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FINAL REPORT

Hydrocarbon Analysis of a Shellfish sample from the Tampa Bay Area.

Submitted To:
Florida Department of
Environmental Protection
Office of Coastal Protection

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We report here results of the hydrocarbon analysis of a clam sample collected November 19, 1993 by Cesar Rodriguez of the Florida Department of Environmental Protection. Three clams were collected at the station which had previously shown the highest tissue hydrocarbon levels (Site 5: N.E. Side of N. Skyway Causeway). These clams were composited and subsampled for analysis. Clams from the control station (Emerson Point) were also analyzed as a referent. All methodologies were identical to those outlined in our previous report (Mote Marine Technical Report # 335).

There were no petroleum related hydrocarbons detected in the clam sample analyzed. The hydrocarbons present were restricted to clusters of biogenic hydrocarbons and were found in both samples analyzed. This indicates that the petroleum hydrocarbons observed in the clams sampled at this station on September 29th have been depurated.

These samples were amended with individual aliphatic and aromatic compounds prior to extraction (recovery surrogates). These surrogates went through the entire analytical process and were determined to have 92% and 90% recovery respectively in the sample from the northeast side of the Skyway. This indicates the efficiency of the extraction method and shows that if there had been hydrocarbons present, we would have detected them.

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FINAL REPORT

Analysis of Shellfish Samples from the Tampa Bay Area Following the August 10th Oil Spill

FL. D.E.P. Contract P.O. #S 3700 431111

Submitted To: Florida Department of Environmental Protection
Office of Coastal Protection

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SUMMARY

Clams from several locations in waters north of Mullet Key were found to be free of oil spill related contamination, while clams from other sites in the same general area were found to have elevated levels of hydrocarbon contamination which was related to the oil spill. Hydrocarbon profiles in the clam samples indicate that these organisms were exposed through the water column and have tissue burdens only of *n*-alkanes, which should depurate rapidly. These levels were not detectable organoleptically (C. Rodriguez, Personal Communication).

The oyster sample collected in John's Pass was found to be grossly contaminated by oil. This organism had significant tissue burdens of not only the *n*-alkanes, but also polycyclic aromatic hydrocarbons and other cyclic compounds. The body burden of these organisms is thought to be of sufficient concentration that detrimental effects are likely to be observed in the impacted population. The oil related components found in this sample are not only potentially toxic to the oysters themselves, but would also be detrimental to any organisms preying on the stressed oysters.

INTRODUCTION

Significant quantities (5 to 9 thousand barrels) of number 6 fuel oil and an undetermined amount of jet fuel were spilled in the mouth of Tampa Bay following the August 10th collision of the freighter *Balsa 37* with the barges *Ocean 255* and *B155*. Shifting winds and current forces moved the majority of the spilled oil out of Tampa Bay and into the Gulf of Mexico in the days following the spill. Changes in wind direction several days later brought oil back to Gulf beaches and the inshore waters north of the mouth of Tampa Bay.

Concern then arose about the impact of oil contamination of shellfish and the potential for effects on both the health of the shellfish community and consumers of shellfish. In an attempt to evaluate if there was oil contamination of selected shellfish beds, 6 composited shellfish samples were collected from areas of possible or known oil impact, and the results of these analyses are presented here. Criteria for public health concerns, established by the U.S. Food and Drug Administration, utilize organoleptic detection as an initiual screening technique to identify oil contaminated shellfish. Using the organoleptic technique, shellfish are considered contaminated if oil is detectable by smell, taste or sight. Chemical analysis by gas chromatography also is used to provide quantitative and qualitative analysis of oil contaminants.

BACKGROUND

In order to set the stage for the results of the shellfish analyses discussed below, we must first provide a very brief overview of factors which may affect the accumulation and depuration of hydrocarbons by bivalves. Most of the research on accumulation of petroleum products by bivalves has been performed using *Mytilus edulis*, though a reasonable amount of research has used the northern clam *Mercenaria mercenaria*. The clams discussed in this report are of the species *Mercenaria campechiensis*, and the oysters were of the species *Crassostrea virginica*. The discussion presented here is based on available literature,

however, it should be understood that some differences in behavior are to be expected between species.

Shellfish can accumulate hydrocarbons from either the dissolved or particulate phases of the water column. Dissolved hydrocarbons are accumulated across the gill surface, particulate hydrocarbons may be ingested and accumulated *via* absorption in the gut (Widdows *et al.* 1982). Maximum tissue concentrations are dependant on the lipid content of the organism, and thus, may vary with age, season, sexual maturity, and will definitely differ from species to species. Actual tissue burdens are determined by both the mode and concentration of exposure. If water column concentrations are high enough, bivalves may stop or slow syphoning in an attempt to minimize exposure (Widdows *et al.* 1982). At lower concentrations or if the hydrocarbons are present with the organisms food, the bivalves continue to filter water and greater exposure may result. It has also been determined that the mode of accumulation can play a role in where (which tissues) hydrocarbons are initially deposited in the organism (Lee *et al.* 1972, Widdows *et al.* 1982), prior to the hydrocarbon burden reaching equilibrium with all available tissue pools.

Once the hydrocarbons have been accumulated, the question becomes, how long will it take to depurate the organisms body burden. Depuration depends on several factors, these include: the total body burden, length of exposure, type of hydrocarbon accumulated, mode of accumulation and the species being studied (Lee 1977). Elimination of accumulated hydrocarbons is essentially entirely due to depuration since bivalves have only a small capacity to metabolize hydrocarbons (Livingstone 1985). If tissue concentrations remain below the level of acute toxicity, depuration will begin as soon as environmental concentrations decrease. Hydrocarbons accumulated from a single short exposure (such as the oil spill) should be depurated relatively quickly (days to months) depending on hydrocarbon type (aliphatic or aromatic) and exposure mode (food or water). Given only an acute exposure, aliphatic hydrocarbons (alkanes) accumulated from the water are reported to depurate quickly, usually in 2 to 6 weeks (Lee 1977). Aromatic hydrocarbons and either type of hydrocarbon accumulated from food tend to be retained in tissues for longer periods of time. Reports of depuration range from 2 weeks to greater than 8 weeks (Lee 1977, Tanacredi and Cardenas 1991). Chronic exposure allows the hydrocarbons to equilibrate with all the lipid pools within an organism and results in longer depuration times.

METHODS

Sampling

We report here results for 5 clam and 1 oyster samples. All clam samples were collected in 2 feet of water by Cesar Rodriguez of the Florida Department of Environmental Protection, on September 29, 1993. Three clams were collected at each of 5 sites, these clams were composited and subsampled for analysis. The coordinates for each sampling station are shown in Table 1, with the results. Site locations were described by Mr. Rodriguez as follows:

Site 1: Emerson Point, off marker #7. Mote # H-93-2090.
Site 2: Bunces Pass, in front of marker #5. Mote # H-93-2091.
Site 3: Madelain Key, east side. Mote # H-93-2092.
Site 4: N.E. Tip of Mullet Key. Mote # H-93-2093.
Site 5: N.E. Side of N. Skyway Causeway. Mote # H-93-2094.

The oyster sample was collected by Mote personnel on August 17th from the root props of Mangroves on the west side of Elinor Island in John's Pass (Mote # H-93-2063). These samples were wrapped in aluminum foil and stored over ice for return to the laboratory. The clams were refrigerated until shucked (<24 hours), the oyster sample was stored frozen. Observations for evidence of oil contamination were made during collection and processing of the shellfish. The oysters were visibly oiled externally, with oil smell evident on opening. None of the clams visually exhibited oil contamination externally, nor internally, they also did not have an evident oiled smell (organoleptic detection).

Extractions

Clam samples were shucked and composited (3 clams per site) using a Virtus bladed homogenizer (model 23). Wet tissue weights for each of the clams were obtained prior to homogenization. Due to an oversight, individual clam shell sizes were not recorded. The oyster cluster was allowed to partially thaw before shucking. Oysters were broken from the cluster and individually shucked and composited. Subsamples of each clam homogenate, and the entire oyster composite, were separately combined with pre-cleaned anhydrous sodium sulfate ($NaSO_4$) and extracted with DCM using a Tekmar Tissumizer. Solvent and tissue were separated using vacuum filtration and the filter cake was re-extracted with fresh DCM (total of 3 extractions per sample). Extracts were reduced in volume using rotary evaporation. The concentrated extract was passed over anhydrous sodium sulfate, taken to just dryness under a stream of nitrogen and redissolved in hexane and submitted to compound class separation.

Compound Class Separations

The hydrocarbon fraction was isolated using combined silica gel - alumina columns. Stationary support materials were extensively cleaned *via* sonication in methanol, DCM and hexane, dried and then activated at 210°C. Upon cooling, the support materials were deactivated 5% with HPLC grade water and stored under hexane. Approximately 12 gm of silica and 6 gm of alumina were sequentially slurry packed into chromatography columns with gentle tapping to ensure uniform packing. The sample (in hexane) was applied to the column

in a small volume (<0.5 ml) and the container sequentially rinsed (2x) with 0.25 ml hexane. The sample was eluted from this column using 10 ml of hexane and 30 ml of 20% DCM in hexane.

Gas Chromatography - Quantitation

The hydrocarbon fraction was taken up in a solution of quantitation standard and analyzed by gas chromatography with flame ionization (GC/FID). All quantitations were performed against these standards which were added at known concentrations just prior to analysis via GC/FID. Selected confirmatory analyses were performed using gas chromatography with mass spectral detection (GC/MS). GC/FID analyses were performed on a Varian 6000 GC with data being collected, stored and analyzed using a P.E./Nelson 2600 Chromatography Data System (DS) software and interfaces. Mass spectral confirmations were performed on a Varian Saturn II Iontrap GC/MS/DS system.

Quality Control/Quality Assurance Procedures

Precision and accuracy of analyses were assessed by analysis of duplicate and spiked samples. Procedural blanks were also run. Extraction efficiency and potential loss of analytes during sample processing were assessed through the use of recovery surrogates added to the sediment or tissue sample prior to extraction. There were two recovery surrogates used, one to simulate alkane recovery (C18:1) and one to simulate PAH recovery (p-terphenyl-d14). The results of these analyses are discussed in Appendix 1.

RESULTS AND DISCUSSION

The results of the shellfish analyses are presented in Table 1. The discussion of these results will treat the clam and oyster samples separately.

Clams

Two of the clam samples had no significant levels of oil related hydrocarbons, just a cluster of biogenic hydrocarbons eluting between C_{20} and C_{22}. The other 3 stations showed small amounts of oil contamination as well as having these biogenic hydrocarbons. Even the most heavily contaminated clam sample showed the presence of only *n*-alkanes, none of the clams appear to be contaminated with the more toxic components of the oil, such as polycyclic aromatic hydrocarbons (PAH). In fact, the hydrocarbon profile observed in the clam tissues is very similar to that observed in water samples collected in areas of Tampa Bay (Sherblom, unpublished). Both the type and concentration of hydrocarbons found in the clams are good news in several respects. The *n*-alkanes are among the least toxic components of the spilled oil, they degrade quickly, and tend to be depurated by bivalves relatively rapidly. This indicates that there should be little damage to the health of the shellfish community, and also fewer effects on potential consumers of contaminated shellfish. These organisms should depurate their remaining body burden, in fact the body burdens observed are likely to be significantly lower than those which would have been observed immediately after the spill, and the tissue concentrations should have dropped significantly in the last month.

Table 1. Hydrocarbons determined in the shellfish samples[1].

	Latitude (N)	Longitude (W)	Biogenic hydrocarbons	Other hydrocarbons
Site 1: 2090.	27°31'57"	82°37'03"	4.7	0.4
Site 1: 2090 Dup.	27°31'57"	82°37'03"	4.7	0.7
Site 2: 2091.	27°38'54"	82°43'45"	9.7	1.1
Site 3: 2092.	27°38'34"	82°42'43"	9.0	2.0
Site 4: 2093.	27°38'24"	82°42'00"	11.2	0.7
Site 5: 2094.	27°39'34"	82°40'15"	3.0	3.7
Site 5: 2094 Dup.	27°39'34"	82°40'15"	3.0	4.9
John's Pass Oyster	27°47'24"	82°46'55"	0.0[2]	30.8
Spiked Clam[3] (2093)	27°38'24"	82°42'00"	11.0	4.3

[1]The Limit of Detection for these analyses was estimated to be 0.2 μg/gm wet.
[2]These peaks were overwhelmed by the petroleum related peaks in this sample, and thus were not quantitated separately.
[3]The hydrocarbon data for this sample are shown only to provide a comparison of the biogenic hydrocarbon values.

Oysters

The oyster sample collected in John's Pass had extremely high tissue levels of petroleum hydrocarbons. This sample was grossly contaminated with not only alkanes, but also significant concentrations of cyclic and aromatic hydrocarbons. While the oyster tissue sample exhibited slightly greater concentrations of the *n*-alkanes relative to the oil, and these alkanes extend to a slightly higher molecular weight, the tissue was also burdened with the more toxic fraction of the oil. The hydrocarbon profile observed in this sample indicates that these oysters were most likely directly exposed to oil slick, rather than having a dissolved phase intermediary.

These results suggest that the level, and probably mode, of exposure for the two types of shellfish examined were significantly different. While the clams reflect accumulation from the aqueous phase, the oyster sample indicates direct oil contamination, potentially in addition to an aqueous phase mode (thus the increased alkane tissue burden relative to that in the oil). It is likely that the oil content of the oysters is at a toxic level for at least some of these organisms, and that this oil fouling will result in changes in the community of oysters populating the John's Pass area. These tissue burdens are also likely to adversely affect organisms feeding on the distressed oysters. This could result in trophic transfer of the contaminating hydrocarbons and adverse affects to other species which had initially avoided significant impacts from the oil spill.

Chromatographic Interpretation

To provide a feel for the hydrocarbons determined in each of the samples and their relationship to the distribution of hydrocarbons in the oil and the water column we present chromatograms of: a dilution of an oil sample collected after the spill in Figure 1; and the water which was in equilibrium with this oil in Figure 2, and the organisms samples in Figures 3-11. Several alkane peaks are labeled either as "Alkanes" or with their carbon chain length shown as a number (C 16 = hexadecane). The area below the chromatogram labeled UCM is the unresolved complex mixture. All the chromatograms are drawn on the same scale and show the detector response from 10 to 60 minutes (GC program 45 • $C_{1\ min}$, 6 • C/min to 285 • $C_{20\ min}$).

We can see that a number of components of the oil were present in the water in contact with it. The interesting and important factors to note are the relative abundances of different components found in these samples. The alkane profile observed in the oil is one of similar peak heights for the alkanes from hexadecane (C_{16}) to Octacosane (C_{28}). The alkane profile observed in the water sample is quite different, these alkanes show a concentration peak centered around Heptacosane to Octacosane (C_{27}-C_{28}), and the alkane series appears to extend at least to hexatriacontane (C_{36}) in the water sample whereas it drops off rapidly above octacosane in the oil. Also the relative abundance of the alkanes to the area of the UCM is much greater in the water sample than it was in the oil sample.

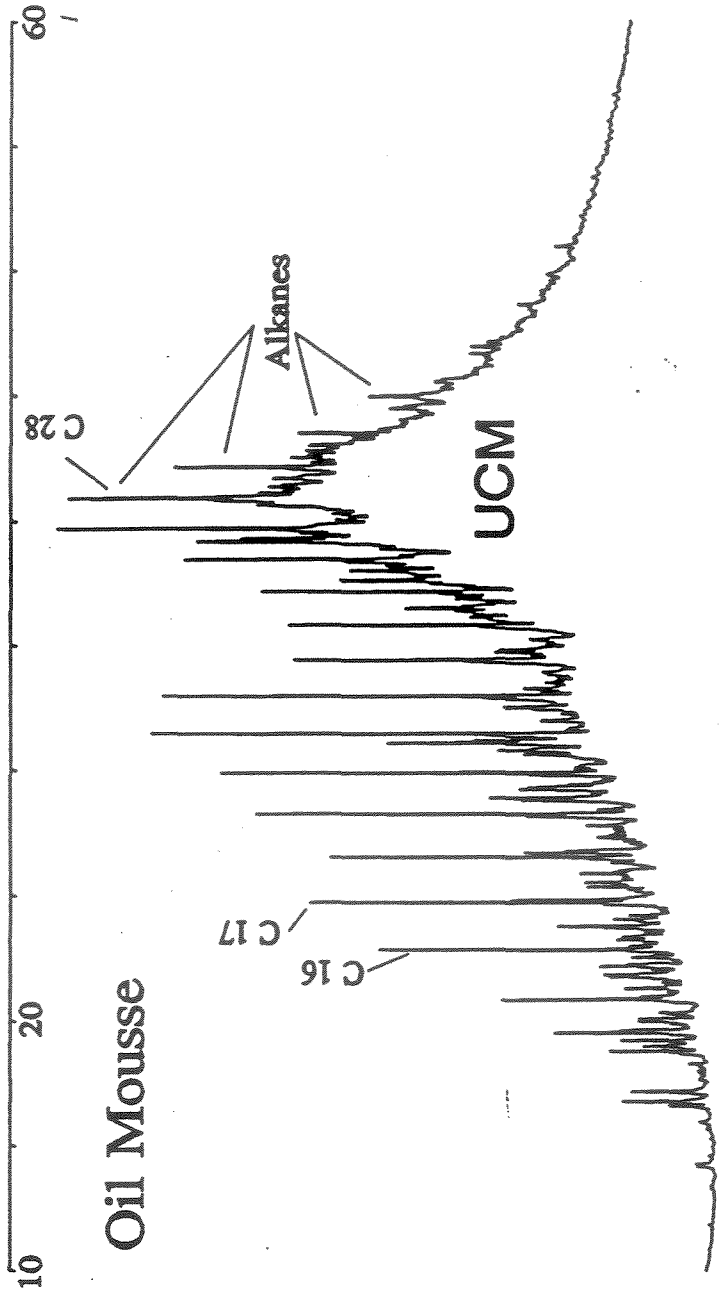

Figure 1. Chromatogram of a diluted oil sample collected off Saint Petersburg Beach on August 17th. Peak labels are discussed in the text.

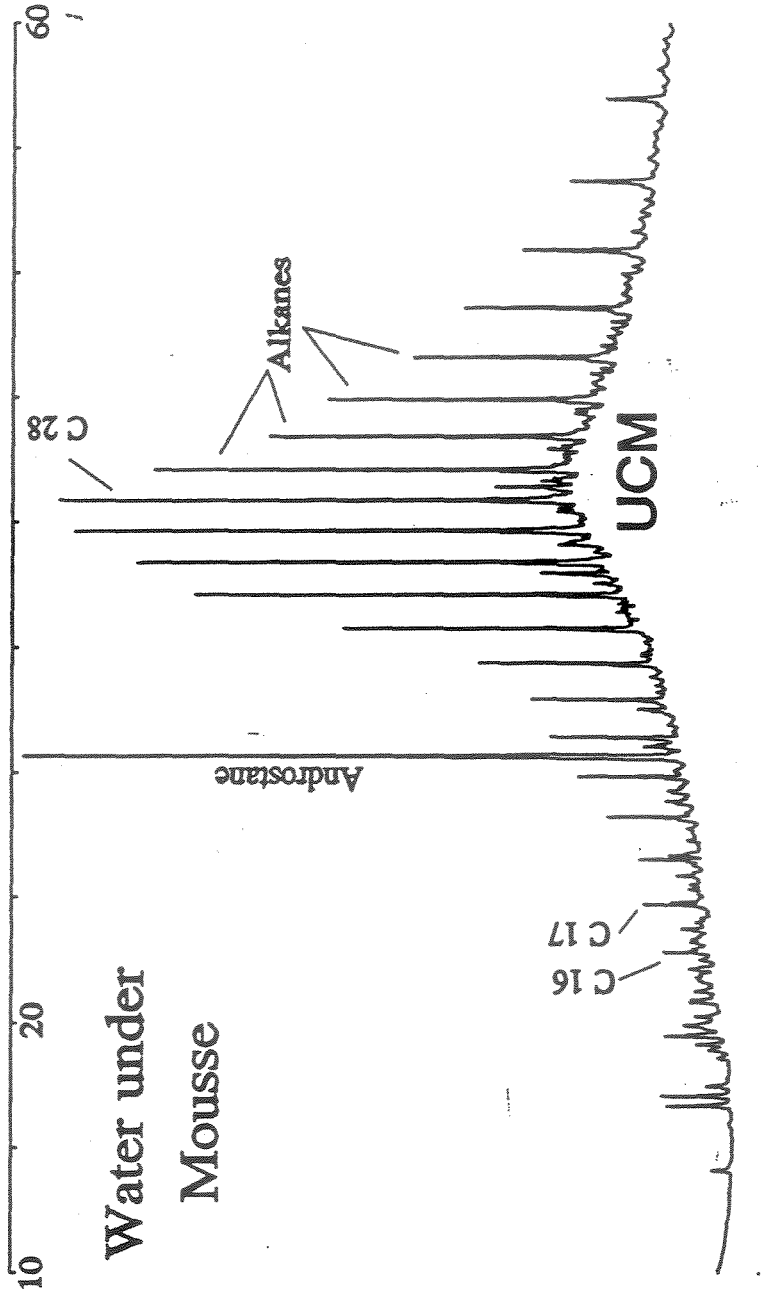

Figure 2. Chromatogram of the hydrocarbons isolated from water under the oil (mousse) sample shown in Figure 1. See text for details.

These differences in the relative abundance of components in the oil versus those in the water would affect the exposure of shellfish which were not directly oiled and be reflected in the hydrocarbon profiles and total concentrations determined in the shellfish samples. Chromatograms of all the shellfish samples analyzed are shown in Figures 3-11. The peak labels in these chromatograms indicate the following compounds: RS 1: 1-Octadecene; QS 1: orthoTerphenyl; QS 2: 1-Docosene; RS 2: paraTerphenyl-D14. Peaks labeled contaminants are phthalates which were inadvertently introduced to the sample extracts during the extraction and workup of these samples. The Biogenic cluster around C_{20} is also noted on each of the clam extract chromatograms.

The appearance of alkanes in some of the clam samples is evident by these chromatograms. The distribution of alkanes found in those clams showing contamination is similar to that observed in the water sample taken from under the oil mousse sample (Figure 2), as well as several water samples collected in the Tampa Bay area after the spill (Sherblom, unpublished). This suggests that these organisms accumulated the alkanes from the water column, and that their oil exposure did not include particulate transport. Exposure to particulate oil contamination would have included many of the less soluble compounds and would cause the tissue burdens to more closely resemble the oil profile.

In the oyster sample (Figure 11) we see not only the alkane signature observed in the clam samples but also other compounds indicative of a more direct contact with the oil. While the oyster sample does also show a higher molecular weight range of alkanes, like the clams, we also found significant concentrations of alkyl-polycyclic aromatic hydrocarbons (PAH). These are the majority of non-alkane peaks observed in the chromatogram shown in Figure 11. These alkyl-PAH are more toxic than the alkanes and indicate that these bivalves were exposed *via* a different mode than were the clams analyzed. Since we know the oysters were collected from a site which was oiled, it then follows that this tissue profile most likely reflects their direct exposure to the oil.

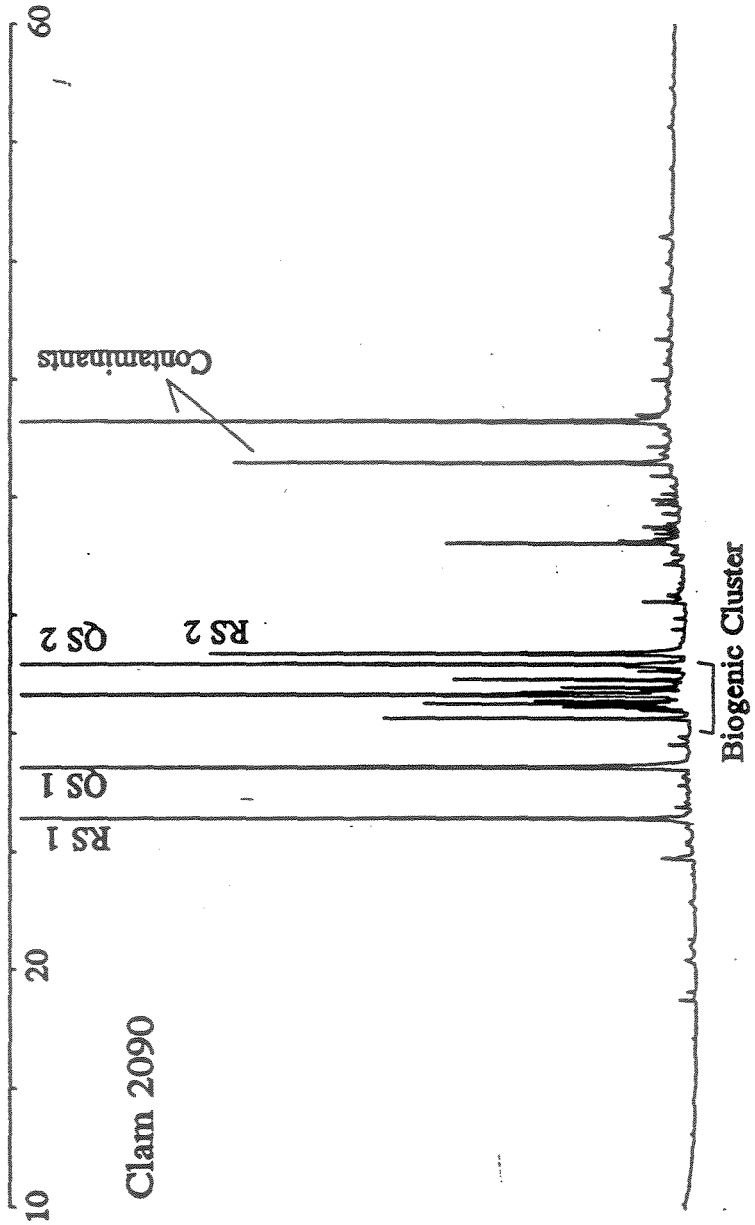

Figure 3. Chromatogram of the hydrocarbon fraction of the extract from clam sample #2090. Peak labels are discussed in the text.

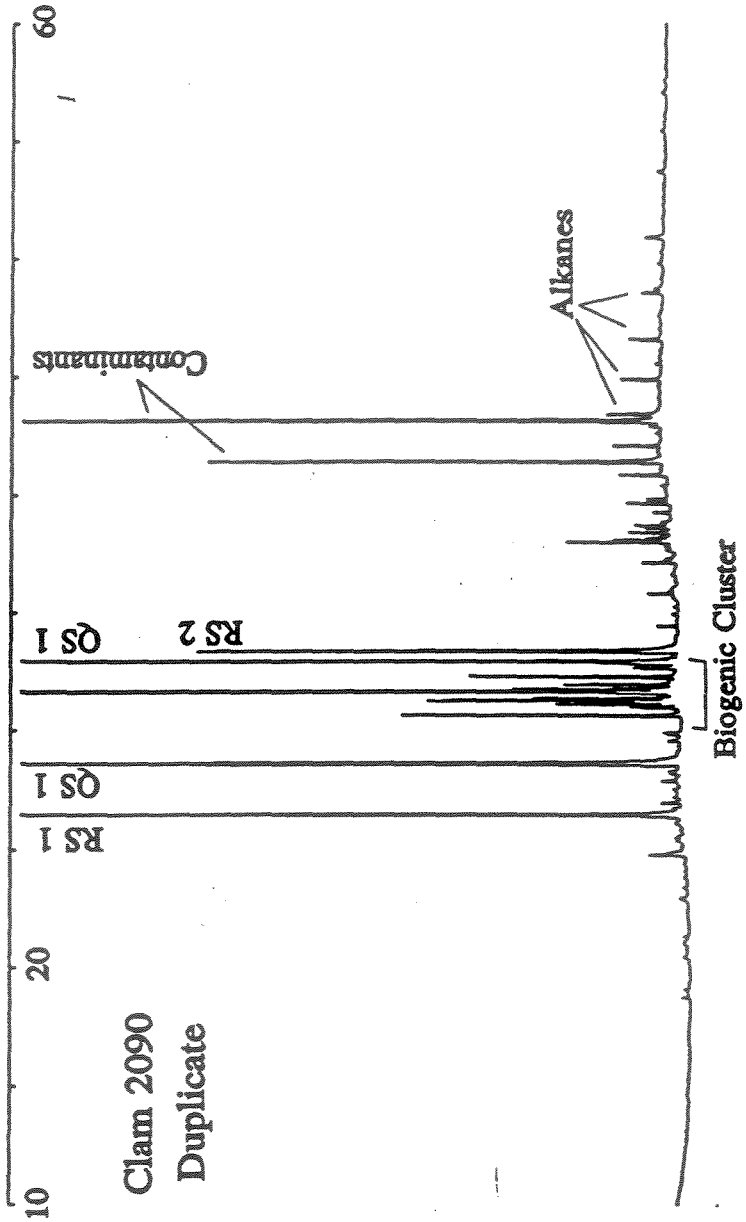

Figure 4. Chromatogram of the duplicate analysis of clam 2090. See text for details.

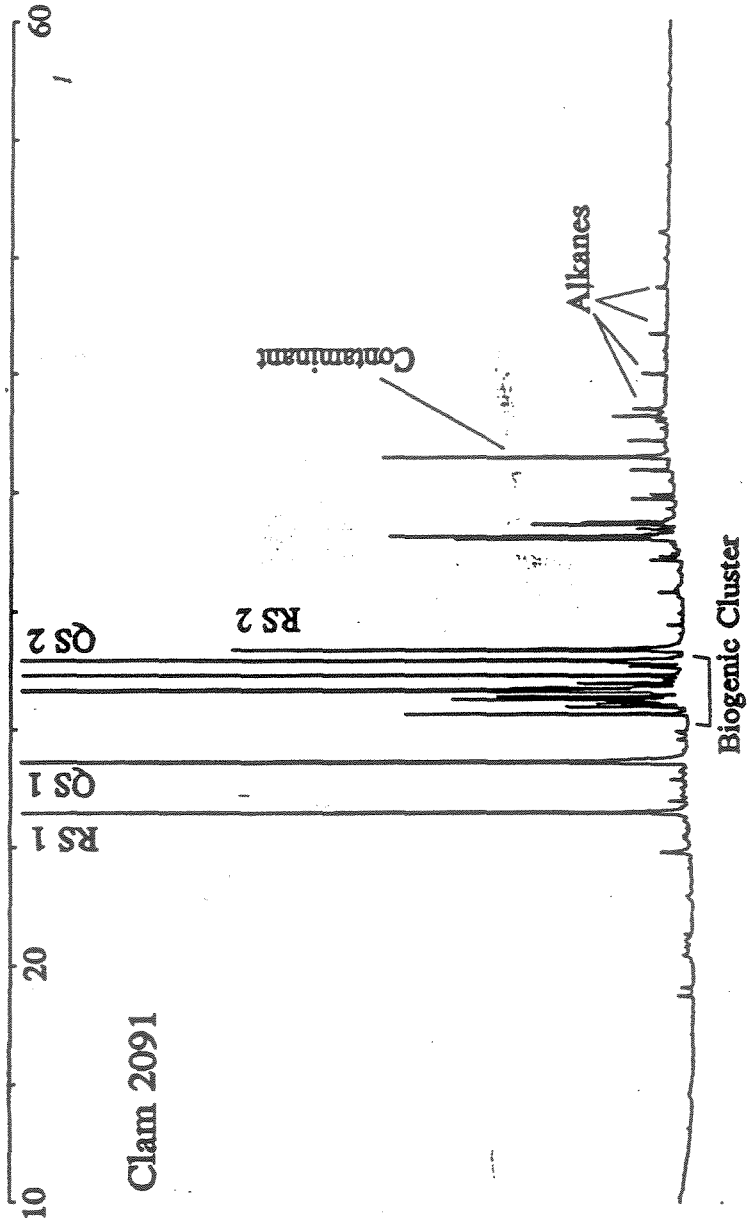

Figure 5. Chromatogram of the hydrocarbons from clam #2091.

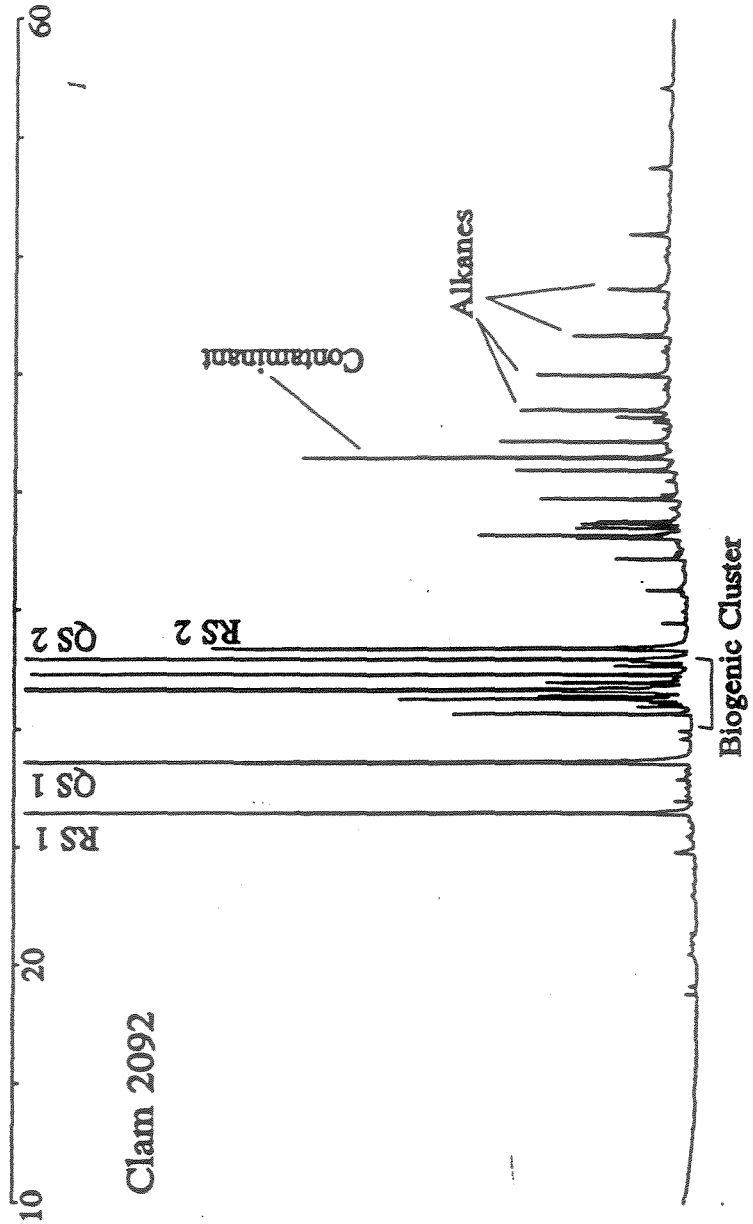

Figure 6. Chromatogram of the hydrocarbons from clam #2092.

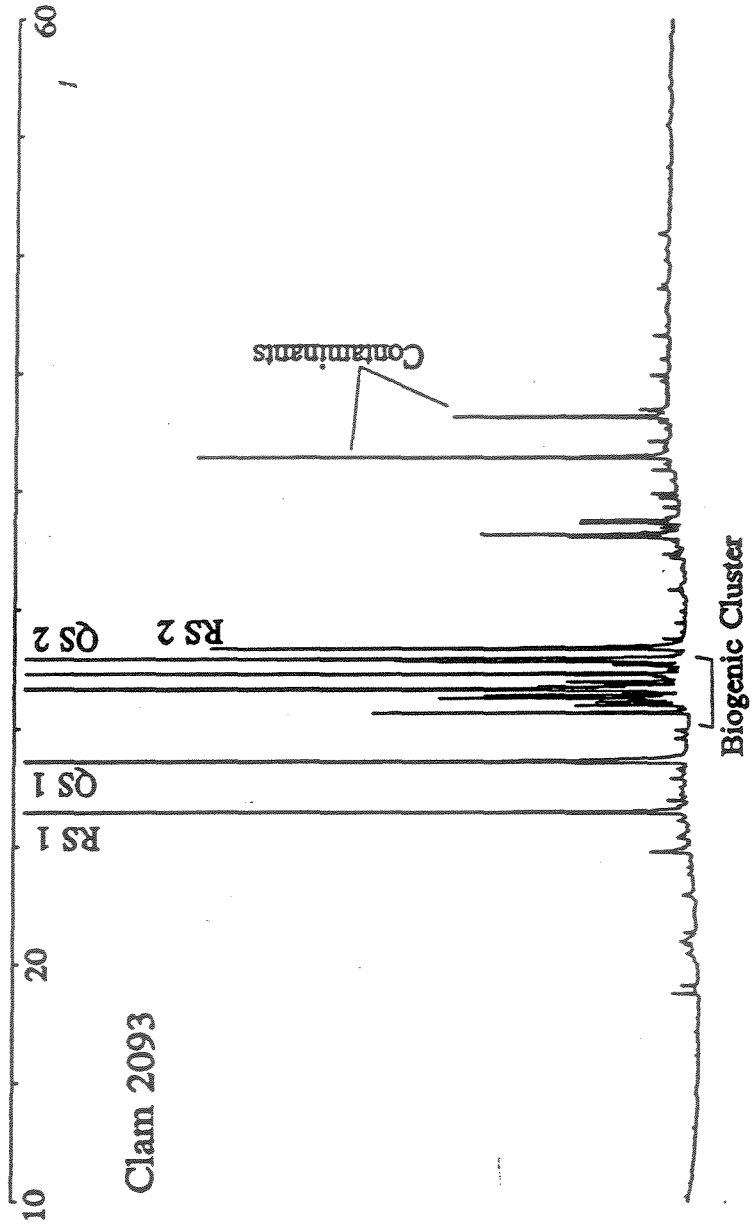

Figure 7. Chromatogram of the hydrocarbons from clam #2093.

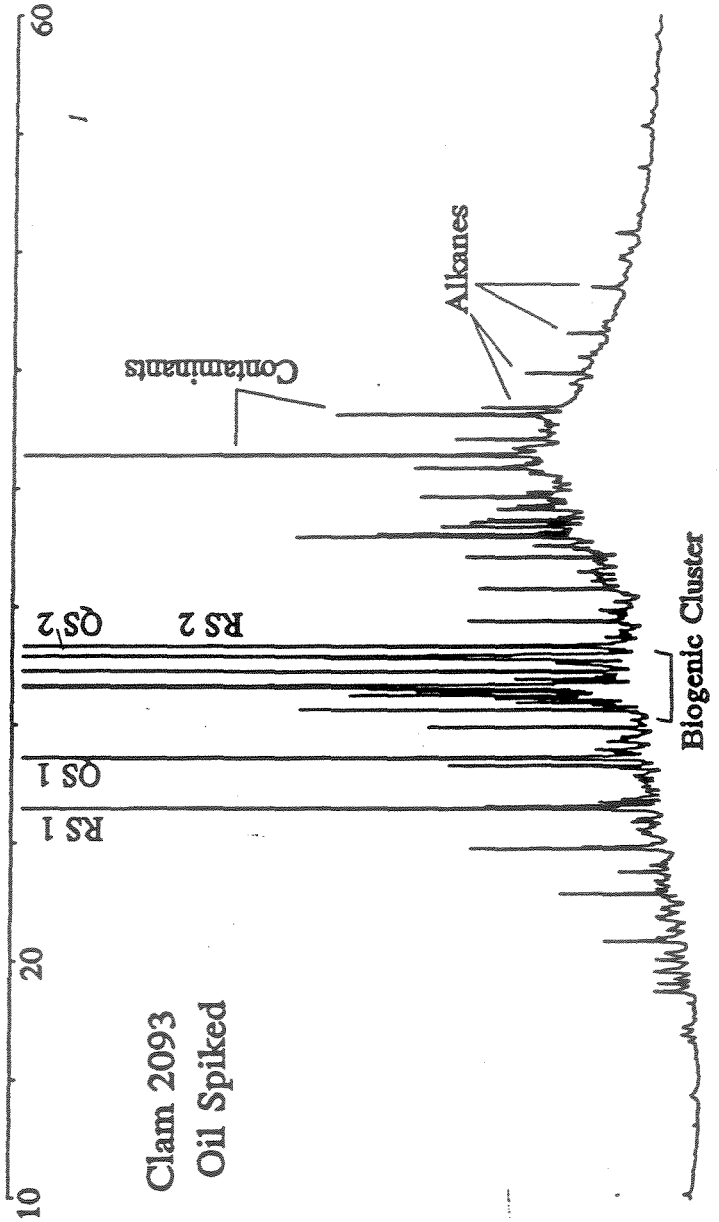

Figure 8. Chromatogram of the hydrocarbon fraction of the extract of a subsample of clam #2093 which had been spiked with oil prior to extraction, see text for details.

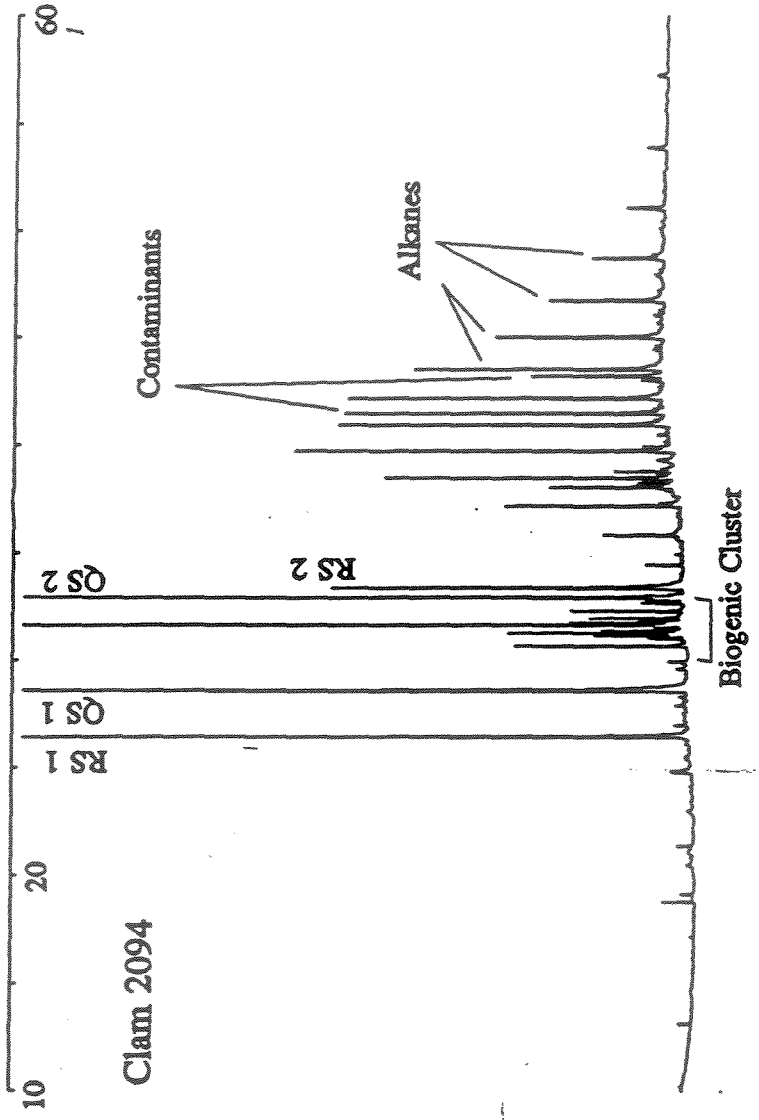

Figure 9. Chromatogram of the hydrocarbons from clam #2094.

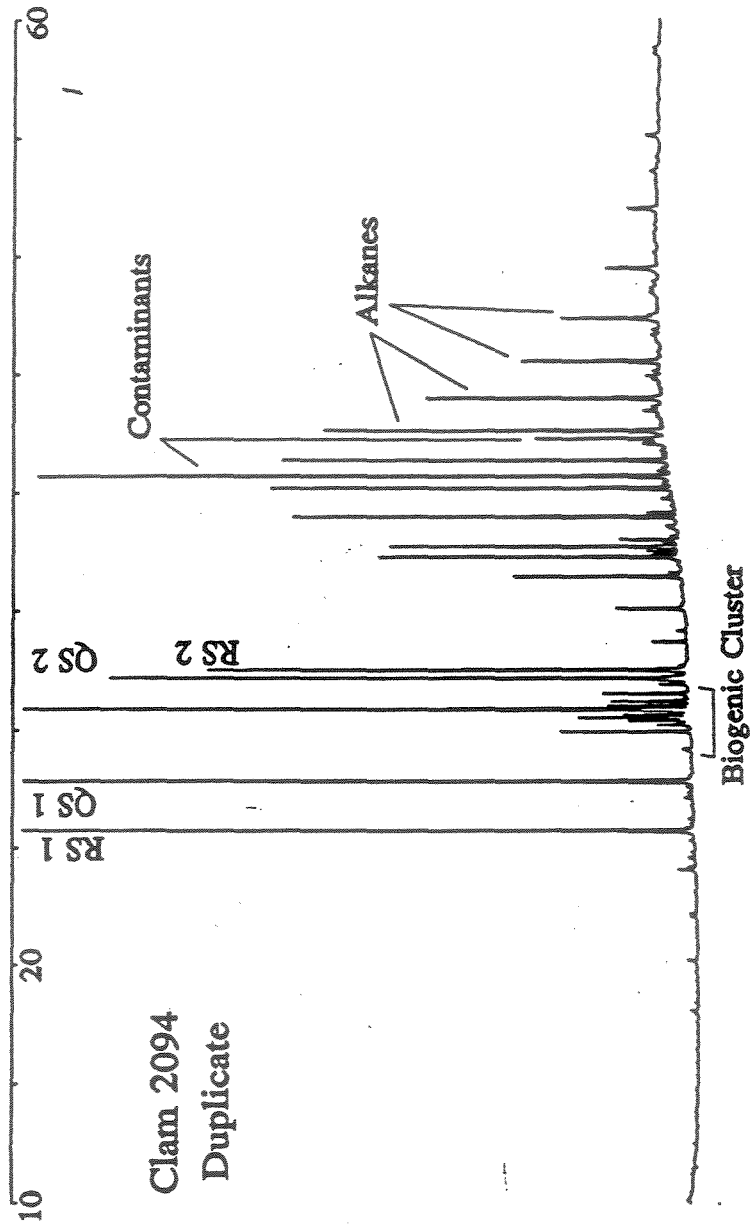

Figure 10. Chromatogram of the duplicate analysis of clam 2094.

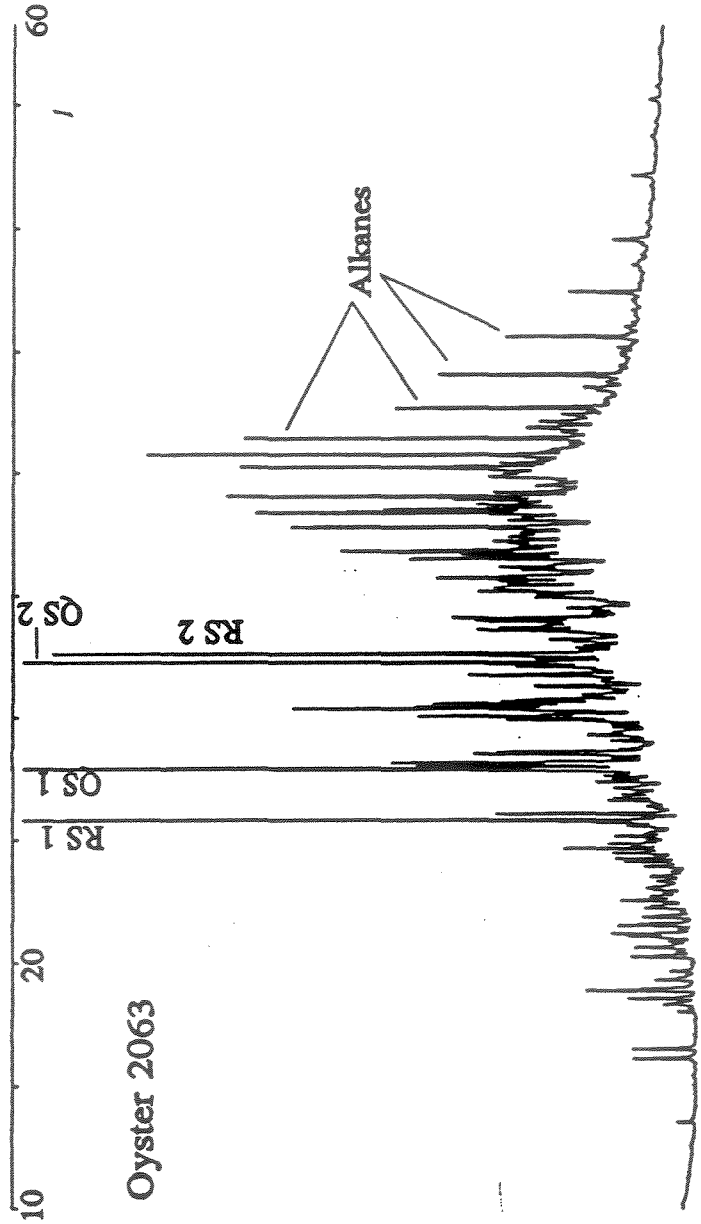

Figure 11. Chromatogram of the hydrocarbons isolated from the oyster sample (#2063). See text for discussion.

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Appendix 1. Discussion of samples analyzed for precision and accuracy.

The biogenic cluster hydrocarbons showed extremely good reproducibility between duplicate analyses of the clam samples, the other hydrocarbons found in these samples were not as reproducible. However, if we compare the results of the total hydrocarbons determined in duplicates we find the two duplicate analyses resulted in total hydrocarbon values which varied by less than 6% and 18%. The sample with greater concentrations of oil hydrocarbons showed greater variability. The limit of detection for these analyses was calculated to be 0.2 μg/gm (wet), this value was calculated using an estimate of a 6 gm wet weight sample. All the shellfish subsamples analyzed in this study were greater than this 6 gm weight. This value is based on the hydrocarbon value one would determine if one had a peak area of 10 times the size of an average "noise" peak in the method blank, divided by a wet weight of 6 gms. Recovery of the compounds spiked into all of the samples ranged from 78 to 111% (except for the spiked sample), indicating there were no unreasonable losses during the analytical workup. Analysis of a spiked clam sample (Clam 2093, Figure 8) shows not only that the method achieved the extraction and analysis of the hydrocarbons in these samples, but also that the molecular weight shift in tissue hydrocarbons was not an artifact of the analytical procedure. The hydrocarbon results presented earlier are repeated in Table 2 along with the data on the recovery of the two recovery surrogates.

Table 2. Recoveries determined in the shellfish samples.

	hydrocarbon μg/gm wet	Biogenic μg/gm wet	% Recovery C18:1	para-Terphenyl
Site 1: 2090.	0.4	4.7	93.6	111.0
Site 1: 2090 Dup.	0.7	4.7	80.0	104.2
Site 2: 2091.	1.1	9.7	87.5	104.6
Site 3: 2092.	2.0	9.0	84.9	108.3
Site 4: 2093.	0.7	11.2	95.4	109.4
Site 5: 2094.	3.7	3.0	78.6	95.6
Site 5: 2094 Dup.	4.9	3.0	97.6	77.9
John's Pass Oyster	30.8	0.0	103.0	107.8
Spiked Clam (2093)	4.3	11.0	90.0	130.0[1]
Procedural Blank			97.5	100.3

[1]There was interference with this peak (coelution) from components of the oil spiked into this sample.