UPTAKE AND LOSS OF PETROLEUM HYDROCARBONS BY THE MUSSEL, *MYTILUS EDULIS,* IN LABORATORY EXPERIMENTS

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ABSTRACT

Petroleum paraffin hydrocarbons (n-$C_{14}H_{30}$ to n-$C_{37}H_{76}$) from No. 2 and No. 5 fuel oils were rapidly incorporated into the mussel, *Mytilus edulis*, in a laboratory system that simulated tides. The mussels were exposed to levels of petroleum hydrocarbons from a surface slick similar to those encountered in the environment after an oil spill. After 14 days in clean seawater, the mussels had lost most of the hydrocarbons from the fuel oils; however, detectable traces of the No. 2 fuel oil still remained after 35 days. Preliminary results from these laboratory studies confirm previous studies of pollutant uptake and loss following actual oil spills.

Petroleum hydrocarbon uptake by the common bay mussel, *Mytilus edulis*, can be readily determined in the laboratory with the analytical chemical methods of solvent extraction, liquid-solid, and gas-liquid chromatography. Mussels lend themselves well to such studies because of 1) their worldwide distribution and ready availability (Davies 1969; Becker et al. 1973); 2) the considerable amount of physiological base line data available (Field 1922); 3) their hardiness as an experimental test organism (Gilfillan 1973); 4) their convenient size, which is small enough to sample adequately and use in the laboratory experimentally but large enough for specific organ dissection (Lee, Sauerheber, and Benson 1972); 5) their position in the intertidal ecosystem as a major pathway for energy transfer utilizing phytoplankton and debris (Ricketts and Calvin 1962); and 6) their known capacity for concentrating various pollutants from their environment (Greffard and Meury 1967; Modin 1969; Zitko 1970; Clark and Finley 1973a).

Earlier studies of hydrocarbon uptake and its effects on marine organisms include those by Griffith (1970) who determined the toxicity of Arabian light crude oil and oil-dispersant mixtures on mussels under tidal conditions, and Lee, Sauerheber, and Benson (1972) who used mineral oil and radio-labeled [^{14}C] heptadecane to study the laboratory uptake, body distribution, and loss of hydrocarbons in mussels. We previously reported on uptake of petroleum hydrocarbons by aquatic organisms from several oil spills (Clark and Finley 1973a). This paper reports the findings of a preliminary laboratory study using two refined petroleum products, a No. 2 fuel oil and a No. 5 fuel oil, in a laboratory system that simulates tidal action.

EXPERIMENTAL METHODS

A tidal aquarium for laboratory studies of the uptake and loss of petroleum by intertidal organisms has been described (Clark and Finley in press) (Figure 1). This system consists of two aquaria set at a 25° angle to represent a beach surface. The first aquarium contained the organisms being exposed to the pollutant, and the second aquarium, where all procedures were duplicated except for the pollutant exposure, contained control organisms. These control organisms served as the base line comparison for mortality studies and hydrocarbon analysis. The flood tide was simulated twice a day by pumping an artificial seawater medium (LaRoche et al. 1970) from a carboy using a timer-equipped, variable-speed pump. The ebb tide was accomplished by siphoning the seawater medium back into the carboys from beneath the surface oil slick in the test tank. Prior to exposure, the mussels (collected in an area distant from known sources of petroleum pollution) were acclimated to the tidal system for 24 h following a previous 48- to 96-h conditioning in the laboratory in an aerated aquarium (Table 1).

In practice, usually two sets of test organisms were used; one set was placed in the intertidal zone, held by glass rods placed horizontally across

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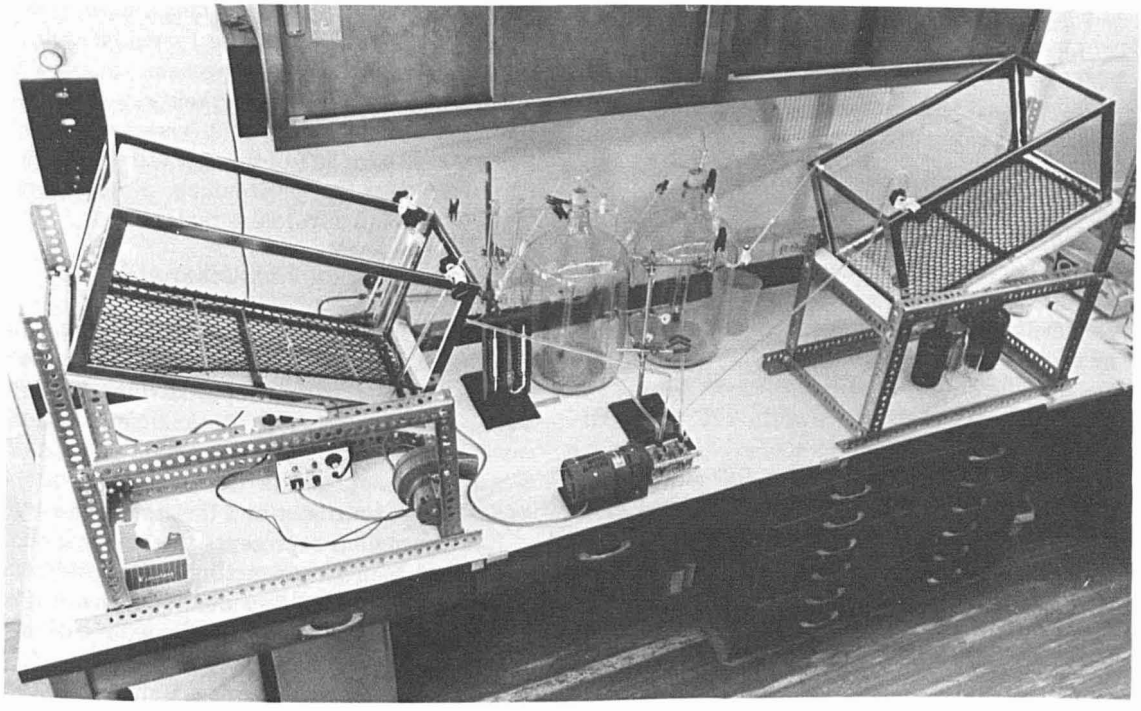

FIGURE 1.—Tidal aquarium system showing the test and control tanks.

the bottom of the sloping aquarium bottom, and exposed to the tidal sweep of the oil floating on the water surface. The second set was placed below the extreme lower level of the tidal sweep so that the organisms were continuously immersed in the medium where they were exposed to dissolved and emulsified fractions of the pollutant but never physically covered with oil.

During the experiment, a known volume of oil was layered onto the 1,700 cm^2 surface of the test aquarium at "high" tide. The system was then allowed to run through several complete tidal cycles in an attempt to simulate the conditions which might be found under a pier or along a beach following a significant oil spill. Two types of refined products were used in the uptake studies, a No. 2 heating fuel oil and a No. 5 heavy burner fuel oil.

TABLE 1.—Data on size of mussels, number of specimens, and experimental conditions.

Item	No. 2 fuel oil	No. 5 fuel oil
Average shell length	56.7 mm	54.7 mm
Number of specimens:		
Controls	36	16
Exposed — Surface slick	18	18
Exposed — Submerged	18	6
Exposure time	48 h	32 h
Complete tidal cycles	4	2.7
Water/air temperature	21°C	20°-22°C
Actual volume of oil applied	100 ml	97 ml
Slick thickness calculated:		
"High tide"	0.55 mm	0.53 mm
"Low tide"	1.53 mm	1.49 mm

At the end of the exposure, the bulk oil was skimmed from the surface at "high" tide, and the organisms were immediately placed in aerated aquaria containing seawater medium. The water was changed daily and the mussels fed a clam-based diet three times a day (LaRoche et al. 1970). Groups of the two sets of mussels were removed from these aquaria for analysis 1, 7, 14, and 35 days after the end of the exposure experiments.

The paraffin hydrocarbon analysis techniques for marine organisms have been described by Clark and Finley (1973b). Mussels were sampled and analyzed in groups of two to six individuals; analysis was run on the combined tissue and body fluids. No hydrocarbon content of the test seawater medium nor of the clean seawater was determined. Care was taken to minimize contamination of the shucked meats from oil that might have adhered to the shell, particularly in the early samples (1 and 7 days). All results have been reduced to "parts-per-million (ppm) dry extract-

ed-weight" basis (10^{-6} g of *n*-paraffin hydrocarbon per gram of the sum of the dried residue plus the solvent extractables).

RESULTS

Mortality Studies

Acute toxicity studies were not intended to be a major portion of this investigation since our paraffin hydrocarbon analysis techniques are used primarily on surviving organisms that have taken up oil pollutants at levels below that detectable by sight or smell.

The percentage of cumulative mortality (Figures 2 and 3) shows an approximate doubling for mussels exposed to the No. 2 fuel oil compared with the mussels exposed to the No. 5 fuel oil, although the duration of exposure was also greater for the No. 2 fuel oil (46 h vs. 32 h). Both the slick-exposed and the submerged specimens in the No. 5 fuel oil had a slightly higher mortality than the controls, but these differences might not be significant because of the small number of organisms used. The No. 2 fuel oil slick-exposed specimens, however, showed a mortality over twice that of the controls. The submerged specimens had a low initial mortality, but after 2 wk mortality had increased to the level of the slick-exposed specimens.

These mortality data provide a comparison of the two petroleum pollutants and of the test and control groups but were not further utilized to compute median tolerance limits which were beyond the scope and objective of these preliminary qualitative studies.

Griffith (1970) reported no mortalities in mussels exposed to aged crude oil for four tidal cycles over a total of 120 h. When the same mussels were placed in clean seawater, their rate of recovery was assessed by noting that byssal reattachment required 18 h for 50% of the exposed organisms but less than 12 h for the controls. No observations beyond 120 h (5 days) were reported.

No. 2 Fuel Oil Studies

Groups of mussels were collected 1, 7, 14, and 35 days after their removal to clean seawater medium. The *n*-paraffin hydrocarbon patterns for one set are presented as an example (Figure 4). If one assumes that the hydrocarbon pattern of the "controls" represents the natural or biogenic paraffin hydrocarbons and that the pattern of the "test" specimens represents the biogenic plus the pollutant, then by subtracting the former from the latter the resulting "residual" pattern might be expected to depict the pollution hydrocarbon pattern of the petroleum product tested. The "residual" paraffin patterns are shown for the No. 2 fuel oil bioassay study for mussels sampled 1 and 7 days (Figure 5) and 14 and 35 days (Figure 6) after removal from the pollutant.

The shape of the residual paraffin patterns for all four sampling periods approximates that of the pollutant below *n*-$C_{27}H_{56}$ and above a residual content level of 0.050 ppm., although individual paraffin hydrocarbons may show variation from the smooth, nearly bell-shaped curve for the No. 2 fuel oil. The quantities of uptake and loss of *n*-paraffin hydrocarbons from the No. 2 fuel oil by the mussels are shown in Figure 7. The uptake after

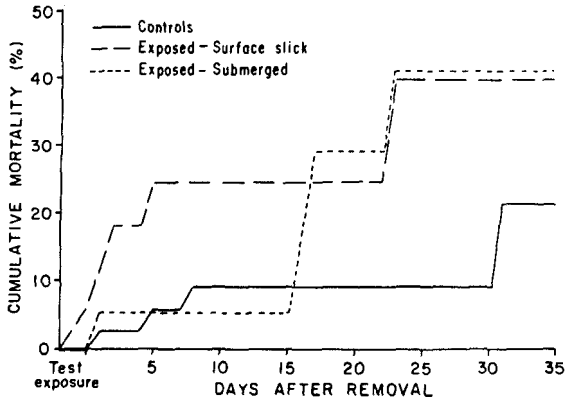

FIGURE 2.—Cumulative mortalities of mussels for 35 days following a 48-h exposure to a No. 2 fuel oil.

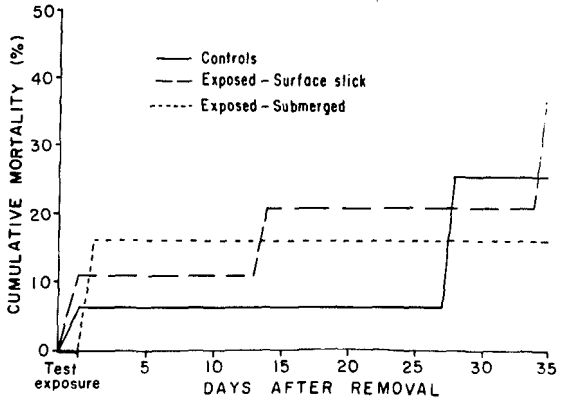

FIGURE 3.—Cumulative mortalities of mussels for 35 days following a 32-h exposure to a No. 5 fuel oil.

48-h exposure plus 1 day in clean medium was 110 ppm. for the slick-exposed specimens compared with only 29 ppm. for the submerged specimens. Within 7 days the residual content had dropped by nearly 75%, after which it declined at a slower rate but was still significantly above background (8 ppm.) after 35 days in the slick-exposed specimens.

No. 5 Fuel Oil Studies

The residual paraffin hydrocarbon pattern (Figure 8) for mussels exposed to a No. 5 fuel oil revealed a definite uptake of pollutant hydrocarbons at the end of the 32-h exposure, and the specimens collected 7 days later from clean seawater medium contained less than 1 ppm. total residual paraffin hydrocarbons attributable to the pollutant (Figure 7).

Gas-liquid chromatography of the saturated hydrocarbon fraction of the exposed mussels revealed a series of branched-chain hydrocarbons from below C_{14} to C_{26}, but of this series only pristane was quantified and included in the calculations. Most unsaturated and aromatic compounds were separated from the saturated fraction at the silica gel/alumina column chromatography stage without further analysis.

DISCUSSION AND CONCLUSIONS

These experiments, although preliminary in nature, provide four basic conclusions: 1) mussels rapidly took up pollution hydrocarbons during exposure; 2) mussels lost pollution hydrocarbons when removed from the test tanks and held in clean seawater (depuration), but significant quantities of No. 2 fuel oil remained for 35 days; 3) the *n*-paraffin residual pattern (exposed levels minus control levels) established for the exposed mussels nearly duplicated the shape of the pollutant hydrocarbon pattern; and 4) these laboratory results confirmed analyses made on shellfish following actual oil spills in the marine environment.

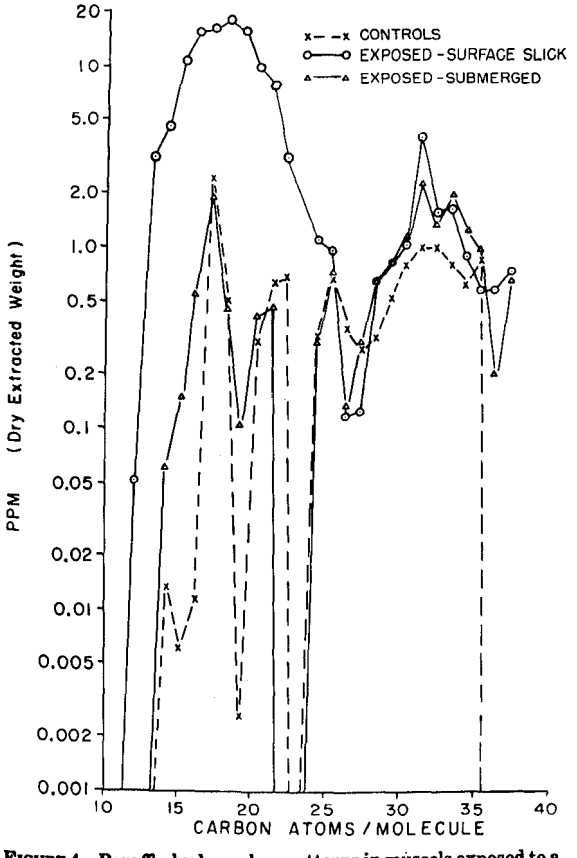

FIGURE 4.—Paraffin hydrocarbon patterns in mussels exposed to a No. 2 fuel oil: 1 day after removal.

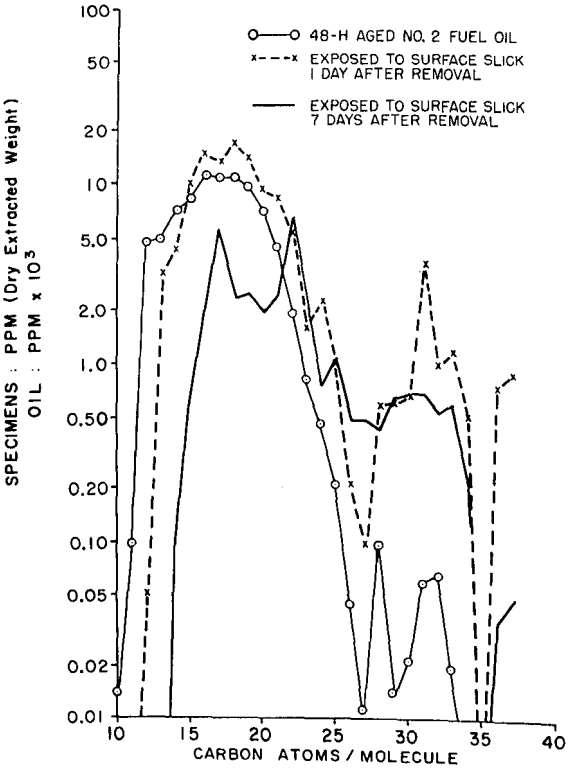

FIGURE 5.—Residual paraffin patterns of mussels exposed to a No. 2 fuel oil: 1 and 7 days after removal.

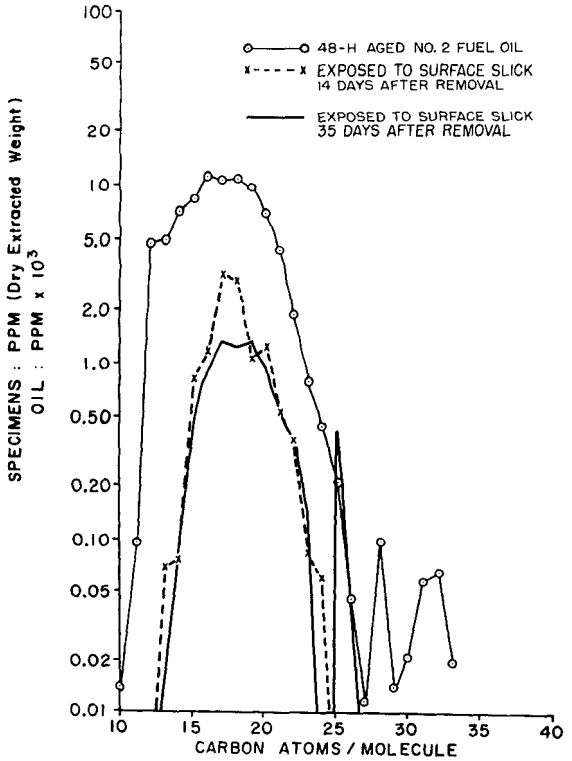

FIGURE 6.–Residual paraffin patterns for mussels exposed to a No. 2 fuel oil: 14 and 35 days after removal.

The mussels rapidly took up a wide range of petroleum paraffin hydrocarbons. Lee, Sauerheber, and Benson (1972) had found this to be the case for mussels where [^{14}C] heptadecane was detected within 15 min. The level of hydrocarbons we found in our mussels exposed to No. 2 fuel oil represents less than maximum uptake because after exposure they were held 24 h in clean seawater before sampling. The No. 5 fuel oil specimens were collected immediately after their 32-h exposure to the oil, and extreme care was exercised to avoid contaminating the tissue with any oil adhering to the outside of the shell when shucking prior to extraction.

Griffith (1970) suggests that the attachment of the byssal threads by the mussel could be affected by the exposure to petroleum. The byssal attachment is made by means of a grooved foot, which is extended from the shell and placed in contact with the substratum. Glandular secretions of collagen mixed with phenolic protein run from the foot groove, become attached to the substratum, and during withdrawal of the foot undergo tanning by the action of polyphenoloxidase (Pujol 1967). It is

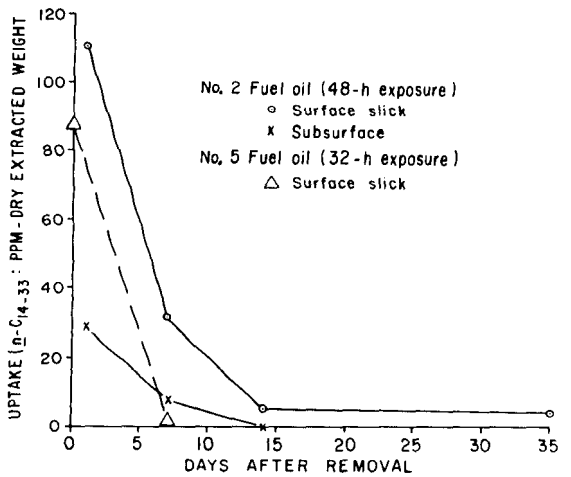

FIGURE 7.–*n*-Paraffin hydrocarbon uptake and loss of fuel oil by mussels.

not certain whether the oil upsets this chemical process or inhibits the muscular actions of the foot necessary to anchor the byssal thread.

When other mollusks such as the American oyster, *Crassostrea virginica*, were exposed to an

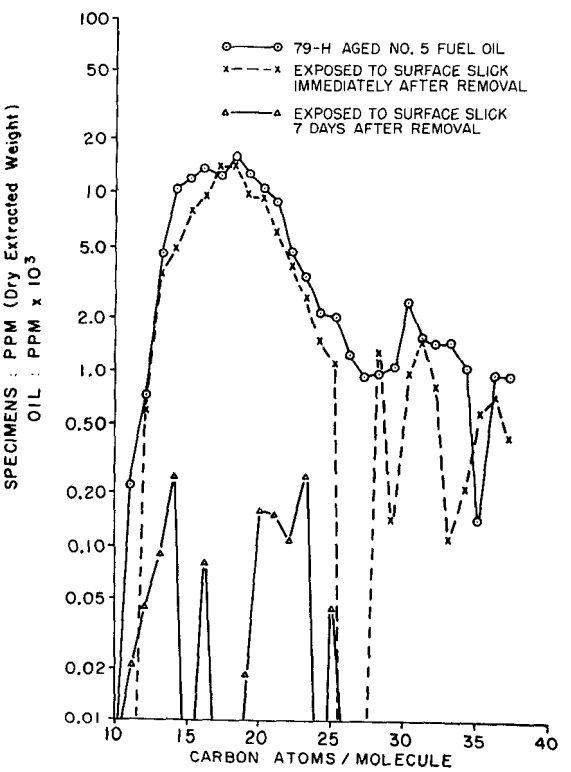

FIGURE 8.–Residual paraffin patterns for mussels exposed to a No. 5 fuel oil.

oil-water mixture, as distinct from our nonagitated surface oil slick under tidal conditions, they also showed rapid uptake. Anderson (1973) found uptake of No. 2 fuel oil to be greatest in the first 24 h with lower uptake at longer periods. By comparison, he found uptake in clams, *Rangia cuneata*, reached a maximum in 72 h, and greater concentrations in the clam tissue were reached than for oysters. Aromatic hydrocarbons showed a greater uptake in the mollusk tissue than saturated forms.

Stegeman and Teal (1973), using a flow-through exposure system, showed that oysters initially took up a No. 2 fuel oil-water mixture in direct relation to the hydrocarbon concentration in the water up to at least 450 μg/liter, above which they remained closed. They also found an enrichment in aromatic fractions compared to the saturated fraction, and under long-term exposure (49 days) the latter fraction showed a progressive decrease in amount with increasing length of exposure.

Under our conditions the No. 5 fuel oil-exposed specimens took up less *n*-paraffin hydrocarbons than the No. 2 fuel oil-exposed mussels, and the former also lost them faster. Both sets of specimens had depurated their residual hydrocarbons by 75% within 1 week, but the No. 2 fuel oil-exposed mussels still contained detectable amounts at the end of 35 days. Lee, Sauerheber, and Benson (1972) found that mussels would discharge over 90% of the incorporated mineral oil after several days, a result they confirmed with labelled *n*-heptadecane.

Anderson (1973) found that oysters lost 94% of the saturated hydrocarbon uptake but only 82% of their aromatics after 13 days when exposed to a No. 2 fuel oil; after 52 days no residual pollutant hydrocarbons were detected at the 0.5-ppm. level (wet weight). Exposure to South Louisiana crude oil showed detectable but low levels of saturates but no detectable aromatics after 27 days depuration. Stegeman and Teal (1973) also found a rapid loss of hydrocarbons from oysters exposed to No. 2 fuel oil-water mixture, but a persistent portion (34 ppm. wet weight above background) remained.

These preliminary experiments did not provide results as to the mechanisms or pathways of petroleum hydrocarbon uptake by the contaminated mussels. The mechanisms of uptake and transport of pollutant hydrocarbons from the environment into organisms may have a very important effect on the degree to which the subsequent depuration is reversible. For instance, hydrocarbons transported across gill membranes in solution or as emulsified droplets enter the bloodstream of fishes very rapidly and can be rapidly depleted on removal of the pollutant (Roubal 1974). On the other hand, hydrocarbons in food sources are resorbed at a different site, which, for the basking shark occurs in the spiral valve where they are transferred to the liver and remain highly persistent (Blumer 1967).

The residual paraffin hydrocarbon patterns showed a strikingly similar pattern to the aged pollutant, yet the organisms appeared healthy and had no visible contamination or oily odor. Lee, Sauerheber, and Benson (1972) indicated that gas-liquid chromatograms of mineral oil in mussels were like the original mineral oil except for some loss of the short-chain paraffin hydrocarbons. Blumer et al.(1970) used gas chromatograms of oysters and scallops contaminated by a No. 2 fuel oil to show that the patterns had the same general features as the chromatogram of the fuel oil.

Anderson (1973) found an *n*-paraffin hydrocarbon pattern in clams similar to the high-aromatic No. 2 fuel oil; however, the maximum hydrocarbon in the clam (n-C_{17}: approximately 1.6 times more than n-C_{16} and 1.9 times n-C_{18}) was one carbon number higher than the pollutant maximum (n-C_{16}: 1.1-1.2 times the adjacent paraffins). Stegeman and Teal (1973) showed gas chromatograms of contaminated oysters having a pattern similar to the pollutant and a maximum *n*-paraffin concentration of C_{18} or C_{19}.

Our presentation of petroleum hydrocarbon uptake as evidenced by *n*-paraffin analyses is not a complete picture of petroleum contamination since it reflects only a portion of the total hydrocarbons and non-hydrocarbons in the oil. Also, the various hydrocarbon components of the petroleum are not necessarily available to the organisms in the water in the same proportion as they exist in the original petroleum. Vaughan (1973) reported an enrichment of methylnaphthalenes compared to n-$C_{12\text{-}20}$ paraffins of 15:1 in oysters exposed to a South Louisiana crude oil (50 μg/ml) in a seven-day bioassay experiment and an enrichment in the water extracts of about 3:1. Further, the *n*-paraffin content of petroleum pollutants in shellfish is often depleted with time relative to that of the source (Stegeman and Teal 1973). Therefore, while our values for pollutant uptake based on *n*-paraffin hydrocarbon analyses yield conservative estimates, they demonstrate that these experimentally simple methods can be

useful. Aromatic hydrocarbons, which are a biogenic rarity yet often a major component of petroleum and its refined products found to be rapidly taken up by marine organisms, may be rapidly lost from contaminated organisms (Lee, Sauerheber and Dobbs 1972; Stegeman and Teal 1973; Anderson 1973). Consequently, the utility of these compounds in marine pollution monitoring programs and in long-term bioassay experiments may be somewhat limited.

We previously reported (Clark and Finley 1973b) uptake by mussels, *M. edulis* and *M. californianus*, of petroleum *n*-paraffins following oil spills in the marine environment. A No. 2 fuel oil spill resulted in considerably greater uptake (10 ppm. of n-C_{17}) than for mussels exposed to Navy Special fuel oil residue (nearly 1 ppm. of n.-C_{17}); however, in both cases it was obvious that the apparently healthy mussels had acquired an *n*-paraffin hydrocarbon pattern like that of the pollutant. By creating an oil slick and a tidal system within an aquarium under laboratory conditions, it is possible to show that these earlier findings can be reproduced. These results add further support to the data of other investigators who used different approaches and analytical techniques.

We did not analyze hydrocarbon levels in specific organs, conduct metabolic studies, or determine aromatic content. Stegeman and Teal (1973) and Anderson (1973) found that aromatic hydrocarbons were often enriched in oysters in preference to the *n*-paraffins.

While we have given percentage loss of pollutant paraffin hydrocarbons in our presentation as well as actual concentration levels (Figure 7), the percentage value is very dependent on both the level of initial pollution exposure and on the lower limit of sensitivity of the experimental method for detecting pollutant uptake near biogenic background concentrations in marine organisms. Thus, one might have two sets of organisms showing similar concentrations of residual pollutant after considerable depuration but with a dramatically different percentage loss as a result of different exposure levels.

The variation of uptake and loss of petroleum hydrocarbons in marine organisms most certainly is related to the magnitude of the exposure—the amount of the pollutant and the duration, as well as physical and chemical properties of the pollutant.

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