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Polynuclear Aromatic Hydrocarbons in Mussels from the Estuary and Northwestern Gulf of St. Lawrence, Canada

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Polynuclear aromatic hydrocarbons (PAH) are generated mainly by natural and anthropogenic incomplete combustions (SUESS 1976). They are also present in fossil fuels (YOUNGBLOOD & BLUMER 1975). Their direct biosynthesis, if it does exist, contributes little to the global PAH burden in the environment (NEFF 1979). Their occurrence in the aquatic environment has been estimated to result mainly from anthropogenic sources (HASE & HITES 1977).

Several studies have shown that mussels (Mytilus sp.) are able to concentrate PAH and have used this bivalve as a quantitative indicator for PAH contamination in coastal environments (DUNN & STICH 1975; DUNN & YOUNG 1976; MIX et al. 1977; GRAHL-NIELSEN et al. 1978; FOSSATO et al. 1979; MARCHAND & CABANE 1980; CLARK & LAW 1981; IOSIFIDOU et al. 1982). In this paper, we report the results of the analysis of the PAH concentration in Mytilus edulis from the Estuary and Northwestern part of the Gulf of St. Lawrence which receive waters from the basin of one of the most industrialized regions of North America.

MATERIALS AND METHODS

Blue mussels (Mytilus edulis L.) were collected within a period of two weeks at the end of May, 1977. Forty-one stations were sampled along the Estuary and Gulf coasts (Fig. 1). Only adult specimens ranging from 3 to 4 cm were collected on rocky shores at the mid-tide level.

In the laboratory, soft tissue was removed from the shell, and byssal thread was discarded. Tissues of 50 individuals from each station were pooled, homogenized, and freeze-dried.

For the extraction and purification of PAH, the method of DUNN (1976) was used. The procedure was conducted under yellow light to avoid possible photodecomposition.

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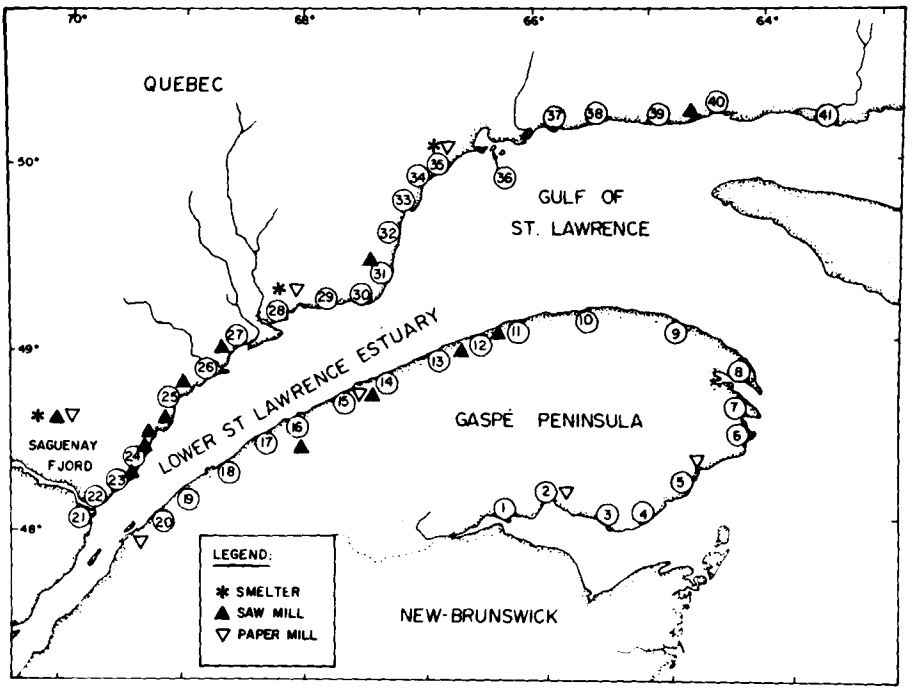

Figure 1. Sampling stations in the Estuary and northwestern Gulf of St. Lawrence

A 5-g aliquot of dry tissue was placed, after rehydration by 5 mL of water, in a flask with 150 mL of ethanol, 7 g of KOH, and two boiling chips. The tissues were extracted by refluxing for 90 min. The extract was then passed quickly through a glass fiber filter (Gelman, Type AE). While still hot, the extract was added to 150 mL water in a funnel, and the flask and filter were rinsed with 50 mL of ethanol. The mixture was extracted 3 times by 200 mL of isooctane, then washed 4 times by 200 mL of 60°C water. The isooctane extract was passed through a column (40 x 400 mm) containing 30 g of Florisil and 60 g of Na_2SO_4 prewashed with 100 mL of isooctane. The column was then washed with 200 mL of isooctane, and the PAH was eluted with 300 mL of benzene.

The combined eluate was evaporated to dryness, and the residue was dissolved in 2 mL of hexadecane. The PAH recovered were measured fluorimetrically in hexadecane.

The PAH in hexadecane was excitated at 365 nm and the emission was read at 430 nm because of the major importance of a peak at this wavelength. Some emission spectra were recorded between 380 and 600 nm. Blank values were systematically subtracted from the sample measurements. The reproducibility of this method (i.e. the coefficient of variation of 5 replicates) was 8%. The results are expressed in arbitrary fluorescence units at 430 nm

reported to the exact dry tissue weight of the sample analyzed. Some samples were analyzed on a gas chromatography-mass-spectrometer (GC-MS) performed on a Ribermag model R10-10B (Instrument S.A.) coupled with a PDP 8 mini computer. The column used was a quartz capillary column (SP 1000) and the temperature was programmed from 100 to 270°C at 5°C/min. Samples for GC-MS analyses were dissolved in hexane.

RESULTS AND DISCUSSION

The emission spectra recorded exhibited peaks at 390, 408, 430 nm and in some cases at 460 nm. However, the relative importance of each of these peaks differs from one sample to another. This indicates the PAH composition of the mussels vary with the sampling location. Thus, the PAH quantification based on the intensity of the fluorescence emission at 430 nm is not an exact measurement of the total amount of PAH in the extracts, but gives a semi-quantitative measurement of the levels of those emitting near this wavelength.

PAH levels in the collected mussels are presented in Table 1. The frequency distribution of the data (Fig. 2) shows that most of them are normally distributed between 7 and 45 units, with a mean of 25 and a standard deviation of 8 units for 35 determinations; only 6 values (stations 21, 22, 25, 26, 27 and 35) exceed 50 units, with the highest reaching 120 units.

An effort was made to try to discern a distribution pattern of the PAH levels in the mussels as a function of the residual surface circulation pattern (EL-SABH 1979). Appropriate coastal zones in the study area, exposed to water of different origins, were chosen, but the results of an analysis of variance were not

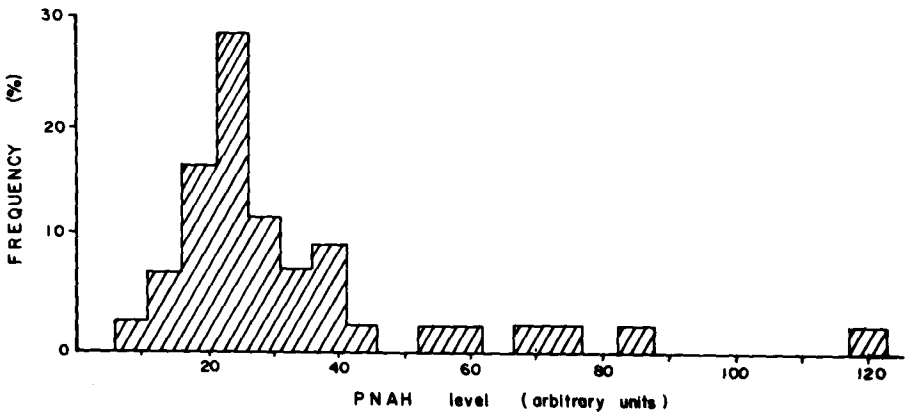

Figure 2. Frequency distribution of the fluorescence emission at 430 nm (arbitrary units) for the 45 samples

Table 1. PAH levels in *M. edulis* from the estuary and northwestern gulf of St.Lawrence.

Station number	Locality	PAH level em: 430 nm; ex: 365 nm (arbitrary units)
1	Pointe Miguasha	19
2	New-Richmond	20
3	Pointe Bonaventure	25
4	Pointe aux Loups marins	25
5	Pointe Newport	24
6	Percé	26
7	St-Georges-de-Malbaie	23
8	Cap-des-Rosiers	31
9	Pointe-à-la-Frégate	23
10	Mont-St-Pierre	16
11	Cap-Rivière-à-Martre	32
12	Ste-Anne-des-Monts	23
13	Capucins	20
14	Ste-Félicité	33
15	Petite Rivière Blanche	23
16	Pointe Mitis	26
17	Ste-Luce	33
18	Bic	38
19	St-Simon-sur-Mer	28
20	Rivière Trois-Pistoles	25
21	Baie Ste-Catherine	84
22	Tadoussac	120
23	Grande Bergeronne	13
24	Rivière Petits-Escoumins	27
25	Forestville (Industrial area)	68
26	Cap Colombier	54
27	Rivière Barthelemy	59
28	Baie-Comeau	39
29	Franquelin	21
30	Godbout	24
31	Baie-Trinité	28
32	Pointe-aux-Anglais	19
33	Baie-des-Homards	17
34	Port-Cartier	15
35	Port-Cartier (Industrial area)	76
36	Ile Grande Basque	45
37	Baie Moisie	20
38	Rivière-au-Bouleau	22
39	Sheldrake	27
40	Rivière Magpie	40
41	Havre St-Pierre	38

statistically significant at the 5% confidence level. Furthermore, no geographical trend in PAH levels appears on the south shore of the Lower St. Lawrence Estuary, which is characterized by the presence of a salinity gradient resulting from the mixing of brackish water, originating from the upper part of the estuary, with the seawater entering from the Gulf. Thus, either there is no difference in the PAH content between the brackish waters and the seawater of the Gulf or the difference is not important enough to be detected by the PAH content of the mussel populations. In the latter case, if brackish waters from the Upper Estuary were to contain higher PAH level, the difference may not have been detected because the PAH are predominantly associated with the suspended matter which settles out before reaching the study area. Our results are similar to those reported by KEIZER et al. (1977), who found no difference in the fluorescence levels in seawater extracts from the Lower Estuary and the Gulf. Moreover, the same authors suggest biogenic origin of the hydrocarbons measured by fluorescence in this area. This and the very low levels of benzo(a)pyrene measured in mussels from some stations which exhibit PAH levels $\leq$ 50 units (PICARD et al. in press), suggest that these PAH levels present in most of the mussels of the estuary and N.W. Gulf of St. Lawrence probably reflect the natural background level of PAH in the environment.

For the six high levels measured we have tried to deduce whether they result from physiological condition of the animals, or from local contamination. The major physiological change, gametogenesis, does not significantly affect the seasonal variation of the benzo(a)pyrene content of a mussel population (MIX et al. 1982). It is probable that other PAH behave in the same way. In addition, since the fluorescence measurements, normally distributed, include samples originating from different biogeographical areas, it is unlikely that physiological conditions could sufficiently affect the PAH content of the mussels to be responsible for the 6 high levels encountered. Thus, it is more probable that environmental PAH concentration account for the high levels measured in the mussels at least for the population from stations 21 and 22.

In order to establish the possible origin of the contamination, a reference sample from the main population (station 13) and another from station 22 were analysed on a GC-MS. Total ion chromatograms are shown in Figure 3. While phenanthrene and fluoranthene are the only PNAH identified at the reference station, the mass spectra of the separated compounds for mussels from station 22 allowed the identification of several unsubstituted PAH in appreciable amounts. Because of their unsubstituted character, it appears that those compounds are of pyrolytic origin (HASE & HITES 1977), perhaps due to the saw mill or smelter activities present on the Saguenay River and Fjord, which flows into the St. Lawrence Estuary at this station.

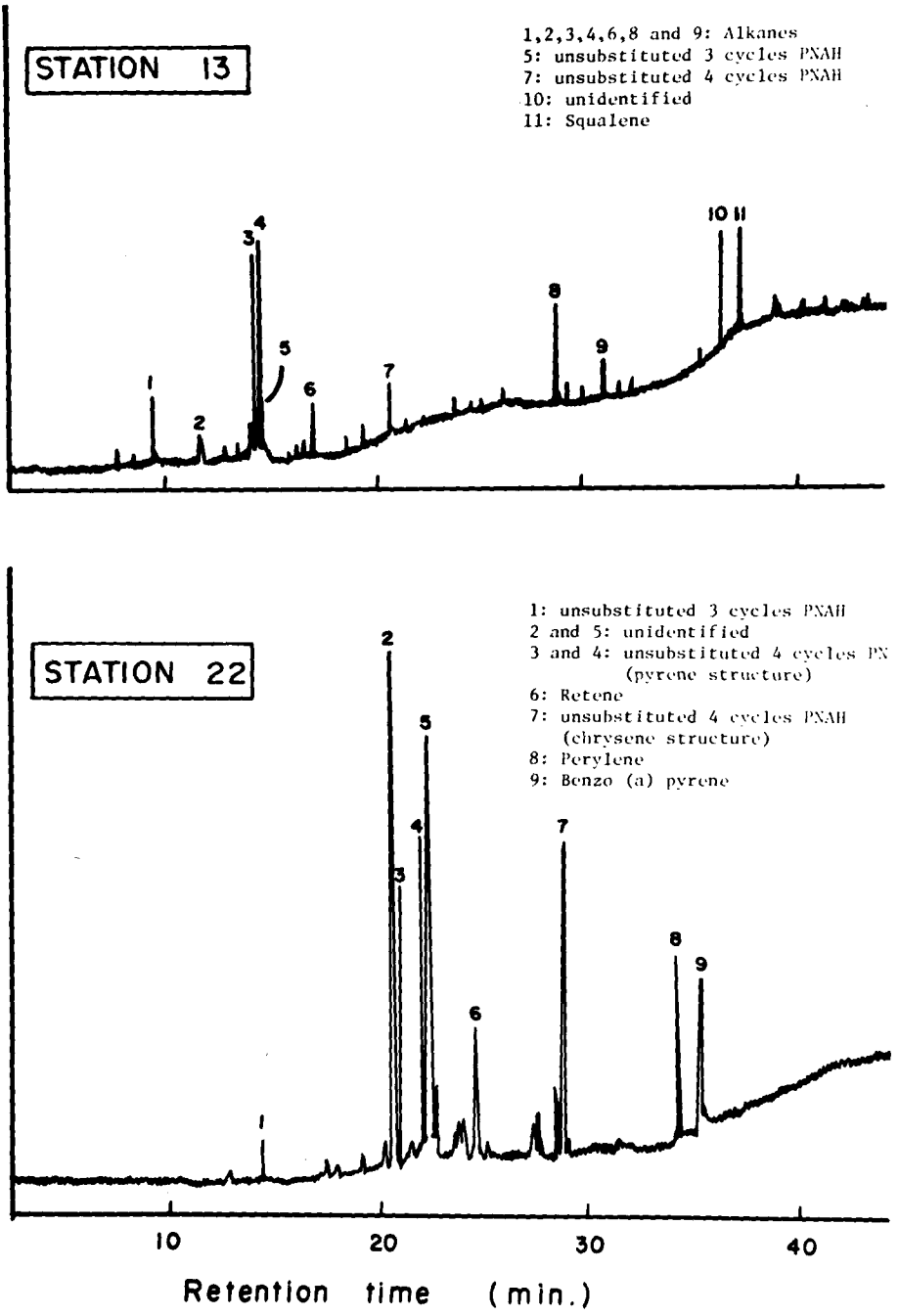

Figure 3. Total ion chromatograms of the mussel extracts from stations 13 and 22

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