Arch. Environ. Contam. Toxicol. 24, 249–257 (1993)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Total Unsaturated Compounds and Polycyclic Aromatic Hydrocarbons in Molluscs Collected from Waters Around Newfoundland

J. Hellou[1], C. Upshall, J. F. Payne, S. Naidu, and M. A. Paranjape

Science Branch, Northwest Atlantic Fisheries Centre, Department of Fisheries and Oceans, P.O. Box 5667, St-John's, Newfoundland, A1C 5X1, Canada

Abstract. Baseline concentrations of total unsaturated compounds extracted from six species of molluscs, collected from waters around Newfoundland and Labrador were determined by ultraviolet/fluorescence spectroscopy (uv/f, chrysene equivalents, IOC recommendation). Extracts of muscle tissue from scallops, *Placopecten magellanicus*, clams, *Mya arenaria*, whelks, *Buccinum undatum* and propeller clams, *Cyrtoderia siliqua* had negligible hydrocarbon concentrations compared to the corresponding visceral mass. The pattern of concentration (GC-MS) of the 16 PAH priority pollutants (EPA recommendation) was similar in the visceral mass of these species and in whole mussels (*Mytilus edulis*) and periwinkles (*Nucella lapillus*). When the 16 PAH were detected (>5 ng/g, dry weight), fluoranthene dominated (<200 ng/g), followed by phenanthrene-anthracene (<50 ng/g). Extracts of the visceral mass of scallops appeared to contain a relatively larger amount of uv/f absorbing hydrocarbons; therefore, a further investigation was undertaken. A C-25 unsaturated hydrocarbon of molecular weight 346 was identified as the major component of the extraction mixture. Overall, hydrocarbon levels were very low in comparison to other geographical regions.

Polycyclic aromatic compounds (PAC) are ubiquitous in nature, where they originate from a variety of anthropogenic sources involving mainly combustion processes and/or the use of petroleum (Neff 1979). Because of atmospheric transport, PAC can be found in areas removed from an immediate point source of contamination (Baek *et al.* 1991). Many PAH have carcinogenic (White 1986) or mutagenic potential (Pahlman and Pelkonen 1987). Sixteen parental PAH have been listed as priority pollutants by the World Health Organisation (WHO), the European Economic Community (EEC) and the US Environmental Agency (EPA). There is very little data regarding hydrocarbons in marine organisms living in waters around Newfoundland and Labrador (Hellou et al 1990; Hellou et al 1991) and none on invertebrates. In the present study, six species of invertebrates, blue mussel, *Mytilus edulis*, northern propeller clam, *Cyrtoderia siliqua*, soft shell clam, *Mya arenaria*, sea or giant scallop, *Placopecten magellanicus*, common whelk, *Buccinum undatum* and common periwinkle, *Littorina littorea* were analyzed for total unsaturated hydrocarbons and for the presence of the 16 PAC priority pollutants. This investigation of the baseline level of hydrocarbons focused on the invertebrates representing the bivalve and gastropod species most commonly found in the Northwest Atlantic, including three commercially important species, mussels, scallops and clams.

In the past, molluscs were thought to lack mixed-function oxygenase (MFO) enzymes (Lee 1972). Since then, cytochrome P-450 and associated MFO activities have been detected in at least 23 species (Livingstone *et al.* 1989). These enzymes are involved in the metabolism of a variety of xenobiotics, where oxidation (phase I), sometimes followed by conjugation (phase II) takes place. Metabolism of hydrocarbons leads to the formation of more polar, water soluble compounds, that can be eliminated by the organisms. However, even though invertebrates can metabolize xenobiotics, they have much lower metabolic rates than vertebrates (Stegeman and Lech 1991). This shortcoming has made molluscs the marine animals of choice for monitoring the accumulation of pollutants in the aquatic environment (Mix 1984). Concentrations of chemicals in molluscs tend to reflect the levels present in the aquatic environment.

Materials and Methods

Six species of molluscs were sampled as available, either by diving in Fortune Bay or by trawling in Bonavista Bay and on the St-Pierre Bank (Figure 1). The sampling in the Bays took place in October-November 1990, while it took place in August 1990 and April 1991 on St-Pierre Bank.

After collection, animals still in their shells, were individually wrapped in aluminum foil and frozen at −40°C on board ship or kept at 0°C when returned to the laboratory by land. Samples were then held at −40°C for a few weeks, the aluminum foil removed before transferring to a petri dish to thaw out. The size of the shells (length × width) was measured in mm, using calipers, while it is reported in Tables 1–4, in cm, for convenience. Soft tissues were then removed and either single or pooled whole animals were used, depending on animal size. Two

[1]To whom correspondence should be addressed.

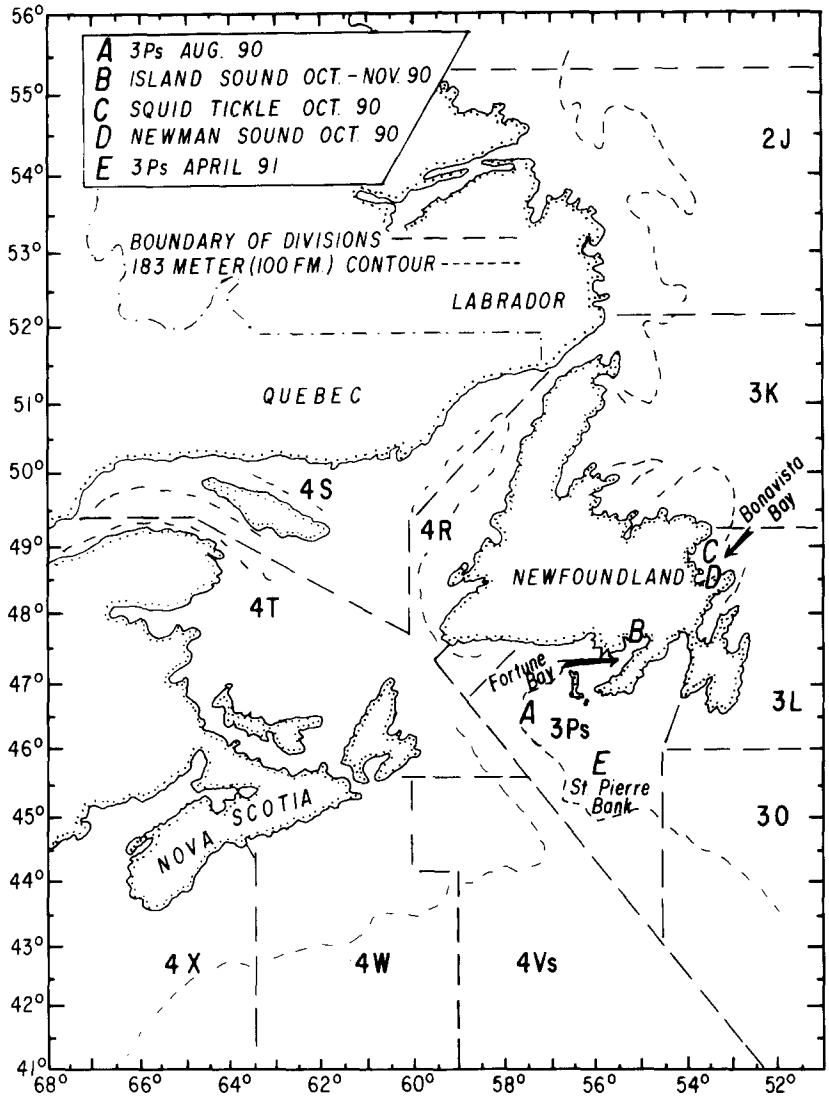

Fig. 1. Map representing the sampling locations: locations A and E, offshore; locations B, C, and D inshore

Table 1. PAC concentrations in mussels (*Mytilus edulis*)

	Size[a] (cm)	H_2O (%)	Chrysene (μg/g)	Venezuelan (μg/g)	Info[b]
1)	5 × 3 (7)	89	0.45	ND	D, whole
2)	5 × 3 (5)	89	1.80	1.14	D, whole
3)	5 × 3 (8)	91	1.68	0.74	C, whole
4)	6 × 3 (5)	86	0.36	0.24	C, whole
5)	6 × 4 (5)	89	0.75	0.39	C, whole
6)	7 × 4 (3)	82	0.21	ND	D, whole
7)	8 × 4 (2)	90	0.38	0.24	B, whole
8)	8 × 4 (2)	90	0.30	ND	D, whole
9)	10 × 6 (1)	89	0.27	ND	B, whole
10)	10 × 6 (1)	88	0.36	ND	B, whole

[a]size (length × width) is followed by number of animals pooled in analysis (in paranthesis)
[b]letters represent inshore locations, sampled in October (Figure 1)
-VM: visceral mass, whole refers to soft tissue, ND: not detected (<0.01 μg/g, dry weight), used a value of 0 for calculating means

Table 2. PAC concentration in scallops (*Placopecten magellanicus*)

	Size[a] (cm)	H_2O (%)	Chrysene (μg/g)	Venezuelan (μg/g)	Info[b]
1)	8 × 8 (1)	78	0.01	ND	B, muscle
2)	10 × 9 (1)	79	0.30	ND	C, muscle
3)	10 × 10 (1)	84	ND	ND	B, muscle
4)	12 × 11 (1)	80	0.09	ND	D, muscle
5)	12 × 12 (1)	83	ND	ND	B, muscle
6)	13 × 13 (1)	81	ND	ND	D, muscle
7)	14 × 14 (1)	83	0.16	ND	B, muscle
8)	14 × 14 (1)	76	ND	ND	C, muscle
9)	14 × 14 (1)	76	ND	ND	A, muscle
10)	15 × 14 (1)	80	ND	ND	C,muscle
11A)	8 × 8 (1)	83	0.01	ND	E, muscle
11B)		83	6.87	6.30	E, VM
12A)	11 × 11 (1)	77	0.03	ND	E, muscle
12B)		72	2.88	5.14	E, VM

[a]size (length × width) is followed by number of animals used in analysis (in paranthesis)
[b]letters represent locations, B, C and D are inshore, sampled in October, A and E are offshore (Figure 1)
-VM: visceral mass, ND: not detected

Table 3. PAC concentrations in clams (*Mya arenaria*) and propeller clams (*Cyrtoderia siliqua*)

	Size[a] (cm)	H_2O (%)	Chrysene (μg/g)	Venezuelan (μg/g)	Info[b]
1)	6 × 4 (1)	90	1.92	4.28	D, muscle
2)	7 × 5 (1)	84	1.05	2.32	D, muscle
3)	8 × 6 (1)	88	1.03	1.90	C, muscle
4)	10 × 8 (1)	88	2.32	5.54	D, muscle
5)	13 × 9 (1)	88	0.85	1.66	C, muscle
6)	15 × 10 (1)	90	0.75	1.16	C, muscle
7A)	9 × 8 (1)	81	ND	ND	E, muscle
7B)		76	0.72	0.57	E, VM
8A)	8 × 7 (1)	82	0.02	ND	E, muscle
8B)		79	0.72	0.37	E, VM
Propeller clams					
9A)	7 × 3 (1)	75	0.10	0.24	E, muscle
9B)		75	0.41	0.32	E, VM
10A)	7 × 3 (1)	79	0.22	0.45	E, muscle
10B)		80	0.52	0.17	E, VM

[a]size (length × width) is followed by number of animals used in analysis (in paranthesis)
[b]letters represent locations, E is offshore, sampled in October (Figure 1)
-VM: visceral mass, ND: not detected

Table 4. PAC concentrations in whelk (*Buccinum undatum*) and periwinkle (*Littorina littorea*)

	Size[a] (cm)	H_2O (%)	Chrysene (μg/g)	Venezuelan (μg/g)	Info[b]
Periwinkles					
1)	2 × 1 (34)	85	ND	ND	D, whole
2)	2 × 1 (30)	76	ND	ND	C, whole
3)	3 × 1 (20)	81	0.84	1.72	D, whole
4)	3 × 1 (27)	79	0.06	0.27	D, whole
5)	3 × 1 (22)	75	0.84	1.87	D, whole
6)	3 × 2 (20)	83	0.44	0.63	B, whole
7)	4 × 2 (18)	74	ND	ND	D, whole
8)	4 × 2 (16)	72	ND	ND	C, whole
Whelks					
9)	8 × 4 (1)	76	ND	ND	A, muscle
10)	9 × 3 (1)	73	0.03	ND	A, muscle
11A)	9 × 5 (1)	74	0.09	ND	A, muscle
11B)		66	0.24	0.11	A, VM
12A)	9 × 3 (1)	72	ND	ND	E, muscle
12B)		70	0.66	0.35	E, VM

[a]size (length × width) is followed by number of animals pooled in analysis (in paranthesis)
[b]letters refer to locations, A and E are offshore (Figure 1)
-VM: visceral mass, ND: not detected

animals from the four larger species were analyzed using muscle tissue and visceral mass (Tables 1–4). The visceral mass analyzed comprised all the soft tissues (internal organs) other than the adductor muscle.

The extraction procedure is a modification of that proposed by Warner (1976). Briefly, caustic digestion of 8–10 g of muscle or 3–5 g of visceral mass is followed by solvent partitioning and a two step purification method using alumina and silica chromatographic columns. Quantitative analysis was done by ultraviolet/fluorescence (uv/f) spectrophotometry and results are reported in terms of chrysene equivalents, using 310 and 360 nm (IOC recommendation), excitation and emission wavelengths, respectively. In a comparative study of the extraction methods, concentrations are reported in terms of Venezuelan crude equivalents (335 and 360 nm), diesel oil equivalents (280 and 305 nm), and phenanthrene equivalents (313 and 374 nm).

Muscle extracts and visceral mass extracts were also analyzed by gas chromatography-mass spectrometry (GC-MS), using a Hewlett-Packard 5890 GC coupled to a Hewlett-Packard Series 5970 mass selective detector and a Hewlett-Packard Series 300, data system, using a CP Sil-5 column (25 m × 0.20 mm i.d.). Temperature programs are indicated under Figures 3, 4, and 5. Extracts collected after the alumina column, containing the saturated as well as unsaturated hydrocarbons, were analyzed by GC-MS in the total ion chromatogram (TIC) mode. Extracts used after the silica column, containing only the unsaturated fraction, were analyzed by GC-MS in the single ion monitoring mode (SIM), where the 10 ions corresponding to the 16 PAH priority pollutants (EPA recommendation) were scanned. GC-MS results are not adjusted (± 20% due to recoveries and response of instrument). The detection limits for the 10 monitored ions were 5 ng/g.

The rotary evaporator was used for evaporating solvents, while in four cases a Kuderna-Danish apparatus was substituted to compare duplicate analyses (not listed in the tables). Blanks, duplicates (10–30% variation) and recoveries (78–106%) were performed after the analysis of 10 to 12 samples. Recoveries were of solvent solutions spiked with chrysene and phenanthrene, respectively. Blanks always had undetectable amounts of PAH. Evaporation using a Kudernah-Danish apparatus gave results that were identical to the rotary evaporator, in two cases and slightly higher in another two (0.45 vs 0.10 and 0.09 vs 0 ppm).

The infra-red (IR) spectrometer was a Perkin-Elmer 1720 Fourier Transform IR, used with a 27 μl KBr cell and CCl_4 as solvent. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Varian Gemini 300. Samples were dissolved in 30 μl of CCl_4 inserted in a micro capillary tube fitted with a micro-cell assembly into a 5 mm NMR tube containing D_2O to facilitate locking. Chemical shifts are reported in reference to tetramethyl silane (TMS = 0 ppm).

Pearson correlations were used to determined the relationship between size and concentrations, in the case of mussels and scallops. Variables were represented by length and chrysene equivalents, the log transformed data was also used. The non-parametric Mann-Whitney test (Rohlf and Sokal, 1981) was used to determine nearshore (grouping locations B, C and D) versus offshore (grouping locations A and E) differences. Results obtained for clams were compared to propeller clams (muscle versus muscle) and results for periwinkles were compared to whelks (whole versus muscle). Concentrations identified as non detected were given a value of 0 in the calculations. The second statistical analyses go against the authors best judgement and philosophy outlined by Warren, 1986. Statistics were not applied to sample sizes of 2.

Results and Discussion

Analyses by Fluorimetry

The PAC concentration obtained for whole mussels, *Mytilus edulis* are presented in Table 1. Levels ranged from 0.21 to 1.80 μg/g (chrysene equivalents, dry weight). Concentrations obtained by uv/f are also reported in terms of Venezuelan crude, for comparison. All samples were obtained from nearshore locations and are listed in order of increasing size, where higher concentrations are associated with smaller animals ($r < 0.05$). Smaller mussels have higher filtration rates and gill area, relative to body size, which may explain the higher contaminants load (Muncaster *et al.* 1990).

PAC concentrations obtained from the muscle tissue of the giant scallop, *Placopecten magellanicus*, ranged from undetectable to 0.30 μg/g (Table 2). The concentrations in the

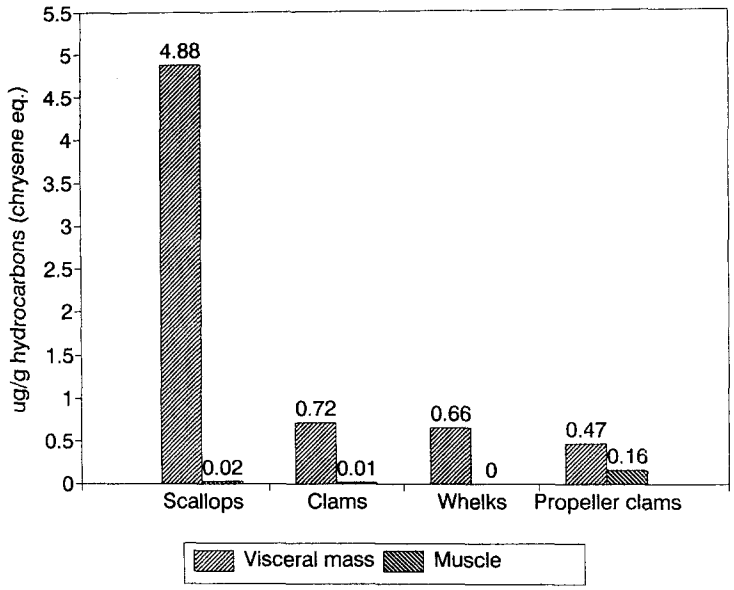

Fig. 2. Mean concentration of unsaturated compounds (uv/f analysis) in muscle and visceral mass of samples collected at location E, in April

visceral mass of two animals caught on St-Pierre Bank were nearly 100 and 700 times higher than in their respective muscle tissues. The higher visceral mass concentration is associated with the smaller animal, although in the case of muscle tissue of scallops caught nearshore, this association is insignificant.

The mean PAC concentration in the muscle of clams *Mya arenaria*, collected nearshore in October was 1.32 μg/g, while levels were almost undetectable (0.09 μg/g) in clams and propeller clams collected offshore in April (Table 3, $p = 0.05$). Jovanovich and Marion (1987) found that the peak in gametogenesis of clams (*Rangia cuneata*) was correlated with the peak in uptake of anthracene and with the lowest depuration rate, leading to a maximum hydrocarbon concentration in tissues. The clams from around Newfoundland reach sexual maturity in June and spawn in July-August (Hawkins 1985). Animals caught nearshore, in October, post-spawning had elevated concentrations compared to the offshore, in April, pre-spawning. This is opposite to the above case and indicates a correlation with location rather than with reproductive stage (Pruell *et al.* 1984).

The concentration observed in muscle tissue of two clams, propeller clams and whelks (Tables 3 and 4, Figure 2) were lower than for their respective visceral mass (<0.01 to 0.22 compared to 0.24 to 0.72 μg/g). Mean levels in whole periwinkles were slightly lower than in whole mussels (0.27 compared to 0.66 μg/g, chrysene units, dry weight). The smaller size of the visceral mass relative to soft tissues, in periwinkles compared to mussels, could explain this difference.

Comparison of Fluorimetry Results

To compare the molluscan species analyzed, the PAC concentrations obtained from animals collected from one location (Figure 1, location E) at one time of year (April) are presented in Figure 2. The data show that the muscle tissue of scallops, clams, whelks and propeller clams is very clean compared to the visceral mass. The concentration in the visceral mass is highest in scallops, the largest animals, while clams, whelks and propeller clams have similar concentrations. Concentrations in the visceral mass reflect the continuous uptake and elimination of xenobiotics compared to mainly accumulation in muscle tissue.

Environmental variables such as temperature, distance from shore and type of sediments affect the concentration of hydrocarbons available to animals. Lower temperatures, nearshore waters and finer sediments of the same general location have higher concentrations of organics. Differences in pumping rate, filtration rate, mobility, sexual maturity (related biochemical changes), lipid content of tissue, age or size of molluscs and efficiency of the MFO enzymes activity can sometimes affect the levels of xenobiotics observed in different molluscan species or within a species.

A comparison of our results with those obtained in other studies is reported in Table 5. Different extraction methods were used in these analyses and although PAC concentrations were all obtained using uv/f spectrophotometry, various standards were employed. Mason (1987) has shown that the choice of standards can bias results (overestimation or underestimation with phenanthrene and chrysene, respectively) obtained for the PAH concentration in mussels (relative to Quatar crude) collected at a relatively polluted site. Mason's comparison of various standards allows us to convert ("nonrigorously") some of the concentrations reported in the literature (Table 5). We can also express all the concentrations in terms of dry weight, by assuming about 80% water in the tissues. However, we can not compare the quality assurance (QA) and quality control (QC) protocol, since it was not always reported. Taking these variables into account, the results obtained in our study point to a very clean environment, comparable to the Arctic (Cretney *et al.* 1987).

Analysis of PAC by uv/f examines the total "hydrocarbon" content of tissues, regardless of their nature (biogenic and/or anthropogenic). Only uv/f absorbing molecules are examined. There is a concern regarding the effect of unsaturated biological materials (such as squalene) on the amount of uv/f absorbance detected. This effect is more pronounced when dealing with samples from a "clean" environment, while it is negligible in the case of contaminated samples (Hellou and Upshall, submitted). However, since there is an effect due to the presence of

Table 5. Comparison of PAC concentrations obtained in molluscs

Species (tissue)	Location	uv/f	Concentration (dry weight)	Ref.
M. edulis	Poland	310/360	160 to 420[a]	A
M. edulis	Sweden	313/374	14 to 69[b]	B
		310/360	1.4 to 6.9[c]	
M. baltica	Sweden	313/374	88 to 141	B
		310/360	8.8 to 14.1[c]	
P. maximus	France	310/360	1.1 to 7.7[d]	C
(gonads)			2.4 to 14.9	
(somatic tissue)			2.1 to 19	
N. minuta	Canadian	331/356	1.1 to 1.4	D
M. calcarea	Arctic		0.5 to 3.5	
S. groen.			0.1 to 3.6	
M. truncata			0.2 to 1.2	
M. gallopro.	South	280/374	10 to 5000	E
	Africa	310/360	2 to 1000	
M. guyanensis	Trinidad	310/360	18 to 81	F

[a]Ekofisk crude equivalents; [b]converted wet concentration to dry (x5); [c]converted phenanthrene (313/374) and Quatar crude (280/374) equivalents to chrysene (310/360) by dividing by 10 and 5, respectively; [d]concentration in muscle tissue, other species have concentrations for whole animals.

References: A-Law and Andrulewicz 1983; B-Broman and Ganning 1985; C-Friocourt et al 1985; D-Cretney et al 1987; E-Mason 1988. F-Singh et al 1992

non-aromatic unsaturated compounds, results obtained by uv/f analysis have to be validated using an alternative spectroscopic technique (Farrington *et al.* 1986).

Analysis by GC-MS, IR and NMR

Samples with hydrocarbon concentrations lower than 1 μg/g (dry weight, chrysene equivalents) displayed the presence of very few peaks in the GC-MS-TIC mode (Figure 3A and B, less and more than 1 μg/g, respectively) and mainly fluoranthene (<200 ng/g, dry weight) and phenanthrene-anthracene (<50 ng/g, dry weight), when analyzed by GC-MS-SIM (Figure 4A and B, less and more than 1 μg/g) for the 16 PAH priority pollutants (DL = 5 ng/g). These values are similar to those reported for molluscs from unpolluted areas (Mix and Schaffer 1983; Pruell *et al.* 1984; Cretney *et al.* 1987).

The visceral mass of scallops with a relatively higher concentration of PAC was suitable for more analyses. The IR spectrum of this fraction did not show the presence of any functional groups in the mixture. The ^{1}H NMR spectrum confirmed the presence of a double bond (5.4 ppm, cyclic or acyclic), while aromatic protons were undetectable (7.0 ppm). Because of the small amount of extract, the ^{13}C NMR spectrum displayed the presence of only two chemical shifts, due to the predominant carbon-types within the mixture. One chemical shift, at 132.2 ppm (δ scale) can be assigned to C-3, 7 and 11 type-carbons and the other at 32.5 ppm to C-4, 5, 9 and 12 type carbons of terpenoid structures. The major unsaturated compound had a retention time between nC-20 and nC-21 (29.5 min, Figure 3B), similar to that reported for C-25 acyclic alkenes found by Prahl and Carpenter (1984) in sediments and reviewed by Rowland and Robson (1990). The following fragments were observed in the MS of the major component (Figure 5): 346 ($C_{25}H_{46}$), 291 ($C_{21}H_{39}$), 233 ($C_{19}H_{33}$), 165 ($C_{12}H_{21}$), 135 ($C_{10}H_{15}$), 109 (C_8H_{13}) and 95 (C_7H_{11}). This fragmentation is identical to that of an acyclic triene found by Barrick *et al.* (1980) in sediments. The foolproof structural identification of this C-25 triene as an isoprenoid hydrocarbon would necessitate the availability of a synthetic standard.

Comparison of Extraction Methods

To determine if the peaks observed in the GC-MS-TIC (Figure 5) of the scallops extracts were due to an extraction artifact, the visceral mass of several scallops was pooled and subsampled prior to extraction using different methods (Ehrhardt *et al.* 1991):

1) extraction at room temperature (20°C) under laboratory light, using Na_2SO_4 and CH_2Cl_2;
2) extraction at room temperature, in the dark, using Na_2SO_4 and CH_2Cl_2;
3) extraction with Soxhlet apparatus, using CH_2Cl_2, for 3 h;
4) KOH hydrolysis, 50°C, 20 h (method used with other samples).

The various extracts were purified by chromatography and the following results obtained (mean dry weight ± standard deviation):

1.37 ± 0.08 μg/g in chrysene units
3.54 ± 0.18 μg/g in Venezuelan crude units
20.78 ± 0.34 μg/g in diesel oil units
67.35 ± 4.11 μg/g in phenanthrene units

The GC-MS profiles were similar using these four extraction methods, pointing to the "legitimacy" of the unsaturated compound detected in the visceral mass of scallops and not to a decomposition product.

Microscopic Content of the Visceral Mass

After extensive spectroscopic analysis of the visceral mass of scallops, the visceral mass extract of clams, whelks and propeller clams were analyzed by GC-MS-SIM, scanning m/e = 233, 291 and 346. These fragment ions were not detected. Therefore, the stomach content of one scallop, propeller clam and clam was examined under the microscope, to determine if a difference could be observed. After filtration through a 20 micron mesh, the following organisms were observed (decreasing abundance):

In scallops:
ciliates > dinoflagellates and dinoflagellate cysts > diatoms and a few crustacean eggs and pollen

In clams:
diatoms and a few dinoflagellate cysts

In propeller clams:
diatoms > ciliates

The following species were identified—diatoms: *Coscinodiscus*, *Thalassiosira* and *Nitzchia*; dinoflagellates: *Protoperidinium*; ciliates (tintinids): *Acanthostomella*, *Parafavella*, *Tintinnopsis* and *Plychocylis*. Whether dinoflagellates play a role in the presence of this C-25 triene would remain to be clarified. The hydrocarbon could have also originated from the degrada-

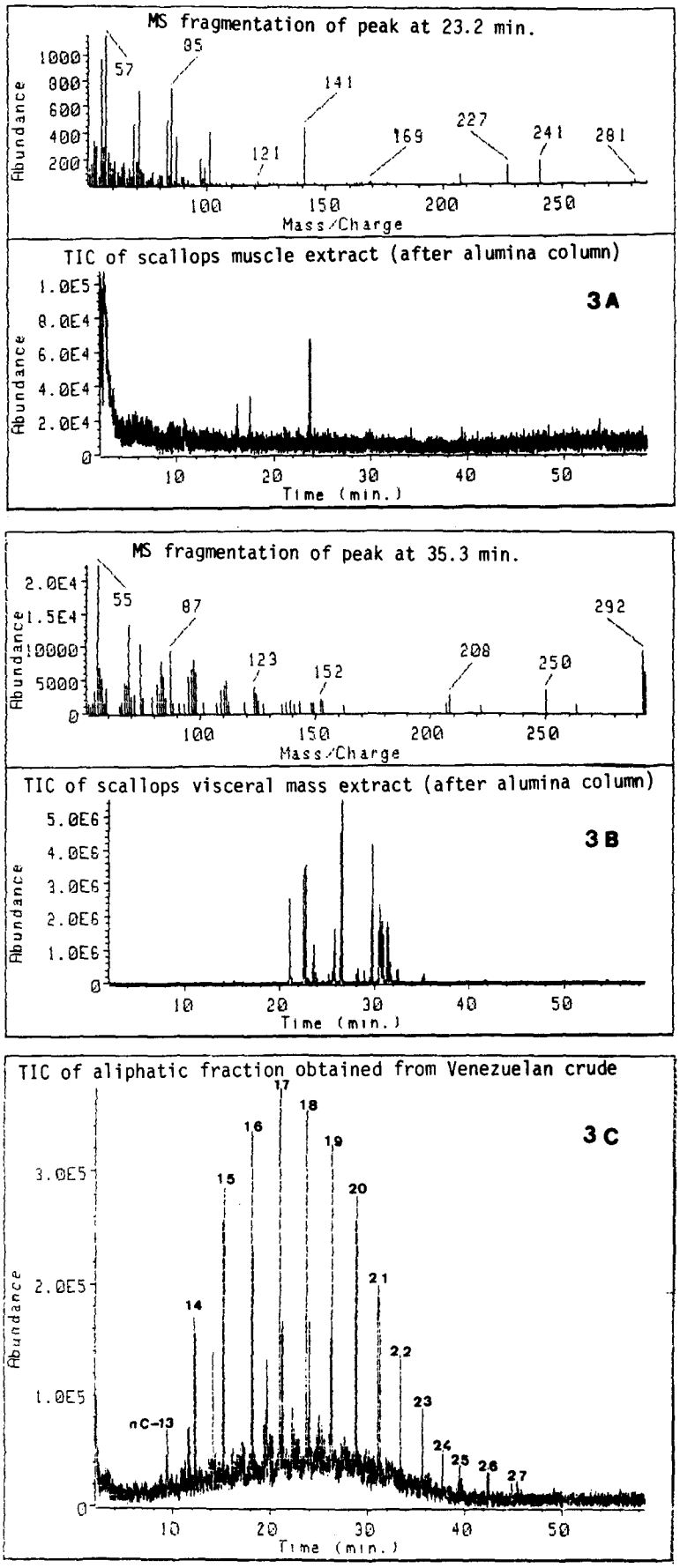

Fig. 3. Examples of total ion chromatograms (TIC): (A) TIC obtained from a muscle extract (after alumina chromatography, <1.0 ppm, chrysene units) and of the MS fragmentation observed for one component; (B) TIC obtained from a visceral mass extract (after alumina chromatography, >1.0 ppm) and of the MS fragmentation observed for one component; (C) TIC obtained for the aliphatic fraction of Venezuelan crude, numbers are of carbons present in linear aliphatic chain. Temperature program: 80°C for 1 min, increasing at 4°C/min, to 250°C, maintained there for 15 min.

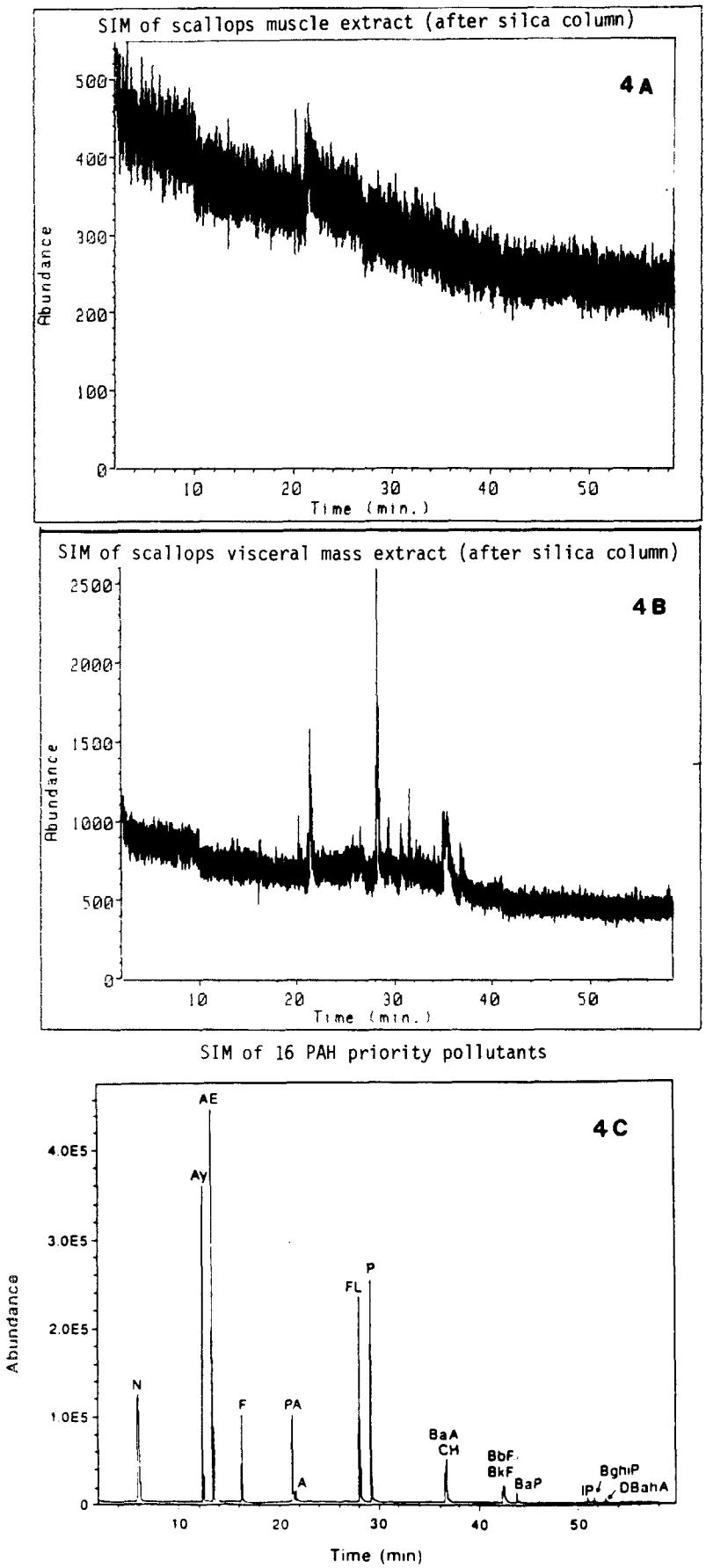

Fig. 4. Examples of single ion monitoring chromatograms (SIM): (A) SIM obtained from a muscle extract (after silica chromatography, <1.0 ppm, chrysene units); (B) SIM obtained from a visceral mass extract (after silica chromatography, >1.0 ppm); (C) SIM of mixture containing the 16 PAH priority pollutants. N: naphthalene, AY: acenaphthylene, AE: acenaphthene, F: fluorene, PA: phenanthrene, A: anthracene, FL: fluoranthene, P: pyrene, BaA: benzo[a]anthracene, CH: chrysene, BbF: benzo[b]fluoranthene, BkF: benzo[k]fluoranthene, BaP: benzo[a]pyrene, Ip: dibenz-[a.h]anthracene, BghiP: benzo[g.h.i.]perylene, DBahA: indeno[1,2,3,c.d]pyrene. Same temperature program as in Figure 3

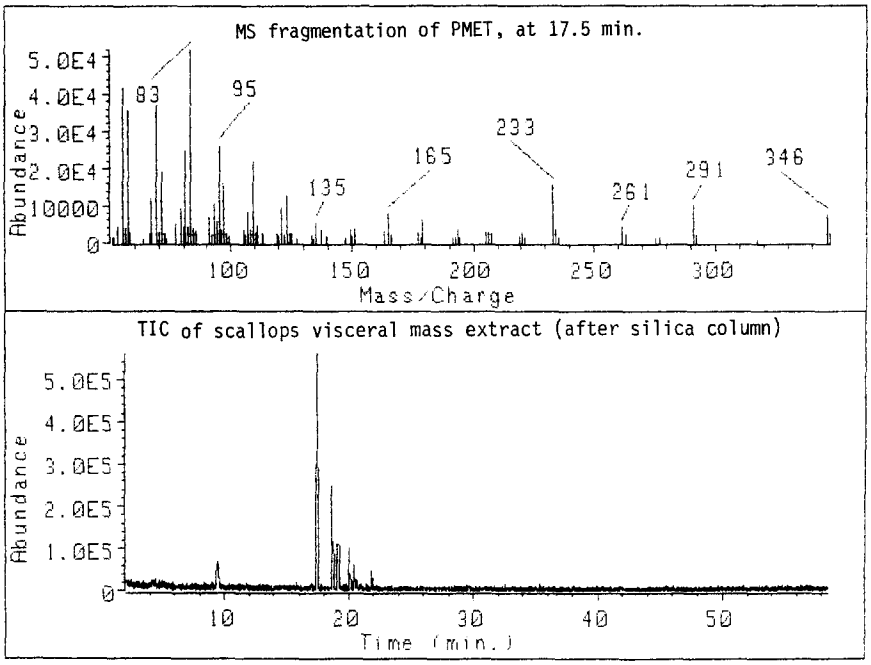

Fig. 5. TIC of scallops visceral mass extract (after silica chromatography) and MS fragmentation observed for the major component (PMET). Temperature program: 150°C for 1 min, increasing at 2°C/min, to 250°C and maintained there for 5 min.

tion of a larger isoprenoid precursor, such as squalene, chlorophyll or carotene, either in the marine environment or within a species consumed by the scallops.

Comparison with Other Studies

The identification of a non-aromatic compound, when analyzing for PAH is not unusual. It emphasizes the importance of examining the components present in a mixture. For example, Boehm and Quinn (1978) identified the presence of a hydrocarbon of molecular weight 342, when examining PAH extracts of the quahog (*Artica islandica*) from Rhode Island Sound. Farrington *et al.* (1986) determined the presence of a series of diaromatic-tetracyclic hydrocarbons in polychaetes collected from New York Bight.

The molluscs investigated in this study cover a broad range of habitats and feeding habits. Mussels are intertidal or subtidal organisms, which attach themselves to various substances and filter feed on suspension material. Scallops are sublittoral, epibenthic and feed on seston. Clams and propeller clams are found burrowed in soft bottoms, where they behave as deposit feeders. The larger (whelk) and smaller (periwinkle) gastropods are mobile animals, living in or on the benthos. Whelks are carnivorous and generally feed by burrowing through prey, while periwinkles are grazers, feeding on algae.

Regardless of the above differences, we have generally observed lower concentrations of uv/f absorbing hydrocarbons in samples collected offshore in April and higher concentrations in samples collected nearshore in October-November. Nearshore and offshore differences have been observed when studying the accumulation of paralytic shellfish poisons (Cembella and Shumway 1989), and phaeopigments (Robinson *et al.* 1989) in scallops (*Placopecten magelanicus*). This variation could reflect a seasonal change due to temperature: higher temperature, lower bioconcentration (Jovanovich and Marion 1987), reproductive stage of a species: spawning occurs between mid-may for the whelk, but extends sometimes into October for some of the scallops (Friocourt *et al.* 1985) and/or mirror the levels of chemicals (biogenic and/or anthropogenic) found in the surrounding waters. The higher accumulation of chemicals in the digestive gland compared to the adductor muscle also has precedent (Jamieson and Chandler 1982) and is important.

Acknowledgments. We would like to thank the technicians of the shellfish section who helped to sample the animals during the cruises and dives, and especially Mr. Frank Cahill. Also, Mark and Fraser Carpenter, of Doryman Marine, Squid Tickle, Newfoundland, for the collection of some of the samples, Marc Hawryluk for preparing samples for microscopic examination, Dr. W. Warren for statistical advice and Dr. W. Cretney for his comments regarding the C-25 unsaturated hydrocarbon. This research was supported in part, by the Green Plan for Toxic Chemicals and the Program for Energy Research and Development (PERD).

References

Baek SO, Field RA, Goldstone ME, Kirk PW, Lester JN, Perry R (1991) A review of atmospheric polycyclic aromatic hydrocarbons: sources, fate and behavior. Water Air Soil Pollut 60:279–300

Barrick RC, Hedges JI, Peterson ML (1980) Hydrocarbon geochemistry of the Puget Sound region I. Sedimentary acyclic hydrocarbons. Geochim Cosmochim Acta 44:1349–1362

Boehm PD, Quinn JG (1978) Benthic hydrocarbons of Rhode Island Sound. Estuar Coast Mar Sci 6:471–494

Broman D, Ganning B (1985) Bivalve molluscs (*Mytilus edulis* and *Macoma baltica*) for monitoring diffuse oil pollution in a northern Baltic Archipelago. Ambio 14:23–28

Cembella AD, Shumway SE (1989) A seasonal and spatial study of the uptake, sequestering and transformation of paralytic shellfish toxins by the giant scallop, *Placopecten magellanicus*. J Shell Res 8:482

Cretney WJ, Green DR, Fowler BR, Humphrey B, Engelhardt FR, Norstrom RJ, Simon M, Fiest DL, Boehm PD (1987) Hydrocar-

bon biogeochemical setting of the Baffin Island Oil Spill experimental sites. III-Biota. Arctic 40 Suppl 1:71–79

Ehrhardt M, Klungsoyr J, Law RJ (1991) Hydrocarbons: Review of methods for analysis in sea water, biota and sediments. Techniques in marine environmental sciences, no. 12. International Council for the Exploration of the Sea, Denmark, 47 pp

Farrington JW, Wakeham SG, Livramento JB, Tripp BW, Teal JM (1986) Aromatic hydrocarbons in New York Bight polychaetes: Ultraviolet fluorescence analyses and gas chromatography/gas-chromatography-mass spectrometry analyses. Environ Sci Technol 20:69–72

Friocourt MP, Bodennec G, Berthou F (1985) Determination of polyaromatic hydrocarbons in scallops (*Pecten maximus*) by uv fluorescence and HPLC combined uv and fluorescence detectors. Bull Environ Cotam Toxicol 34:228–238

Hawkins CM (1985) The soft-shell clam. Underwater World factsheet. Published by Communications Directorate. Department of Fisheries and Oceans, Canada. Catalogue no. Fs 41-33

Hellou J, Stenson G, Ni I-H, Payne JF (1990) Polycyclic aromatic hydrocarbons in muscle tissue of marine mammals from the Northwest Atlantic. Mar Pollut Bull 21:469–473

Hellou J, Upshall C, Ni I-H, Payne JF, Huang YS (1991) Polycyclic aromatic hydrocarbons in harp seals (*Phoca groenlandica*) from the Northwest Atlantic. Arch Environ Contam Toxicol 21:135–140

Jamieson GS, Chandler RA (1982) Paralytic shellfish poison in sea scallops (*Placopecten magellanicus*) in the West Atlantic. Can J Fish Aquat Sci 40:313–318

Jovanovich MC, Marcou KR (1987) Seasonal variation in uptake and depuration of anthracene by the brackish water clam *Rangia cuneata*. Mar Bio 95:395–403

Law R, Andrulewicz E (1983) Hydrocarbons in water, sediments and mussels from the southern Baltic Sea. Mar Pollut Bull 14:289–293

Lee RF, Sauerheber R, Benson AA (1972) Petroleum hydrocarbons: uptake and discharge by the marine mussel *Mytilus edulis*. Science 177:344–346

Livingstone DR, Kirchin MA, Wiseman A (1989) Cytochrome P-450 and oxidative metabolism in molluscs. Xenobiotica 19:1041–1062

Mason RP (1987) A comparison of fluorescense and GC for the determination of petroleum hydrocarbons in mussels. Mar Pollut Bull 18:528–533

———(1988) Hydrocarbons in mussels around the Cape Peninsula, South Africa. S Afr J Mar Sci 7:139–151

Mix MC (1984) Polycyclic aromatic hydrocarbons in the aquatic environment: Occurence and biological monitoring. Rev Environ Toxicol 50:51–102

Mix MC, Schaffer RL (1983) Concentrations of unsubstituted polynuclear aromatic hydrocarbons in Bay mussels (*Mytilus edulis*) from Oregon, USA. Mar Environ Res 9:193–209

Muncaster BW, Hebert PDN, Lazar R (1990) Biological and physical factors affecting the body burden of organic contaminants in freshwater mussels. Arch Environ Contam Toxicol 19:25–34

Neff JM (1979) Polycyclic aromatic hydrocarbons in the aquatic environment: sources, fates and biological effects. Applied Science Publishers Ltd, London, 262 pp

Pahlman R, Pelkonen O (1987) Mutagenicity studies of different polycyclic aromatic hydrocarbons. The significance of enzymatic factors and molecular structures. Carcinogenesis 8:773–778

Prahl FG, Carpenter R (1984) Hydrocarbons in Washington coastal sediments. Est Coast Shelf Sci 18:703–720

Pruell RJ, Hoffman EJ, Quinn JG (1984) Total hydrocarbons and polycyclic aromatic hydrocarbons and synthetic organic compounds in the hard shell clam, *Merceneria merceneria*, purchased at commercial seafood stores. Mar Environ Res 11:163–181

Robinson WE, Langton RW, Boggs CC (1989) Chlorophyllous pigment and lipid stores in the digestive glands of inshore and offshore populations of the deep-sea scallop *Placopecten magellanicus*. Mar Ecol Prog Ser 52:181–192

Rowland SJ, Robson JN (1990) The widespread occurence of highly branched acyclic C_{20}, C_{25}, and C_{30} hydrocarbons in recent sediments and biota, a review. Mar Environ Res 30:191–216

Singh JG, Chang-Yen I, Stoute VA, Chatergoon L (1992) Hydrocarbon levels in edible fish, crabs and mussels from the marine environment of Trinidad. Mar Pollut Bull 24:270–272

Sokal RR, Rohlf FJ (1981) Biometry, WH Freeman, San Francisco 859 pp

Stegeman JJ, Lech JJ (1991) Cytochrome P-450 monooxygenase systems in aquatic species: Carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ Health Perspect 90:101–109

Warner JS (1976) Determination of aliphatic and aromatic hydrocarbons in marine organisms. Anal Chem 48:578–583

Warren WG (1986) Statistical analysis and microcomputers in forestry research. Proceedings of division VI. General subject. International Union of Forest Research Organisation, 18th World Congress, Ljubljana, Yugoslavia, pp 1–11

White KL (1986) An overview of immunotoxicology and carcinogenic polycyclic aromatic hydrocarbons. Environ Carcinogen Rev C4:163–202

Manuscript received July 31, 1992 and in revised form September 24, 1992.