ARCTIC
VOL. 40, SUPP. 1 (1987) P. 71-79

Hydrocarbon Biogeochemical Setting of the Baffin Island Oil Spill Experimental Sites. III. Biota

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(Received 12 June 1986; accepted in revised form 24 March 1987)

ABSTRACT. A baseline for petroleum residues in the Cape Hatt region of Baffin Island in arctic Canada was obtained in anticipation of controlled oil releases of the Baffin Island Oil Spill (BIOS) Project. Tissue hydrocarbons in a variety of arctic marine species were dominated by biogenic hydrocarbons. UV/F analysis of tissues indicated an upper limit of petroleum residues in the low to sub $\mu g \cdot g^{-1}$ concentration range. PAHs were detected in samples in the low $ng \cdot g^{-1}$ concentration range and revealed a distribution of the combustion type. The hydrocarbon baseline in the BIOS study area was found to be as low as might be found anywhere on earth and therefore ideally suited to the BIOS study.

Key words: BIOS, arctic marine Canada, hydrocarbon baseline, organisms

RÉSUMÉ. On a obtenu un niveau de référence pour les résidus de pétrole dans la région du cap Hatt de l'île Baffin dans l'Arctique canadien, en prévision des déversements de pétrole faits sous contrôle pour le projet BIOS (projet de déversement de pétrole à l'île Baffin). Les hydrocarbures contenus dans les tissus de différentes espèces marines de l'Arctique se composaient surtout d'hydrocarbures dus à la biogenèse. Des analyses de tissus par fluorescence aux U.V. ont révélé que les résidus de pétrole présentaient une limite supérieure de l'ordre d'une fraction de $\mu g \cdot g^{-1}$ à quelques $\mu g \cdot g^{-1}$. Dans des échantillons, on a détecté des concentrations de HPA de l'ordre d'une fraction de $ng \cdot g^{-1}$ et ces hydrocarbures montraient une distribution caractéristique des produits de combustion. On a trouvé que le niveau de référence des hydrocarbures dans la zone d'étude du projet BIOS présentait des concentrations qui sont parmi les plus faibles qu'on puisse trouver dans le monde, et convenait donc parfaitement à l'étude du projet BIOS.

Mots clés: BIOS, Arctique marin canadien, niveau de référence d'hydrocarbures, organismes

Traduit pour le journal par Nésida Loyer.

INTRODUCTION

This paper is part III in a group of three that appear together in *Arctic* and describe aspects of the hydrocarbon biogeochemistry of the Cape Hatt marine area of Baffin Island. It is concerned mainly with the hydrocarbons found in a variety of plants and animals. The group of three papers forms part of a series in a special issue of *Arctic* that describes the Baffin Island Oil Spill (BIOS) Project.

A main goal of the studies described in this paper was to seek evidence based on the composition of hydrocarbons found in the biota of petroleum inputs to the study area that might compromise the goals of the BIOS experiment. Another goal was to quantify the background levels of hydrocarbons as a baseline for the subsequent experimental oil releases of the BIOS Project. Plants and animals were chosen to represent different trophic levels and feeding strategies. The bivalves were of particular importance to the survey because these have been used extensively as sentinel organisms for a variety of marine pollutants, including petroleum or petroleum-derived hydrocarbons (Farrington *et al.*, 1983).

METHODS

Field Methods

Organism Sampling: In September 1980 a wide variety of plants and animals was collected by divers in Z-Lagoon and Bays 9, 10 and 11. Samples were collected at a water depth of up to 20 m wherever organisms of a particular species seemed abundant. Collection was done by hand or airlift from various depths at various times (see footnotes to Table 4). Bivalves were collected and sealed in plastic bags underwater prior to transfer to land. Starfish and seaweeds were treated in the same manner after it was observed that samples were being placed unprotected into diver support craft and exposed to oily sea water within. In the shore-based laboratory, organism samples were removed from the plastic bags and wrapped in cleaned aluminum foil, which was sealed with masking tape. The aluminum foil packages were stored and transported frozen within new plastic bags.

Prior to the experimental oil releases in August 1981, animals were sampled by divers from the 3 and 7 m tissue plots in Bays 9 and 10 and the 7 m tissue plots in Bays 7 and 11. To diminish the probability of contamination certain precautions were observed. Divers used clean gloves to collect organisms that were hand picked. Hand-picked organisms and those collected using an airlift were deposited in clean nylon wash bags. The mesh bags were sealed below the surface in plastic bags. Organisms were separated from mud and rocks, sorted by species, sealed in aluminum foil with adhesive tape and frozen in the field laboratory. The aluminum foil packages, as before, were placed in plastic bags for storage and transportation.

Laboratory Methods

Extraction of Hydrocarbons by Steam Distillation (Ackman and Noble, 1973; Veith and Kiwus, 1977; Donkin and Evans,

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1984): The method and apparatus used were patterned closely after those of Veith and Kiwus (1977). The tissue samples were digested for 2 h with hot 0.5 M potassium hydroxide and steam distilled overnight. A broad variety of samples collected in 1980 was analyzed by the steam distillation method. The method was validated using blanks spiked with a variety of alkanes and aromatic hydrocarbons and a naturally contaminated sample (Boehm, 1981).

Extraction of Hydrocarbons by Caustic Digestion and Solvent Partitioning (Warner, 1976; Boehm, 1982): The organisms collected in 1981 and later analyzed were limited to five species of bivalves, a sea urchin and a polychaete worm. The bivalves were analysed at ERCO (Energy Resources Company) and the other organisms at CWS (Canadian Wildlife Service). The extraction procedure was similar to that described in Boehm (1982).

Bivalve tissues (guts, muscle, gills) were removed from shells with solvent-rinsed utensils while still frozen. Samples weighing more than about 10 g wet weight were homogenized with a Virtis tissue homogenizer. A 10 g subsample of the homogenate together with a smaller sample were withdrawn for hydrocarbon and dry weight determinations respectively. Except for a small subsample for dry weight determination, the entire homogenate of samples weighing <10 g was used for hydrocarbon determinations. In a 50 ml centrifuge tube with a Teflon-lined screw cap, the tissue homogenate (with added androstane and o-terphenyl for samples undergoing GC/FID or GC/MS analyses) was digested overnight with 5 N KOH solution (20 ml, methanol:water [1:1]) at 50°C in a shaker water bath. After cooling in a refrigerator, the digestate was extracted by vortex mixing with four 10 ml portions of hexane or until the extract was colourless. Separation of phases was facilitated by centrifugation. The extract was dried and concentrated and its residual weight determined as previously described.

Extensive pooling from different tissue plots of samples of the polychaete *Pectinaria granulosa* and the bivalve *Nuculana minuta* was necessary to obtain sufficient tissue for analysis. The analyses were performed essentially according to the procedure for bivalve tissue homogenate described above. The digestate, however, was extracted with dichloromethane:hexane (1:1).

Liquid Chromatography of Hydrocarbon Extracts: Tissue extracts prepared by ERCO from samples collected in 1981 were cleaned up for UV/F analysis on alumina columns, as described in part I (Cretney *et al.*, 1987) for the sediment extracts. Two sizes of columns were used, one consisting of 6.5 g of 7.5% water-deactivated alumina and the other 25 g, depending on the amount of tissue extracted. Elution with hexane (25 and 75 ml respectively) and exchange of that solvent for cyclohexane provided an isolate suitable for UV/F analysis.

After UV/F analyses had been completed, in preparation for GC/FID and GC/MS analysis half portions of each of the extracts from most tissue plots were pooled. The pooling was done by combining the extracts from the five plots on a given depth stratum for each bay and sampling period. The cyclohexane solvent was reduced in volume and displaced with hexane by rotoevaporation. The pooled extracts were fractionated by silica gel/alumina chromatography, as described in part I for sediment sample extracts. Some extracts from individual tissue plots selected for GC/FID and GC/MS analyses were also chromatographed without the above pooling.

Tissue extracts prepared by CWS from samples collected in 1981 were placed on 1% water-deactivated clean-up columns (40 g, Fisher A950 neutral alumina) that had been slurry packed using dichloromethane:hexane (100 ml, 1:1). The eluates obtained on elution with 140 ml of the same solvent mixture were rotoevaporated to about 2 ml, transferred to a 5 ml centrifuge tube and made up to 5 ml with hexane. The hexane solutions were subsampled for UV/F, GC/FID and GC/MS analyses. Further chromatographic separation was not done.

UV/F Analysis: The technique used at the ERCO laboratory was the same as described for sediments in part I (Cretney *et al.*, 1987).

The technique used at CWS differed somewhat. The emission intensity at 350 nm with excitation at 300 nm of a sample extract diluted into the linear range was measured using a Turner fluorometer (model 430). A reference sample of Lagomedio crude oil was used to prepare calibration standards in hexane. Concentrations in Lagomedio crude oil equivalents were determined from emission intensity measurements and the calibration curve. A method precision of 4% was determined by quadruplicate analysis of a moderately oiled sea urchin sample.

CG/FID Analysis: The technique used at ERCO was the same as described for sediment in part I (Cretney *et al.*, 1987).

At CWS, sample extracts that had been cleaned up by alumina chromatography were combined to obtain sufficient material for GC/FID (and GC/MS) analyses. The GC/FID analyses were done using a Hewlett-Packard 5840 gas chromatograph equipped with an autoinjector and flame ionization detector. A 30 m long, bonded phase fused silica column (DB-5, 0.25 mm i.d., 0.1 μm film, J&W Scientific) was used. Injections were made automatically in the splitless mode and the oven was programmed from 60 to 300°C at 5 $C^{\circ} \cdot min^{-1}$ with helium gas as the carrier. Only pristane and phytane were quantified in the samples obtained before the experimental oil releases.

GC/MS Analysis: The technique used at ERCO was the same as described for sediments in part I (Cretney *et al.*, 1987).

At CWS, the GC/MS analyses were performed using a Hewlett-Packard 5985B gas chromatograph-mass spectrometer equipped with a 30 m long fused silica column coated with SE 54 (0.25 mm i.d., J&W Scientific) that was threaded up to the ion source. The carrier gas was helium and injections were made manually in the splitless mode. The source temperature was 200°C and ionization was effected at 70 eV. The oven was programmed from 60 to 300°C at 5 $C^{\circ} \cdot min^{-1}$. A selected ion scanning mode was used in which the chromatogram was divided into five time segments in each of which a different group of seven ions was scanned with a dwell time of 10 ms per ion. The molecular ions were used for quantification with o-terphenyl as the internal quantification standard. Instrument calibration was performed using a procedure similar to the ERCO one. A method precision of 9% was determined by quadruplicate analysis of a moderately oiled sea urchin sample.

Quality Control and Assurance (QC/QA) and Laboratory Intercalibration

The QC/QA program maintained by ERCO was described in part I (Cretney *et al.*, 1987).

The Canadian Wildlife Service Laboratory also maintained a QC/QA program equivalent to that of ERCO for the analysis of organism samples. As noted above, the CWS procedures were similar to those used by ERCO. In order to assess the comparability of data from the two laboratories, an intercalibration

exercise was undertaken. Accordingly, a set of samples was prepared by a chemist at CWS who was otherwise not involved in the project, and replicate subsamples were presented to the two laboratories. Two samples were prepared by adding to a clam (*M. truncata*) and an urchin (*S. droebachiensis*) extract known amounts of Lagomedio crude oil. A third sample consisted of an urchin tissue homogenate from animals collected in the first post-oil release sampling period in Bay 10. The final sample consisted of a solution of Lagomedio crude oil in hexane.

RESULTS

Intercalibration results

Comparison of the UV/F measured concentrations of oil in hexane with the prepared concentration showed that the ERCO and CWS procedures produced comparable and accurate estimates (Table 1). Comparison of the measured concentrations of the oil-spiked urchin and clam extracts indicated that the ERCO method underestimated and the CWS method overestimated the quantity of oil present. In this regard, however, it was interesting that the concentration determined by ERCO for a naturally oiled urchin homogenate was greater than that determined by CWS.

TABLE 1. Results of intercalibration exercise between ERCO and CWS-UV/F determinations

Sample:	Urchin homogenate	Spiked clam extract	Spiked urchin extract	Oil-spiked hexane
	Concentration ($\mu g \cdot g^{-1}$)[a]	Concentration ($\mu g \cdot ml^{-1}$)		
Actual	—	29.2	20.7	34.1
ERCO	218.0	21.2, 20.8[b]	9.4, 11.7[b]	27.5
CWS	93.1	75.2, 72.0[b]	38.8, 39.0[b]	31.7,33.7[b]

[a]Dry weight basis.
[b]Duplicate determination.

The GC/MS results (Table 2) have the greatest congruence for the more highly alkylated members of the various aromatic groups. The urchin homogenate provided the least analytical difference in this respect. Countering this agreeable similarity was the dissimilarity shown by the C_0- and C_1-naphthalenes and fluorenes.

A probable explanation lies in the additional separation and evaporation steps employed in the ERCO method and the dissimilarity in volatility between the standard o-terphenyl and the C_0- and C_1-naphthalenes and fluorenes. The ERCO procedure would be expected to show greater losses of the more volatile compounds than the CWS procedure.

Evidence supporting this expectation was provided by the CWS workers who analyzed the Lagomedio crude oil sample used to spike the clam extract for the aromatic compounds in Table 2. The concentrations of individual aromatics in the whole oil were determined as o-terphenyl equivalents. There was no evidence of loss of the more volatile compounds with respect to the less volatile ones during the workup of the clam extract. Such a loss would have resulted in a systematic decrease in the total oil concentration estimates with increasing compound volatility.

TABLE 2. Results of intercalibration exercise between ERCO and CWS-GC/MS determination

Sample:	Urchin homogenate		Spiked clam extract	
	Concentration ($\mu g \cdot g^{-1}$)[a,b]		Concentration ($\mu g \cdot ml^{-1}$)[b]	
Compound	ERCO	CWS	ERCO	CWS
Naphthalene(N)	n.d.	0.23,0.47	0.0025	0.027,0.020
C_1N	n.d.	0.36,0.35	0.0018	0.056,0.057
C_2N	0.13	0.41,0.43	0.012	0.060,0.066
C_3N	0.21	0.64,0.56	0.019	0.098,0.070
C_4N	0.16	n.a., n.a.	0.0093	0.063,0.045
Fluorene(F)	n.d.	0.033,0.055	n.d.	0.0049,0.0049
C_1F	n.d.	0.059,0.046	n.d.	0.0078,0.0075
C_2F	0.030	0.11,0.088	0.0031	0.014,0.012
C_3F	0.090	n.a., n.a.	0.0031	n.a., n.a.
Dibenzothiophene(DBT)	0.060	0.062,0.041	0.0016	0.0090,0.0073
C_1DBT	0.12	0.16,0.16	0.012	0.027,0.028
C_2DBT	0.16	0.25,0.28	0.022	0.045,0.044
C_3DBT	0.22	0.29,0.28	0.025	0.047,0.050
Phenanthrene(P)	0.090	0.18,0.40	0.0046	0.027,0.029
C_1P	0.14	0.26,0.26	0.012	0.037,0.043
C_2P	0.24	0.25,0.24	0.014	0.050,0.054

[a]Dry weight basis.
[b]In o-terphenyl equivalents.
[c]Duplicate determinations.
n.d. = not detected; detection limit = 0.001 $\mu g \cdot ml^{-1}$.
n.a. = not analyzed.

The clam extract was spiked to give a total oil concentration of 0.029 $\mu g \cdot ml^{-1}$, which compares favourably with the mean total oil concentrations calculated at CWS by averaging the oil concentrations calculated for each of the aromatics in Table 2. The mean concentrations (±standard deviations) for the two subsamples were 0.041 ±0.012 $mg \cdot ml^{-1}$ and 0.036 ± 0.012 $\mu g \cdot ml^{-1}$ respectively.

The results of the intercalibration exercises were considered acceptable in view of the degree of intercompatibility of aromatic compound analyses that have been obtained by leading laboratories in intercalibration exercises with tissue homogenates (Galloway *et al.*, 1983; Wise *et al.*, 1980).

Study Results

UV/F Analysis of Tissues of Marine Organisms: As noted above, UV/F analyses were not done on organisms collected in 1980. Samples were collected in 1981 prior to the oil releases, however, to establish a baseline (Table 3). Since the baseline was made for the purpose of comparison with the post-oil release results, no attempt was made to determine the nature of the fluorescent compounds measured. For the seven species of organisms studied, the results made it clear that the differences from bay to bay for each species were minimal. The highest concentrations among the species were found for the urchins *Strongylocentrotus droebachiensis*. The urchins were also found to contain the highest concentrations of hydrocarbons determined by GC/FID and gravimetric analyses (see below). The UV/F concentrations appeared to reflect the hydrocarbon concentrations, although the answer to whether the fluorescence arose from biogenic materials or xenobiotic aromatics is unknown.

GC/FID and Gravimetric Analysis of Organism Samples Collected in 1980: A survey of the hydrocarbon concentrations and compositions in a variety of organisms from Bays 9, 10 and

TABLE 3. Concentrations of hydrocarbons in organism samples by UV/F spectrophotometry — 1981 samples

Species	Stratum	Plot	Concentration ($\mu g \cdot g^{-1}$)[a] Bay 7[b]	Bay 9[c]	Bay 10[d]	Bay 11[e]
Astarte borealis	7m	1	5.8	n.a.	0.29	0.75
		2	1.9	0.46	0.31	0.26
		3	1.6	0.91	0.37	0.30
		4	1.0	0.95	0.57	0.97
		5	n.a.	0.98	0.64	0.23
Geometric mean:			2.0	0.79	0.41	0.42
95% confidence interval:			[0.63,6.7]	[0.44,1.4]	[0.26,0.65]	[0.19,0.95]
Macoma calcarea	7m	1	0.92	1.2	2.5	n.a.
		2	1.2	1.2	3.5	2.1
		3	0.91	0.42	0.9	2.5
		4	1.1	0.55	1.5	8.1
		5	1.2	0.51	2.5	0.5
Geometric mean:			1.1	0.70	2.0	2.1
95% confidence interval:			[0.89,1.3]	[0.37,1.3]	[1.0,3.8]	[0.35,13]
Mya truncata	7m	1	0.3	0.37	0.72	0.55
		2	0.28	0.44	0.56	0.44
		3	0.53	0.31	0.67	0.43
		4	0.36	0.19	0.40	0.40
		5	0.24	0.44	0.53	0.33
Geometric mean:			0.33	0.33	0.56	0.42
95% confidence interval:			[0.23,0.48]	[0.22,0.52]	[0.42,0.75]	[0.34,0.53]
Mya truncata	3m	6	n.s.	0.23	0.71	n.s.
		7	n.s.	0.50	0.60	n.s.
		8	n.s.	0.33	0.74	n.s.
		9	n.s.	0.50	1.2	n.s.
		10	n.s.	0.45	0.73	n.s.
Geometric mean:			—	0.38	0.77	—
95% confidence interval:			—	[0.25,0.58]	[0.55,1.1]	—
Nuculana minuta	7m	1-5	1.2	1.3	1.4	1.1
Pectinaria granulosa	7m	1-5	17.7	4.7	2.6	6.6
Serripes groenlandica	7m	1	n.a.	0.51	1.6	n.a.
		2	n.a.	0.10	0.8	n.a.
		3	1.2	1.3	0.6	11.6
		4	1.2	0.3	3.6	n.a.
		5	0.9	1.1	1.1	n.a.
Geometric mean:			1.1	0.47	1.2	11.6
95% confidence interval:			[0.72,1.6]	[0.13,1.7]	[0.53,3.0]	—
Strongylocentrotus droebachiensis	7m	1	11.5	9.5	8.9	8.8
		2	17.5	12.9	18.0	8.8
		3	9.0	20.0	45.6	9.1
		4	10.2	21.9	40.4	38.4
		5	15.6	19.0	8.9	11.7
Geometric mean:			12	16	19	13
95% confidence interval:			[8.7,18]	[10,25]	[7.2,51]	[5.7,18]

[a]8% weathered Lagomedio crude oil equivalents (measured at emission wavelength of 356 nm) per unit dry weight of tissue.
[b]Sampled for all species on 17 August 1981.
[c]Sampled for *M. truncata*, *P. granulosa* and *S. droebachiensis* on 7-9 August 1981 and for *A. borealis*, *M. calcarea*, *N. minuta* and *S. groenlandica* on 8 August 1981.
[d]Sampled for all species on 14 August 1981.
[e]Sampled for *A. borealis*, *M. calcarea*, *N. minuta* and *S. groenlandica* on 13 August 1981 and *M. truncata*, *P. granulosa* and *S. droebachiensis* on 12 August 1981.
n.a. = not analyzed.
n.s. = not sampled.

11 and Z-Lagoon was undertaken as an aspect of the selection process in choosing the study organisms for the oil release experiments. The organisms were not randomly chosen and included a variety of size classes, because the intent was to achieve an illustrative rather than representative sampling of the species populations.

The concentrations of hydrocarbon-containing fractions f_1 and f_2 were found to vary more than an order of magnitude for the species studied (Figs. 1 and 2). Generally, the ranges of concentrations for the individual species were not greatly smaller than the overall concentration range. Nevertheless, the urchin *S. droebachiensis* clearly showed the highest concentrations in the f_1 and f_2 fractions by either GC/FID or gravimetric analyses. There were no obvious differences in f_1 or f_2 concentrations in animals from different bays.

The use of logarithmic scales in Figures 1 and 2 proved to be more useful than the use of linear scales to display the results. The use of the latter produced scattergrams with extreme data compression near the origin and no obvious relationship between the two methods of analysis.

The GC/FID analyses also provided compositional detail about the compounds in the organisms, giving a qualitative basis to assess the organism samples for the presence of oil contamination. Most of the samples show no qualitative evidence of oil contamination by GC/FID. As noted above, a few samples were believed to have been contaminated by oil during sampling. These samples, however, were not sufficiently contaminated to be quantitatively distinguished from the uncontaminated samples (Figs. 1 and 2). As might have been expected from the quantitative results, the GC/FID traces of f_1 and f_2 did not give unambiguous evidence of contamination. Four suspect samples of the 17 starfish samples collected did show equivocal evidence of oil contamination based on their gas chromatograms, and this finding was confirmed in the case of two that were selected for GC/MS analysis (see below).

An interesting feature of some of the chromatograms of the f_1 fraction from the starfish was a fairly complex mixture of components in the range of 9-12 carbon-containing hydrocarbons (Fig 3a). The f_1 fractions of the seaweed *Fucus* sp. also exhibited the same feature (Fig. 3b). Indeed, the entire chromatograms appeared to consist of peaks from a similar suite of compounds, although the relative size of some corresponding peaks differed considerably. The chromatograms of the f_2 fractions of the starfish (Fig. 4a) and seaweed (Fig. 4b) also appeared to have many peaks in common.

The gas chromatograms of the f_1 fractions from *Mya truncata*, although they had in common many identical component peaks, showed a highly varied chemical composition. The chromatograms of the f_2 fractions showed a similar diversity. The variability probably arose mainly from changes in the relative concentration of the low, intermediate and high volatility ranges of biogenic components in the gas chromatograms (Fig. 5).

The gas chromatograms of the f_1 and f_2 fractions of the urchin *Strongylocentrotus droebachiensis* were more uniform in appearance from sample to sample than those of the *Mya*. The urchin samples were pooled, which probably accounts for the uniformity. The chromatograms for f_1 (Fig. 6a) and f_2 (Fig. 7a) of a sample from Bay 9 were typical of the set in displaying unmistakable biogenic constituents. The chromatograms (Figs. 6b and 7b) obtained for the seaweed *Laminaria* sp. were very similar to those of the urchin.

GC/MS Analysis of Organism Samples Collected in 1980:

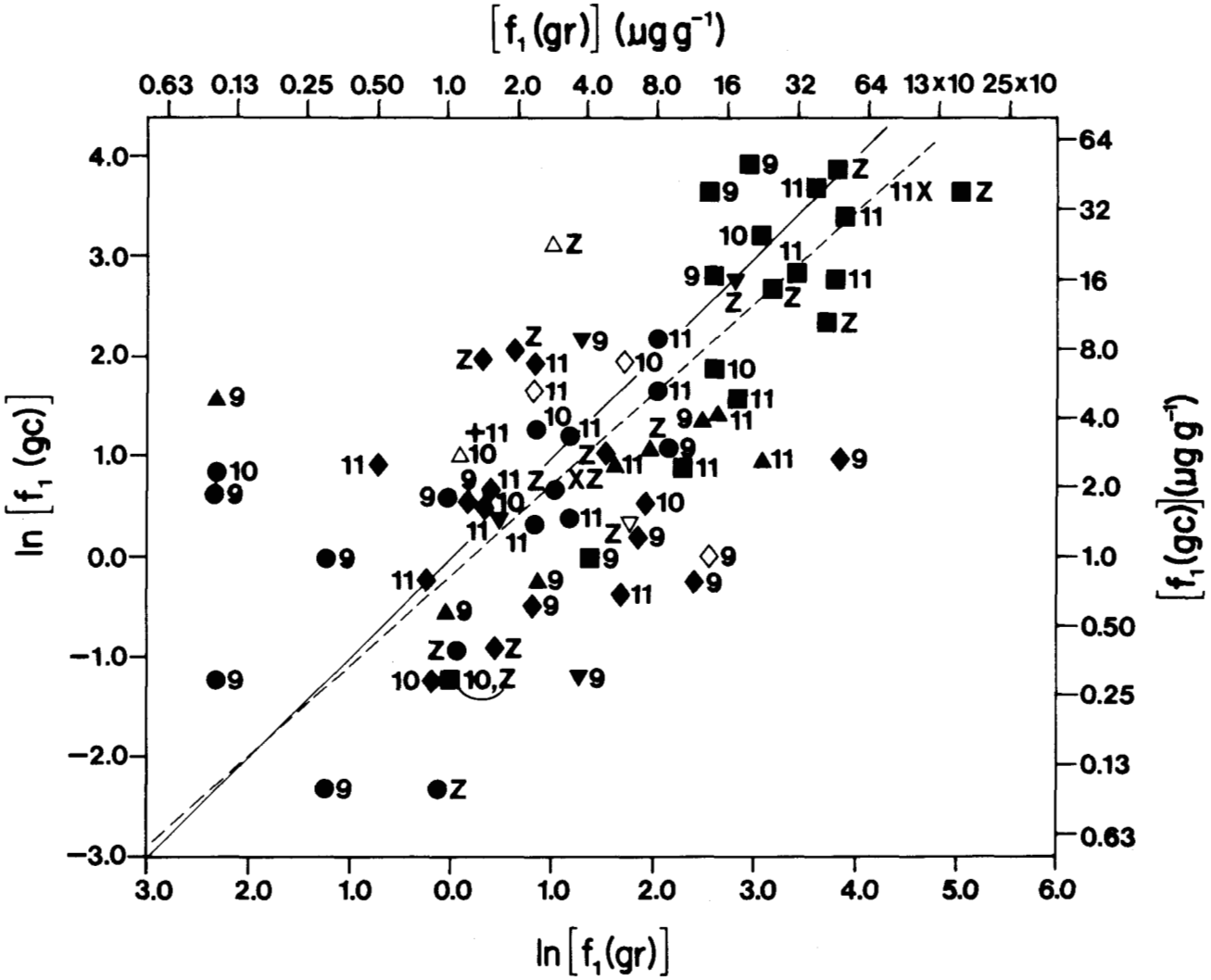

FIG. 1. Scattergram showing the f_1 concentrations as determined by gas chromatography and weighing for a variety of plants and animals from Bays 9, 10, 11 and Z-Lagoon. Concentrations are in $\mu g \cdot g^{-1}$ dry weight of tissue. (———) axis of geometric means, (----) reduced major axis, (●) *Leptosterias polaris*, (◆) *Mya truncata*, (▲) *Psolus fabricii* or *Psolus* sp., (■) *Strongylocentrotus droebachiensis*, (▼) *Serripes groenlandica*, (□) *Astarte borealis*, (+) *Agarum sp.*, (◇) *Fucus* sp., (△) *Laminaria* sp., (×) *Myoxocephalus scorpius*, (▽) Scallop.

Samples for GC/MS analysis were selected with an emphasis on the starfish because some had been accidentally contaminated with oil. GC/MS analysis confirmed the presence of petrogenic aromatics in starfish and algae samples that the GC/FID analysis indicated were oiled (Table 4). Entire families (having zero to four alkyl carbon-containing substituents) of benzenes, naphthalenes, phenanthrenes and fluorenes were found to be present. The bivalves *Mya* and *Serripes* presented an interesting contrast. Although aromatic hydrocarbons were detected in them, the compounds were the unsubstituted or parental forms of the families.

DISCUSSION

The gravimetric and gas chromatographic analyses provide two estimates of the concentrations in organisms of hydrocarbons (Figs. 1 and 2) that may be combined to give a third estimate. The geometric mean is preferable for this combining to the arithmetic mean because of the proportionality between the mean and the standard deviation that is generally observed for trace constituents in environmental samples (Eberhardt *et al.*, 1976). The choice of one of the three as the best estimate depends on the nature of the samples being analyzed. Characteristics of the samples that are important in making the choice include the volatility range of the compounds in the mixture, the response factors for the individual components and the amount and nature of non-hydrocarbon material.

The geometric mean is probably the best estimate for the samples analyzed in this study because each method tends to compensate for deficiencies in the other, although there is the uncertainty in the calibration of the GC for unknown mixtures. The gas chromatographic method discriminates against compounds that are less volatile than *n*-tritriacontane (nC_{33}) or are thermally unstable, while the gravimetric method does not. The gravimetric method discriminates more against compounds more volatile than *n*-octadecane (nC_{18}) than does the gas chromatographic method. Thus, for samples consisting of similar amounts of compounds in all volatility ranges, the geometric mean will likely provide the best estimate of the three.

In Figures 1 and 2, points on any line perpendicular to the solid diagonal line have the same geometric mean. The disper-

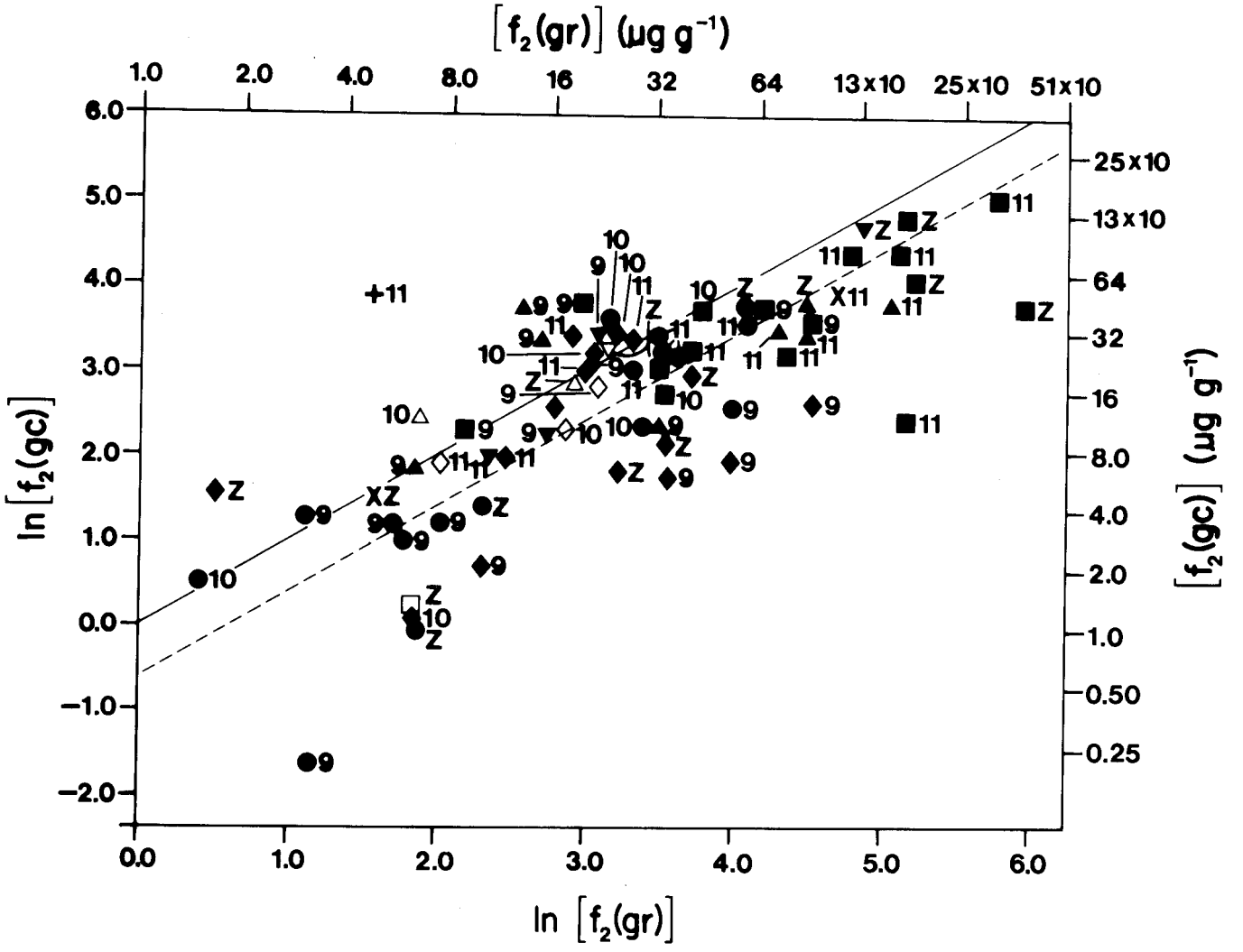

FIG. 2. Scattergram showing f_2 concentrations as determined by capillary gas chromatography and weighing for a variety of plants and animals from Bays 9, 10, 11 and Z-Lagoon. Concentrations are in $\mu g \cdot g^{-1}$ dry weight of tissue. See caption to Figure 1 for explanation of symbols.

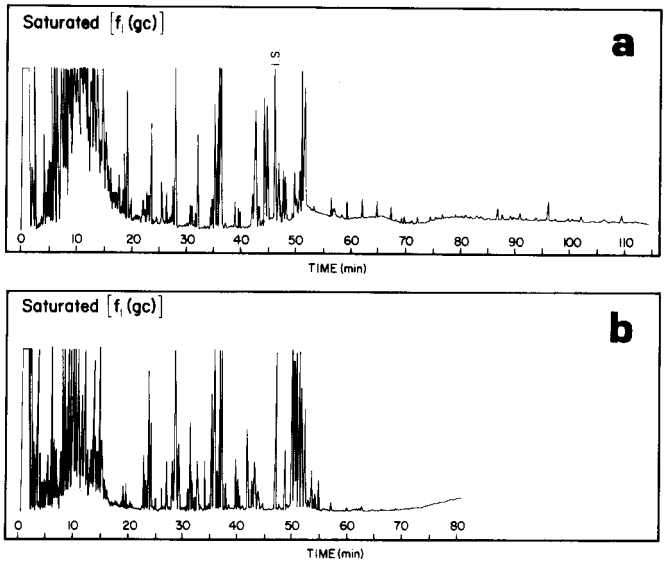

FIG. 3. Capillary gas chromatograms of the f_1 fraction: (a) of an extract from an uncontaminated sample of *Leptosterias polaris* from Bay 10, and (b) of an extract from a sample of *Fucus* from Bay 11.

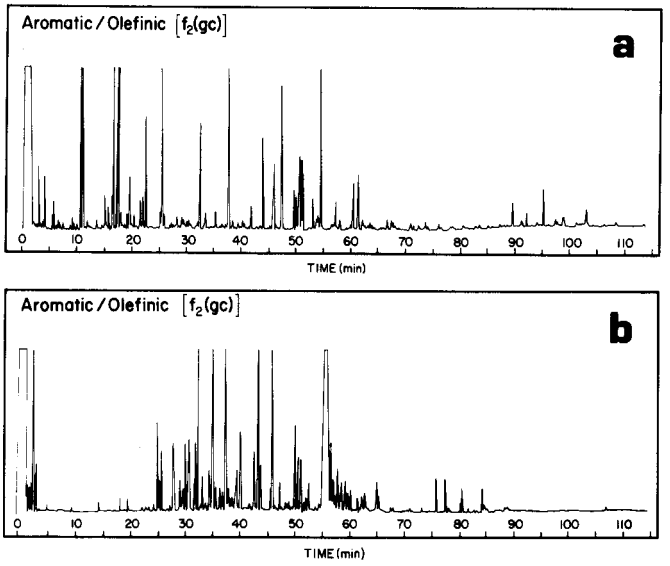

FIG. 4. Capillary gas chromatograms of the f_2 fraction: (a) of an extract from an uncontaminated sample of *Letposterias polaris* from Bay 10, and (b) of an extract from a sample of *Fucus* from Bay 11.

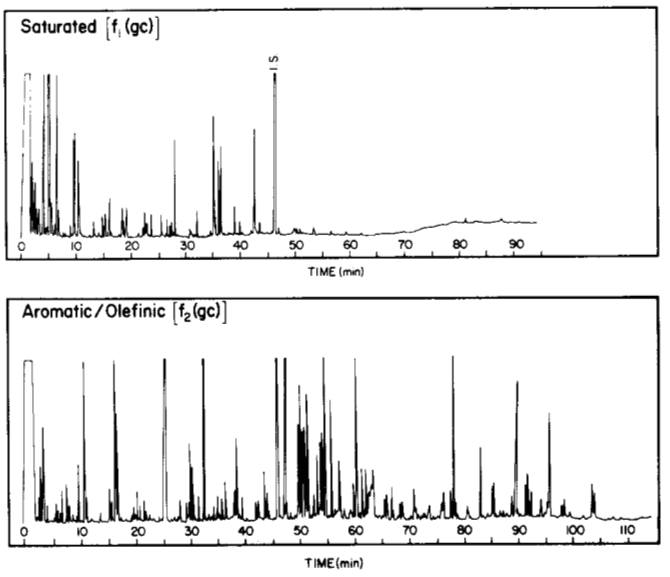

FIG. 5. Capillary gas chromatograms of the f_1 and f_2 fractions of an extract from a sample of *Mya truncata* from Bay 11.

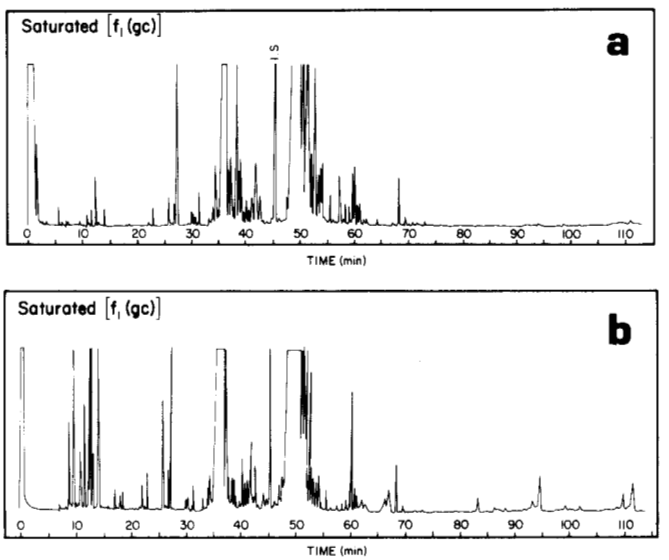

FIG. 6. Capillary gas chromatograms of the f_1 fraction: (a) of an extract from a sample of *Strongylocentrotus droebachiensis* from Bay 9, and (b) of an extract from a sample of *Laminaria* from Z-lagoon.

sion of points along perpendicular lines is caused by variation in amounts of compounds of various volatilities or stabilities, as noted above. Ideally, the data points should be homogeneously distributed about the solid diagonal line. The reduced major axis (dashed line), however, is the line found by regression to best fit the data (Ricker, 1973). Although the reduced major axis and axis of geometric means do not coincide, as would be expected under ideal circumstances, the agreement is quite good. The relative placement of the two axes in Figure 2 indicates that the gas chromatographic method uniformly underestimates the amount of material present with respect to the gravimetric method. In Figure 1, the placement of the lines indicates that the two methods give uniform results for the lower concentrations, but the gas chromatographic method increasingly underestimates with increasing concentrations.

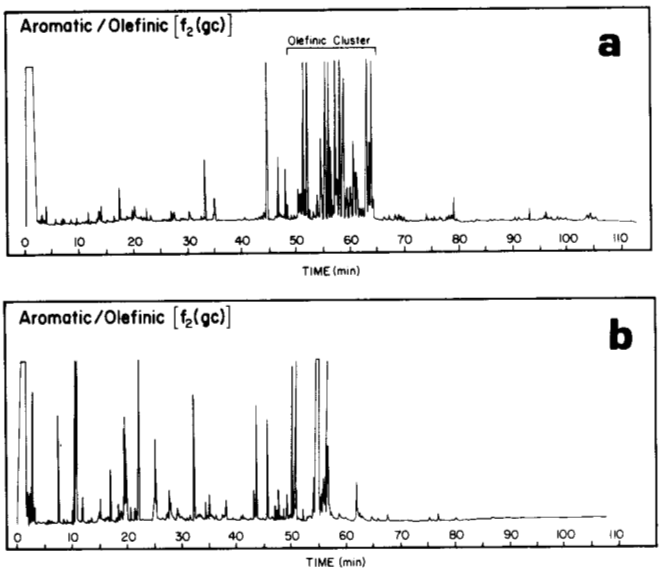

FIG. 7. Capillary gas chromatograms of the f_2 fraction: (a) of an extract from a sample of *Strongylocentrotus droebachiensis* from Bay 9, and (b) of an extract from a sample of *Laminaria* from Z-Lagoon.

TABLE 4. Concentrations of aromatic hydrocarbons in organisms by GC/MS — 1980 samples

		Concentration ($ng \cdot g^{-1}$)[a]						
Species	Location[b]	Np[c]	AB	B	F	Ph	Fl/Py	BP
Mya truncata	Bay 9	7	n.d.	n.d.	n.d.	3	n.d.	n.d.
	Bay 10	n.d.	n.d.	n.d.	n.d.	8	n.d.	n.d.
	Bay 11	n.d.	n.d.	n.d.	n.d.	1	n.d.	n.d.
	Z-lagoon	5	n.d.	n.d.	n.d.	6	n.d.	n.d.
Serripes groenlandica	Bay 9	2	n.d.	n.d.	n.d.	10	4	n.d.
Leptosterias polaris	Bay 9	28	11	n.d.	n.d.	n.d.	n.d.	n.d.
		1270	1300	100	n.d.	n.d.	n.d.	n.d.
		8	3	n.d.	n.d.	n.d.	n.d.	n.d.
		5100	3500	360	130	40	n.d.	n.d.
	Bay 10	140	190	11	n.d.	5	19	14
	Z-lagoon	140	220	8	n.d.	10	n.d.	n.d.
Agarum sp.	Bay 11	150	10	8	2	11	5	n.d.
Fucus sp.	Bay 10	6700	1700	200	70	490	n.d.	n.d.
Laminaria sp.	Bay 10	20	10	2	1	8	n.d.	n.d.

[a]Weight of aromatic hydrocarbon per unit weight of dry tissue.
[b]For GC/MS (and generally for GC/FID) analyses, all organism collections were done by diver on 10, 13, 8 and 16 August 1980 in Bays 9, 10, 11 and Z-Lagoon respectively. In the three bays sampling was generally within the north and south boundaries of the experimental zone. For Bay 9, sampling depths were 9-12 m for all organisms except *Fucus* sp., 4 m and *Psolus* sp., 17 m. For Bay 10 sampling depths were 4 m for *Fucus* sp., 5 m for *Laminaria* sp. and *L. polaris* and 7 m for *M. truncata* and *S. droebachiensis*. For Bay 11 all organisms were from 15-20 m except *Agarum* sp., 20 m; *Fucus* sp., 3 m and *S. groenlandica*, 5 m.
[c]Np = naphthalenes: m/z 128, 142, 156, 170; AB = alkylbenzenes: m/z 120, 134, 148; B = biphenyl: m/z 154; F = fluorenes: m/z 166, 180, 194, 208; Ph = phenanthrenes: m/z 178, 192, 206, 220, 234; Fl/Py = fluoranthene/pyrene: m/z 202; BP = benzopyrenes: m/z 252.
n.d. = not detected.

The comparison of the quantitative results adds confidence to both methods as means of determining the quantities of hydrocarbons in plants and animals. Either method should provide a good basis of comparison with data from other pristine areas of the world or from the post-spill period of the BIOS experiment.

The review in Clarke and Macleod (1977) provides a good source of information concerning the hydrocarbon content of a variety of organisms from uncontaminated and oil-contaminated sites around the world. The range of concentration found in this study for the f_1 and f_2 hydrocarbons compares favourably with the ranges reported for organisms from sites uncontaminated by oil.

With respect to the goals of the BIOS experiment, major differences in hydrocarbon content among the bays could have complicated the interpretation of the results, particularly if petroleum oil was shown to give high levels in one or other of the bays. The quantitative results provided no evidence of a difference in hydrocarbons in species of plants or animals among the bays.

The UV/F analyses of various organism samples suggest that the levels of oil residues in subtidal organisms of the study area are as low as have been reported anywhere in the world. Clarke and MacLeod (1977) present a useful compilation of UV/F data for comparison purposes. Although differences in methodology make precise comparisons impossible, even among data obtained using UV/F methods, the concentrations determined by UV/F for the Cape Hatt marine organisms are far less than those determined for similar organisms exposed to oil, either in the laboratory or in nature, and are similar to those from presumably uncontaminated areas. Although the compounds contributing to the fluorescence of tissue extracts were not determined, they probably comprise mainly natural materials that survive the extraction and purification techniques but are not necessarily aromatic hydrocarbons. The difficulty in making comparisons is underscored by the results of the intercalibration between CWS and ERCO. The small differences in the methods used by the two laboratories, although they did not contribute to an appreciable concentration difference for the reference sample solution of Lagomedio crude oil, did translate into two- to fourfold differences in the case of the spiked tissue extracts and the homogenate of oil-exposed urchins.

The low ppb range of aromatic hydrocarbon concentrations in the bivalve extracts measured by GC/MS compares favourably with the lowest concentrations found elsewhere in bivalves (Clarke and MacLeod, 1977; Pancirov and Brown, 1977; Mix and Schaffer, 1983; Farrington *et al.*, 1983). Most data on aromatic hydrocarbons have been gathered for the blue mussel *Mytilus edulis*, which was unavailable in the study zone, so only a general comparison can be made. *Mya*, *Serripes* and *Mytilus*, however, are all filter feeders. It should also be noted that the data presented here pertain only to the short ice-free summer period. Some temporal variability may be anticipated based on observations elsewhere (Farrington *et al.*, 1983), although the long ice-bound winter period in the Arctic and the otherwise compressed seasons made extrapolations from the temperate zones of doubtful validity.

The wide range of aromatic hydrocarbon concentrations observed for the starfish and seaweed is consistent with the observed oiling of certain of the samples during collection before appropriate procedures were in place. Only the samples with the lowest concentrations are likely to be representative of the environment.

A comparison of the GC/FID composition of the hydrocarbon-containing fractions of the various species reveals a probable food chain relationship between *S. droebachiensis* and *Laminaria* sp. and between *L. polaris* and *Fucus* sp. The f_1 fractions from each of these pairs, in particular, appear to be made up of almost identical suites of compounds. It seems reasonable to assume that the animals may be grazing on the plants, although there may be intermediaries in the food chain transfer process.

In explanation of the variation between the data sets from the gas chromatographic and gravimetric determinations, it was suggested that most of the variation was due to gross compositional differences from sample to sample. A comparison of the compositional variability within species gives further support to the suggestion. For example, some of the f_1 fractions of *L. polaris* are characterized by a relatively large amount of a fairly complex set of compounds in the decane to tetradecane range. In others, the feature is almost completely absent. Although recovery of this hydrocarbon range by the methods used is expected to be less efficient and more variable than that of less volatile components (Engelhardt *et al.*, 1982), the variation observed seems far greater than can be ascribed to procedural reasons and likely reflects a substantial natural variability in lower molecular weight components relative to the higher molecular weight ones. While being retained fairly efficiently by the GC/FID procedure, the low molecular weight components will likely be lost almost entirely during the evaporation-to-dryness step necessary for the gravimetric analysis. Hence, for a given gravimetric concentration quite different GC/FID concentrations might be expected, assuming there are no compensating differences in non-chromatographable components. The same low molecular weight compounds seem to be present in *Fucus* sp., and so the variation of this component in starfish may simply reflect variation in diet.

The GC/MS analysis of the tissue aromatic hydrocarbons provides useful information pertaining to their probable sources. The *Mya truncata* samples had aromatic hydrocarbon distributions that were in all cases strongly dominated by the parent aromatic hydrocarbons. This result suggests, as in the case of the sedimentary hydrocarbons, that the main source of contaminating aromatic hydrocarbons is atmospheric fallout of aerosols transported from the lower latitudes. Since there is evidence (Farrington *et al.*, 1983) that petroleum hydrocarbons discharged into coastal areas may be more biologically available to bivalves than pyrogenic hydrocarbons, the finding of the pyrogenic distribution of aromatic hydrocarbons underscores the probable absence of significant petroleum inputs to the study area. As expected, the samples of starfish and seaweed that were contaminated during collection contain aromatic hydrocarbon distributions typical of petroleum oils.

CONCLUSIONS

The analyses of biota from the BIOS study sites provided evidence of the absence of prerelease petroleum oil contamination of the area. For the purposes of the BIOS biological experiments, it was clear that the study organisms and populations would not already be petroleum oil adapted and stressed prior to the experimental oil releases.

ACKNOWLEDGEMENTS

Refer to part I (Cretney *et al.*, 1987) for acknowledgements of help and guidance.

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