Aromatic Hydrocarbons in Aquatic Fauna

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A rapid method for screening of aquatic fauna for the presence of polynuclear aromatic hydrocarbons is described. The mixture of hydrocarbons is separated from lipids by column chromatography on alumina, characterized and quantitated by fluorometry. Pyrene is employed as a fluorescence standard. The method can be used to detect contamination of aquatic fauna by crude oil, fuel oils, and creosote oil. Creosote oil was detected in samples of shellfish.

The occurrence of polynuclear aromatic hydrocarbons in marine fauna was reviewed by SUESS (1970), and the literature dealing with the contamination of aquatic fauna by petroleum is quite extensive. A rapid analytical technique, suitable for monitoring of polynuclear aromatic hydrocarbons in biological samples is not available, and most of the determinations of oil in biological samples were carried out by gas chromatography which is not a particularly sensitive technique for the detection of aromatic hydrocarbons. A fluorometric method for the detection of Bunker C oil in marine fauna has been published (ZITKO 1970), but, since the encountered levels of oil were very high, the measurements were carried out on crude extracts without any cleanup.

Some of the levels of petroleum hydrocarbons in aquatic fauna, reported in the literature, are summarized in Table 1. The concentrations are expressed on lipid basis for easier comparisons. It can be seen that the sensitivity of an analytical technique used for baseline monitoring should be at least 100 μg/g.

Creosote oil is derived from coal tar and is used mainly as timber preservative. Creosote oil contains polynuclear aromatic hydrocarbons such as anthracene, phenanthrene, fluorene, pyrene, etc. The contamination of aquatic fauna by creosote oil has not been reported in the literature.

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TABLE 1.

Levels of petroleum hydrocarbons in aquatic fauna.

Organism	Petroleum hydrocarbons, µg/g lipid*	Reference
Mussel	13300 - 39900	GREFFARD and MEURY 1967
Oyster	605	SMITH Jr. 1954
Scallop	133 - 934	BLUMER et al. 1970
Oyster	4600	BLUMER et al. 1970
Oyster	15740	EHRHARDT 1972
Shellfish	193 - 1070	FARRINGTON and QUINN 1973
Clam	6670	ZITKO 1970
Periwinkle	2000 - 33350	ZITKO 1970
Barnacle	60000	MORRIS 1973a
Crustacea	1000 - 2600	MORRIS 1973b
Bluefish	771	SMITH Jr. 1954
Trout	20000	MACKIE et al. 1972
Trout	1900 - 5500	ACKMAN 1973

*The reported concentrations were expressed on lipid basis assuming a moisture content of 80% and lipid content of 1.5% of wet weight.

EXPERIMENTAL

Samples. Commercial marine lipids (herring oil, cod liver oil, and seal oil) were obtained from the manufacturers. Additional samples of lipids were isolated by extracting freeze-dried tissues of fish and shellfish with spectrograde hexane (Fisher Scientific H-334) or diethyl ether (Fisher Scientific E-193). Eels (*Anguilla rostrata*) were taken in the St. John River estuary, and sea ravens (*Hemitripterus americanus*) in the St. Croix River estuary, New Brunswick, Canada. Winter flounder (*Pseudopleuronectes americanus*) originated from both localities. All the above samples were obtained in the fall of 1972 and were stored at -20°C. Mussels (*Mytilus edulis*) were collected from the wharf of the Biological Station, St. Andrews, N.B., and samples of periwinkles (*Littorina littorea*) and whelks (*Buccinum undatum* and *Neptunea decemcostata*) were taken in the vicinity of the wharf, and in Passamaquoddy Bay, New Brunswick. A sample of clams (*Mya arenaria*) was also obtained from this location. These samples were collected in the fall of 1974 and processed immediately after collection. Extracted lipids were stored under nitrogen at 0°C.

Samples of oils were obtained from refineries

and from several oil spills. The sample of creosote oil was the same as that used previously (ZITKO et al. 1969).

Cleanup. Alumina (Fisher Scientific A-540, activated 4 h at 800°C and deactivated by adding 5% water, 4 g) was charged to a 45 x 0.5 cm glass column. The column was percolated with spectrograde hexane until 6 mℓ of effluent was collected. The sample (100 mg lipid or 3-200 µg oil) was then applied to the column in 1-1.5 mℓ of hexane, washed into the column with 1.5 mℓ of hexane, and the column was percolated with additional hexane. The receiver was changed when the total effluent volume reached 9 mℓ, and 15 mℓ of effluent was collected. The first 9 mℓ of hexane was discarded, and the UV spectrum of the 15 mℓ fraction was recorded. The volume of this fraction was adjusted so that the absorbance in 1 cm cells did not exceed 0.1 between 300 and 400 nm. Many fractions could be concentrated on a rotatory evaporator at room temperature and the final volume was adjusted to 5 mℓ. Fluorescence emission spectra were then recorded.

Elution patterns of crude and Bunker C oil were determined by collecting 3 mℓ fractions of the effluent. In the case of creosote oil, one 5 and one 10 mℓ fraction were collected.

Instrumental techniques. Ultraviolet and fluorescence spectra were recorded on a Beckman DK-2A and a Perkin-Elmer MPF-2A instrument, respectively. In the latter case the excitation and emission slit width was 10 nm. Unlesss indicated otherwise, fluorescence was excited at 310 nm and the emission spectrum between 320 and 460 nm was recorded. The sensitivity of the instrument was adjusted to give a fluorescence emission of 300 units/ µg/mℓ for solutions of pyrene (Eastman Organic Chemicals 3627, lot 681) in spectrograde hexane. The fluorescence emission of pyrene was excited at 333 nm and measured at the emission maximum (390 nm). The fluorescence emission of samples was expressed as the concentration of pyrene in µg/mℓ which gives the same emission as a 1 µg/mℓ solution of sample. In the case of lipids, the amount applied to the alumina column was used to calculate the concentration.

A Finnigan Model 1015D quadrupole instrument was used to record mass spectra of some of the samples. The samples were introduced by a direct probe and the spectra were scanned from 30 to 560 mass units while the temperature of the probe was raised from ambient to 100°C. The electron energy was 20 eV.

RESULTS AND DISCUSSION

Pyrene as a standard in fluorescence measurements. Pyrene is a suitable standard for the measurement of fluorescence emission of oils. The fluorescence emission maximum of pyrene is approximately in the center of the range of fluorescence emission maxima of oils, and diluted pyrene solutions (0.1 - 0.3 µg/mℓ) are stable for at least 7 days when stored in subdued light.

The fluorescence emission of oils may then be expressed in "pyrene units" (concentration of a solution of pyrene with the same fluorescence emission). This allows one to maintain constant instrument sensitivity and to make comparisons between oils and between laboratories. Three samples of crude oil had a fluorescence emission of 0.074±0.007 pyrene units/µg/mℓ (mean ± standard deviation) at 370 nm. The fluorescence emission of five samples of Bunker C oil was 0.140±0.028 and that of creosote oil was 0.059 pyrene units/µg/mℓ.

The fluorescence emission of lipids may also be expressed in pyrene units/µg/mℓ and, if the appearance of the emission spectrum indicates the presence of crude or Bunker C oil (a broad maximum between 350 and 380 nm), or creosote oil (one or two maxima between 340 and 360 nm and maxima at 402 and 426 nm), the concentration of oil in µg/g lipid may be calculated by multiplying by 10^6 and dividing by the fluorescence emission of the appropriate oil in pyrene units/µg/mℓ.

Chromatography on alumina. The alumina columns used in this work retain up to 100 mg of lipids. A prewashing of the columns with 6 mℓ hexane is required to remove fluorescent impurities, emitting fluorescence between 320 and 360 nm. As can be seen from Figure 1, most of crude and Bunker C oil is eluted in 9 mℓ of effluent, but it is necessary to collect 15 mℓ to achieve a practically complete elution. It can also be seen that some fractionation of the oils takes place on the column. The elution patterns of creosote oil will be discussed later.

The average recovery of crude, Bunker C, and creosote oil was 69.3, 66.4, and 89.0%, respectively, and was not affected by the presence of lipids.

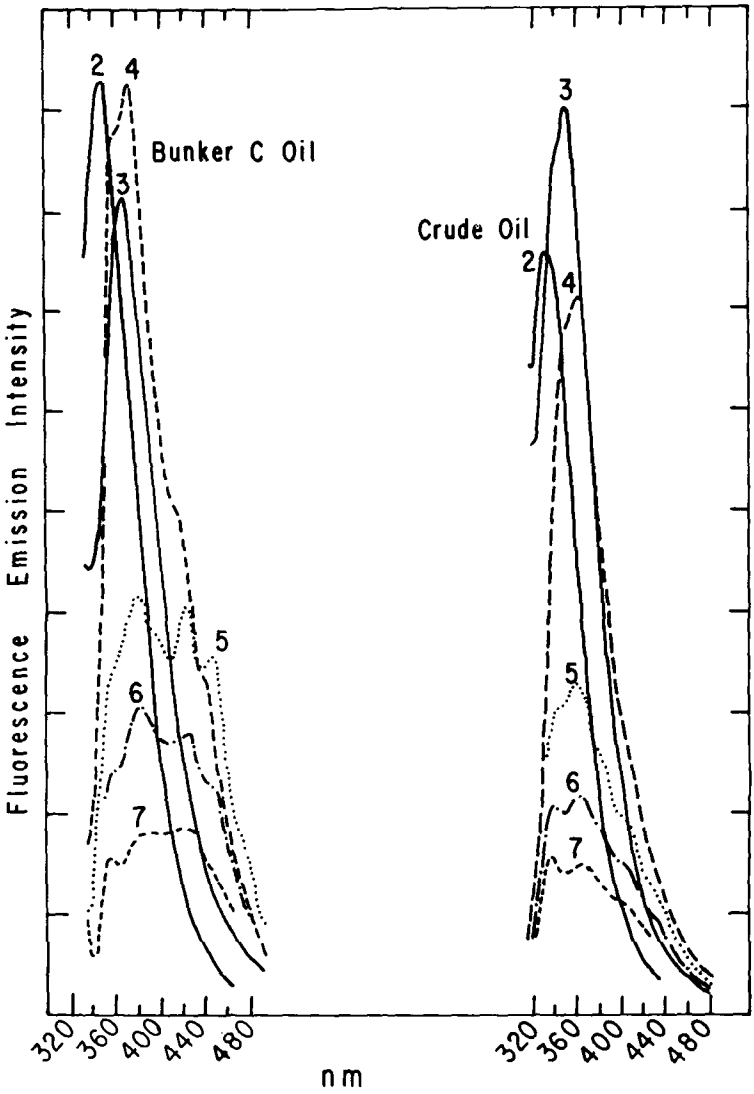

Fig. 1. Fluorescence emission spectra of oil fractions eluted from the alumina column. Each fraction consists of 3 mℓ effluent. Fraction 1 (first 3 mℓ after sample application) had emission spectrum of solvent blank. Spectrum of fraction 3 was recorded at 50% sensitivity.

Fluorescence emission spectra of herring lipids spiked with Bunker C oil are presented in Figure 2. A Bunker C oil concentration of 50 µg/g is still easily detectable in the cleaned-up samples (Fig. 2A). Without the cleanup, a Bunker C oil concentration of 100 µg/g results in an overall increase of fluorescence emission of the lipid solution, but the emission spectrum of Bunker C oil is not recognizable (Fig. 2B), and the detection limit of Bunker C oil without cleanup is approximately 1000 µg/g. Taking into consideration the fluorescence emission intensity of crude and creosote oil, the respective detection limits would be approximately two times higher than those of Bunker C oil. Relative standard deviations of triplicate analyses of herring lipid blank and spiked sample (50 µg/g) were 3.7 and 3.1%, respectively.

Fluorescence emission of extracts of aquatic fauna. The described technique was applied to lipids extracted from aquatic fauna and the results are summarized in Table 2.

TABLE 2

Fluorescence emission of cleaned-up extracts of aquatic fauna.

Sample	Fluorescence emission at 370 nm, (pyrene units/µg/mℓ) x 10^6
Commercial herring oil	2.12
" seal oil (3 samples)	2.16±0.5*
" cod liver oil	1.33
Winter flounder, muscle	14.8
" " liver	19.5
Sea raven, liver	4.15
Eel, muscle	6.14
" liver	6.14
Mussels	61.7
Periwinkles, Biological Station	192
" Passamaquoddy Bay	27.1
Whelks, Biological Station	20.9
" Passamaquoddy Bay	11.9
Clams	27.1

*Standard deviation

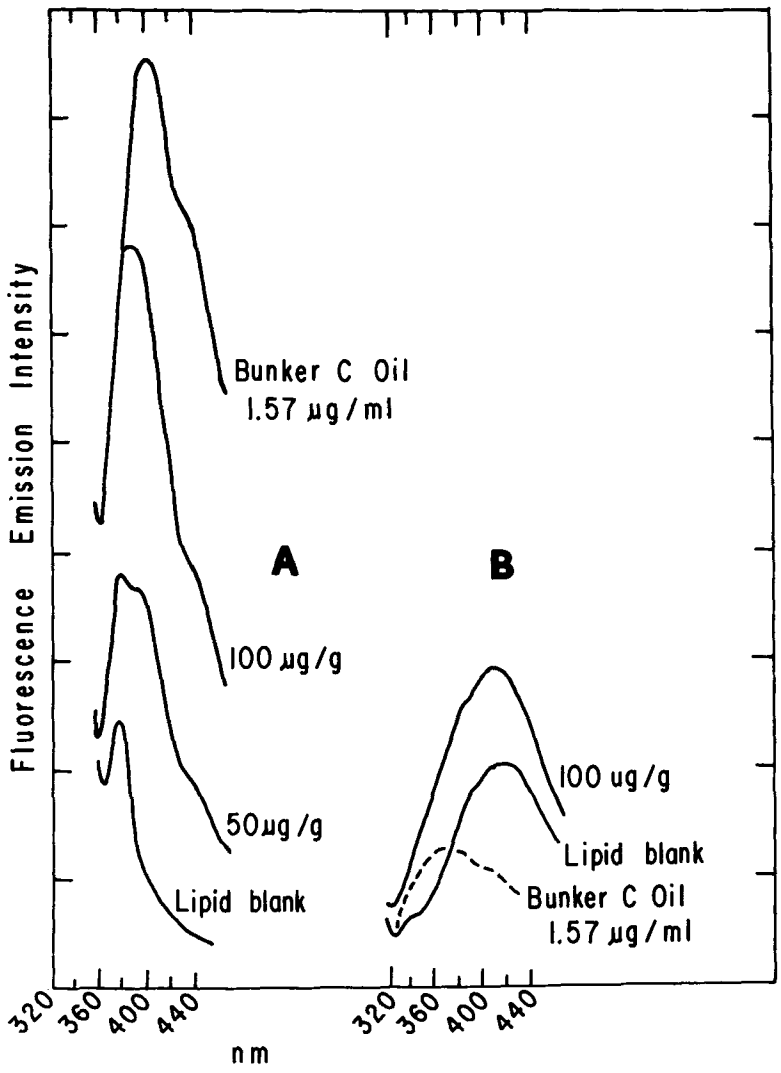

Fig. 2. Fluorescence emission spectra of herring lipid, spiked with Bunker C oil.
A - cleaned-up extracts,
B - extracts without cleanup.

With the exception of winter flounders, the fluorescence emission of the cleaned-up extracts of seals and fish is much lower than that of the shellfish. The weight of the material eluted from the alumina column was 1-3 mg, except for the winter flounder liver extract, where this amount was 10 mg. The UV spectrum winter flounder extracts had absorption maxima at 250 nm, but the fluorescence emission spectra could not be attributed with certainty to polynuclear aromatic hydrocarbons. On the other hand, the high fluorescence emission of the cleaned-up extracts of shellfish is, at least partly, due to contamination with creosote oil.

Detection and identification of creosote oil. UV spectra of some of the cleaned-up extracts of shellfish are presented in Figure 3. The presence of polynuclear aromatic hydrocarbons is indicated by several absorption maxima in the extracts of periwinkles and mussels (Fig. 3, curve 1 and 2, respectively). Both these UV and the fluorescence spectra very closely resemble those of creosote oil (Fig. 4). The fractionation patterns of creosote oil and the shellfish extracts on the alumina column are also identical, compounds with the fluorescence emission maxima at 402 and 426 nm being in both cases eluted in the 10 mℓ fraction (Fig. 4). Mass spectra of the "periwinkles, Biological Station" sample and of creosote oil were also very similar and contained molecular ions at masses of 178, 192, and 202 ($C_{14}H_{10}$, $C_{15}H_{12}$, and $C_{16}H_{10}$) in the 5 mℓ fraction, and molecular ions at masses of 202, 228, 252, and 278 ($C_{16}H_{10}$, $C_{18}H_{12}$, $C_{20}H_{12}$, and $C_{22}H_{14}$) in the 10 mℓ fraction. In addition, the 5 mℓ fraction of the periwinkle extract contained ions at masses of 328, 342, 356, and 402, 416, 430 ($C_{26}H_{16}$, $C_{27}H_{18}$, $C_{28}H_{20}$, and $C_{32}H_{18}$, $C_{33}H_{20}$, $C_{34}H_{22}$). The origin of these polynuclear aromatic hydrocarbons is not known.

The emission spectra of the cleaned-up extracts of whelks, periwinkles from Passamaquoddy Bay, and clams clearly indicated contamination with creosote oil which, in these cases, was not visible from the UV spectra (see for example Fig. 3, curves 3-5).

The concentration of creosote oil, calculated from the data in Table 2 is 1046, 3254 and 459, 354 and 202, and 459 µg/g lipid in mussels, periwinkles, whelks, and clams, respectively. It is interesting to note the difference in creosote oil concentration in periwinkles and whelks collected at the same localities. This may indicate that polynuclear aromatic hydrocarbons are not

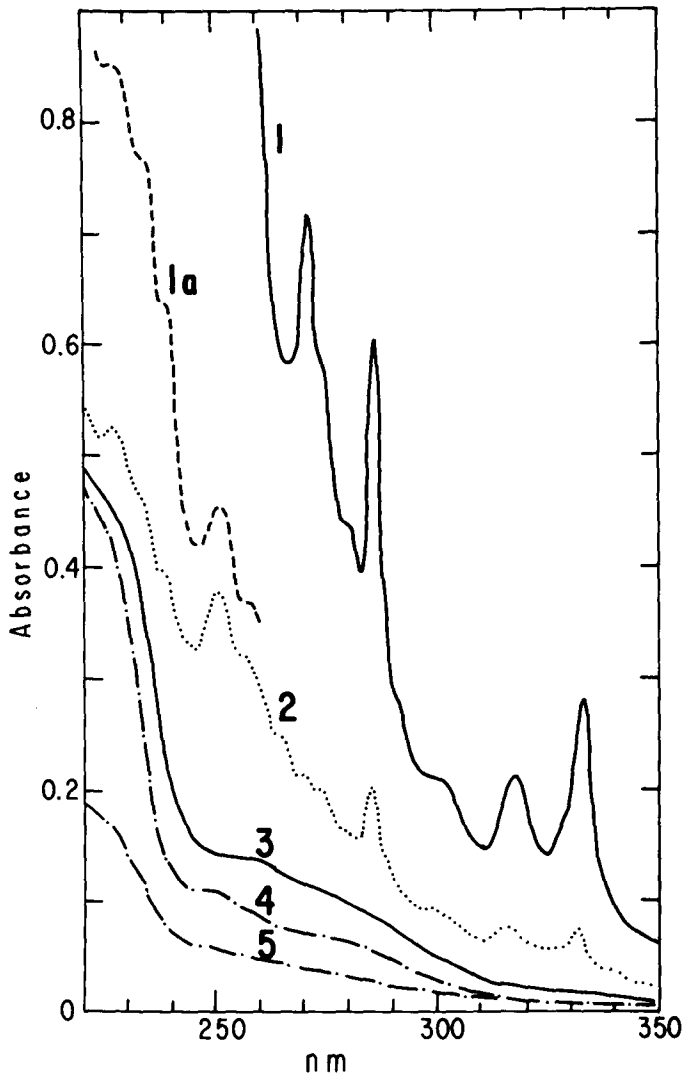

Fig. 3. UV spectra of cleaned-up extracts of periwinkles from Biological Station (1,1a), and Passamaquoddy Bay (3), whelks from these locations (4 and 5, respectively), and mussels (2).

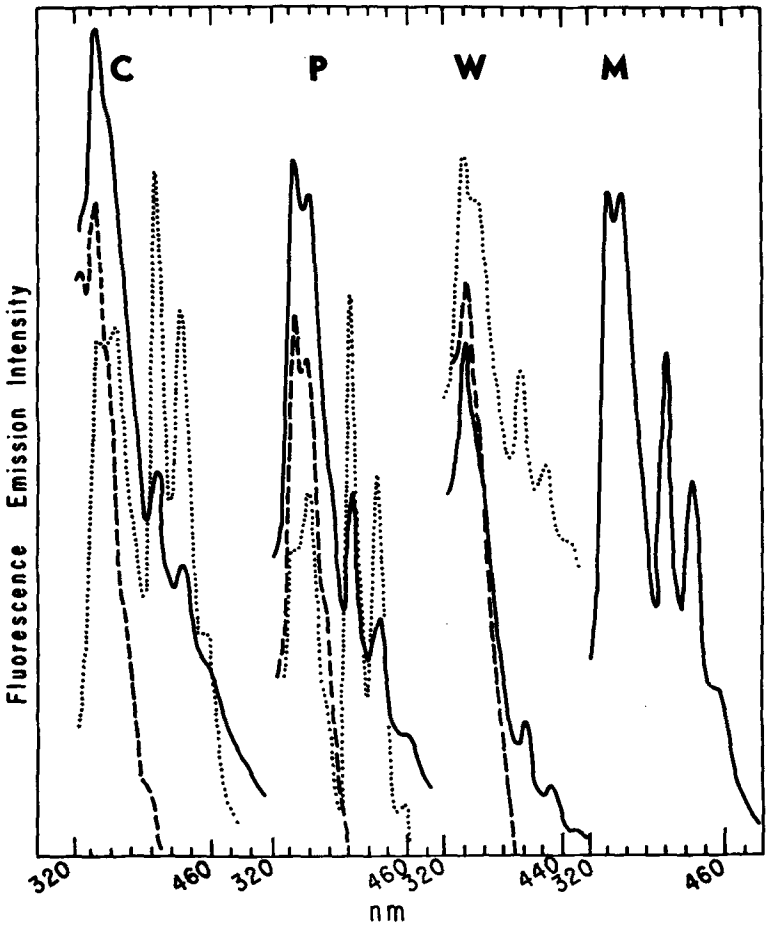

Fig. 4. Fluorescence emission spectra of creosote oil (C), periwinkles (P) and whelks (W) from Biological Station, and mussels (M); whole fraction (———), first 5 mℓ (----), and following 10 mℓ of effluent (•••) from the alumina column. Only a qualitative presentation.

accumulated along the food chains. It should also be mentioned that while the Station's wharf is periodically repaired with creosote oil-treated timber, there are no sources of creosote oil in the vicinity of the sampling site in Passamaquoddy Bay. The contamination of coastal areas by creosote oil could be quite widespread and a detailed survey should be carried out.

CONCLUSIONS

The detection limit of crude, Bunker C, and creosote oil in the described method is approximately 100, 50, and 100 µg/g lipid, respectively. Polynuclear aromatic hydrocarbons of these oils were not detectable in samples of fish and seals. Creosote oil in concentrations ranging from 202 to 3254 µg/g lipid was detected in the samples of shellfish.

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