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Determination of Polyaromatic Hydrocarbons in Scallops (*Pecten maximus*) by UV Fluorescence and HPLC Combined with UV and Fluorescence Detectors

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The use of filter-feeding bivalves as environmental biomonitors has received widespread attention during the last years. Their way of life made them extremly vulnerable to both acute and chronic exposure to environmental contaminants. Their population are cosmopolitan, sedentary, filter-feeding, relatively stable and extensive enough to be sampled repeatedly. Furthermore, they consist of commercially valuable sea-food species. Thus, the measurement of chemical contamination is of interest for public health consideration. The utility of this class of animals for monitoring the presence of marine contaminants was advocated in the "Mussel Watch" program (1981) previously introduced by Goldberg et al. (1978) and described later (Farrington et al. 1983). Most of the studies about hydrocarbons were conducted on mussel, oyster and clam samples polluted artificially in laboratory experiments (Clark and Finley 1975) or exposed to accidental contamination (Fossato and Siviero 1974 ; Di Salvo et al. 1975 ; Wolfe et al. 1981 ; Friocourt et al. 1981 ; Laseter et al. 1981). Many data from literature obtained on mussel and oyster samples from 1970 to 1978 were reviewed by Marchand and Cabane 1980.

During the last few years, the northern coasts of Brittany (France) were heavily polluted with several oil spills : Amoco Cadiz (1978, 230 000 t of Iranian and Arabian crudes). Gino (1979, 40 000 t carbon black), Tanio (1980, 10 000 t no 2 fuel oil). This area of considerable economic importance for its oyster and scallop production is heavily dependant on the quality of its coastline and inshore waters. The most important scallop beds in Europe are mainly in Scotland, Ireland and France. Almost one third of the production of France comes from Brittany and especially the Baie of St Brieuc and the Rade of Brest. After the Amoco Cadiz oil spill, studies have been conducted on oysters from defined areas to determine the fate and effects of the hydrocarbons. No work was done on scallop samples. In fact there are only a few data in literature concerning scallop tissues (Alzieu 1981 for pesticide contents).
The purpose of this paper is to provide information on the level of hydrocarbon pollution in areas concerned with scallop breeding, it is an attempt to use these organisms as biomonitors.

MATERIALS AND METHODS

Adult specimen of *Pecten maximus*, two to three years old, were dredged on four geographical sites (Baie de St Brieuc, Carantec, Brest, Camaret) from September 1980 to February 1982 (figure 1). They were collected with care to avoid contamination.

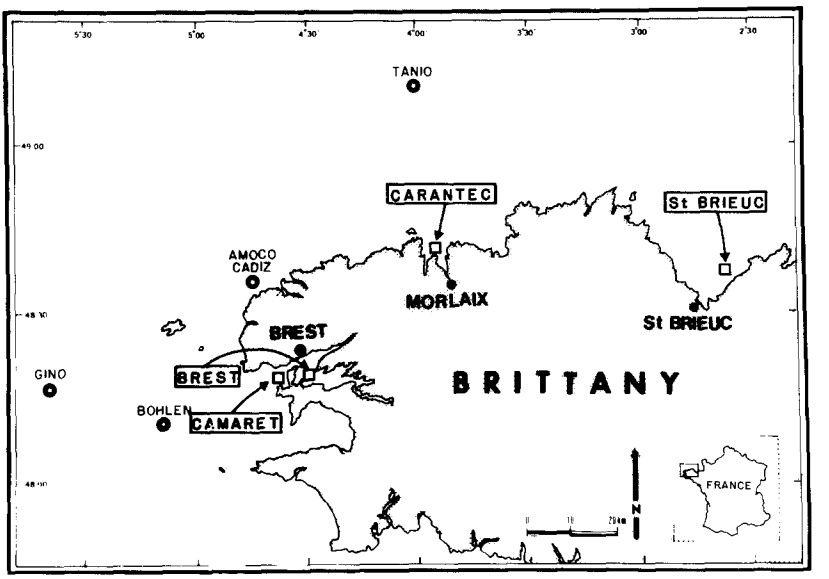

Figure 1 . Locations of scallop sampling areas along the coasts of Brittany.

As soon as collected, the organisms were kept frozen in aluminium foil. Prior to analysis, the different organs (adductor muscles, gonads, somatic tissues) were fractionnated, homogeneized and freeze-dried. Wet and dry weights were determined. About 5 to 10g (dry weight) of scallop tissues were studied according to the analytical process previously described (Friocourt et al. 1981, Berthou and Friocourt 1982) slightly modified (figure 2).

UV-spectrofluorimetry (UV-SF) was performed on a Perkin-Elmer device (Norwalk, USA) model 3000 ; analytical conditions were those according to MARPOLMON measurement procedure (IOC Marine Pollution Monitoring Program). Hexane extracts were placed in quartz UV cell and fluorescence intensity was measured at 360 nm with excitation at 310 nm. Results were reported in chrysene equivalents with a standard curve of chrysene solutions in hexane (10 mg/l).

Materials and analytical conditions used for gas chromatography (GC) and mass spectrometry (MS) were those previously described (Berthou et al. 1981). High performance liquid chromatography (HPLC) quantification and cleaning-up steps were performed on Lichrosorb-NH_2 using a non-polar solvent n-heptane according to the procedure previously developed (Berthou and Friocourt, 1981).

In these conditions, PAH were separated according to the number of condensed aromatic rings independently of alkyl substituents. By coupling UV and fluorescence detectors in series, much information is obtained. UV profile is representative of all PAH classes and a semi-quantification can be performed by detection at 254 nm using d-12 chrysene as internal standard. In these conditions the response factors are not very different from one compound to another ; an estimation by ring classes is possible. The specificity of the fluorescence detection depends on the selected excitation and emission wavelengths. The chosen set was that recommended by Lee et al. 1981 : excitation 360 nm, emission 420 nm, indicative of high molecular weight PAH.

All the results given further are determined in µg/g dry weight (ppm). In case of UV-SF or HPLC measurements, they are expressed in chrysene equivalents.

The gonad index is defined as the ratio of dry gonad weight on dry body weight (gonads + somatic tissues + muscle) (Shafee and Lucas 1980).

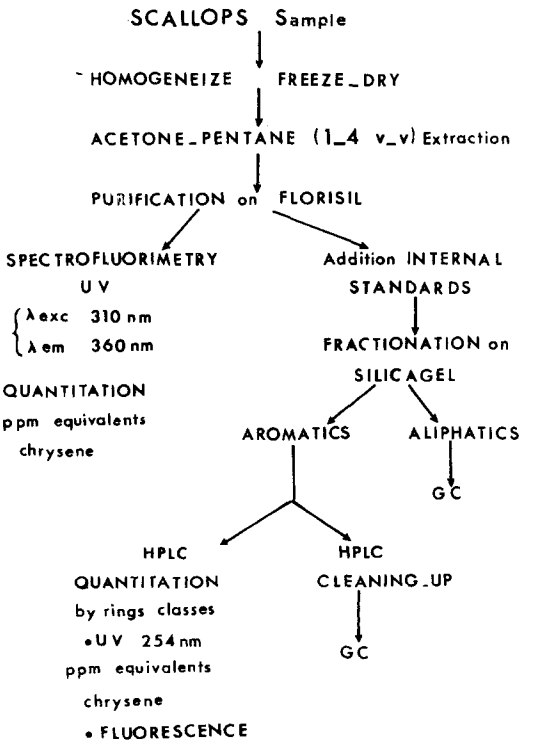

Figure 2 . Analytical process.

RESULTS AND DISCUSSION

Great care was taken to discern a distribution pattern of the PAH levels in the scallop tissues as a function of the different nature of these tissues. So, at first, gonads, adductor muscle and somatic tissues were separately quantified as the size of the organs was sufficient to allow such a separation.

Table 1 : Total aromatic hydrocarbons (PAH) determined by UV-SF in scallop tissues (ppm dry weight, chrysene equivalents).
Gonad index : ratio of dry gonad weight on dry body weight.

Sites	Sampling dates	Gonad index	tissues	PAH
St Brieuc	13.02.81	0.041	gonads	7.3
			somatic tissues	9.9
			muscles	3.6
	03.04.81	0.085	gonads	5.9
			somatic tissues	5.4
			muscles	2.7
	26.05.81	0.159	gonads	2.4
			somatic tissues	2.1
			muscles	1.1
	26.06.81	0.226	gonads	8.3
			somatic tissues	18.8
			muscles	5.3
Brest	12.02.81	0.130	gonads	14.9
			somatic tissues	19.3
			muscles	3.8
Camaret	15.04.81	0.116	gonads	12.7
			somatic tissues	16.6
			muscles	7.7

The data obtained on six samples by means of UV-SF are listed in table 1. In a first approximation, the levels in gonads were nearly twofold that observed in muscles : 8.6 ± 4.5 (SD) ppm and 4.0 ± 2.2 ppm. This result was expected as the lipid content is higher in gonads than in muscles. In four samples, the PAH burden of the somatic tissues was more than in gonads (16.1 ± 4.3 ppm and 10.8 ± 3.6 ppm). In the two other samples, it was nearly the same. This result is an agreement with that observed on mussel samples by Di Salvo et al. 1975. Both aliphatic and aromatic hydrocarbon levels were higher in somatic tissues than in gonads. It would have been interesting to correlate our data with the different lipid contents. It seems that it is not the only factor involved in the accumulation of hydrocarbons. Afterwards, considering the data obtained and the fact that the gonads were atrophied during several months and could not be easily separated from the somatic tissues, in further analytical processes only adductor muscles were separately studied from the other organs of scallops.

Table 2 : PAH levels in scallop tissues determined by UV-SF and HPLC (UV, 254 nm) expressed in ppm dry weight, chrysene equivalent (G : gonads, ST : somatic tissues, M : muscles).
X : mean value calculated as the ratio of PAH levels on weight :

$$X = \frac{(G + ST)}{P} + \frac{(M)}{P'}$$

- : not determined.

sites	sampling dates	gonad index	UV-SF G+ST	UV-SF M	UV-SF X	HPLC G+ST	HPLC M	HPLC X
St Brieuc	15.09.80	0.052	2.8	0.9	1.7	3.3	0.8	2.0
	14.10.80	0.024	3.1	1.3	2.0	3.3	0.8	1.2
	18.11.80	0.016	15.2	5.2	9.5	11.7	4.7	9.3
	13.02.81	0.041	9.6	3.6	5.7	7.4	2.8	5
	03.04.81	0.085	5.5	2.7	4.1	2.8	3.1	5.7
	26.05.81	0.159	2.1	1.1	1.8	1.1	0.7	0.3
	23.06.81	0.226	14.1	5.3	9.9	13.6	3.7	9.5
	30.09.81	0.025	2.8	1.9	2.2	-	-	-
	15.01.82	0.027	8.5	3.1	5.8	-	-	-
Brest	12.02.81	0.130	18.1	3.8	11.2	24.1	3.6	13.9
Camaret	15.04.81	0.116	15.7	7.7	8.3	15.1	9.3	13.5
	02.02.82	0.212	23	3.1	9.7	-	-	-
Carantec	15.02.81	0.051	19	4.3	9.8	-	-	-
	06.10.81	0.051	5.8	1.8	3.2	4.6	1.6	3.0
	15.01.82	0.045	6.3	3.1	4.3	-	-	-
	15.02.82	0.043	19	3.5	6.6	-	-	-

In table 2 are given the results obtained both by UV-SF and HPLC on sixteen samples collected over seventeen months on four different areas. The average value observed in muscles was nearly the third of that determined in gonads and somatic tissues quantified all together (3.2 ± 1.8 ppm, 10.7 ± 7 ppm, n = 16).

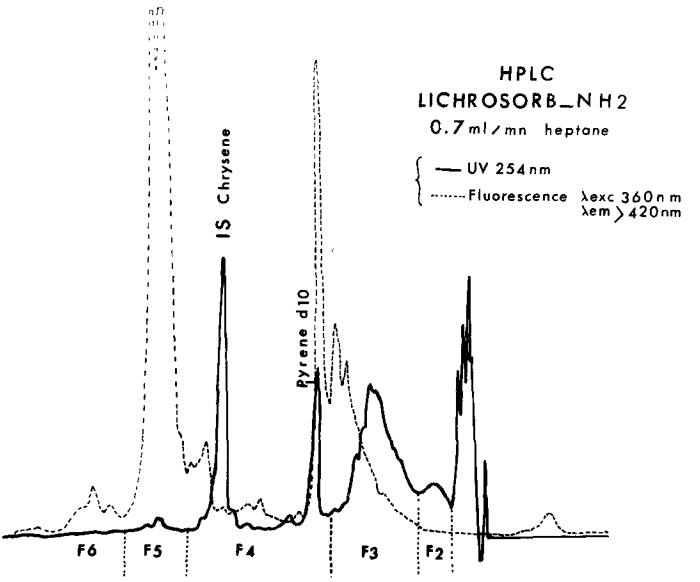

Figure 3. HPLC profiles of gonads and somatic tissues of scallops collected in baie de St Brieuc. F2, F3, F4, F5, F6 correspond to 2, 3, 4, 5 and 6 rings PAH regions.

Table 3. Two, three, five-rings PAH levels in scallop samples determined by HPLC (UV, 254 nm) expressed in ppm dry weight, chrysene equivalents. (G : gonads, ST : somatic tissues, M : muscle).

Sites	Sampling dates	2-rings G+ST	2-rings M	3-rings G+ST	3-rings M	5-rings G+ST
St Brieuc	15-09-80	2.07	0.5	1.1	0.25	0.8
	14-10-80	2.1	0.5	0.95	0.25	0.6
	18-11-80	6	2.5	4.3	0.5	0.15
	13-02-81	4.4	1.8	2.9	1.0	--
	03-04-81	3.7	2.1	2.6	1.0	0.2
	26-05-81	0.7	0.4	0.02	0.25	0.4
	23-06-81	6.5	2.2	5.8	1.4	--
Brest	12-02-81	4.4	1.8	2.9	1.0	
Camaret	15-04-81	9	5.4	5.9	3.1	
Carantec	06-10-81	2.8	0.95	1.7	0.6	

The HPLC measures were in good agreement (0.93 , n = 10). All the liquid chromatographic profiles from the different scallop samples were similar. They mainly consisted of numerous two and three rings compounds (F2, F3, figure 3). The fractions F4 and F5 seemed to be very poor. They were not easily quantified in UV : the fraction F4 contained the two aromatic internal standards used d-10 pyrene and d-12 chrysene which might have hidden a few compounds ; the five rings PAH had not a good response at 254 nm. The table 3 contains the PAH levels estimated in fractions F2, F3, F5 of muscles on one side and both gonads and somatic tissues on the other side. They were lower in muscle than in the whole gonads-somatic tissues. It did not seem to exist a striking selectivity in the compound found. They were identified by MS and mainly consisted of tri-alkylated naphtalene, mono, di, tri, tetra alkylated phenanthrene or anthracene, traces of mono methyl dibenzothiophene (DBT), di, tri, tetra alky DBT.

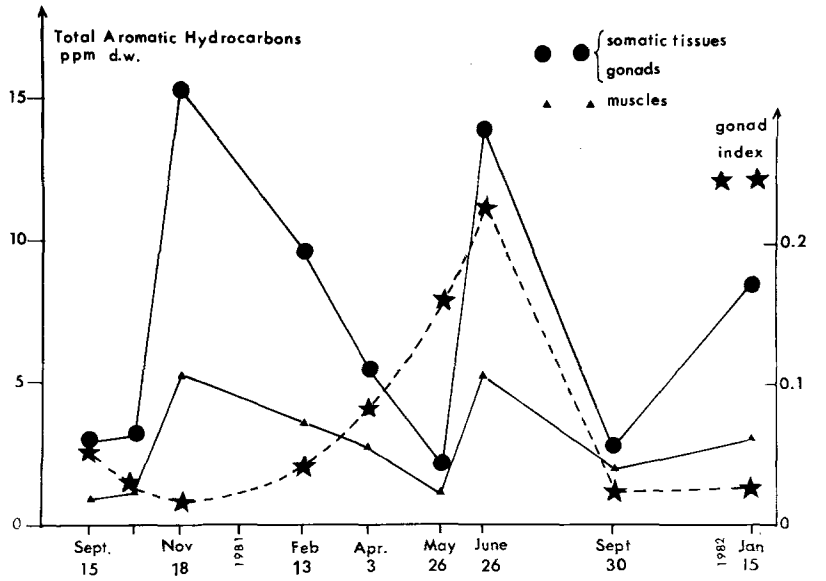

Figure 4 . Temporal fluctuactions of PAH concentrations determined by UV-SF and gonad index of Scallops sampled in baie de St Brieuc.

The temporal variability of the PAH concentrations on the scale of several months was investigated by analyses of nine samples collected on Baie de St Brieuc over a seventeen months period from September 1980 to January 1982. In fact, there are several possible reasons for temporal fluctuations as changes in biological and biochemical activities (filtration rate, spawning activities), changes in the environmental temperature of sea water. The breeding cycle of the scallop (*Pecten maximus*) living in this area begins now to be well-known (Bergeron and Buestel, 1978). The gonads are becoming mature from February to June At the end of

this month, they are the largest ; then, as the sea water temperature is increasing and reaches about 15°C the gamets are emitted There are several spawnings ; at the end of July, the gonads are completely atrophied until next February : they are in the resting stage.
The data obtained by UV-SF method and the gonad index of each sample were reported on table 2 and figure 4.The main features were the observation of two peak values in November 1980 (5.2 ppm in muscle, 15.2 ppm in the whole gonads and somatic tissues) and June 1981 (5.3 ppm and 14.1 ppm) preceded with two minima in October 1980 (1.3 ppm and 3.1 ppm) and May 1981 (1.1 ppm and 2.1 ppm). So the PAH levels in the whole organism followed the same evolution within a yearly period. Our first proposal was to link the amounts of PAH with the maturity state of gonads and lipid content. In fact, this might have been true in the samples collected in October 1980 and June 1981. On the opposite way, the values observed in November 1980 and May 1981 did not follow this hypothesis : the maximum of PAH was corresponding to a minimum of the gonad index value and reciprocally. So, in samples collected from November 1980 to February 1981, the maxima in PAH contents may be due to the influence of environmental conditions. In fact this sampling period was that of winter months featured with storms and strong winds which release the hydrocarbons trapped in sediments, remains from chronic imputs as tanker discharges and ancient oil spills (Amoco Cadiz, Tanio), natural and anthropogenic incomplete combustions of forest fires and fossil fuels.

Table 4 : PAH levels in scallop tissues sampled on different commercial sites (ppm dry weight, chrysene equivalents , X : mean value : gonads + somatic tissues + muscle).

Sites	sampling dates	gonad index	UV-SF X	HPLC
St Brieuc	13.02.81	0.041	5.7	5.0
Brest	12.02.81	0.130	11.2	13.9
Carantec	15.02.81	0.051	9.8	--
St Brieuc	03.04.81	0.085	4.1	5.7
Camaret	15.04.81	0.116	8.3	13.5
St Brieuc	14.10.80	0.024	2.0	1.2
St Brieuc	30.09.81	0.025	2.2	--
Carantec	06.10.81	0.051	3.2	3.0

In order to estimate the degree of coastal pollution in four commercial sites, the PAH burden of eight samples collected at nearly the same period of the year were compared (table 4). The gonads of the collected samples were in a comparable physiological state either in the resting phase or at the beginning of the spawning phase. But, as only a few samples were available, the conclusions

given were only limited observations. In February, samples collected in Brest and Carantec were twice more polluted than the one from Baie de St Brieuc. In April, the same observation could be made from the data obtained in Baie de St Brieuc and Camaret. These results were confirmed with the measures carried out on samples collected in Baie de St Brieuc and Carantec on October 1981.

In all cases, whatever the period of the year, the samples from Baie de St Brieuc were the least polluted. These results were in agreement with those obtained in our laboratory on oyster samples collected both in Baie de st Brieuc and Carantec area. They were explained with the geomorphological features of the considered places : the Baie de St Brieuc is widely open on the Channel while Carantec, near Morlaix, located on a narrow estuary is polluted by the neighbouring towns and the remains of Amoco Cadiz and Tanio oil spills. Brest area (Brest and Camaret) is located on a semi-enclosed bay spoiled with chronic industrial outputs and agricultural discharges. This was confirmed by GC-MS studies which exhibited the occurence of some pesticides as polychlorobiphenyls (PCBs).

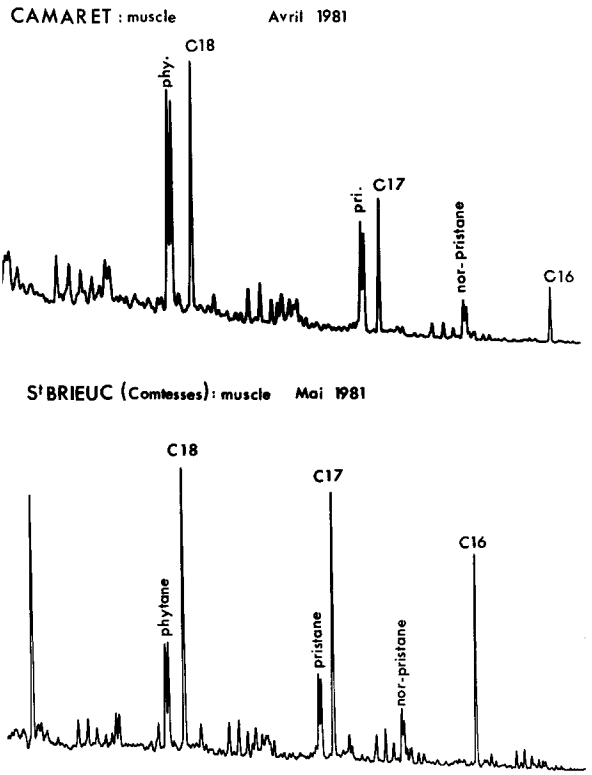

Figure 5 . Sections of gas chromatograms of aliphatic fractions of scallop muscles collected in Camaret, St Brieuc areas. Conditions : column : 50 m x 0.3 mm I.D. 0.15 µm OV-1 ; temperature programm : 0.4 °C/minute from 60°C.

The results of GC quantification of aliphatic fractions will not be given there as biogenic and other aliphatic hydrocarbons were quantified all together. The GC profiles of the samples collected in St Brieuc, Camaret, Carantec, Brest exhibited the presence of double peaks of isoprenoids : nor-pristane, pristane and phytane corresponding to the resolution of diastereoisomeric pairs (figure 5). In case of pristane, for example, the leading peak of the doublet consists of the two enantiomers RR and SS of fossil origin while the rear peak consists of the mesoform (RS : SR) of biogenic origin (Berthou and Friocourt 1981). This was demonstrated to be an unmistakable sign of pollution.

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