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Hydrocarbons in Benthic Algae from the Eastern Gulf of Mexico

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(Abbreviated title: Hydrocarbons in benthic algae)

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Abstract

Thirty-six benthic algae were collected from the continental shelf along the eastern Gulf of Mexico to compare hydrocarbon content and distribution with specimens collected in earlier studies and to determine effectiveness of benthic algae as petroleum pollution indicators. The algae contain 82.83143 ppm aliphatic hydrocarbons by weight with much lesser quantities of aromatic hydrocarbons (11.8 ± 22.7 ppm). The aliphatics of red algae are composed almost entirely of $n\text{-C}_{17}$ (70-95%, of aliphatic weight), whereas green algae have varying amounts of n -alkanes in the range of $n\text{-C}_{15}$ to $n\text{-C}_{19}$ with homologous series of odd carbon number n -alkenes. Phytadienes occur as high as 740 ppm dry wt in the green algae and do not appear to be related to time or place of collection or to influence of pollution. The one brown algal species contains predominantly $n\text{-C}_{15}$. Ca. 30% of the samples appear to be polluted with partially degraded petroleum residues, but no pollution of recent derivation was detected in any specimen.

Introduction

Hydrocarbons constitute a minor but ubiquitous component of all marine organisms. Because of chemical stability and wide structural differences, hydrocarbons make good environmental indicators and are relevant to chemotaxonomy. With release of fossil fuels into the marine environment, it is increasingly important to differentiate biogenic and fossil fuel hydrocarbons in order accurately to assess the extent of oil pollution. Knowledge of the natural hydrocarbon background is limited and must be expanded in all facets of the marine environment to include indicator species where possible. Lobsters, clams, crabs, barnacles, mussels, snails and other organisms (Clark et al., 1973, Clark and Finley, 1973; Mayo et al., 1974; Ehrhardt and Heinemann, 1974; Barrington and Medeiros, 1975; Teal, 1976) have been used successfully in this capacity. Berner et al. (1976) have suggested several indicator species suitable in various coastal areas. Marine algae and zooplankton, however, represent the bulk of marine biomass and get incorporated into other marine organisms and into the sediments. Loss or damage to this important food source could be devastating. This work extends that of other investigators in the knowledge of hydrocarbons in benthic algae and their value as petroleum pollution monitors (Clark et al., 1973; Youngblood et al., 1971; DiSalvo et al., 1976; Clark and Blumer, 1967). These algae, however, come from the warmer waters of the Gulf of Mexico.

Materials and Methods

Thirty-six benthic algae were collected along five transects in the northeastern Gulf of Mexico continental shelf on the R.V. Bellows and the R.V. Gyre. Collections were made over three seasons. The first collection was in June, 1975. The second in October, 1975 and the third in January, 1976. Table 1 lists locations of sample sites and transects, period collected and algae species. Transect I extends east-west from Ft. Myers, Florida in water depths of 120 feet.

TABLE 1

Transect II sites lie off Tampa, Florida in water depths of 100-200 feet.

Transect III, known as the "middle grounds," begins at a point midway between Tampa and St. Marks, Florida in depths 80 feet extending to 138 feet. Transect IV extends in a west-southwest direction from St. Marks, Florida with average water depths of 120 feet. Transect V stations lie southwest of Panama City, Florida in average water depths of 120 feet. Transect VI extends east-southeasterly from Pascagoula, Mississippi. High turbidity readings in this area prevents the high algal biomass seen at other transects, therefore, few samples were collected along this transect where water depths ranged from 120-144 feet. Collections were made primarily by divers, but some collections were made by dredge and trawl. The algae was sorted and individual species identified, placed in acid-washed glass containers and frozen immediately. All precautions were taken to minimize hydrocarbon contamination during collection and sorting. The samples remained frozen until ready for analysis.

The frozen algae were allowed to thaw and were washed with distilled water. They were homogenized in methanol using a stainless steel Waring "

Table 1

Benthic Algae. Algal Taxa and Collection Stations

Sample #	Location	Species	class
<u>June 1975</u>			
<u>Transect I</u>			
IA-A-i-B-6	26°25.5'N, 82°59.5'W	<u>Halimeda</u> sp.	Chlorophyceae
IA-A+B-7	26°25.5'N, 82°59.5'W	<u>Rhodomenia</u> sp.	Rhodophyceae
IA-A+B-9	26°25.5'N, 82°59.5'W	<u>Cystodictyon pavonium</u>	Chlorophyceae
<u>Transect II</u>			
IIA-A+B-1	27°50'N, 83°31'W	<u>Laurencia corallopsis</u> ,	Rhodophyceae
		<u>Gracilaria cylindrical</u> +	Rhodophyceae
		<u>blodgettii</u>	Rhodophyceae
IIA-A+B-2	27°50'N, 83°31'W	<u>Gracilaria mammilaris</u>	Rhodophyceae
IIA-A+B-3	27°50'N, 83°31'W	<u>Eucheuma</u> sp.	Rhodophyceae
062-A-17	27°49'N, 55°90'W	<u>Caulerpa sertularoides</u>	Chlorophyceae
<u>Transect III</u>			
IIIA-A+B-2	28°29'N, 84°21'W	<u>Codium</u> sp.	Chlorophyceae
047-A-3	28°34'N, 84°20'12"W	<u>Codium repens</u>	Chlorophyceae
047-A-5a	28°34'N, 84°20'12"W	<u>Halimeda discoidea</u>	Chlorophyceae
146-B-1	28°41'N, 84°24'W	<u>Kallymenia perforata</u> +	Rhodophyceae
		<u>Dictyota dichotoma</u>	Phaeophyceae
147-B-5	28°40'N, 84°13'W	<u>Halimeda discoidea</u>	Chlorophyceae
247-A-2	28°36'16"N, 84°15'40"W	<u>Codium repens</u>	Chlorophyceae
251-B-25	28°33'N, 84°16'W	<u>Halimeda discoidea</u>	Chlorophyceae
<u>October 1975</u>			
<u>Transect II</u>			
11.1-A-12	27°50'N, 83°31'W	<u>Halymenia</u> sp.	Rhodophyceae
062-A-5	27°49'55"N, 83°31'10"W	<u>Caulerpa sertularoides</u>	Chlorophyceae
064-A-3	27°50'N, 83°25'W	<u>Gracilaria blodgettii</u> +	Rhodophyceae
		<u>compressa</u>	Rhodophyceae
064-B-3	27°50'N, 83°25'W	<u>Gracilaria blodgettii</u>	Rhodophyceae

Table 1 Continued

Benthic Algae. Algal Taxa and Collection Stations

Sample #	Location	Species	Class
<u>Transect III</u>			
IIIA-A-10	28°29'N, 84°21'W	<u>Caulerpa</u> sp.	Chlorophyceae
047-A-5b	28°34'N, 84°20'12"W	<u>Halimeda discoidea</u>	Chlorophyceae
146-B-14	28°41'N, 84°23'40"W	<u>Dictyota dichotoma</u>	Phaeophyceae
147-A-2	28°38'18"N, 84°13'54"W	<u>Halimeda discoidea</u>	Chlorophyceae
151--A-3	28°32'07"N, 84°18'24"W	<u>Laurencia intricata</u>	Rhodophyceae
247-A-4	28°36'16"N, 84°15'40"W	<u>Codium repens</u>	Chlorophyceae
251-A-3	28°32'40"N, 84°16'03"W	<u>Halimeda discoidea</u>	Chlorophyceae
<u>Transect IV</u>			
IWA-A- 6	29°04'N, 85°14'W	<u>Codium</u> sp.	Chlorophyceae
<u>Transect V</u>			
VA-A- 12	29°50'N, 86°05.5'W	<u>Pryssonnelia rubra</u>	Rhodophyceae
<u>January, 1976</u>			
<u>Transect 11</u>			
11A-A-17	27°50'N, 83°31'1)	<u>Codium</u> sp.	Chlorophyceae
062-A-1	27°49'55"N, 83°31'10"W	<u>Codium isthmocladium</u>	Chlorophyceae
064-A-9	27°50'N, 83°25'W	<u>Eucheuma isiforme</u>	Rhodophyceae
<u>Transect III</u>			
047-A-25	28°34'N, 84°20'12"W	<u>Codium carolinianum</u>	Chlorophyceae
146-.1-1	28°41'N, 84°23'40"W	<u>Codium carolinianum</u>	Chlorophyceae
147-A-2	28°38'18"S, 84°13'54"W	<u>Halimeda discoidea</u>	Chlorophyceae
151-A-1	28°32'07"N, 84°18'24"W	<u>Codium carolinianum</u>	Chlorophyceae
247-A-27	28°36'16"N, 84°15'40"W	<u>Codium carolinianum</u>	Chlorophyceae
251-A-10	28°32'40"N, 84°16'03"W	<u>Codium carolinianum</u>	Chlorophyceae

blender and extracted in a Soxhlet apparatus with Methanol-Benzene (1:1) for 12 hours. The Methanol-Benzene was removed and replaced with fresh benzene. Extraction continued in the Soxhlet apparatus for 12 additional hours. The extracts were combined and washed with acidic (PH 4) water. An aliquot of the lipid was dried and weighed; the remainder was saponified by refluxing overnight with 0.5 N KOH-MeOH. The nonsaponifiable fraction obtained by benzene extraction of the methanolic solution was fractionated on a glass column packed with activity I silica gel beneath activity I neutral alumina (2:1 by volume). The column packing to sample ratio was always greater than 50:1. The column was eluted with two column volumes of hexane to yield an aliphatic hydrocarbon fraction; two column volumes of benzene eluted the aromatic and polyolefinic hydrocarbons. The two fractions were taken to dryness and analysed on a Perkin-Elmer Model 990 gas chromatograph using 2 m x 3.2 mm stainless steel columns packed with 4% FFAP and 3% SE-30 on Anakrom AS, 90/100 mesh. Data synthesis was performed with a Perkin-Elmer PEP-1 data system. Tentative identification of peaks was by comparison of retention times on both GLC phases with those of standards of n-alkanes, pristane, phytane, n-alkenes and aromatic compounds. Such identification techniques failed to yield identities of components of the aromatic fraction which were usually at very low concentrations.

Further characterization of compounds was accomplished by hydrogenation. At atmospheric pressure, hydrogen was bubbled into aliquots of all samples suspected of containing olefinic material. PiO_2 was added to the sample in hexane and the sample stirred for 20 minutes under hydrogen taking care to maintain the initial 1 ml volume of hexane. The sample was then rechromatographed. Normal alkane identities were

confirmed if retention times agreed with those of standards on FFAP and SE-30. Shifts in retention times after hydrogenation on both columns also further characterized those hydrocarbons having double bonds.

Results

The data in Table 2 suggests that the predominant hydrocarbon material in these benthic algae is aliphatic. In some cases (e.g. IA-A+B-7, IIA-A+B-2, 062-A-5, 146-B-14, VA-A-12) the aromatics constitute as much as 1/3 the weight of aliphatics. However hydrogenation of some of these aromatic fractions indicates that much of this material may be polyolefinic hydrocarbon material. Of the aliphatic components, the vast majority consists of n-alkanes occurring in the range of C₁₅ to C₂₃ with constituents evident occasionally as high as C₃₂ or as low as C₁₃ n-alkanes. The gravimetric data displayed in Table 2 demonstrates several similarities of the various algal species. With a few exceptions the values for aliphatic, aromatic and n-alkanes fall respectively within one order of magnitude. The Caulerpa sp. from Transect III, October 1975 has an unusually high aliphatic weight, but an n-alkane concentration about average for remaining species. Only the Dichtyota sp. from the same transect and sampling period contains grossly elevated values of all three parameters surpassing most species by a factor of 10.

TABLE 2

Gas chromatographic data reveals rather simple distributions of algal aliphatic hydrocarbons. Either n-C₁₇, n-C₁₅ or n-C₁₉ predominates the chromatogram in every instance as seen in Table 2 which is in agreement with results of Clark and Blumer (1967) and Youngblood et al. (1971). In addition to these low molecular weight saturated materials, Table 2 also shows a lesser but not insignificant contribution of the n-alkenes C_{17:1} and C_{19:1} and some higher molecular weight (n-C₂₃₊) alkane material.

TABLE 2
Benthic Algae. Chromatographic Parameters

Sample # June '75	Tr/Cl ^a	Hydrocarbon classes ppm dry wt. ^b			Aliphatic Components, ppm dry wt.		
		Aliphatics	Aromatics	n-Alkanes	Major alkanes, a lkenes ^c	Phytadienes, K.I. 1922,1953,1979,2004	Homologous_n-alkene group, K.I. 1712, 1914,2116,2320,2524
IA-A+B-6	I/G	3.8	1.0	2.5	17(1.8),19(0.24)		
IA-A+B-7	I/R	35.	11.3	30.	17(27.),19:1(0.75)	2.0	
IA-A+B-8	I/G	68.	8.7	35.	17(32.)	1.3	
IIA-A+B-1	11/R	38.	12.	25.	17(19.8),15(0.72)	8.5	
IIA-A+B-2	II/R	171.	8.	130.	17(12),15(4.3)	23.	
IIA-A+B-3	11/R	50.	7.	19.	17(17.5)	29.	
C62-A-17	IX/G	153.	11.5	95.	19(46.3),17(38.0), 19:1(6.3),17:1(4.2)		44.9
IIIA-A+B-2	III/G	21.	1.1	16.1	17(13.4),19(1.39), 17:1(0.56)		
C47-A-3	III/G	51.	1.1	39.	17(35.),19(2.6)		
C47-A-5a	111/G	96.	6.7	84.	17(46.),19(21.), 23(5.1),15(2.9)		2.9
146-B-1	III/R+B	143	14.0	133.	17(93.),15(33)		
147-B-5	III/G	72.	11.6	62.	17(25.),19(10.0), 23(2.9)		
247-A-2	III/G	44	5.	38.	17(34.),19(2.3)	3.0	2.9
251-B-25	III/G	31.	4.3	26.	17(15.9),19(7.3), 23(0.87)	1.5	-
Oct. '75							
IIA-A-12	II/R	43.	5.7	40.	17(25.)		
C62-A-5	11/G	31.	11.5	79.7	19(11.7),17(5.1), 19:1(1.39)		14.4
C64-A-3	11/R	61.	13.6	56.	17(49.)		
C64-B-3	II/R	66.	0.9	64.	17(62.)	2.	
IIIA-A-10	III/G	850.	26.	42.	17(22.),21(3.0), 23(3.82)	740.	18.6
C47-A-5b	111/G	80	10.4	49.	17(57),23(3.6), 19(2.4)	23.	15.4
146-B-14	111/B	330.	140.	220.	15(198.),19(13.6), 17(4.9)		

TABLE 2 Continued

Sample #	Tr/Cl ^a	Hydrocarbon classes ppm dry wt. b			Aliphatic Components, ppm dry wt.	
		Aliphatics	Aromatics	n-Alkanes	Major alkenes, alkenes	Monoterpenes, s.i. 1922, 1953, 1979, 2004 K.I. 1712, 1914, 2116, 2320, 2524
147-A-2	III/G	22.	4.	19.	17:17.7), 19(1.72), 17:1(1.71)	-
151-A-3	III/R	20.	50.	15.	17:12.8), 17:1(0.67)	-
247-A-4	III/G	92.	10.1	75.	17:62.8), 19(6.4)	2.6
251-A-B	III/G	39.	2.5	26.	17:19.8), 19(2.2), 25(1.9)	-
IV-A-6	IV/G	33.	2.6	16.7	17:4.1), 19(1.41)	4.5
VA-A-12	V/R	6.5	3.0	5.3	17:8.1), 17:1(0.47)	-
Jan. 76						
IIA-A-17	II/G	4.2	5.3	3.1	17(2.6)	-
052-A-1	II/G	4.4	3.3	3.4	17(2.8), 19 ° 5)	0.5
054-A-9	II/R	26.	2.7	34.	17(34.)	0.3
047-A-25	III/G	30.	3.5	18.4	17(16.6), 19(0.96)	1.0
145-A-1	III/G	80.	10.3	47.	17(42.), 19(2.5), 15(1.16)	5.
47-A-2	I I/G	39.	9.3	24.	17(22.), 19(6.8), 29(2.1)	-
151-A-1	III/G	67	3	57	17(51.), 19(3.1), 15(0.50)	3
247-A-27	II/G	41.	3.1	24.	17(20.), 19(1.11)	2.5
251-A-1	II/G	28.	1.3	24.	17(22.), 19(1.23), 23(0.24), 15(0.21)	1.8

^aTranssect and algal class (R-red, G-green, B-brown) buty determined from gas chromatographic data using standards to interpolate response factors. Major n-alkanes are denoted by C No., n-alkenes by C No. and number of double bonds. Monoalkenes are those whose gas chromatograms match those of authentic n-alkenes with terminal unsaturation. Concentrations in ppm dry weight are in parentheses.

Pristane and phytane occur in very low concentrations in a great number of specimens as can be seen in the $\text{pristane}/n\text{-C}_{17}$ and $\text{phytane}/n\text{-C}_{18}$ ratios of Table 3. The occurrence of **pristane** is not unusual in plants and has been noted in other algal species (Clark and Blumer, 1967; Youngblood et al., 1971). Of rarer occurrence in plants is **phytane** which has been identified in extreme trace quantities in a few **algal** samples (notably Burns and Teal, 1973), Youngblood et al. (1971) did not detect **phytane** in any of the algae collected in the Cape Cod area. The presence of phytane does not necessarily **signal** the presence of petroleum contamination in algal specimens, however it is one of the many indicators used to detect petroleum hydrocarbons.

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Possibly related biochemically to the occurrence of phytane in certain of the **algae** is the presence of four peaks between ca. K.I. 1900 and 2000 in the **aliphatic chromatograms** of many of the algal species which may be typified by that in Figure 1. These components are tentatively identified as **phytadienes**. Blumer and Thomas (1965) and Blumer et al. (1965) have suggested that **phytadienes** are present in some zooplankton and fish, but may occur as artifacts from any number of laboratory procedures commonly associated with hydrocarbon analysis. Since the precursor of **phytadienes** is assumed to be **phytol**, part of the chlorophyll molecule, their creation by saponification and/or adsorption chromatography would seem feasible in algal extracts. **Phytadienes** might also result from decomposition of **plant l-issue**. However the divers responsible for specimen collection were exceedingly careful to sample only live, healthy specimens so this source seems unlikely. Blumer and Thomas (1965) report that the four **phytadienes** elute gas chromatographically from Carbowax between K.I. 1900 and 2000 and can be hydro-

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TABLE 3
Benthic Algae. Parameters of Pollution Interest

Sample	Tr/Cl ^a	Pristane ECY7----	Phytane n-C18	odd even C<20	odd even C>21	$\Sigma C<20$ $\Sigma C>21$	Indications of Pollution ^b
June 75							
IA-A+ B-6	I/G	0.011	0.0	27.	3.0	10.9	No
IA-A+ B-7	I/R	0.0	0.0	150.	1.0	51.	V. slightly, if at all
IA-A+ B-8	I/G	0.0	0.0		0.9	15.	V. slightly
IIA-A+B-1	11/R	0.005	2.5	44.	1.0	7.9	Definitely, considerable HMW unresolved matl.
IIA-A+B-2	11/R	0.005	1.4		0.9	11.5	Yes
IIA-A+B-3	11/R	0.003	0.5	106.	0.9	19.2	V. slightly, if at all
062-A-17	11/G	0.003	0.0	20.	2.0	37.	Yes
IIIA-A+B-2	111/G	0.010	0.0	9.8	1.4	19.8	?
047-A-3	111/G	0.002	0.0	39.	3.0	105.	No
047-A-5a	111/G	0.003	0.6	86.	5.5	9.6	No
145-B-1	III/R+B	0.002	0.0	?	1.5	107.	V. slightly, if at all
147-B-5	111/G	0.004	0.7	78.1	1.4	3.0	Definitely, considerable HMW unresolved matl.
247-A-2	111/G	0.001	0.5	73.	0.8	103.	No
251-B-25	111/G	0.002	0.0	73.	5.8	16.3	No
Oct. 75							
11A-A-12	11/R	0.003	0.8	60.	1.0	16.5	Yes
062-A-5	11/G	0.0	0.0	64.	2.8	14.4	No
064-A-3	11/R	0.003	0.0	46.	1.6	13.7	V. slightly, if at all
064-B-3	11/R	0.002	0.0	156.	0.5	84.	V. slightly, if at all
IIJ-A-A-10	111/G	0.040	0.2	7.4	1.1	4.2	?
047-A-5b	111/G	0.001	0.0	11.9	7.1	12.2	No
146-B-14	111/B	0.101	0.0	260.	0.8	703.	No
147-A-2	111/G	0.004		78.	7.4	10.5	No
151-A-3	111/R	0.0	0.0	72.		32.	No
247-A-4	111/G	0.002	0.0	67.	1.3	22.	V. definitely, tremendous HMW unresolved matl.

TABLE 3 Continued

Sample	Tr/Cl ^a	<u>Pristane</u> <u>n-C17</u>	<u>Phytane</u> <u>n-C18</u>	<u>odd</u> <u>even</u> C<20	<u>odd</u> <u>even</u> C>21	$\frac{\Sigma C<20}{\Sigma C>21}$	Indications of Pollution ^b
251-A-3	111/G	0.003		81.	-	7.5	No
IVA-A-6	IV/G	0.004	0.0	40.	1.5	50.	V. slightly, if at all
VA-A-12	V/R	0.017	0.0	28.	1.3	18.4	No
Jan. 76							
11A-A-17	11/G	0.017		22.	2.4	13.9	No
062-A-1	11/G	0.014	0.4	36.	1.3	16.2	No
064-A-9	11/R					v. large	No
047-A-25	111/G	0.002	0.0	42.	1.7	74.	No
146-A-1	111/G	0.002	0.0	46.		2\$.	Definitely, considerable unresolved HMW matl.
147-A-2	111/G	0.004	0.0	61.	4.7	7.8	--
151-A-1	III/G	0.001	0.0	61.	0.5	53.	--
247-A-27	111/G	0.005	0.3	51.	0.9	12.6	Definitely, considerable unresolved HMW matl.
25I-A-10	III/G	0.002	0.0	75.	2.4	44.	No

^aTransect and algal class (R-red, G-green, B-brown)

^bThe presence of high molecular weight n-alkanes of carbon preference index = (i.e. $\frac{\Sigma \text{odds}}{\Sigma \text{evens}} = 1$) with a large unresolved envelope were the primary factors indicating pollution in these algae.

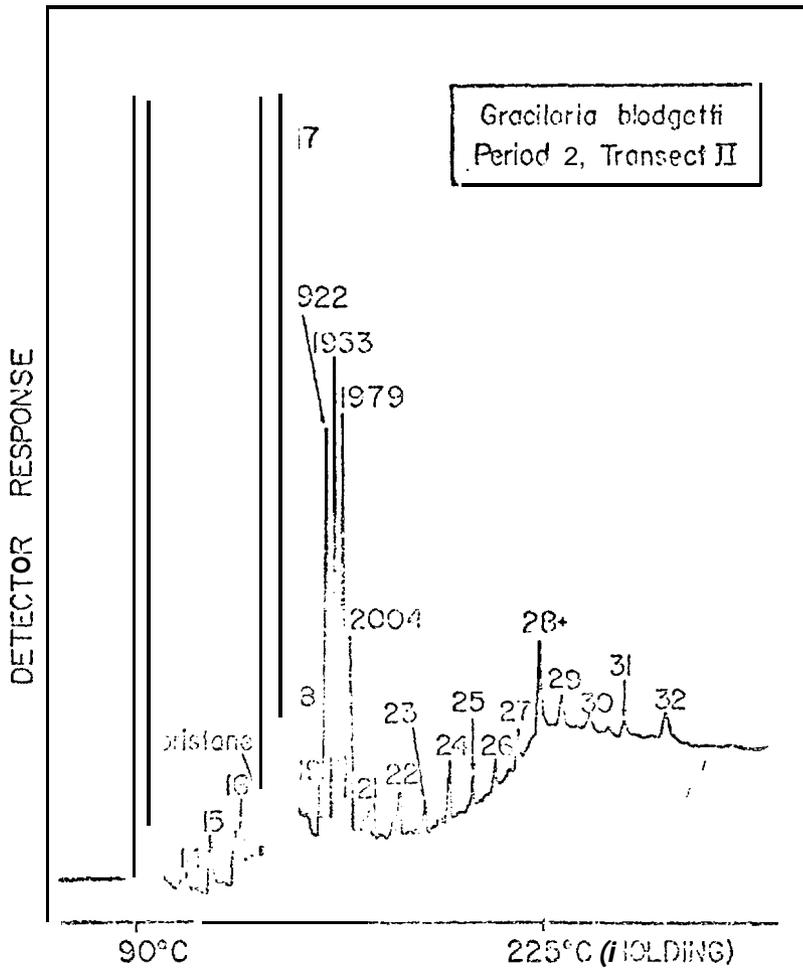


Figure 1

generated to phytane. This was the clue to our identification of phytadienes in the benthic algae. FFAP, being a modified Carbowax, we felt should give results similar to Carbowax. Two samples containing an abundance of the series of four peaks, Caulerpa sp. and Eucheuma sp., were chosen to verify the presence of phytadienes in benthic algae.

Untreated lipid extracts of these two algae were analyzed directly by gas chromatography, bypassing saponification and adsorption chromatography. The four peaks were present but disappeared with a simultaneous increase in the amount of phytane after the raw lipid was hydrogenated in agreement with results obtained with the aliphatic hydrocarbon fractions. Chromatograms of Eucheuma sp. lipid aliquots and aliphatic hydrocarbon fractions before and after hydrogenation are shown in Figures 2 and 3. The appearance of these four peaks in both treated and untreated extracts indicates clearly that phytadienes do not occur in algal extracts merely as laboratory artifacts.

Figs 2 & 3

As further proof of identification and of natural occurrence of phytadienes the assumption was made that chlorophyll represents at most ca. 2% dry wt of some algae and that phytol represents ca. 33% of chlorophyll. A quantity of purified phytol equivalent to 50 times that which would occur in the plant tissue used in the previous experiment was chromatographed on fully activated alumina and silica gel. Approximately 30 ppm (μg phytadienes/g phytol) of material resulted in the aliphatic fraction eluting at exactly the Kovats Indices of the suspect 4 peaks in the algae. As shown in Table 4, the amount of phytadienes occurring as artifacts from phytol dehydration would in most cases be insignificant and probably could not account for the levels of phytadienes shown in Table 2. Phytadienes evidently do occur naturally in benthic algae accounting for as much as 740 ppm dry wt.

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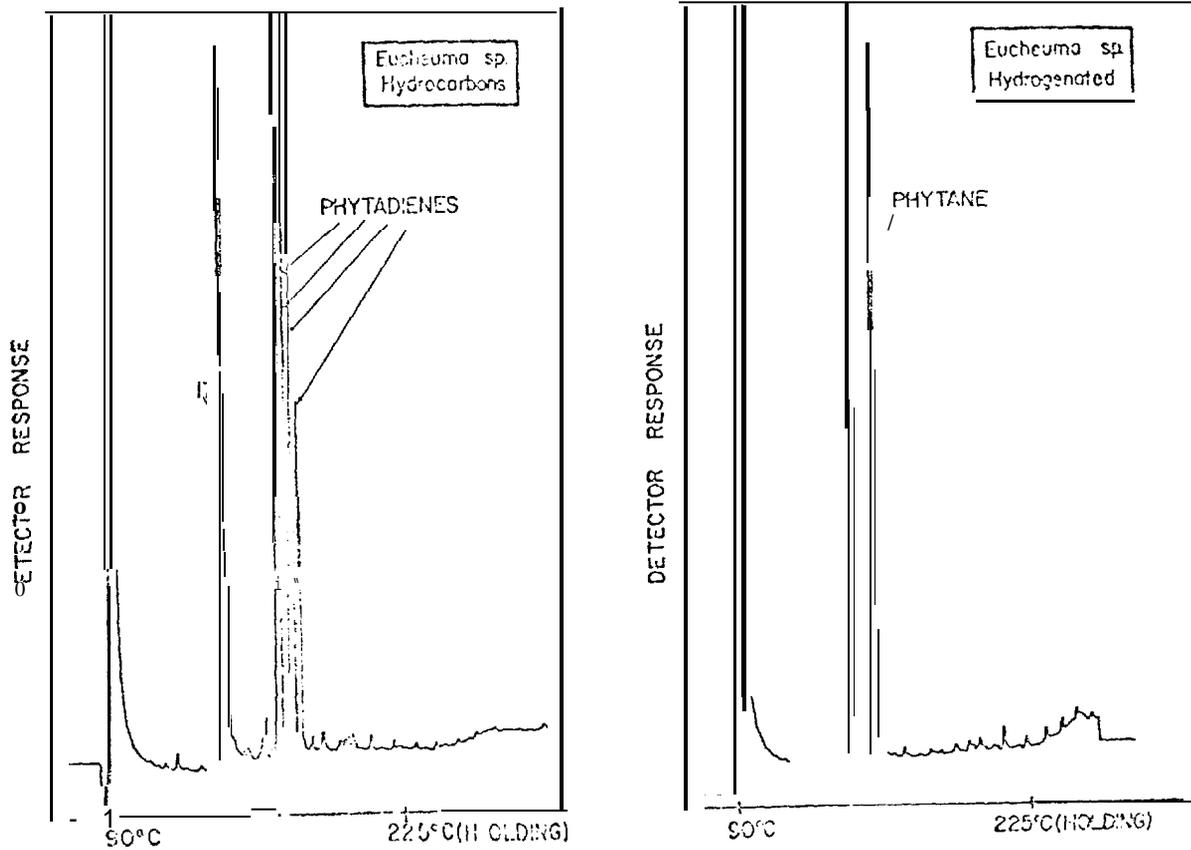


Figure 2

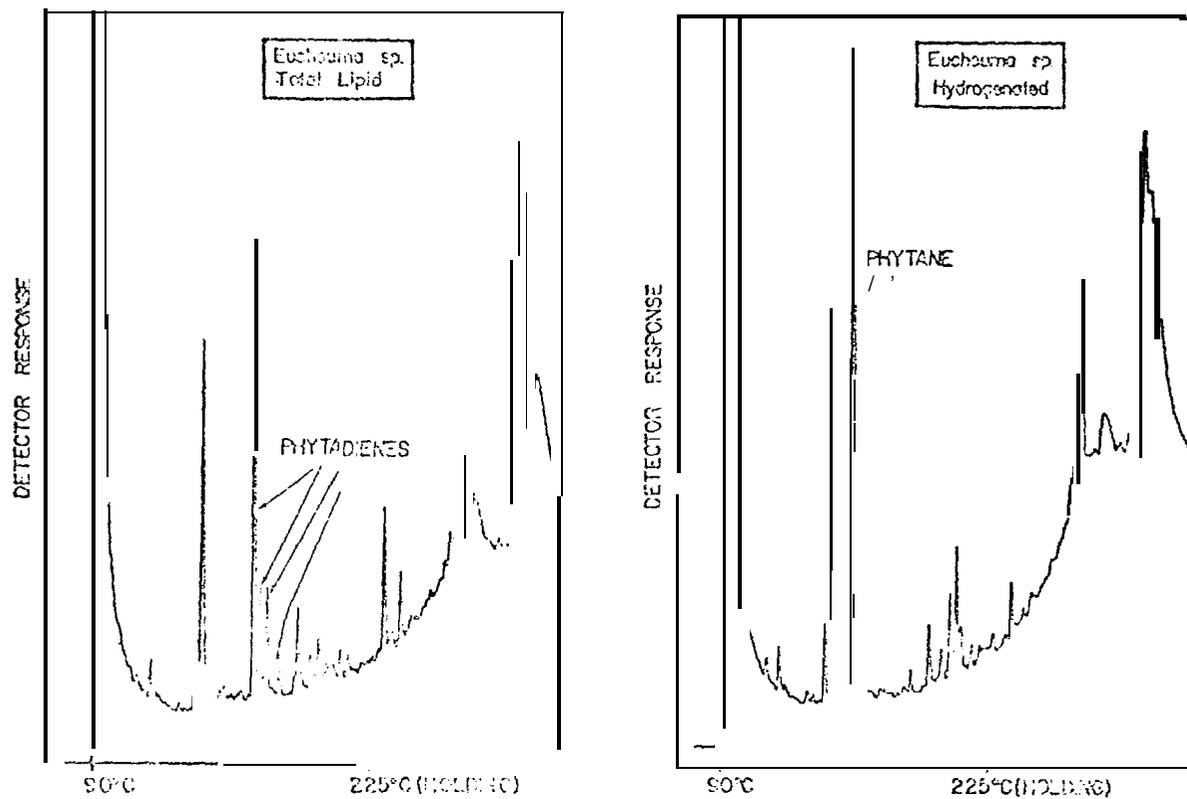


Figure 3

TABLE 4

Phytadienes in Algal Extracts

<u>Sample</u>	<u>Total µg of phytadienes^a</u>	<u>Total µg of phytane after hydrogenation</u>
<u>Caulerpa</u> sp.		
IIIA-A-10 lipid ^b	74	208
III-A-10 aliphatics ^b	288	301
<u>Eucheuma</u> sp.		
IIA-A+B-3 lipid ^b	193	247
IIA-A+B-3 aliphatics ^b	136	96
Phytol treated with		
alumina-silica gel ^c	22	12.6

^aPhytadienes are the four compounds with K.I.ca 1922, 1953, 1979 and 2004.

^bweights are calculated to represent the same initial weight of alga.

^cPhytol treated was 50X greater than would normally be found in the weight of algae analyzed for this experiment.

Fig. 4

Another interesting aspect of the **aliphatic** hydrocarbons in **benthic** algae is the presence of a homologous series of **n-alkenes** eluting just after the odd-C-number **n-alkanes** on FFAP with Kovats Indices of **ca.** 1712, 1915, 2117, 2320 and 2524. The series can be seen in Figure 4 showing the **aliphatics** of a **Halimeda** sp. Table 2 notes the presence and concentration of these components. In some samples (notably No. 062-A-17) these unsaturates constitute a sizeable fraction of the total **aliphatics**. Though upon hydrogenation the peaks disappear and increase the adjacent **n-alkane** peak area by equivalent amounts, they are not **1-n-alkenes** which **elute** at higher Kovats Indices. Accompanying this homologous series are two and possibly three lesser series peaking at the **C₁₉** and **C₂₁** homologies. These series **elute** approximately 45, 65 and 95 Kovats units higher than the saturated homologies on FFAP. All these series may be the **poly-olefins** reported by Youngblood *et al.* (1971) though occurring in these samples in much lower concentrations.

Chromatograms from certain of the algae indicate a possible petroleum origin for some hydrocarbons. Though the impact of oil pollution is usually detected in the lower molecular weight (**LMW**) range of the **aliphatic** materials, severely weathered **petroleum** products or tarry residues will produce a more complex suite of **aliphatics** resulting primarily in a high molecular weight (**HMW**) unresolved complex mixture (**UCM**). The **UCM** consisting of cyclic and branched materials used as an indicator of petroleum pollution (Blumer and Sass, 1972; Zafiriou, 1973; Barrington, *et al.*, 1976; Teal, 1976) resists microbial degradation more effectively than the **n-alkane** assemblage so tends to remain in polluted sediments and organisms after the **n-alkanes** have been dissipated (ZoBell, 1970; Blumer *et al.*, 1973; Lee, 1976). This large hump of unresolved material is

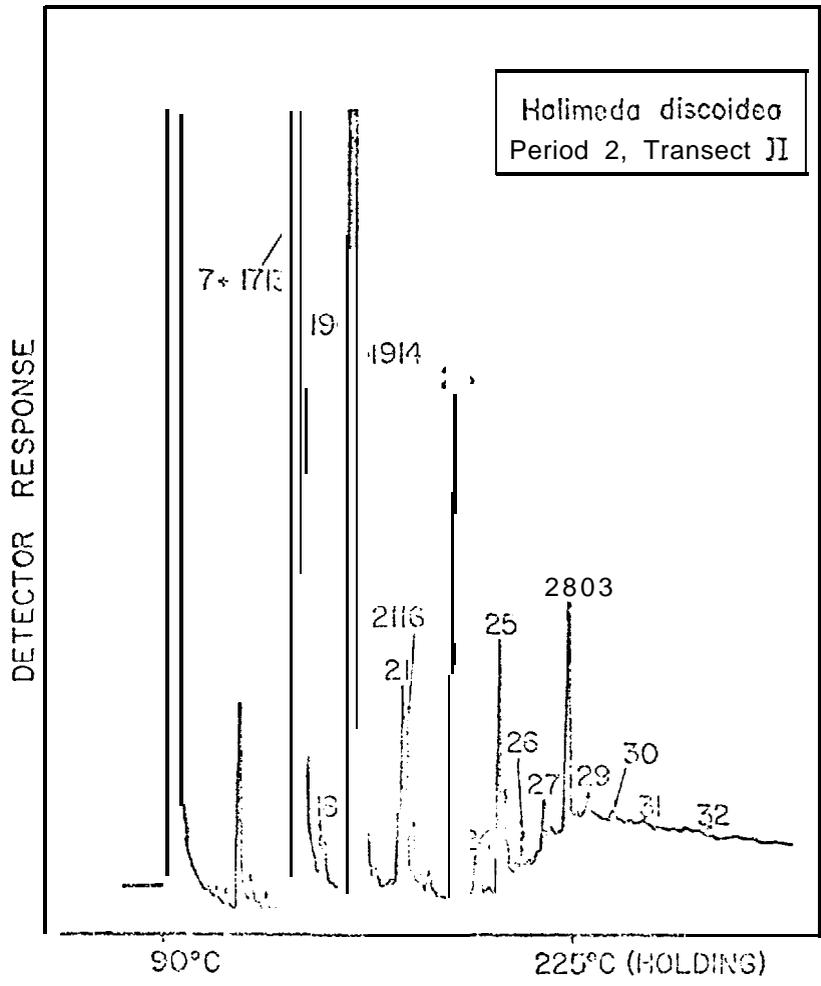


Figure 4

apparent in several of the chromatograms. An example is shown in Figure 5. Accompanying the UCM in most cases is a suite of high molecular weight *n*-alkanes with no pronounced odd/even preference which could be the remnants of petroleum *n*-alkanes also of no odd/even preference (Petrovic' and Vitorovic, 1976). Youngblood et al. (1971) also noted a suite of *n*-alkanes in benthic algae outside the range of C₁₅ to C₁₉ but did not explore the possibility of a petroleum source. In Table 3 are listings of the ratio of the HMW alkanes to LMW alkanes and the ratio of odd/even members in each fraction. In all specimens where the HMW fraction constitutes a major portion of the *n*-alkanes, this suite occurs with little preference for odd-carbon number. In sharp contrast the odd/even ratio in low molecular weight *n*-alkanes is invariably very high. The presence of large quantities of the UCM (not quantitated in Table 3) and a large measure of HMW material of little carbon No. preference was used as the indication of pollutant hydrocarbon suggested in Table 3. Pristane and phytane quantities were too small to be of much value in ascribing a pollutant source to the hydrocarbons. Approximately 30% of the samples collected had at least a positive indication of degraded petroleum hydrocarbons based upon this criteria.

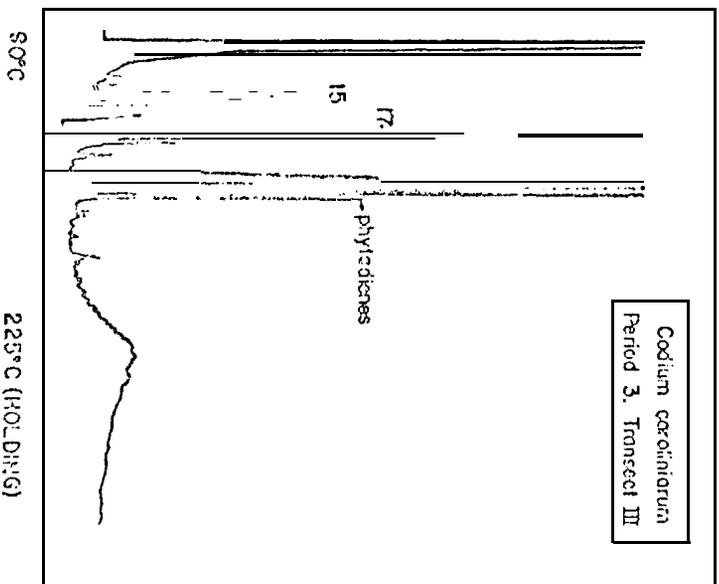
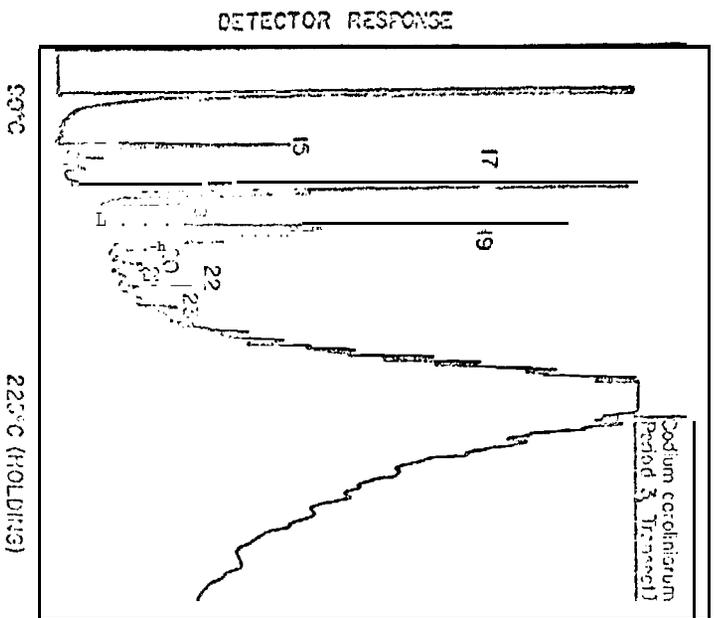


Figure 5

Discussion

Rhodophyta

Among the Chlorophyta, **Phaeophyta** and **Rhodophyta**, the representatives of Rhodophyta present the least diversity in hydrocarbon distribution. Though some amounts of **pristane**, phytane and phytadienes were noted, the gas chromatograms of the red algae are dominated by the **n-alkane**, C_{17} , constituting 70-95% of the total **aliphatic** fraction. No other **n-alkane** or **n-alkene** in the range of C_{15} to C_{19} ever occurs at a concentration even 10% that of C_{17} . Another characteristic of the red algae is the presence of quantities of high molecular weight **n-alkane** material with an absence of any odd carbon number dominance. This high molecular weight (**HMW**) suite of hydrocarbons with no odd or even carbon number preference in most red algal species is accompanied by little of the **UCM** thereby indicating a non-petroleum and probably a **biogenic** source for these hydrocarbons. It has been suggested that **epiphytic bacteria** may be responsible for **HMW n-alkanes**. In a **Sargassum** study (Burns and Teal, 1973) this possibility was dismissed as a source of the **HMW** material occurring in that alga. The idea of epiphytic organisms on the red algal species probably cannot be entirely discarded as a possible contributor.

Chlorophyta

The green algae (Chlorophyta) with aliphatic hydrocarbons consisting primarily of the **n-alkanes** from $n-C_{15}$ to $n-C_{23}$ are marked by a very **complex assortment** of hydrocarbon distributions. In one species, **Caulerpa sertularoides**, the predominant **n-alkane** is $n-C_{19}$ as opposed to the more common predominance of $n-C_{17}$ in other species of red and green algae. Even where $n-C_{19}$ does not occur in the highest concentrations, it and other low molecular weight alkanes and alkenes represent a large fraction

of the total aliphatic hydrocarbons. Nearly all of the samples contain quantities of phytadienes ranging as high as 740 ppm by wt. in one Caulerpa sp. As stated previously the presence of these phytadienes may be related biochemically to the presence of phytane. However this relationship is not immediately apparent when one looks at the levels of phytadienes vs levels of phytane. In most specimens they both occur, but there are also instances where either appears alone. If chemical stress were responsible for the presence of the phytadienes then one might expect to find a relationship between phytadienes and the presence of petroleum-like hydrocarbons; however a comparison of Tables 2 and 3 and an examination of Figure 5 shows that no strong relationship exists. Further the production of phytadienes does not appear to be seasonally affected. Where the same species was collected at more than one season or more than one station (e.g. Codium repens) the phytadienes though varying in concentration are almost always present. Examining the data in Table 2 gives a clear indication that phytadiene synthesis is a characteristic of several species of green algae.

The homologous series of odd-carbon number n-alkenes with K.I. of 1712-2524 occur exclusively in the green algal specimens collected for this study. This trend of unsaturated hydrocarbon synthesis is supported in examining the chromatograms for other unsaturated species. Besides the phytadienes, the 1-n-alkenes, C_{17:1} and C_{19:1} are among the major aliphatic components in many of the green algal species. The Codium spp. are perhaps unique among the greens in containing so little unsaturated hydrocarbons excluding phytadienes. The Codium fragile analyzed by Youngblood et al. (1971) also noted a very low quantity of unsaturated hydrocarbons when compared to other green algal species.

Sufficient numbers of species of the algal genus, Codium, were collected to investigate variability among closely related species.. Both Codium repens and carolinianum give almost identical hydrocarbon chromatograms each having a large n-C₁₅ with the dominant n-C₁₇. However Codium isthmocladium and all the Codium of unidentified species contained but traces of n-C₁₅. Thus, the major hydrocarbon constituents probably are too variable to permit their use as a taxonomic aid much below the class level of algae.

The best opportunity for observing seasonal variations comes with the green alga, Halimeda discoidea which was collected in all three seasons. Intra-seasonal variability for this species is as great as inter-seasonal variability both in hydrocarbon levels and distribution. Overall hydrocarbon distributions in both the red and green algae remain about the same regardless of season. No decline or increase could be seen in levels of phytadienes or other unsaturated hydrocarbon components as a function of sampling period. It probably can safely be said that seasonal hydrocarbon differences in benthic algae in the Gulf of Mexico are minimal.

Phaeophyta

The brown algae are perhaps most unique in containing a hydrocarbon distribution that easily sets them apart from the greens and reds. The predominance of n-C₁₅ found in Dictyota dichotoma has been seen in Sargassum (Burns and Teal, 1973 and Lytle and Lytle, unpublished data) and other brown algae (Youngblood et al., 1971). Neither the pure strain, No. 146-B-14 or the one mixed with Kallymenia sp. (No. 146-B-1) display any tendency to synthesis of high molecular weight hydrocarbons or to unsaturated hydrocarbons.

Pollution

The presence of hydrocarbon materials indicative of highly degraded petroleum appears to occur almost randomly. Polluted and non-polluted specimens of the same species and from the same sample location and period (see Fig. 5) graphically illustrate this point. These petroleum residues are probably entrapped tar particles which have been found throughout the Gulf of Mexico and other oceanic waters as a result of natural seeps, oil spills and tanker wastes dumped at sea (Horn *et al.*, 1970; Butler *et al.*, 1973; Jeffrey *et al.*, 1974; McGowan *et al.*, 1974; Butler and Harris, 1975; Wade *et al.*, 1976). Though most of the larger tar particles are seen in surface samples, micro-fine particles may occur throughout the water column and could account for those algal samples having tarry residues but no visible evidence of such. Teal (1976) also noted the incorporation of relatively undegraded tar particles into benthic organisms. The residues in the algae would appear to lie at some midpoint in degradation between pelagic tars found in the Gulf of Mexico with little UCM material and much *n*-alkane material and abyssal tars with mostly UCM indicating a high degree of degradation (Jeffrey, *et al.*, 1974). The intermediate depth of algal collections tends to support this contention. No petroleum pollution of recent derivation was found in these algal specimens, and none has been found in the sediments off the coast of Florida where these specimens were collected (Gearing *et al.*, 1976). Unfortunately sediments that have shown a detectable degree of recent petroleum pollution off the Mississippi coast and on the continental slope of Mississippi-Alabama-Florida (Gearing *et al.*, 1976; Lytle and Lytle, 1977) are not areas suitable to the growth of benthic algae so therefore they are not suitable pollution indicators in these areas.

The only **trend** in pollution that might be significant occurred along Transect II off of **Clearwater**, Florida. During collection period **1** all specimens showed **signs** of pollution, during period 2, 75% showed pollution and during period 3, none. This seeming decline is coincident with the migration of **the** loop current in the Gulf further from the Florida coastline which *might* be transporting various Mississippi **River-** derived and other materials across the shelf at this transect (Maul, 1977). With the limited data this appears to be speculative at best.

Conclusions

In general these Gulf of Mexico algal specimens support previous work on hydrocarbon classification. Red, green and brown algae can readily be distinguished by their **aliphatic** hydrocarbon distributions. Reds containing mostly $\underline{n-C}_{17}$ and browns mostly $\underline{n-C}_{15}$ (only one species tested) synthesize relatively few hydrocarbon constituents when compared to the greens, which contain several **n-alkanes** in large abundance and also series of unsaturated hydrocarbons. The presence of **phytadienes** in several species sometimes as dominant components deserves further attention. With the exception of some highly degraded petroleum residues, hydrocarbon analysis of benthic algae documents the relative freedom from pollution enjoyed by the Florida coastal zone.

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