Polycyclic Aromatic Hydrocarbon Accumulation and Sensory Evaluation of Lobsters *(Homarus americaus)* Exposed to Diesel Oil at Arnold's Cove, Newfoundland

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POLYCYCLIC AROMATIC HYDROCARBON ACCUMULATION AND SENSORY EVALUATION OF LOBSTERS (HOMARUS AMERICANUS) EXPOSED TO DIESEL OIL AT ARNOLD'S COVE, NEWFOUNDLAND

by

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ABSTRACT

Williams, U. P., J. W. Kiceniuk, and J. R. Botta. 1985. Polycyclic aromatic hydrocarbon accumulation and sensory evaluation of lobsters (Homarus americanus) exposed to diesel oil at Arnold's Cove, Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 1402: iv + 13 p.

The hepatopancreas of 20 lobsters were analyzed by high performance liquid chromatography for the presence of polycyclic aromatic hydrocarbons (PAH's). Ten of the lobsters had been exposed to a diesel oil spill in Arnold's Cove, Newfoundland for a period of less than 10 hours. Ten lobsters were taken from a nearby harbour to serve as control samples. Lobsters from both sites were subjected to a taste panel to determine if the quality of the lobster meat was affected in any manner by the diesel exposure.

One way analysis of variance indicated a significant increase in the concentration of naphthalene, phenanthrene and pyrene in the lobster from the exposed site. There was no significant difference in the quality of the lobster meat with respect to site.

RÉSUMÉ

Williams, U. P., J. W. Kiceniuk, and J. R. Botta. 1985. Polycyclic aromatic hydrocarbon accumulation and sensory evaluation of lobsters (Homarus americanus) exposed to diesel oil at Arnold's Cove, Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 1402: iv + 13 p.

On a analysé, au moyen de la chromatographie en phase liquide haute performance, l'hépatopancréas de 20 homards pour déceler la présence d'hydrocarbures aromatiques polycycliques (HAP). Dix homards ont été soumis à un déversement de gas-oil dans l'anse Arnold (Terre-Neuve) pendant moins de 10 heures. Dix homards one été capturés dans un port situé tout près pour servir de témoins. Les homards provenant des deux endroits ont été présentés à un jury de dégustation pour déterminer si la qualité de la chair de homard était altérée de quelque façon par la présence de gas-oil.

Une analyse simple de la variance a montré qu'il y avait une augmentation notable de la concentration de naphtalène, de phénanthrène et de pyrène chez les homards provenant de l'endroit exposé. In n'y a pas eu de différence importante de la qualité de la chair de homard selon l'emplacement.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) can enter the marine environment through a number of different sources such as oil spills, industrial effluents, storm drain runoff, fallout from air pollution and cresoted wharves and pilings (Dunn and Fee, 1979). PAH contamination of marine shellfish inhabiting the nearshore environment has been known for some time and has been well documented in the literature (Dunn and Fee, 1979; McLeese and Metcalfe, 1979; Uthe et al., 1984).

On April 28, 1984 ten gallons of diesel oil were spilled into a small harbour at Arnold's Cove, Newfoundland. Lobsters were being held in floating holding cages awaiting sale and shipment to market. Although this spill was small and the lobsters were removed from the immediate area of the spill, exposure may have persisted for as long as 10 hours. Concern was expressed by fishermen in the area about the possibility by tainting of the lobsters.

The present investigation was initiated to determine if conditions at the spill site contributed to accumulation of PAH within the hepatopancreas and to ascertain what effect, if any, exposure had on the suitability of the lobster for human consumption. Freshly caught lobsters from Fairhaven, Newfoundland were sampled to provide an estimate of background levels of PAH in the lobster.

MATERIALS AND METHODS

Lobsters, which were being held in holding pens, were collected live from fishermen at Arnold's Cove and Fairhaven. The hepatopancreas was excised from each sample and PAH'S were extracted with total lipids and estimated by a method modified from Floch et al. (1957) and Bligh and Dyer (1959). One gram of hepatopancreas was homogenized in an Omni Mixer homogenizer in methanol (10 x sample volume) for 1 minute followed by chloroform (20 x sample volume) for 2 minutes. The homogenate was then filtered through Whatman #1 filter paper and transferred to a 125 ml Erlenmeyer flask. The residue was then re-homogenized for 3 minutes with chloroform-methanol mixture (2:1), filtered and pooled with the first extract. Potassium chloride (0.88%) was added to the total extract (¼ volume of total extract), allowed to settle for 10 minutes and the supernatant was decanted. The methanol:water (1:1, of ¼ vol.) washes followed and the top layers were removed following each wash. Five grams of anhydrous sodium sulphate was added and the extract was permitted to stand for 30 minutes. At this point the extract was filtered through glass wool and evaporated under reduced pressure (37°C). The extracts were dried to a constant weight, taken up in 1 ml HPLC grade hexane and analyzed for PAH by high performance liquid chromatography (see Table 1).

Chromatographic instrumentation included two Beckman pumps (Model 110A), one Beckman Controller (Model 420), a Beckman Dual Wavelength UV Detector (Model 152) and a Perkin-Elmer Data Station (Model 3600).

Chromatography was carried out on a Nucleosil NH_2 column (5 µm, 5 mm id, 25 cm L) with injection volume varying from 15-50 ul. The solvent consisted of

100% hexane for 12 minutes followed by a column backflush for 11.8 minutes with a combination of 95% hexane and 5% methyl-t-butyl-ether. The solvent flow rate was 4.0 ml/min throughout the entire run.

A total of eight groups of polycyclic aromatic hydrocarbon (PAH) standards (benzene, naphthalene, fluorene, phenanthrene, pyrene, benzo[a]pyrene, indeno(1,2,3-cd)pyrene and dibenz(a,h)anthracene) were used as external standards and peaks were identified on the basis of retention times. Quantitation was determined by the comparison of the absorbance (254 nm) of the extracts with those of the standards. Concentrations represent standard equivalents of each PAH class (i.e. monoaromatics, diaromatics etc.,) and do not represent the complete PAH composition of the hepatopancreas.

A sample of the diesel oil spilled at Arnold's Cove was obtained from the tanker truck that was refueling the trawler and a class analysis was done on HPLC (see Fig. 2).

A panel of 49 different judges participated in a sensory evaluation of the quality of the various lobster samples. Batches of two lobsters (from the same location) were immersed in 7.3 liters of boiling tap water (containing 50 g salt). Twenty-minutes later the boiling water was drained and approximately 7 liters of cold tap water was placed in the pot. At this time the lobsters were immediately removed from the pot. The cooked lobster meat (mainly tails, but some claws were used) was immediately removed from the shell and placed in covered glass petri dishes. The lobster was served hot using an electric warming tray. Evaluations were conducted in individually partitioned booths with daylight fluorescent lighting. The judges used room temperature tap water for rinsing their mouths between samples.

Each judge was presented with three samples and asked to identify which of the three samples were different from the other two (Triangle test). The judges received either two samples from Arnold's Cove and one from Fairhaven and one from Arnold's Cove and two from Fairhaven (see Table 2 for questionnaire). Any particular judge evaluated all tails or all claws and all samples were evaluated within 15 min of cooking.

RESULTS

There were 20 correct identifications out of 49 triangle tests (Table 3). This is not significant at the 5% level (Larmond, 1982). Of the 20 correct identifications, one judge had no preference, 10 judges preferred the Fairhaven lobster and 9 judges preferred the Arnold's Cove lobster (Table 3). Most of the 20 judges indicated that the difference was slight with a few indicating it was moderate. The judges usually selected the odd sample on the basis of flavor or texture.

One way analysis of variance indicated a significant difference between groups for napahthalene, phenanthrene and pyrene (see Fig. 1). All PAH's were detected in both groups except for pyrene, which was not present in the Fairhaven control site, and benzo[a]pyrene, which was not present in the Arnold's Cove samples (see Table 4 and 5).

A sample of the diesel oil spilled at Arnold's Cove was obtained and a class analysis was done on LC (see Fig. 2). The diesel consisted of monocyclic, dicyclic, tricyclic aromatics and fluorene type compounds.

DISCUSSION

Upon examination of the data it was apparent that there was no difference in monocyclic aromatics between sites. This could be due to an inability to resolve monocyclic aromatics from squalene as well as a high level of naturally occurring monocyclics in the environment. The possibility of accidental oil spillages and intermittent boating activities may also contribute to the similiarity in monocyclic aromatics concentrations between sites.

Some larger PAHs were detected in some of the lobsters from both sites. These are not accumulated in the hepatopancreas as a result of exposure to the diesel but can be attributed to natural variability from site to site and from lobster to lobster. The pyrene present in the lobsters from Arnold's Cove was not accumulated as a result of exposure to the diesel oil as no pyrene type compounds were present in the oil itself (see Fig. 2), therefore, the pyrene came from sources other than the diesel oil spill. McLeese and Metcalfe (1979) suggested that some sites yield more contaminated lobster than do others and Dunn and Fee (1979) reported considerable variability in PAH levels in lobster tails among sampling sites.

The Arnold's Cove lobsters were exposed to diesel oil for only 10 hours (M. A. Barnes, pers. comm.) and PAHs accumulated in the hepatopancreas within this relatively short exposure time. This was not entirely unexpected as McLeese and Metcalf (1979) reported a rapid accumulated of cresote in lobster hepatopancreas and this accumulated at a rate of 85 ug/g lipid/h when the exposure concentration was 0.3 mg/l. Uthe et al. (1984) reported levels of PAH in lobster hepatopancreas 35 times higher than in tails. The hepatopancreas performs some of the same functions as the liver does in vertebrates (i.e. food absorbed from the gut is transformed to storage products) and lipophilic contaminants tend to accumulate in the hepatopancreas lipids.

Dunn and Fee (1979) reported that contamination of commercial shellfish by benzo[a]pyrene appeared to be widespread, however, this does not appear to be the case in this study as only 1 lobster from both sites had any detectable levels of B[a]P. This indicates that the lobsters in this area were not exposed to significant levels of B[a]P.

The results obtained from the taste evaluation indicated that of all the correctly identified samples none were classified as unacceptable. Exposure to the diesel did not taint the lobster enough to make it unacceptable but only enough to give it a slightly different taste. Even though the results regarding preference and acceptance were reported and discussed, it must be remembered that these data resulted from secondary questions and should be interpreted more cautiously than those resulting from the primary question, i.e. is there a difference?

There were significantly higher levels of PAH in lobster hepatopancreas from Arnold's Cove as compared to Fairhaven even with the low amount of diesel spilled and the short exposure time. The sensory evaluation indicated that correctly identified lobster, whether it was from Arnold's Cove or Fairhaven, were still very much liked by the judges.

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Table 1. Protocol for extraction of lipids from Lobster hepatopancreas.

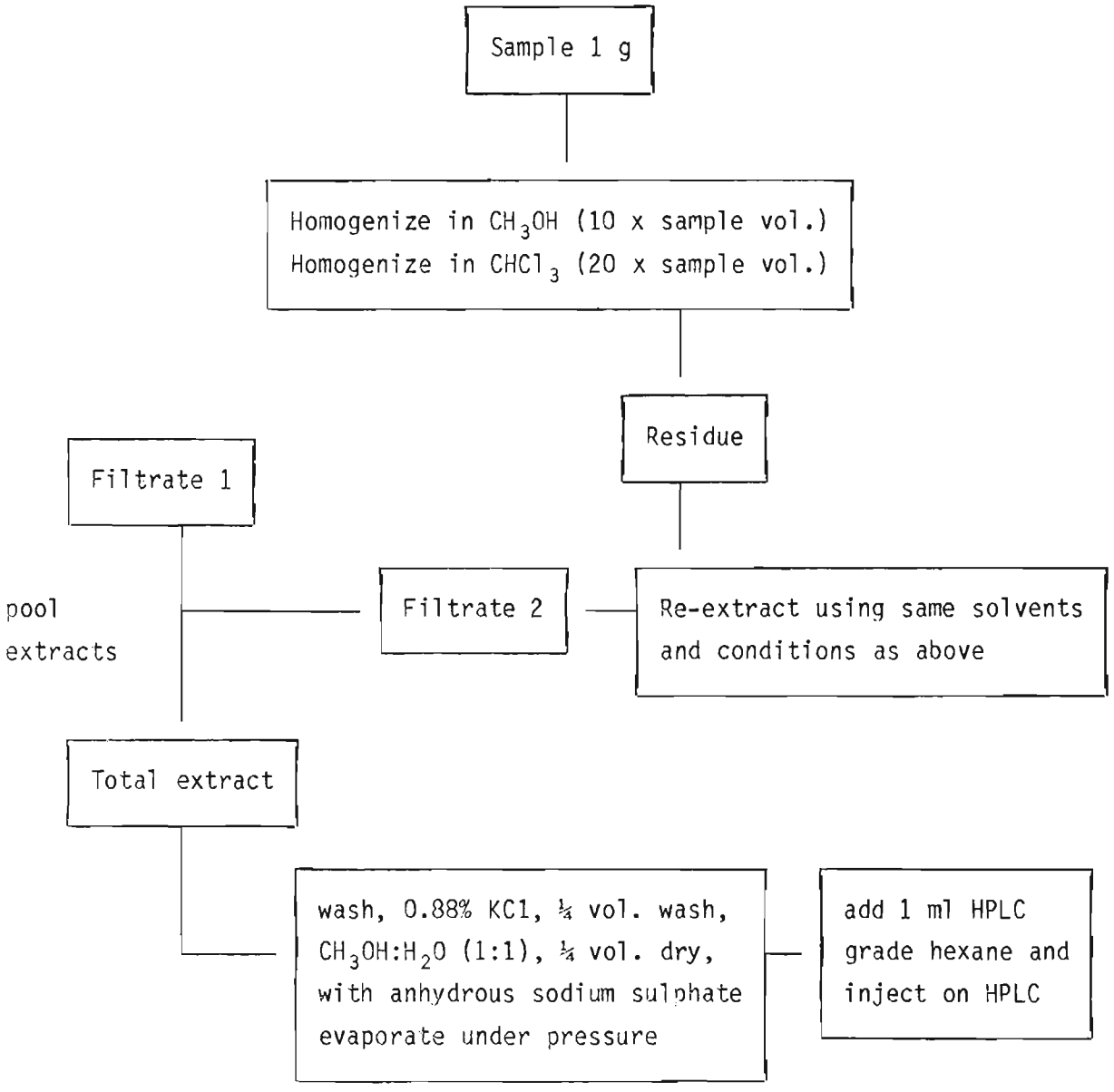

Table 2. Form used to evaluate sensory quality of cooked lobster claws or tails.

NAME: _____ DATE: _____

PRODUCT: _____

Two of these three samples are identical, the third is different.

1. Evaluate the samples in order indicated and identify the different sample.

Code	Check different sample
_____	_____
_____	_____
_____	_____

2. Indicate the degree of difference between the duplicate sample and the different sample.

Slight _____

Moderate _____

Much _____

Extreme _____

3. Is the different sample acceptable? _____
   Are the duplicate samples acceptable? _____

4. Is the different sample more acceptable _____
   Are the duplicate samples more acceptable? _____

5. Is the difference related to: Appearance _____

   Flavor _____

   Odor _____

   Texture _____

6. Comments:

Table 3. Results of sensory evaluation triangle test.

# Of test	# Of correct identification	Diesel exposed samples	Control samples	No reference	# Rated acceptable
49	20 n.s.	5 sl. 2 mod. --- 9=Total	5 sl. 2 mod. 1 much 1 ext. --- 10=Total	1	20

n.s.=non-signficant.
sl.=slight difference.
mod.=moderate difference.
ext.=extreme difference.

Table 4. Arnold's Cove spill site (PAH in ppm).

Sample #	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Benzene	0.034		0.032	0.061	0.023	0.017	0.026		0.027	0.033
Naphthalene	1.968	2.392	2.985	2.858	2.452	2.473	1.852	1.304	1.799	1.690
Fluorene	0.631	0.553	0.370	0.679	0.638	0.532	0.439	0.289	0.376	0.391
Phenanthrene	0.667	1.321	0.875	0.883	0.989	0.473	0.609	0.451	0.491	0.471
Pyrene		0.151		0.373	0.376		0.192	0.181		
B[a]P										
Ideno(1,2,3-cd)-pyrene										
Dibenz(a,h)-anthracene						1.683				

Table 5. Fairhaven Control site (PAH in ppm).

Sample #	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Benzene	0.019	0.049	0.020	0.038	0.077		0.028	0.050	0.016	
Naphthalene			2.256						1.646	
Fluorene			1.380		0.467	0.296				
Phenanthrene	0.184	0.665	1.202	0.163	0.264	0.182	0.126	0.335	0.203	0.094
Pyrene										
B[a]P			0.517							
Ideno(1,2,3-cd)-pyrene										
Dibenz(a,h)-anthracene	1.658						1.326			

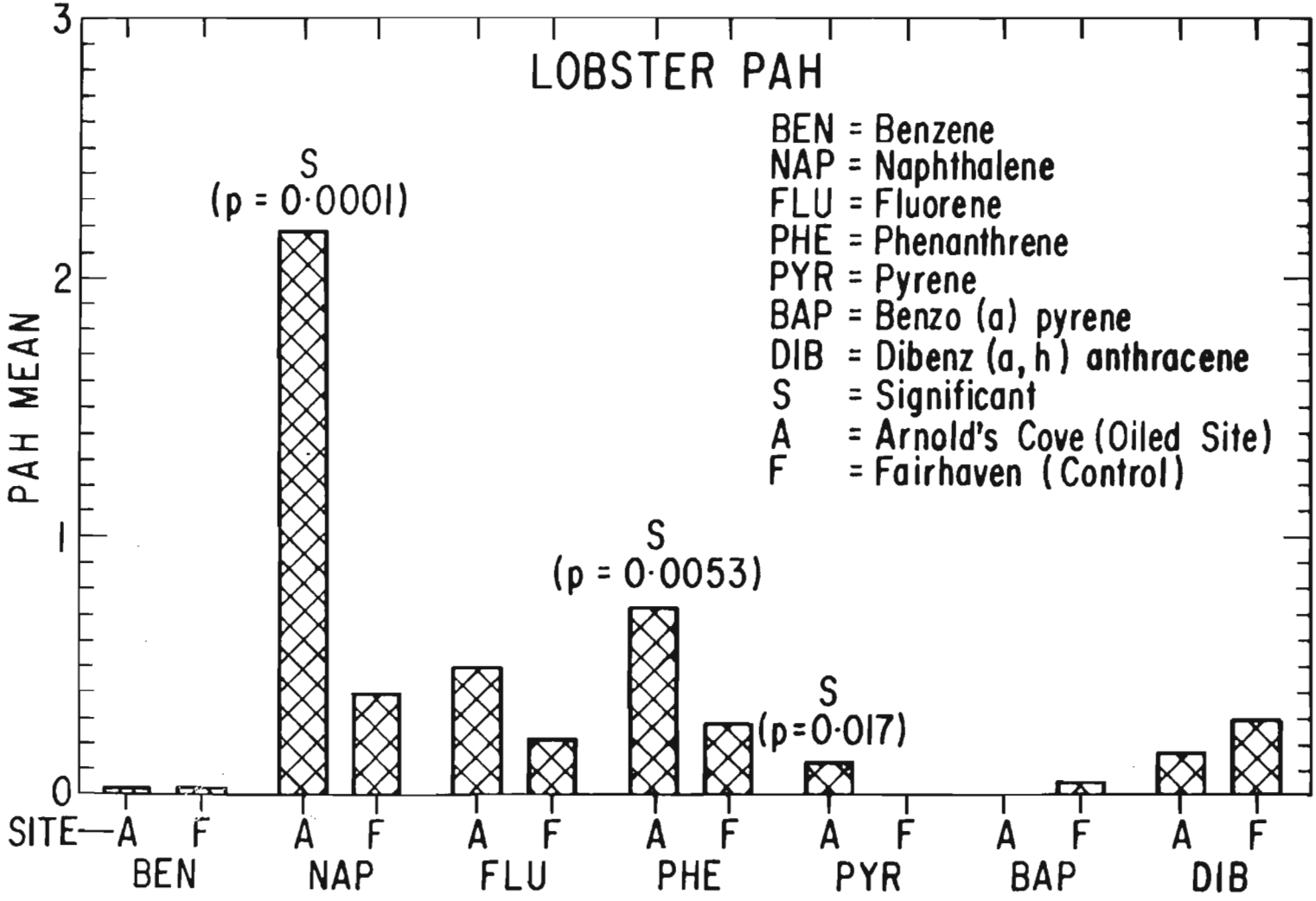

Fig. 1. Comparison of the concentration of PAHs in lobster hepatopancreas between the oiled and control sites.

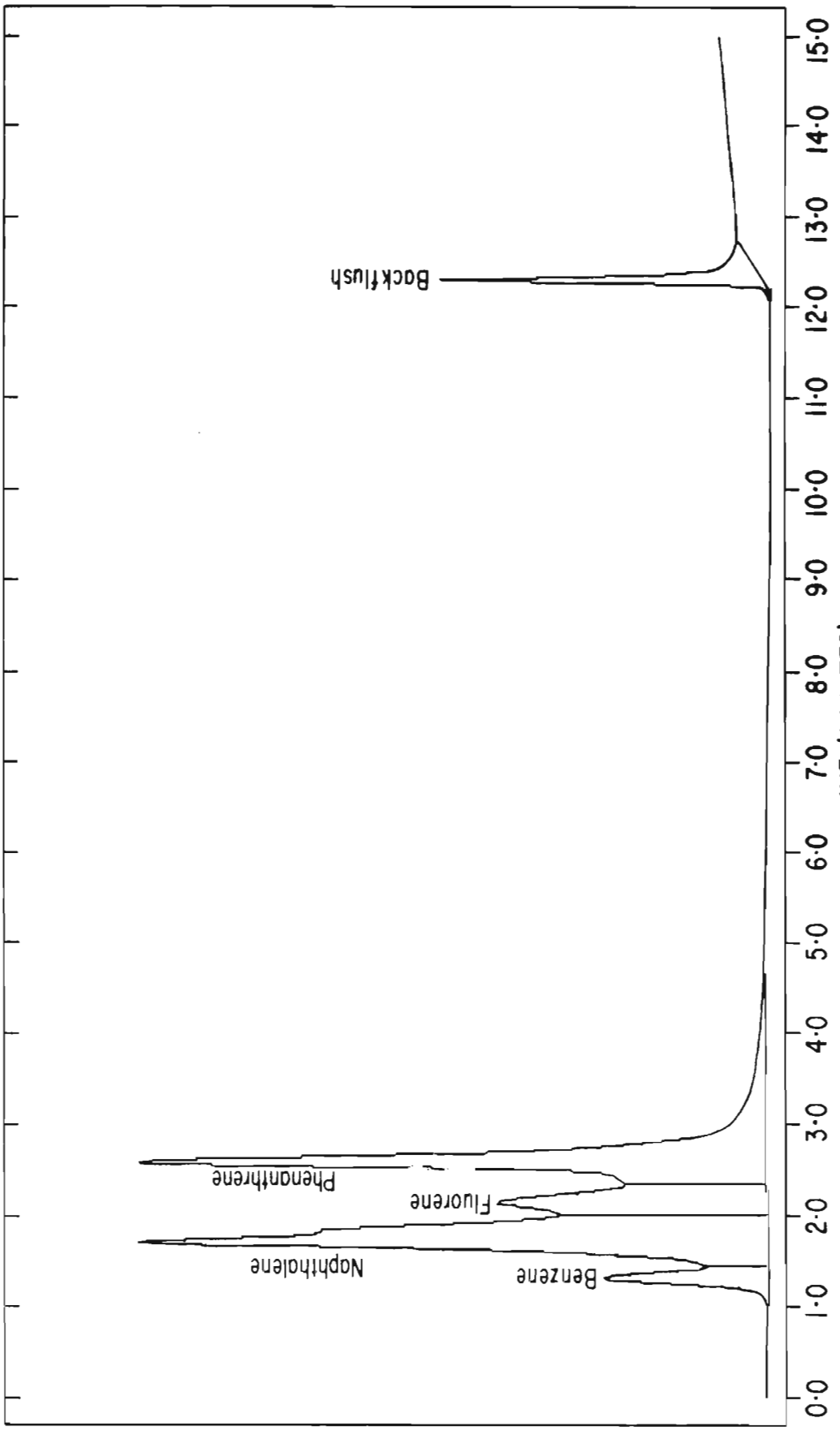

Fig. 2. Diesel oil from Arnold's Cove spill site.

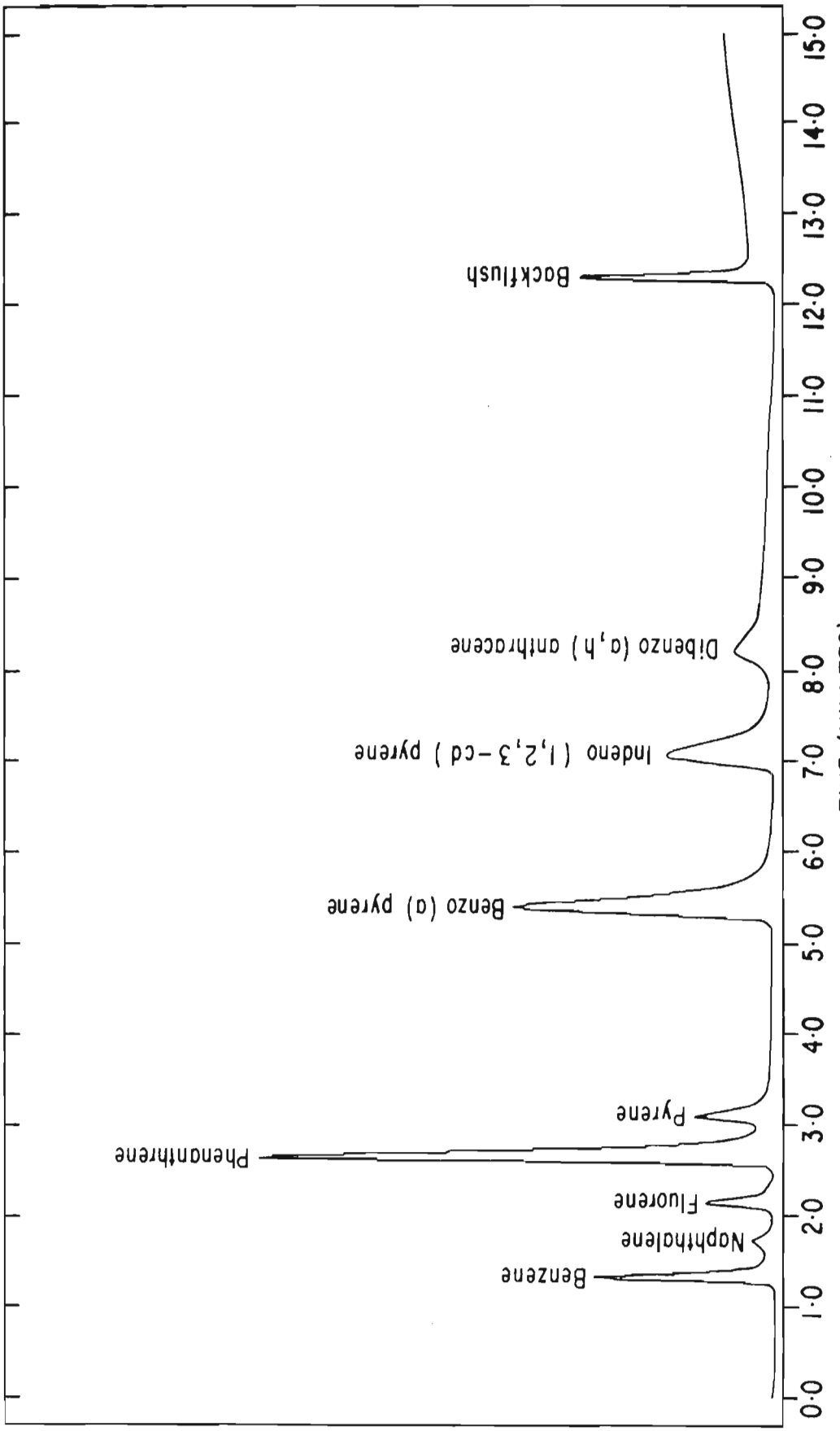

Fig. 3. PAH standards.

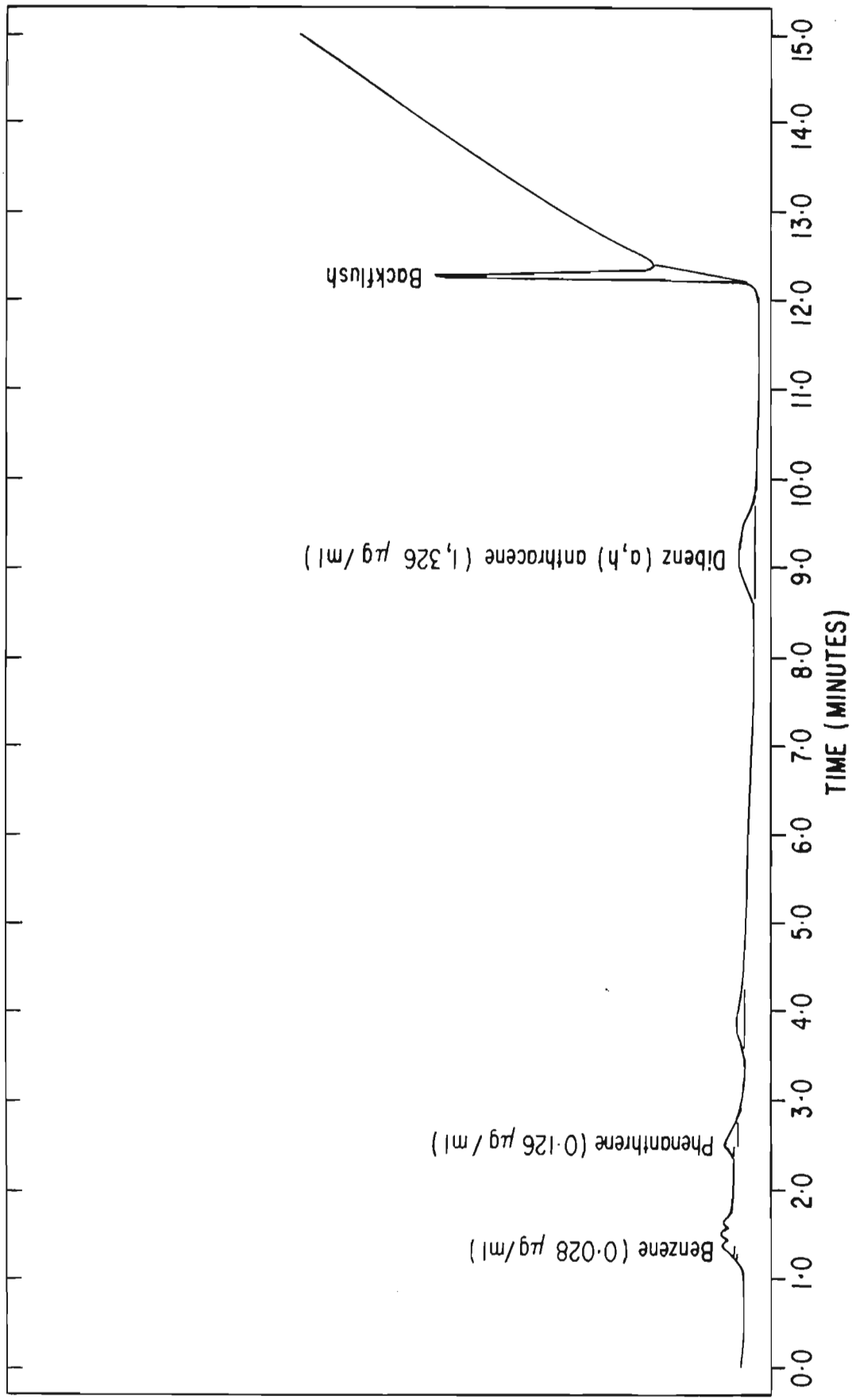

Fig. 4. Hepatopancreas extract from Fairhaven control site (Lobster #7).

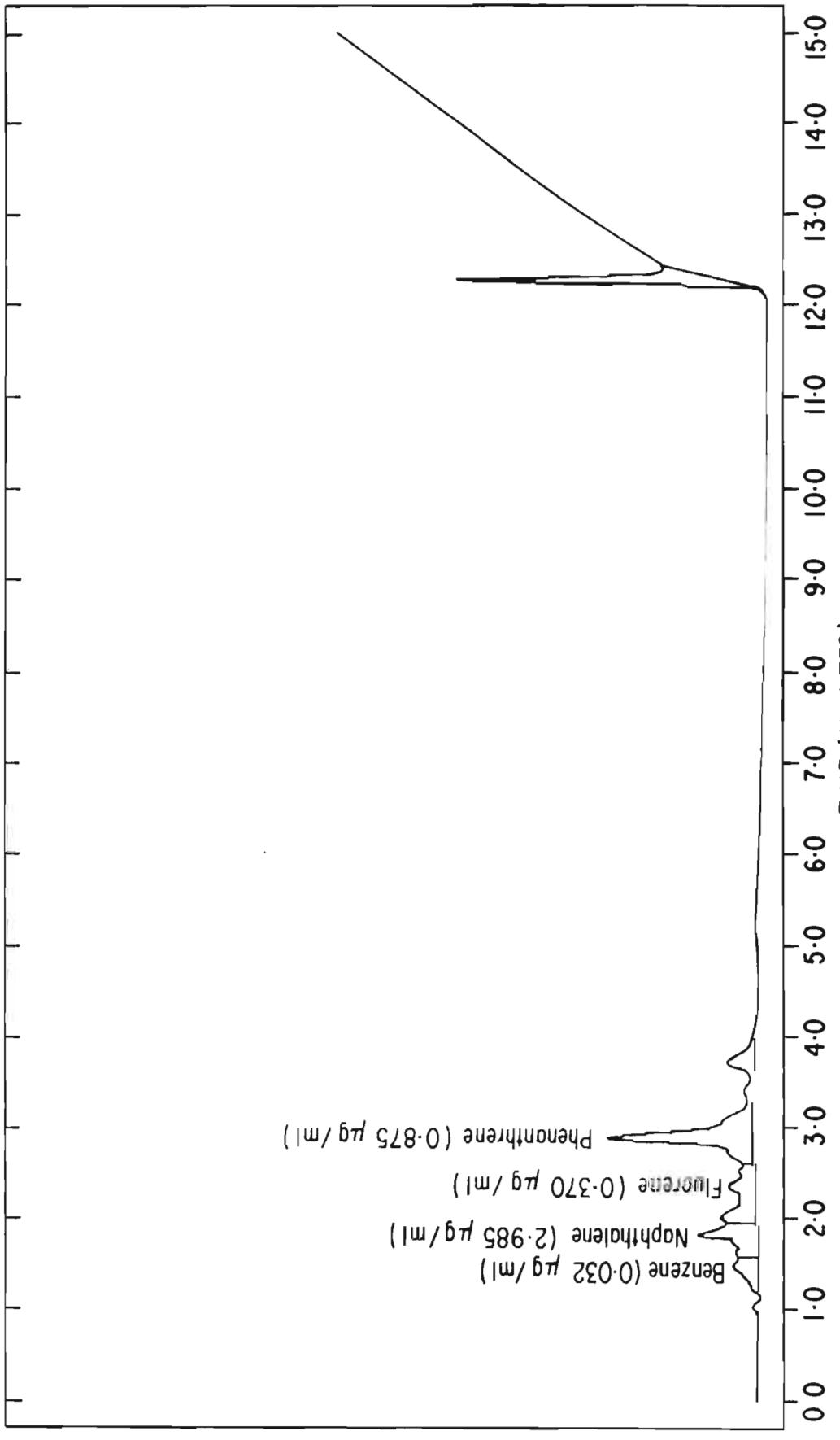

Fig. 5. Hepatopancreas extract from Arnold's Cove spill site (Lobster # 3).