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Alkanes in Barnacles (*Balanus tintinnabulum*) from the Buccaneer Oilfield

Brian S. Middleditch, Evelyn S. Chang, and Brenda Basile
Department of Biophysical Sciences, University of Houston, Houston, Tex. 77004

The uptake and discharge of petroleum hydrocarbons by several shellfish has been studied. Organisms which have been described as useful indicators of oil pollution include barnacles (SHIMKIN et al. 1951, CLARK 1973), clams (ANDERSON 1973), mussels (LEE et al. 1972, CLARK 1973), and oysters (CAHNMANN and KURATSUME 1957, BLUMER 1971, EHRHARDT 1972, ANDERSON 1973, TEAL and STEGMAN 1973). Most of these studies were performed on animals exposed to high concentrations of hydrocarbons in the laboratory or following oil spills at sea. Little is known concerning the effects of continuous exposure of sessile marine organisms to low concentrations of petroleum hydrocarbons in the vicinity of offshore oilfields.

We have previously reported that about 200g per day of alkanes are present in brine discharged from each of two production platforms in the Buccaneer oilfield in the NW Gulf of Mexico (MIDDLEDITCH et al. 1978). We have also found that petroleum alkanes are present at concentrations up to 43 ppb in surface seawater samples from the vicinity of the oilfield (MIDDLEDITCH et al. 1979a), and that some plankton samples collected at the air/sea interface contain C_{20} to C_{30} alkanes which are probably derived from petroleum (MIDDLEDITCH et al. 1979b). The most abundant organisms attached to the legs of the production platforms and well jackets is the barnacle Balanus tintinnabulum. Alkane levels in the flesh and shells of these organisms are now reported.

METHODS

The locations of the two production platforms and 13 well jackets in the Buccaneer oilfield are shown in Fig. 1. Barnacles were collected for us by personnel from the National Marine Fisheries Service from both production platforms and ten of the well jackets in September, 1976, January, 1977, and March, 1977, (Table 1). The samples were frozen on board ship to minimize bacterial contamination.

In the laboratory, the clusters of barnacles were barnacles were broken up using degreased pliers. The shells were broken open to remove the flesh. Shell and flesh samples were examined separately.

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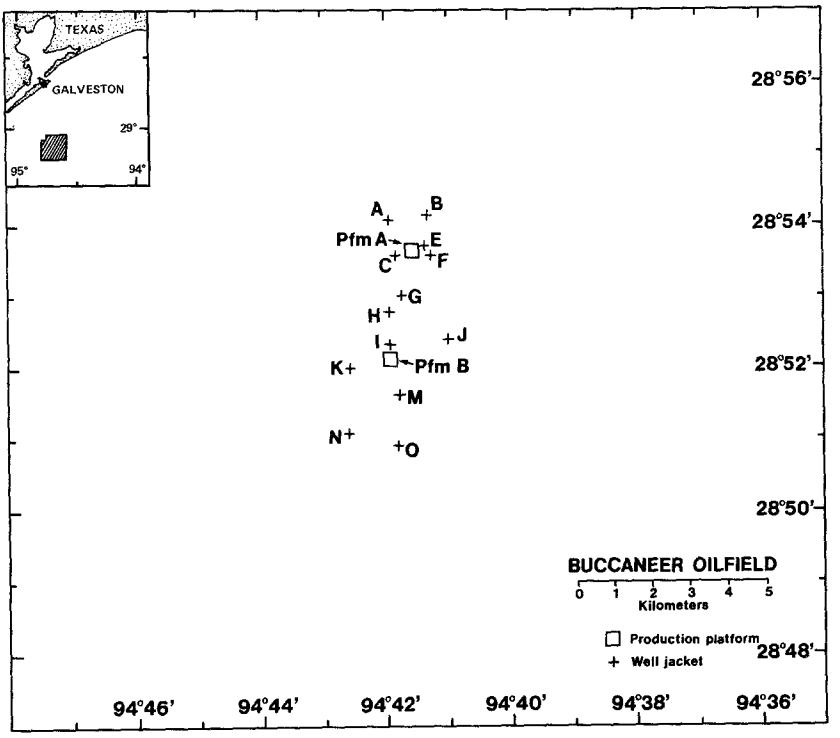

Figure 1. Production platforms and well jackets in the Buccaneer oilfield

TABLE 1

Tabulation of barnacle samples

Sample	Location*	Depth**	Material	Total alkanes (ppm)
I	Pfm A	surface	flesh	1.09
II	Pfm A	surface	shell	0.29
III	Pfm A	10 m	flesh	0.74
IV	Pfm A	10 m	shell	0.26
V	Pfm B	surface	flesh	1.29
VI	Pfm B	surface	shell	0.13
VII	Pfm B	10 m	flesh	0.74
VIII	Pfm B	10 m	shell	0.28
IX	Jkt I	surface	flesh	0.83
X	Jkt I	surface	shell	0.43
XI	Jkt I	10 m	flesh	0.70
XII	Jkt I	10 m	shell	0.19
XIII	Jkt A	surface	flesh	1.07
XIV	Jkt A	surface	shell	0.22
XV	Jkt A	10 m	flesh	0.65
XVI	Jkt A	10 m	shell	0.24
XVII	Jkt K	surface	flesh	3.28
XVIII	Jkt K	surface	shell	0.20
XIX	Jkt K	10 m	flesh	1.53
XX	Jkt K	10 m	shell	0.62
XXI	Jkt H	surface	flesh	1.16
XXII	Jkt H	surface	shell	0.21
XXIII	Jkt H	10 m	flesh	0.62
XXIV	Jkt H	10 m	shell	0.60
XXV	Jkt C	surface	flesh	1.09
XXVI	Jkt C	surface	shell	0.31
XXVII	Jkt C	10 m	flesh	0.33
XXVIII	Jkt C	10 m	shell	0.21
XXIX	Jkt F	surface	flesh	0.72
XXX	Jkt F	surface	shell	0.17
XXXI	Jkt F	10 m	flesh	0.82
XXXII	Jkt F	10 m	shell	0.13
XXXIII	Jkt G	surface	flesh	0.82
XXXIV	Jkt G	surface	shell	0.20
XXXV	Jkt G	10 m	flesh	2.50
XXXVI	Jkt G	10 m	shell	0.31
XXXVII	Pfm A	surface	flesh	0.94
XXXVIII	Pfm A	surface	shell	0.20
XXXIX	Pfm A	13 m	flesh	1.42
XL	Pfm A	13 m	shell	0.11
XLI	Jkt O	surface	flesh	0.56
XLII	Jkt O	surface	shell	0.12
XLIII	Jkt O	13 m	flesh	0.96
XLIV	Jkt O	13 m	shell	0.08

TABLE 1 (continued)

Sample	Location*	Depth**	Material	Total alkanes (ppm)
XLV	Jkt I	12 m	flesh	0.66
XLVI	Jkt I	12 m	shell	0.24
XLVII	Jkt K	11 m	flesh	0.53
XLVIII	Jkt K	11 m	shell	0.17
XLIX	Jkt N	7.5 m	flesh	1.13
L	Jkt N	7.5 m	shell	0.18
LI	Jkt N	15 m	flesh	1.36
LII	Jkt N	15 m	shell	0.10
LIII	Jkt M	15 m	flesh	0.80
LIV	Jkt M	15 m	shell	0.10
LV	Jkt M	7.5 m	flesh	0.46
LVI	Jkt M	7.5 m	shell	0.16

* Locations of platforms and well jackets are shown in Fig. 1

**Water is approximately 20 m deep throughout area

The shell samples were completely cleaned of flesh, but no attempt was made to remove attached organisms. Each shell sample was transferred to a 43 x 130 mm glass extraction thimble in a modified soxhlet apparatus (Toe-Pre 807). n-[$^2H_{42}$]Eicosane and n-[$^2H_{66}$]dotriacontane were added as internal standards for quantitation (MIDDLEDITCH and BASILE 1976). The same sample was extracted with cyclohexane (300 ml) for 6 hr, and the extract was reduced in volume using a Buchi/Brinkman Rotavapor R rotating evaporator prior to chromatography.

The flesh samples were extracted in the manner previously described for plankton samples (MIDDLEDITCH et al. 1979b). The tissue was homogenized, saponified by heating with sodium hydroxide, extracted with diethyl ether, dried over sodium sulfate, and reduced in volume prior to chromatography.

An alkane fraction was obtained from each extract by chromatography on activated silica gel. This fraction was eluted in 40 ml of cyclohexane from a 1 x 20 cm column. Some lipid-rich samples, particularly those from flesh, overloaded the column and lipids were eluted with the alkanes. In such cases, indicated by a yellow coloration of the alkane fraction or anomalous peaks during gas chromatography (GC) column chromatography was repeated. The alkane fraction was reduced in volume to 50 to 100 µl prior to analysis by GC.

Gas chromatography was performed using Perkin-Elmer 3920B instruments equipped with 2 m x 6 mm silanized glass columns containing 1% OV-1 on Supelcoport (100-120 mesh), programed from 100 to 300° at 4° per min, and flame ionization detectors. The

injector and detector temperatures were, respectively, 250 and 300°.

The alkanes and deuteriated alkanes were completely separated by GC, so a mass spectrometer was not required for their selective detection and quantitation. To confirm the identities of individual compounds, however, some samples were examined by combined gas chromatography - mass spectrometry (GC-MS). A Hewlett-Packard 5982A instrument was used under conditions similar to those employed by GC, except that the column temperature was limited to 270° since the instrument was equipped with a silicone membrane molecular separator.

RESULTS AND DISCUSSION

Concentrations of alkanes in flesh and shell samples are given in Table 1. Alkanes lighter that n-dodecane are too volatile for accurate quantitation by the procedures employed. The only branched alkanes detected were pristane and phytane. The term "total alkanes," therefore, refers only to C_{12} to C_{36} n-alkanes, pristane, and phytane.

Flesh from submerged samples

The major alkanes, n-pentadecane, n-heptadecane, n-nonadecane, n-heneicosane, and pristane appear to be indigenous to the barnacles. Of the 17 samples in this category which were analyzed, only three (III, VII, XI) contained petroleum-like alkanes in the region C_{22} to C_{30}, with concentration maxima around C_{25}.

Flesh from surface samples

These samples were also dominated by biogenic alkanes. However, four (I, IX, XXV, XXXIII) of the nine samples in this category collected on September 25, 1976, contained petroleum-like alkanes. There was no evidence of such compounds in samples collected during January and March, 1977. The presence of oil-derived alkanes in flesh from surface samples and its absence from submerged samples is not unexpected, since oil tends to float at the air/sea interface. It is of interest to note that we found comparatively high concentrations of oil in surface water samples from the Buccaneer oilfield on August 31, and September 1, 1976. It appears that some of this oil was retained by the barnacles at least until September 23, but it had dissipated by the time the next barnacle collection was made in January, 1977.

Shells from submerged samples

These samples contained lower concentrations of the biogenic alkanes found in flesh samples. The relative concentrations of pristane and phytane were particularly low. All 17 of these samples contained petroleum-like alkanes, usually in concentra-

tions greater than those of the biogenic alkanes. Alkanes found in shell samples may derive from organisms attached to the shells.

Shells from surface samples

The alkane content (biogenic and petroleum) is similar to that of the submerged samples.

Comparison of alkane concentrations

The mean alkane concentration in flesh samples was 1.03 ± 0.61 ppm, and in shell samples was 0.23 ± 0.13 ppm. The t-test revealed that there was a significant difference between the alkane concentrations of these two groups of L($p<0.002$; $n= 28$) sample.

The shell samples collected in September, 1976 ($n= 18$) contained 0.28 ± 0.14 ppm of alkanes, while those collected during January-March, 1977 ($n=10$) contained 0.15 ± 0.05 ppm. Again, the t-test revealed that the difference in concentration was significant L($p<0.002$). This difference probably reflects a difference in species composition and/or biomass of attached biota between the two seasons.

Careful examination of the data obtained showed that there was no significant difference in alkane concentration between the following groups of specimens:

(i) from production platforms or well jackets, (ii) from the surface or submerged, and (iii)for flesh samples, those collected at different seasons.

CONCLUSIONS

Our results indicate that barnacles growing near the surface ingest oil-derived alkanes when the oil concentration in the water was relatively high. When the alkane concentration in the water is reduced, however, the ingested alkanes may be excreted. These observations are consistent with those of others (see above).

Shells from specimens collected both at the surface and below all contain petroleum-like alkanes although, as noted, it is possible that such compounds derive from organisms attached to the shells. Further work is required to determine whether this assumption is correct.

It is generally considered that petroleum hydrocarbons are not concentrated during passage through food webs. Since these compounds are present at higher concentrations in flesh from some barnacles than in the surrounding water, it would be of interest to determine the hydrocarbon content of fish which graze on the barnacles.

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