolved complex mixture (UCM) was determine by planimetry and related to the nonadecane standard. Identification of individual n-alkanes was done comparing retention times of the sample with the retention times of a prepared mixture of known n-alkanes. The PAHs were quantified with respect to a chrysene standard using a Hitachi F-2000 Fluorescence Spectrophotometer, set at an excitation wavelength of 310 nm and an emission wavelength of 360 nm.

Results and discussion

During the contamination period of this experiment, the concentration of hydrocarbons in the tissues of *C. rhizophorae* increased from 4.1 µg/g (sd=2.1) to 388.6 µg/g (sd=8.3). At the end of the depuration period, the concentrations were 5.62 µg/g (sd=0.4) (figure 1). Figure 2 shows accumulation and depuration patterns of the n-alkanes, branched alkanes, UCM and PAHs. Chromatograms of the aliphatic fractions demonstrate a rather fast increase in the concentration of the hydrocarbons during the contamination period (figure 3). Chromatograms of the aliphatic fraction of samples collected during the depuration period are shown in figure 4.

The rapid rates of accumulation and depuration shown in this work agree with those reported by Stegeman and Teal (1973), Anderson (1974), Fossato and Canzonier (1976), Pruell *et al* (1986) and others. The resolvable hydrocarbons fraction in the DWD used to contaminate the oysters was composed of 27% n-alkanes and 73% branched alkanes. However, the proportions of this fraction in the most polluted oysters were 18% for n-alkanes and 82 % for

Table I: Chromatographic conditions for the analysis of aliphatic fractions

Instrument **Column**	**Hewlett-Packard 5890A** **Capillary fused silica, 5 % phenyl-methyl silicone**
Film thickness	0.17 µm
Internal diameter	0.31 mm
Column length	25 m
Injector temperature	250 °C
Detector temperature	300 °C
Initial temperature	60 °C
Final temperature	300 °C
Rate	4 °C/mn
Carrier gas	He 1.5 Ml/mn
Auxiliary gas	He 28.5 Ml/mn

branched alkanes. This difference can be explained by a faster release of n-alkanes. This agree with the results of Stegeman and Teal (1973) and Lee (1972) in studies with *Crassostrea virginica* and *Mytilus edulis*. They documented a rapid lost of the n-alkane fractions compared to other groups of hydrocarbons.

The presence of UCM in the tissues of the oysters is of particular interest as the diesel oil and the DWD used to contaminate them do not exhibit a detectable amounts of UCM (figure 5). This observation can be considered as indirect evidence showing that the oysters (or their associated bacteria) are capable of modification of the aliphatic fraction to yield a mixture of components eluting as UCM. The accumulation pattern of the UCM fraction differed from the drastic increase and reduction in concentration of the other fractions (figure 2). It is evident that the UCM persisted during the first 20 days of the depuration period. Continuous transformation of the resolvable fraction into the UCM fraction could explain this persistence. The occurrence of metabolic transformation of hydrocarbons by vertebrates, invertebrates and bacteria is documented (Lee *et al.* 1977, 1978; Delaune *et al.* 1980; Livingstone, 1985, Stegeman, 1985).

CONCLUSION

The mangrove oyster *Crassostrea rhizophorae* can accumulate petrogenic hydrocarbons at a rapid rate, but can achieve depuration in approximately two weeks. It is apparent that the oysters eliminate n-alkanes at a faster rate than branched alkanes. Our results also suggest that the oysters are capable of modifications of the resolvable fraction into components eluting as UCM.

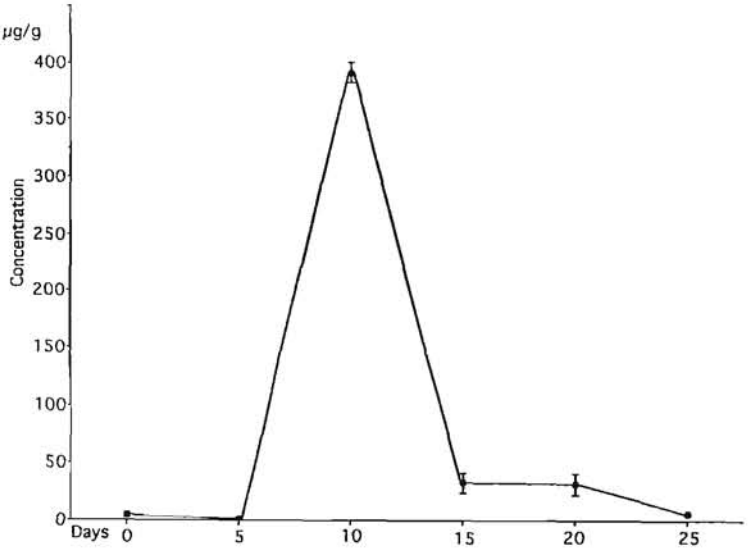

Figure 1: Accumulation and depuration of hydrocarbons.
Bars = S.D. ; n = 4 samples with approximately 20 oysters per sample

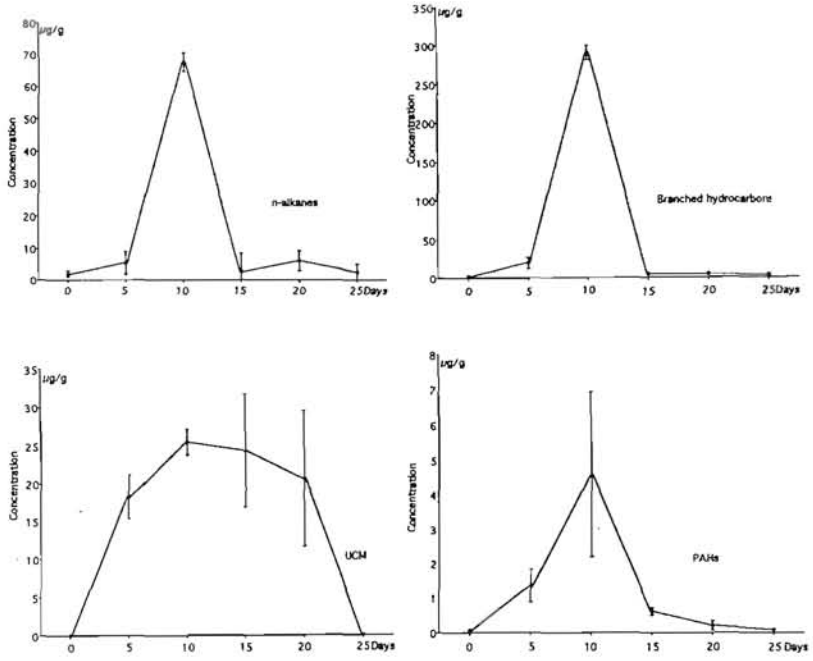

Figure 2: Accumulation and depuration patterns of n-alkanes, branched alkanes, UCM and PAHs

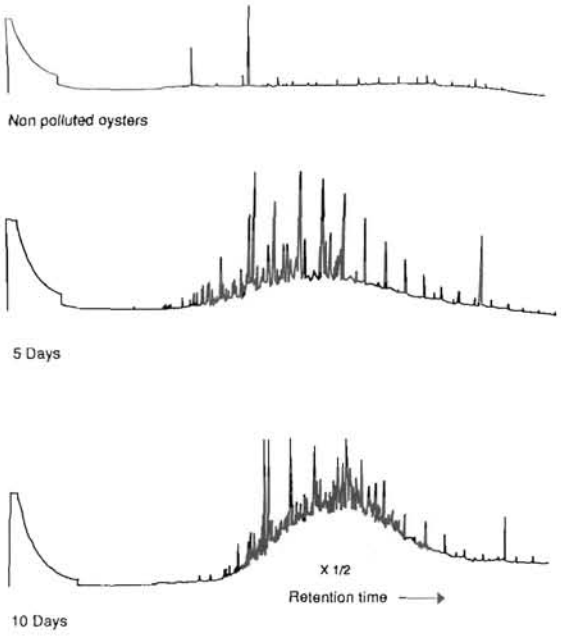

Figure 3: Chromatograms of the aliphatic fraction of samples collected during the contamination period.The aven of the 10 days chromatogram has been reduced by a factor of 2 for presentation

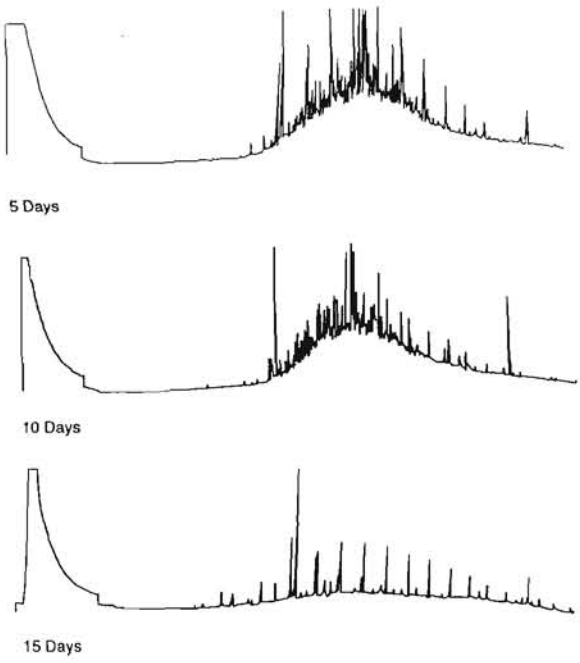

Figure 4: Chromatograms of the aliphatic fraction of samples collected during the depuration period

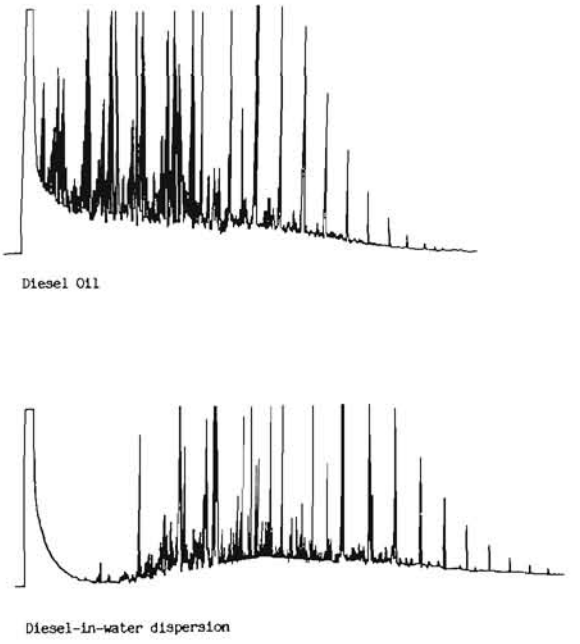

Figure 5: Chromatograms of the aliphatic fraction of diesel oil and diesel in water dispersions. The temperature rate for the diesel oil chromatogram was set at 6 °C/mn. Whereas for the diesel in water dispersion was set at 4 °C/mn

REFERENCES

Anderson J.W., J.M. Neff, B.A. Cox, H.D. Tatem, and G.M. Hightower, 1974. Characteristics of dispersion and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. *Mar. Biol.* **27**, 75-88.

Ballou T.G., R.E. Dodge, S.G. Hess, A.H. Knap and T.D. Sleter, 1987. Effect of dispersed and undispersed crude oil on mangroves, seagrasses and corals. API. Publication No. 4460. Health and Environmental Science Department.

Bohem P.D., J.C. Quinn, 1977. The persistence of chronically accumulated hydrocarbons in the hard shell clam *Mercenaria mercenaria. Mar. Biol.* **44**, 227-233.

CARIPOL. Manual de CARIPOL para la vigilancia de la contaminación por petróleo. CARIPOL IOCARIBE 1984 (Unpublished).

Cocchieri R.A., A. Arnese and A.M. Minicucci, 1990. Polycyclic aromatic hydrocarbons in marine organisms from italian central mediterranean coasts. *Mar. Poll. Bull.* **21,** 15-18.

Delaune R.D., G.A. Hambrick and W.H. Patrick Jr., 1980. Degradation of hydrocarbons in oxidized and reduced sediments. *Mar. Poll. Bull.* **11**, 103-106.

DiSalvo H.L., H.E. Guard, and L. Hunter, 1975. Tissue hydrocarbon burden of mussels as potential monitor of environmental insult. *Environ. Sci. Tech.* **9**, 247-251.

Ehrhardt, M., 1972. Petroleum hydrocarbons in oysters from Galveston bay. *Environ. Poll.* **3**, 256-271.

Farrington J.W., A.C. Davis, N.M. Frew, and K.S. Rabin, 1982. No. 2 Fuel oil compounds in *Mytilus edulis*. Retention and release after an oil spill. *Mar. Biol.* **66**, 15-26.

Fossato V.U., E. Siviero, 1974. Oil pollution monitoring in the Lagoon of Venice using the mussel *Mytilus galloprovincialis. Mar. Biol.* **25**, 1-6.

Fossato V.U., 1975. Elimination of hydrocarbons by mussels. *Mar. Poll. Bull.* **6**, 7-10.

Fossato V.U., W.J. Canzonier, 1976. Hydrocarbons uptake and loss by the mussel *Mytilus edulis. Mar. Biol.* **36**, 243-250.

Lee R.F., R. Saurheber and A.A. Benson, 1972. Petroleum hydrocarbons: Uptake and discharge by the marine mussel *Mytilus edulis. Science.* **177**, 344-346.

Lee R.F., E. Furlong and S. Singer, 1977. Metabolism of hydrocarbons in marine invertebrates: Arylhydrocarbons hydroxylase from the tissues of the blue crab *Callinectes sapidus,* and the polichaete worm *Nereis* sp. In: Pollutants effects on marine organisms. (ed. C.S. Giam), 111-124.

Lee R.F., M. Takahashi and J. Burs, 1978. Short effects of oil on plankton in controlled ecosystems. From the Conference on Assessment of Ecological Impact of oil spill. American Institute of Biological Science, 635-649.

Livingstone D.R. 1985. Responses of the detoxication/toxication enzyme systems of mollusks to organic pollutants and xenobiotics. *Mar. Poll. Bull*, **16,**158-164.

Nasci C., V.U. Fossato, 1982. Studies on physiology of mussels and their ability in accumulating hydrocarbons and chlorinated hydrocarbons. *Environ. Tech. Lett.* **3,** 273-280.

Pruell R.J., J.L. Lake, W.R. Davis and J.G. Quinn, 1986. Uptake and depuration of organic contaminants by blue mussels (*Mytilus edulis*) Exposed to Environmentally Contaminated Sediment. *Mar. Biol.* **91**, 497-508.

Stegeman J.J., J.M. Teal, 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster *Crassostrea virginica. Mar. Biol.* **22,** 37-44.

Stegeman J.J. 1985. Benzo[a]pyrene oxidation and microsomal enzyme activity in the mussel (*Mytilus edulis*) and other bivalve mollusk species from the western north atlantic. *Mar. Biol.* **89,** 21-20.