

HYDROCARBONS FROM ZOOPLANKTON OF THE EASTERN GULF OF MEXICO

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INTRODUCTION

The sale of oil and gas leases along the entire U. S. outer-continental shelf (OCS) and heightened public awareness of the potential harmful impact of petroleum-related activities, resulted in the initiation of environmental baseline and monitoring studies in the lease areas, under the sponsorship of the U. S. Department of Interior, Bureau of Land Management. The first of these studies was the MAFLA (Mississippi-Alabama-Florida) program in the northeast Gulf of Mexico. To date, four sets of seasonal samples have been collected and analyzed, the last three of which were identical with regard to locations, measurements and techniques. My laboratory participated in the program by analyzing for hydrocarbons in water, suspended particulate, neuston and zooplankton. The latter samples are the subject of this report.

METHODS

Zooplankton were collected by oblique tows using 0.5 m, 202 μ m nets with 5:1 length to width ratio. The zooplankton were removed from the cod end (without washing the net), placed in glass vials with Teflon-lined caps and frozen. In the laboratory, samples were thawed and foreign material was removed under a 30 power dissecting microscope. A known weight of oven dried (50°C) samples was refluxed in a 1:1 mixture of benzene and methanolic KOH for four hours.

The mixture was then filtered through pre-combusted Whatman GF/F filters to remove debris and the benzene layer was removed from the filtrate following addition of one part of distilled water. After two additional extractions of the aqueous phase with benzene, the extract was reduced to

dryness and weighed. The residue was taken up in hexane and applied to a prewashed alumina/silica gel column (1:5 v/v ratio, activity one) and eluted with two column volumes of hexane (saturated or non-polar hydrocarbon fraction) and two column volumes of benzene (unsaturated/aromatic or polar hydrocarbon fraction). The hexane fraction was reduced to small volume and the benzene fraction dried and taken up in a small volume of hexane for gas chromatographic (GC) analysis.

Primary GC analysis was done with 2.2 mm I.D. x 2 m stainless steel columns packed with 4% FFAP on Gas Chrom Z, 80/100 mesh. Retention times were converted to retention indices utilizing known standards of n-alkanes. Peak areas were automatically integrated and converted to weight by applying GC response factors calculated from quantitative normal and isoprenoid alkanes and aromatics. These calculations as well as calculations of peak ratios, odd-even preference, wt.% composition and concentration were done by a computer program which produced both paper and magnetic tape output for submission to a central data bank.

Glassware was washed in detergent, soaked in acid, rinsed with distilled water and oven dried. Solvents were doubly distilled. Periodic blanks were run and rejected if material with retention index greater than 1200 was present.

RESULTS AND DISCUSSION

A series of 15 stations along four transects in the MAFLA area (Figure 1) were sampled in June/July 1975, September 1975 and January/February 1976. The zooplankton biomass collected averaged 91 mg dry weight/ins in summer,

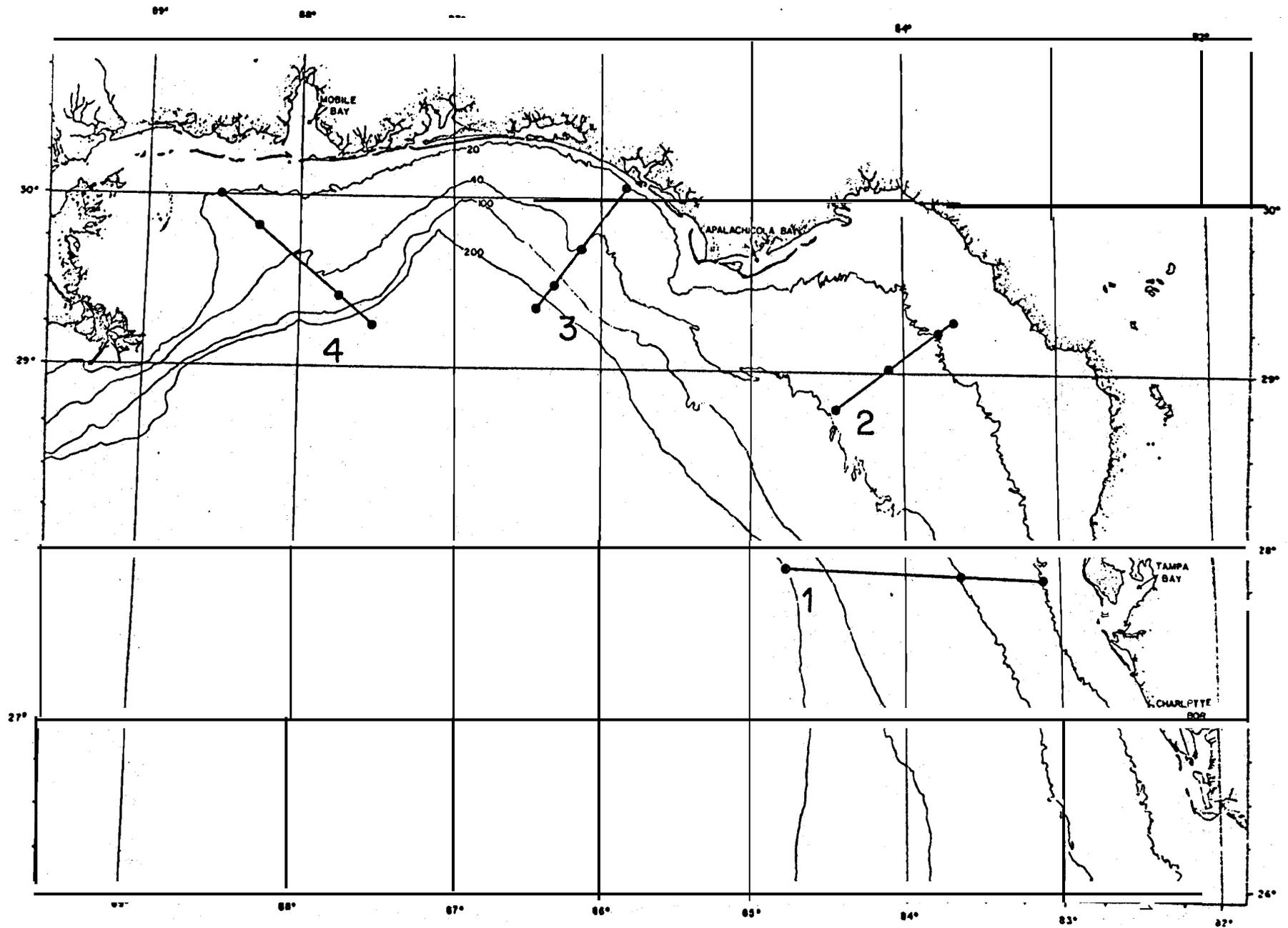


Fig. 1 Transect and station locations in the MAFLA area

18 mg dry weight /m³ in fall and 13 mg dry weight/m³ in winter (Table 1). Total lipid content was nearly constant at 38-50 mg/g dry wt. The total hydrocarbon content (sum of all integratable peaks in both hexane and benzene fractions) averaged 212 µg/g dry wt. in summer, 135 µg/g dry wt. in fall and 719 µg/g dry wt. in winter. In laboratory studies, Lee, et al. (1971), determined that the total lipid content of a *Calanus* sp. was related to the concentration of phytoplankton carbon fed to it. At 100 g of phytoplankton carbon per liter, the copepod contained 120 mg/g of total lipid. The lower total lipid in zooplankton from the MAFLA area may be a reflection of a low standing stock of phytoplankton. The concentration of Chlorophyll a averaged less than 0.5 µg/l (Iverson, 1976) and concentration of POC averaged less than 200 µg/l (Knauer, 1976) during the three sampling periods.

Visual inspection of chromatograms from summer 1975 indicated that the zooplankton hydrocarbons fell into three compositional patterns, which were differentiated primarily by the unsaturated/aromatic fraction. The same groupings recurred in fall and winter. The first group, A (Figure 2), is characterized by high concentrations of pristane and variable amounts of n-alkanes in the C₂₁-C₃₂ region. (Blumer, et al., 1963). The higher n-alkanes are generally not as abundant as in this sample. Two peaks with retention indices of 1950 and 1976 appear frequently. These may be the phytadienes originally reported by Blumer and Thomas (1965). The benzene fraction of group A samples contained a group of peaks with retention indices from 2000 to 3200. There was considerable variation in the composition from station to station and season to season but the retention index range mentioned above was not exceeded. The concentration of total hydrocarbon

TABLE 1: Gravimetric Data - Seasonal

	<u>Summer</u>	<u>Fall</u>	<u>Winter</u>
Zooplankton Biomass mg dry wt./m ³	91	18	13
Total lipid extract mg/g dry wt.	49.9	37.7	49.8
Total hydrocarbon µg/g dry wt.	212	135	719
Total hydrocarbon µg/m ³	19.3	2.4	9.4

- Fig. 2 Zooplankton hydrocarbons, Group A
- A. Station 1102, hexane fraction, summer 1975
 - B. Station 1102, benzene fraction, summer 1975
 - c. Station 1415, benzene fraction, fall 1975
 - D. Station 1102, benzene fraction, winter 1976

Fig. 2A

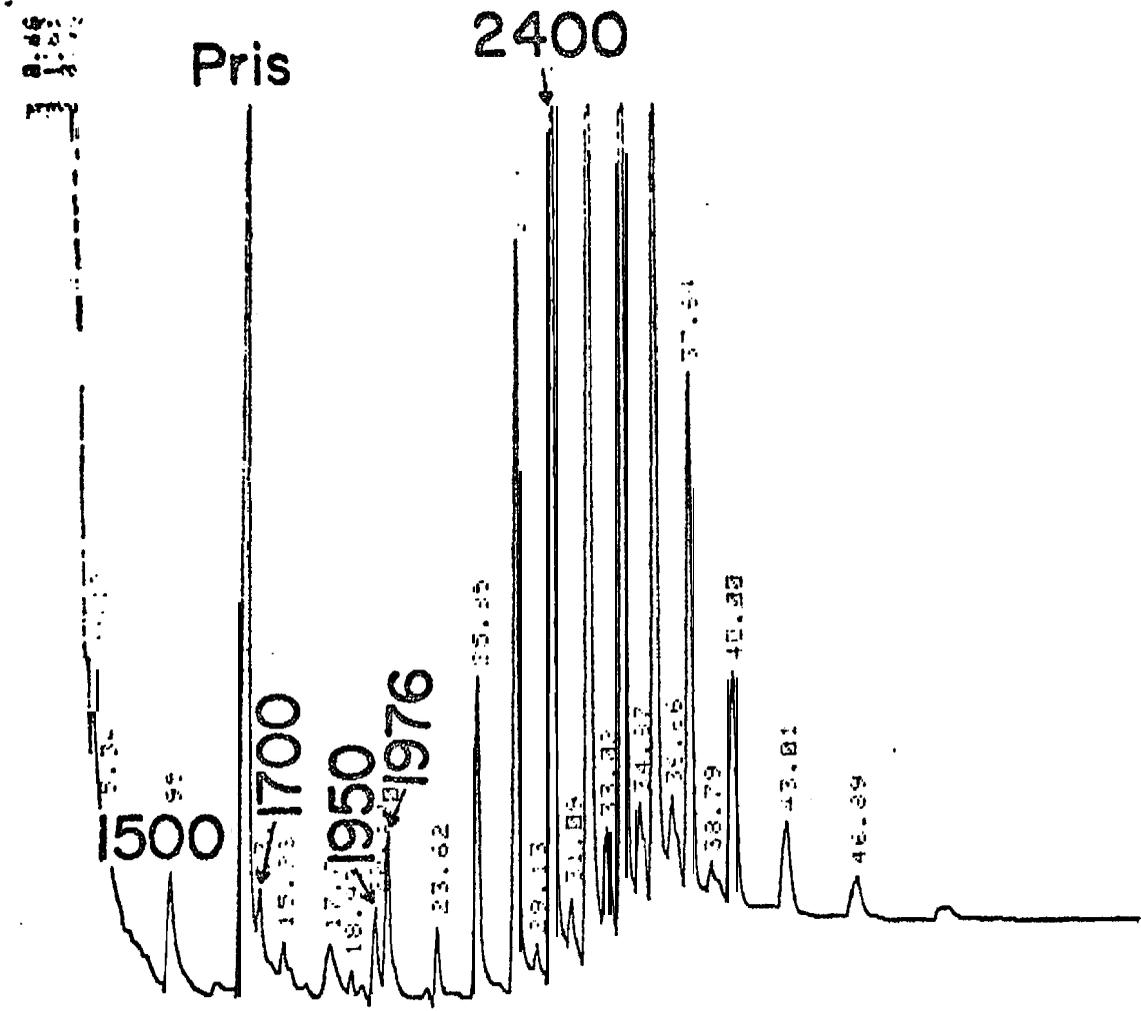
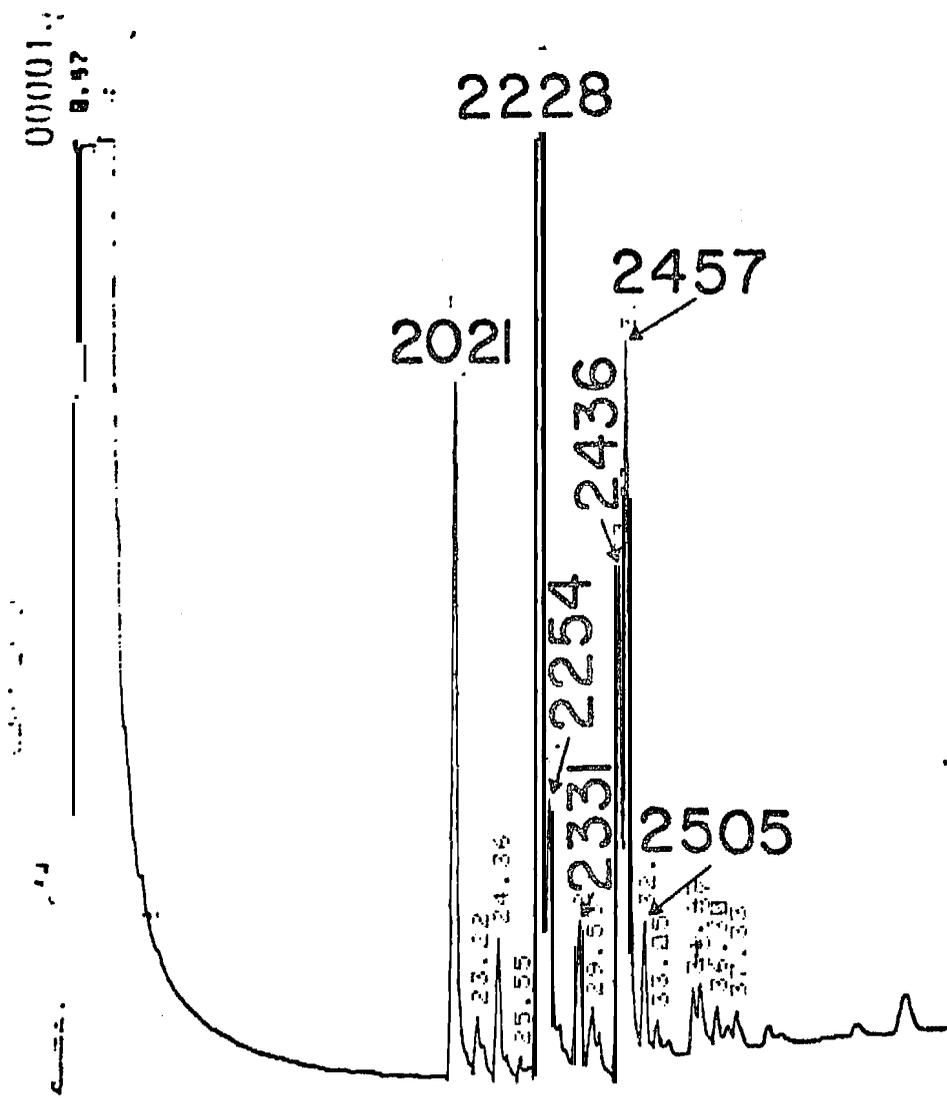


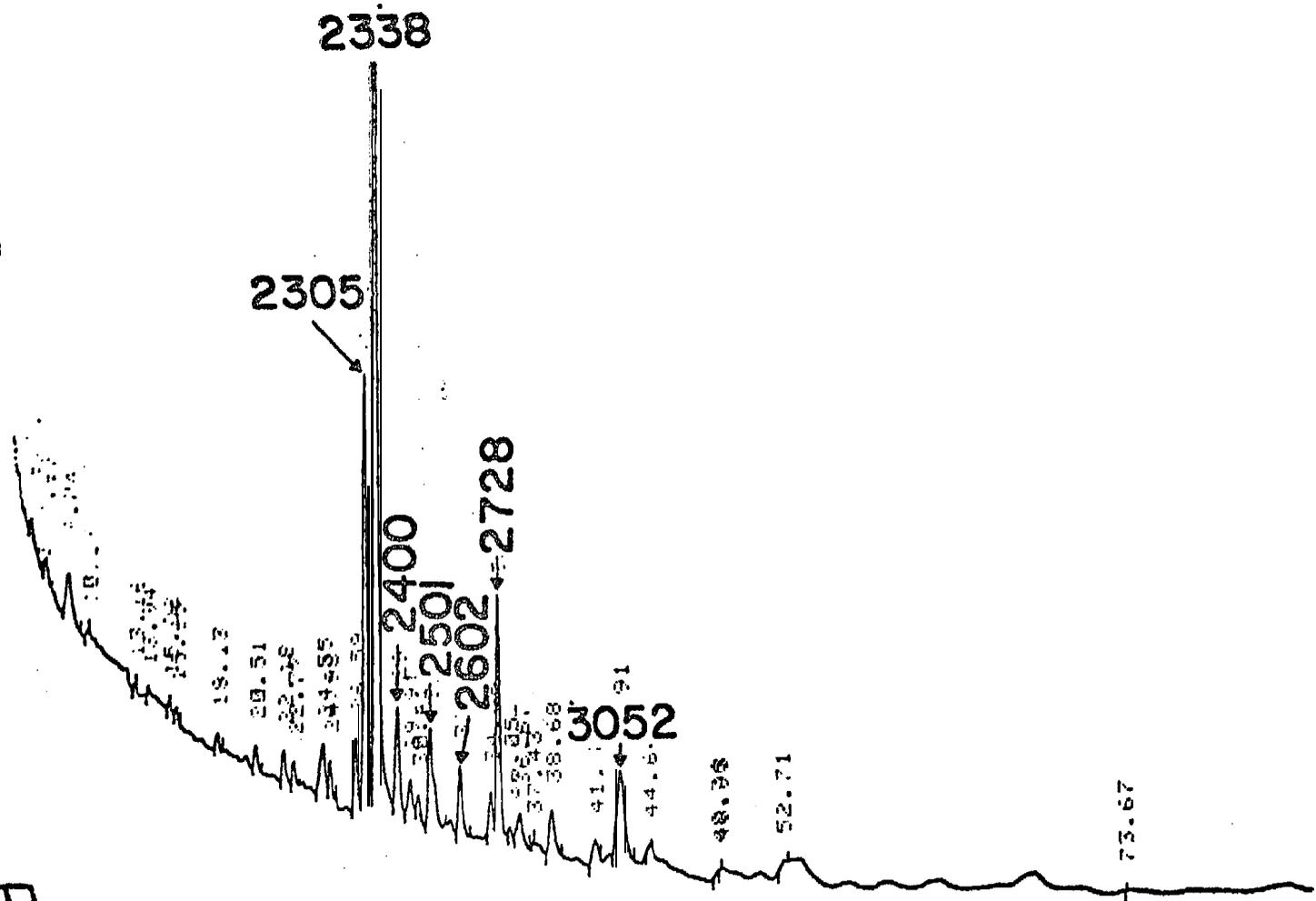
Fig 2B



0.52

0.52

Fig. 2c



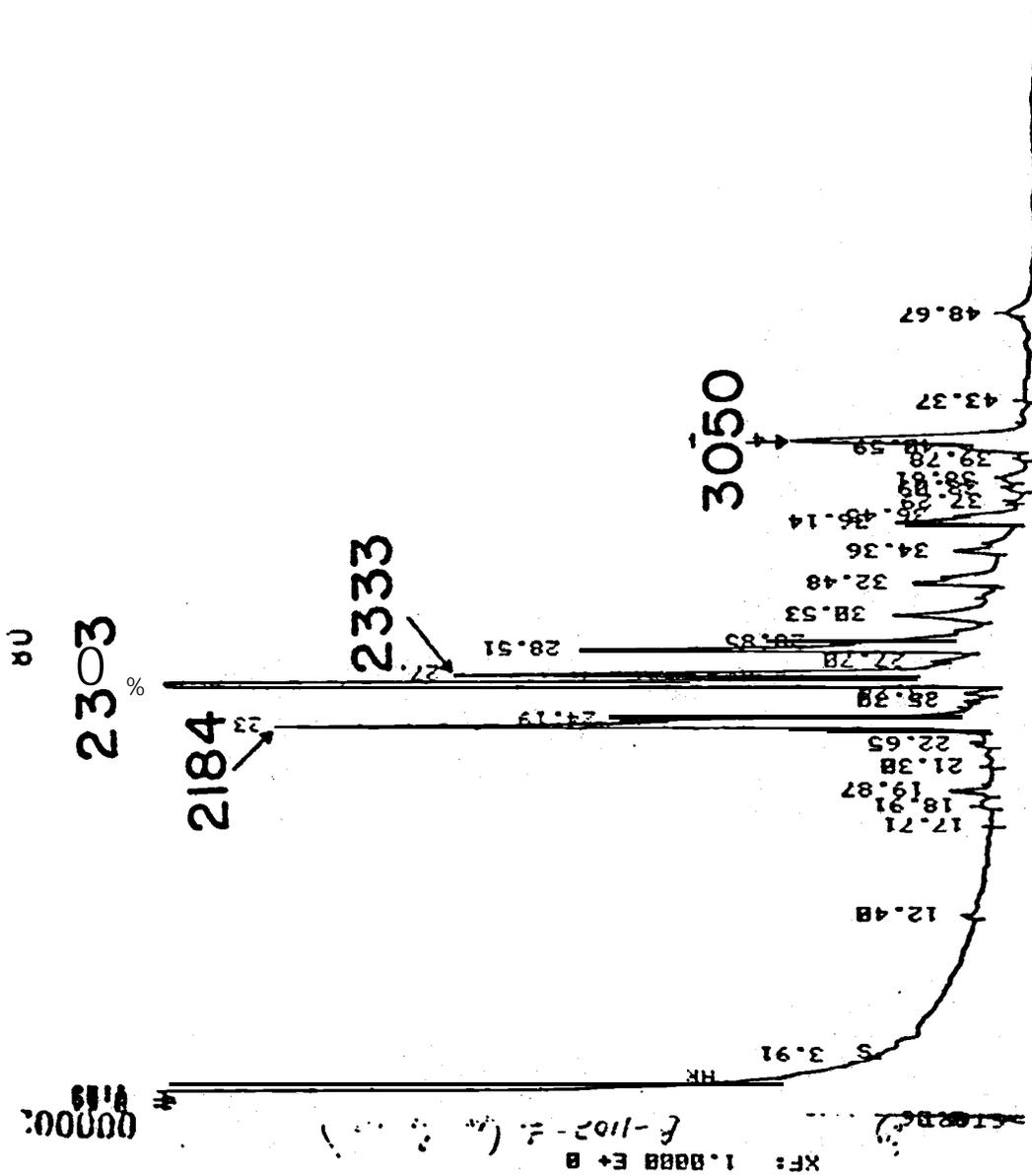


Fig 20

averaged 250 $\mu\text{g/g}$ dry wt. A peak in the benzene fraction at RI ~ 3055 corresponds to squalene (Blumer, 1967). This peak has at least one other component which is resolved from squalene on a non-polar column (SP2100).

The second group, B (Figure 3) contained very low amounts of hydrocarbons, primarily pristane in the hexane fraction and a peak at RI=2350 in the benzene fraction. The total hydrocarbon content averaged 29 $\mu\text{g/g}$ dry wt.

The last group, C (Figure 4), is most interesting. The hexane fractions were much like those of group B, containing pristane and little else. The benzene fractions contained a group of peaks in the 2000-3200 retention index range although they were generally fewer in number and lower in concentration than those in Group A. The interesting feature is the group of peaks with retention index 3400 and greater, to an estimated 4000. The same peaks seem to be recurring in this RI range; a pair at 3415 and 3450, a pair at ~ 3600 and a very large peak at ~ 3800 . Total hydrocarbon content was 640 $\mu\text{g/g}$ drywt. The higher retention index peaks in the benzene fraction account for the bulk of the total hydrocarbon weight. The identity of these components is still a subject of investigation.

The three zooplankton hydrocarbon groupings recurred in each of the three sampling periods. In summer (Figure 5) the C group was most abundant, occupying the offshore stations in Transects II, III and IV. The A group occurred in Transect I and two stations of Transect II while the B grouping was limited to the inshore stations of Transects II and IV. In fall (Figure 6) the B group was dominant and occupied all the inshore stations. The C group appeared offshore in Transects I and III, while the A group appeared only at the two outermost stations on Transect IV. In winter, (Figure 7) the B group was not

D
B

Fig. 3. Zooplankton hydrocarbons, Group B

A. Station 1205, hexane fraction, summer 1975

B. Station 1205, benzene fraction, summer 1975

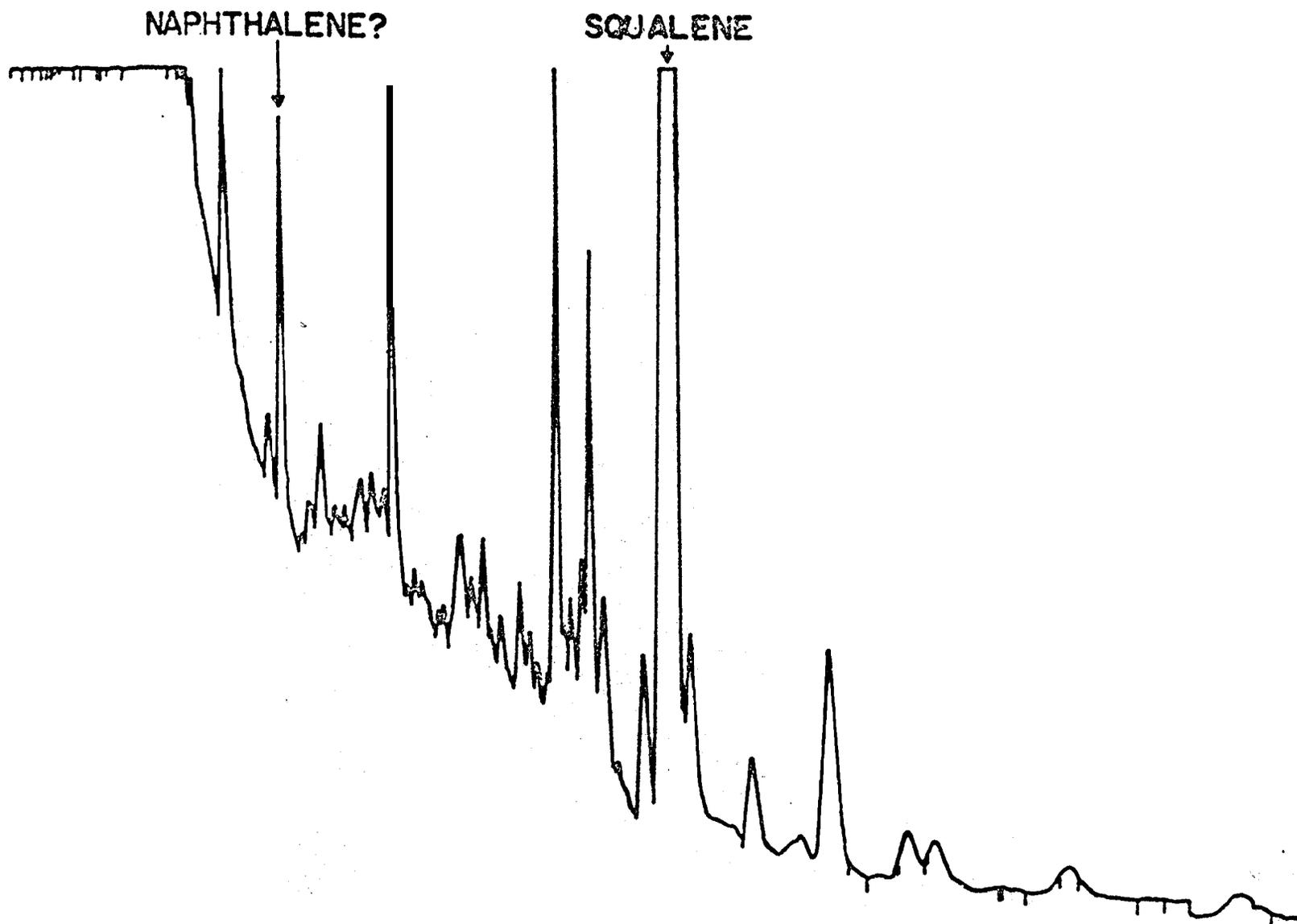


Figure 2d

- Figure 3a. Dissolved hydrocarbon distribution, fall 1975.
- 3b. Station 2, aliphatic fraction, dissolved hydrocarbons, fall 1975.
 - 3c. Station 15, aliphatic fraction, dissolved hydrocarbons, fall 1975.
 - 3d. Station 1, unsaturated/aromatic fraction, dissolved hydrocarbons, fall 1975.
 - 3e. Station 2, unsaturated/aromatic fraction, dissolved hydrocarbons, fall 1975.

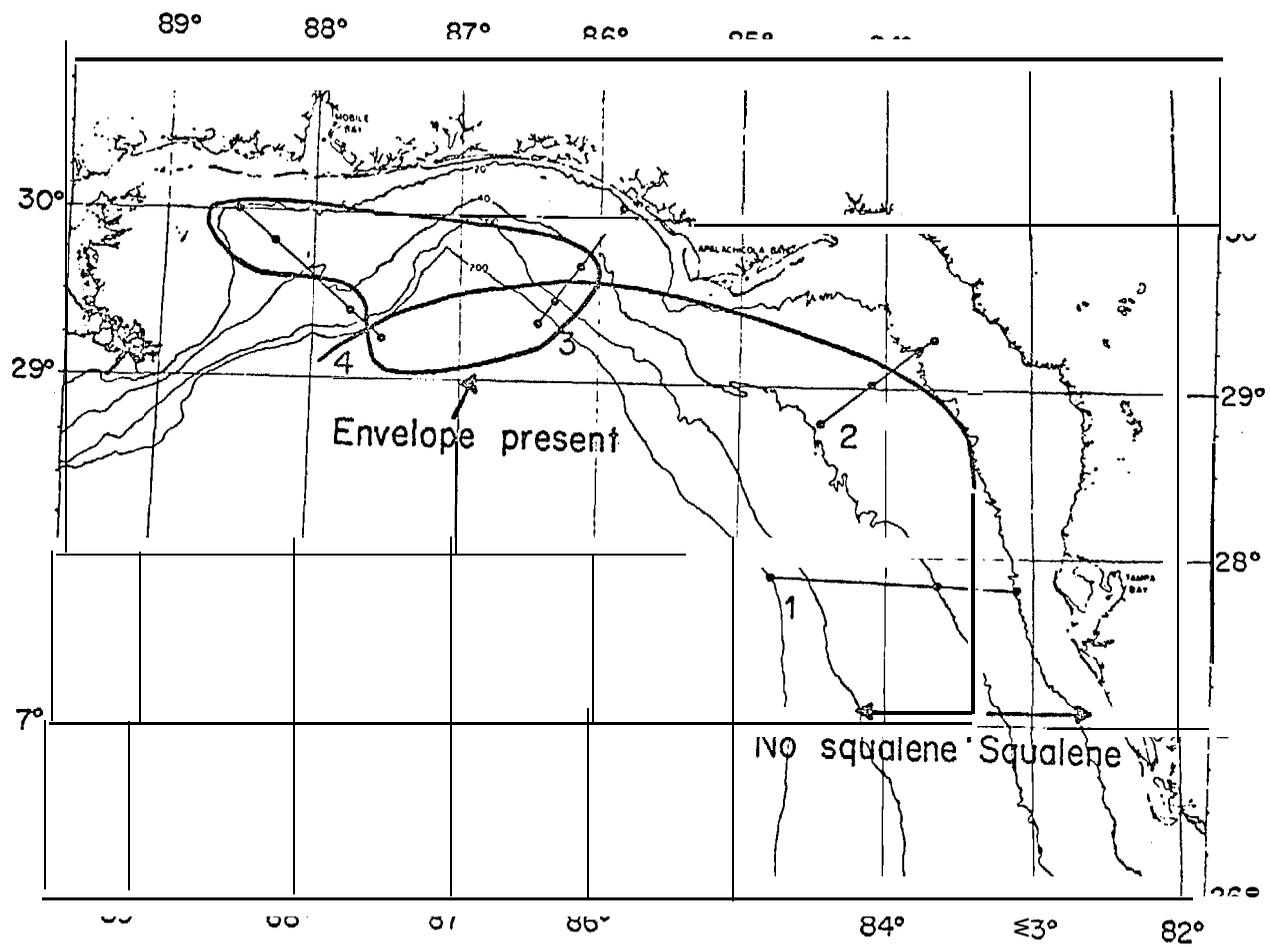


Figure 3a.

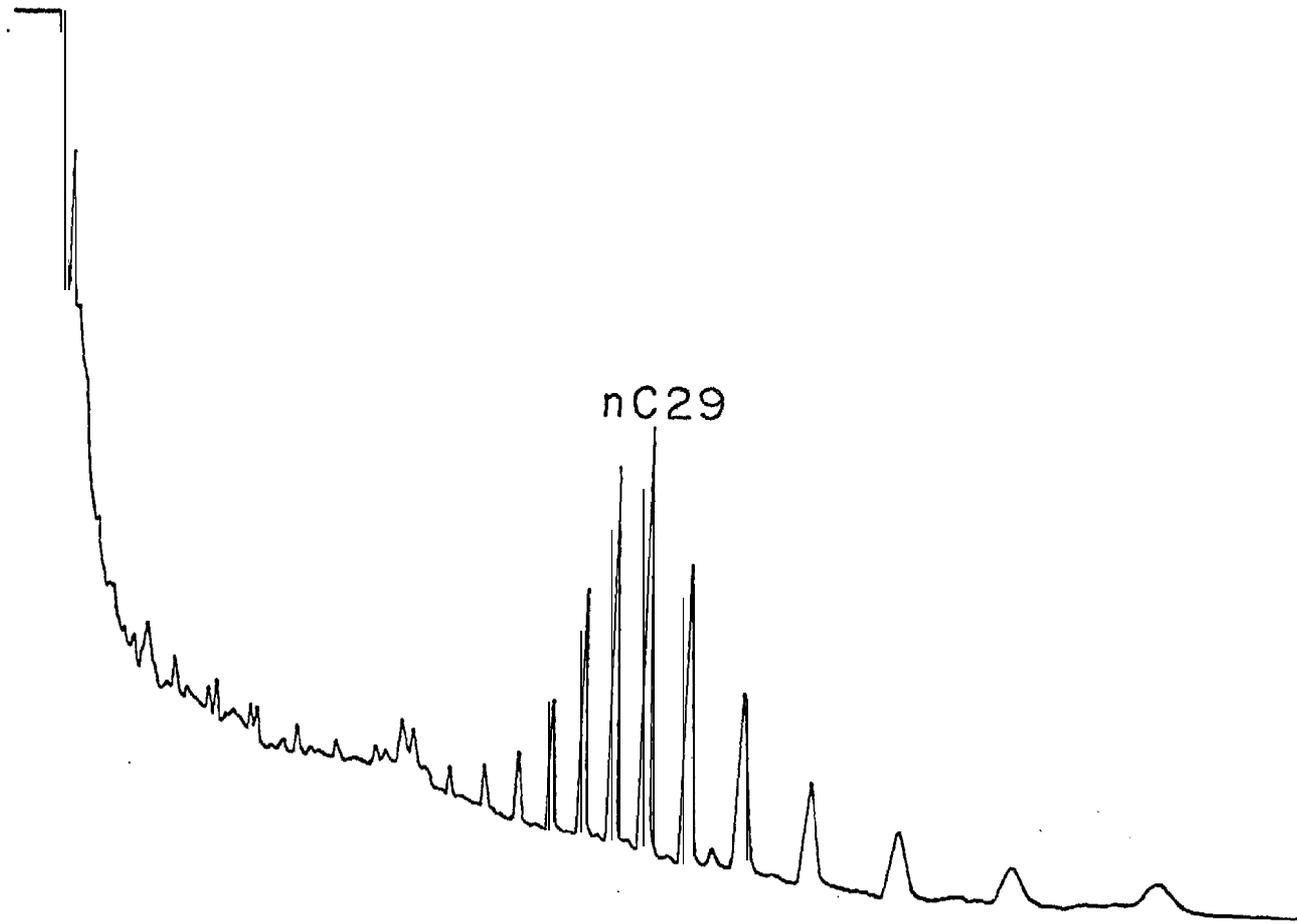


Figure 3b

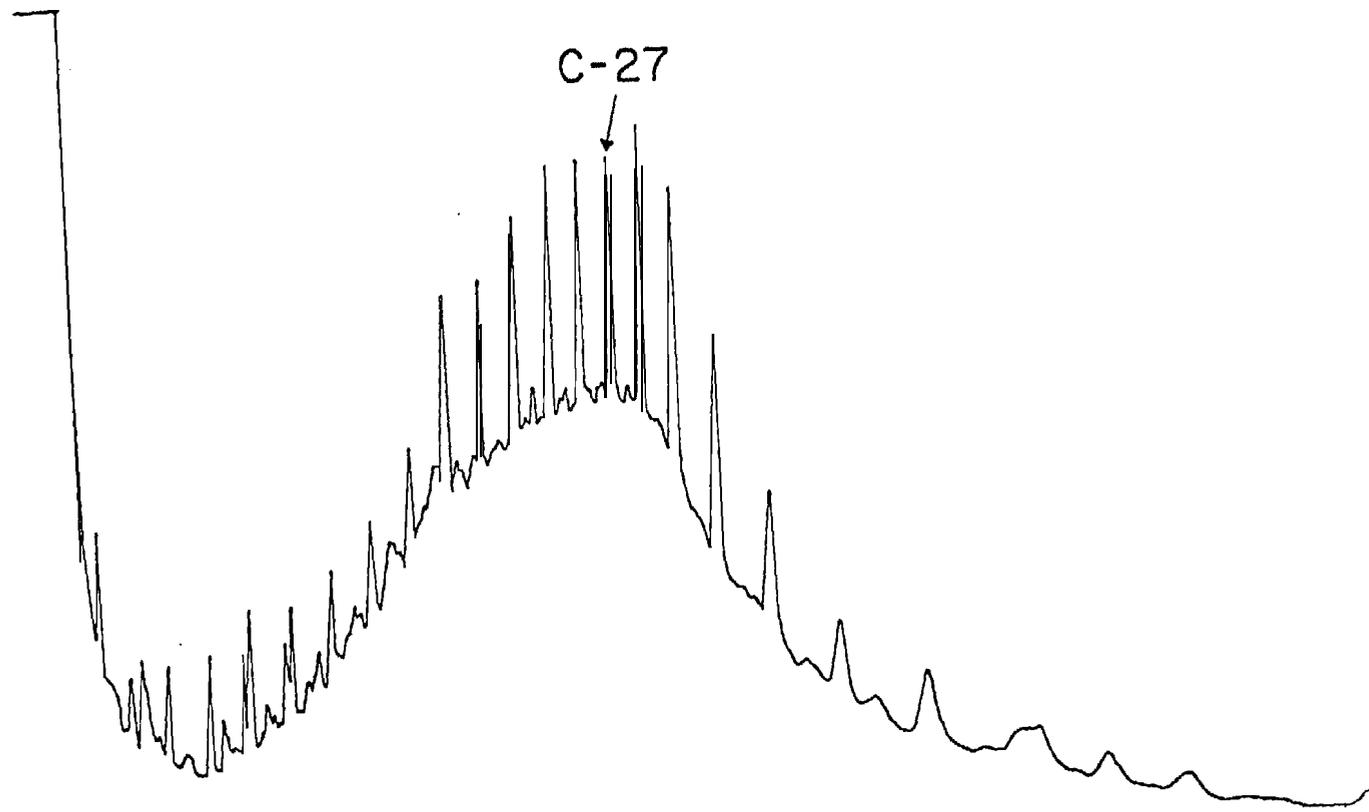
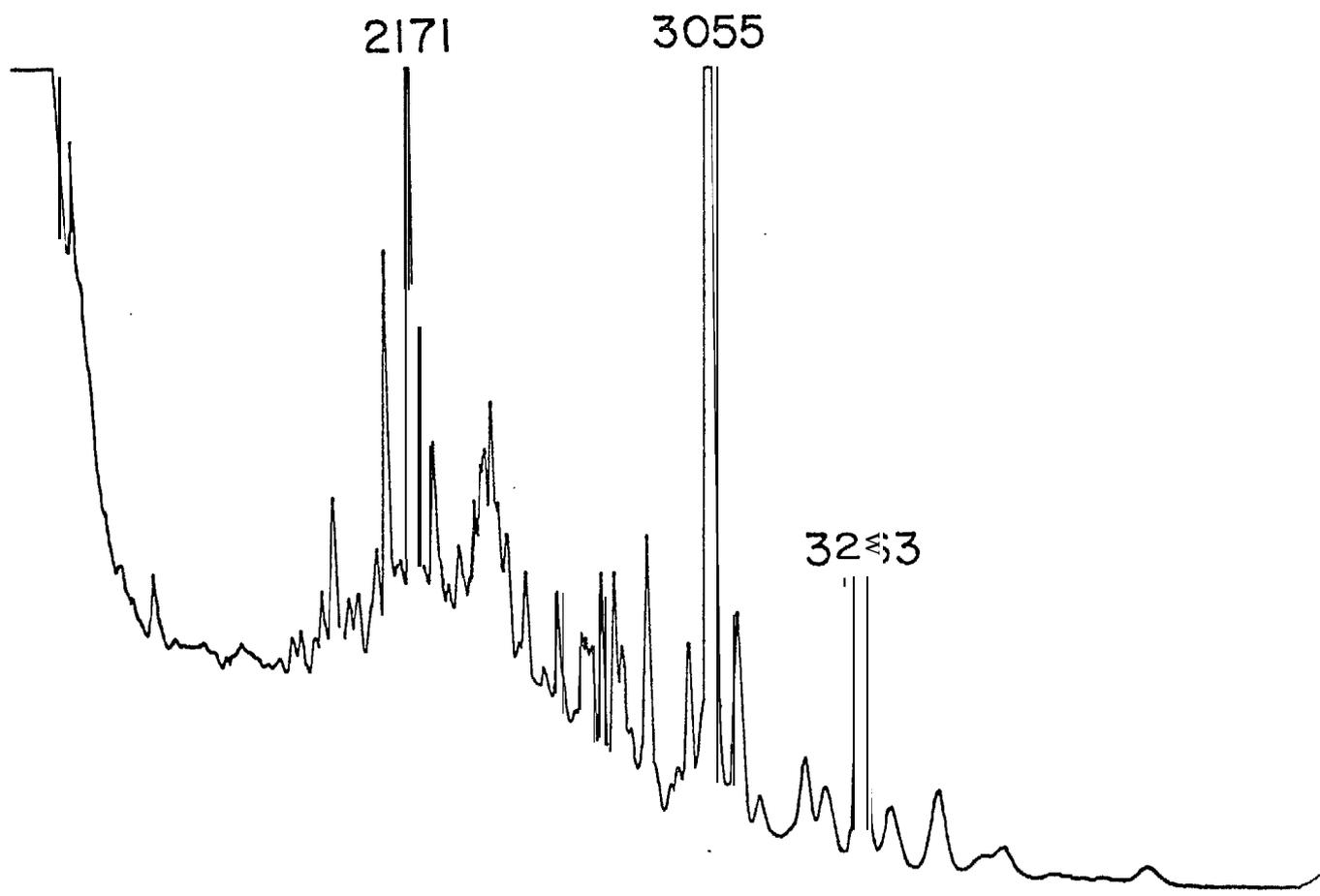


Figure 3c



eur 3d

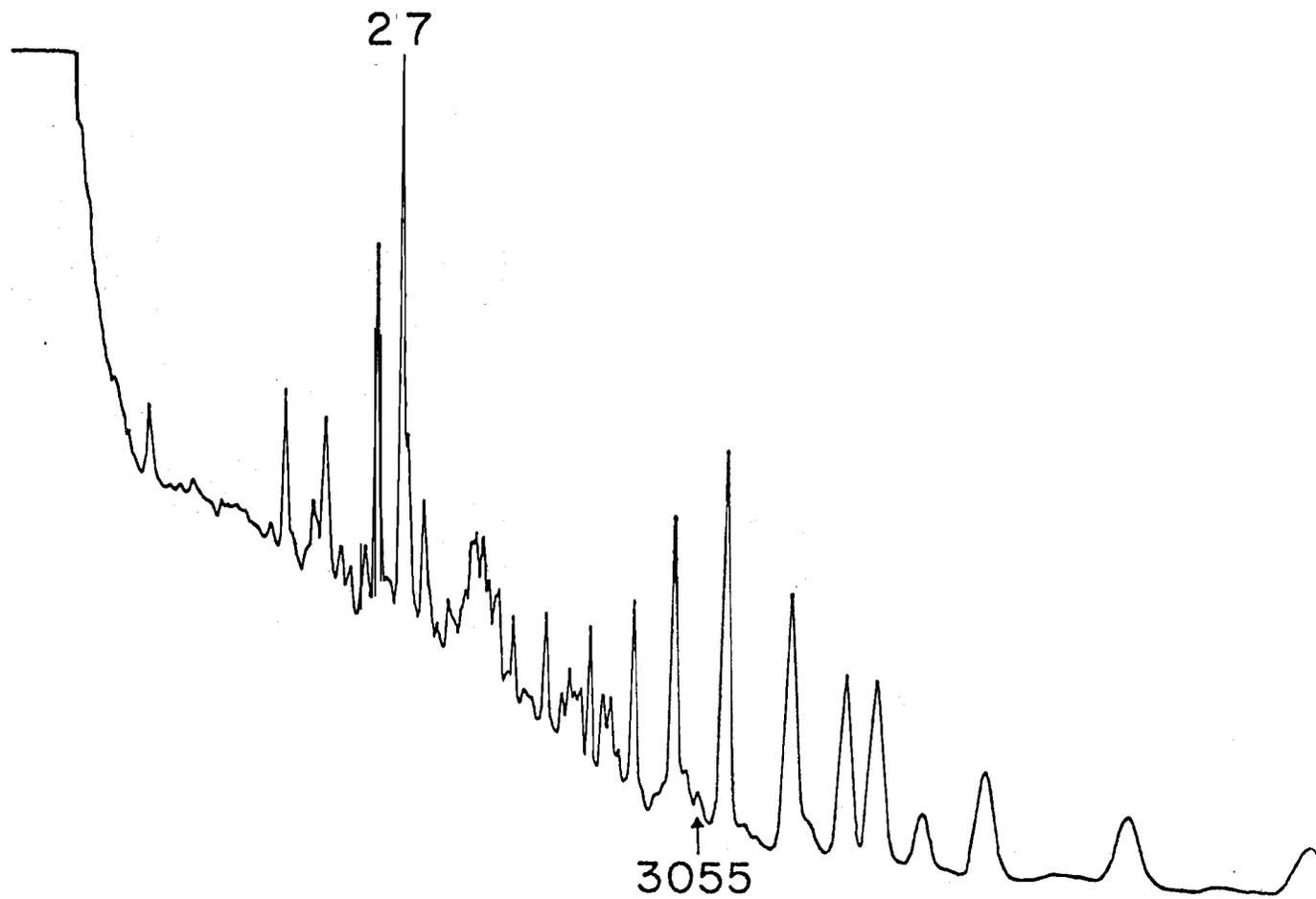


Figure 3e

- Figure 4a. Dissolved hydrocarbon distribution, winter 1976.
- 4b. Station 1, aliphatic fraction, dissolved hydrocarbons, winter 1976.
 - 4c. Station 10, aliphatic fraction, dissolved hydrocarbons, winter 1976.
 - 4d. Station 1, unsaturated/aromatic fraction, dissolved hydrocarbons, winter 1976.

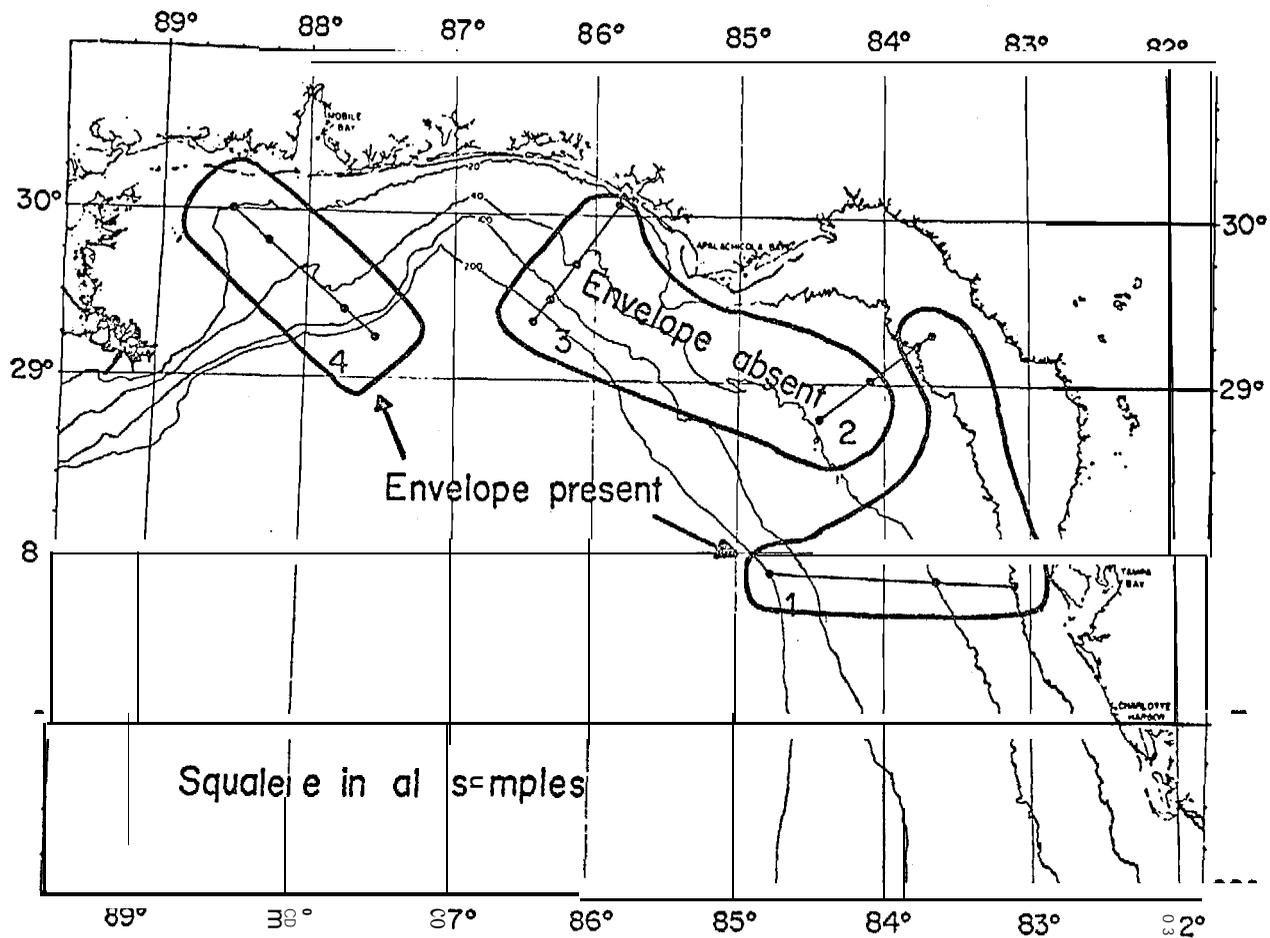


Figure 4a

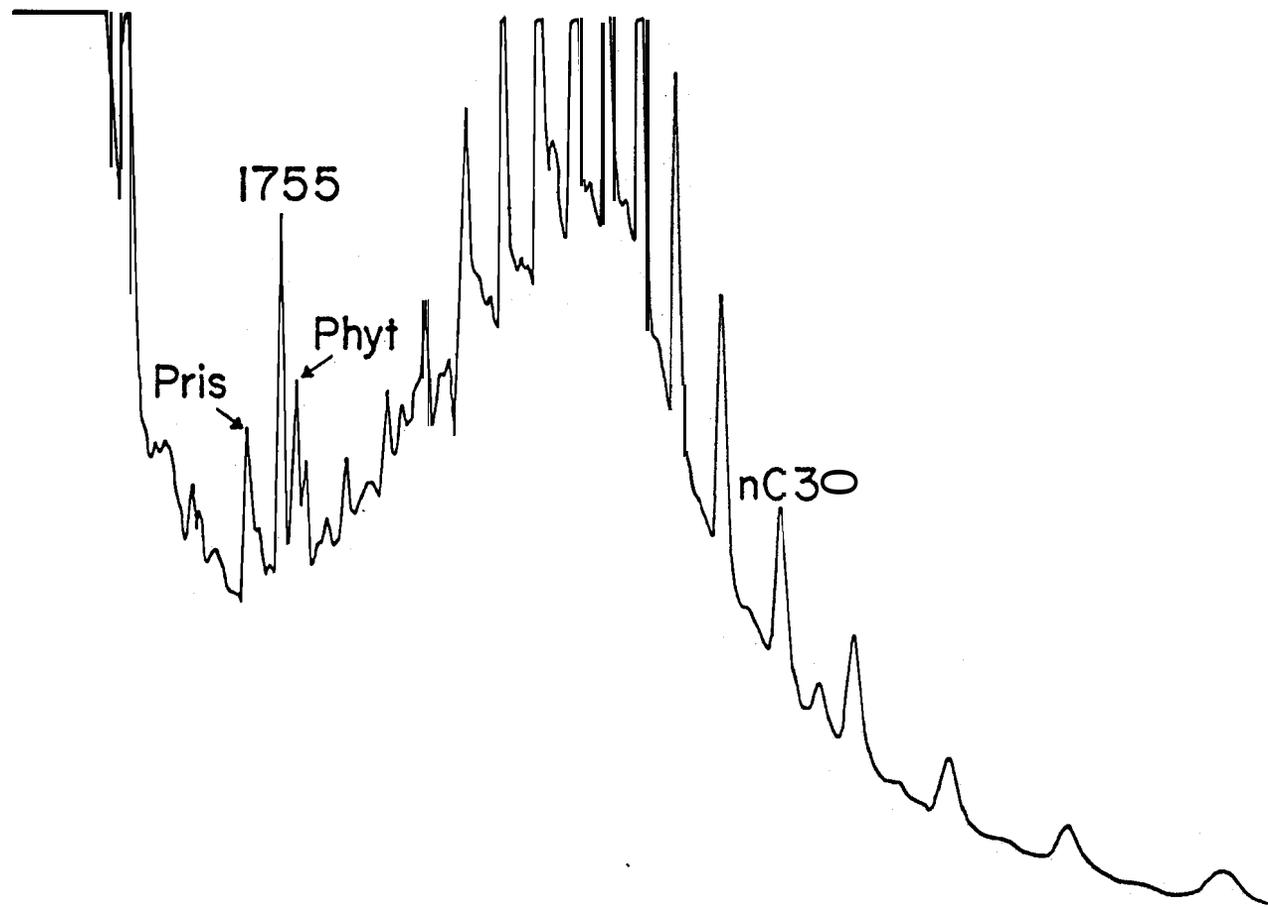


Figure 4b

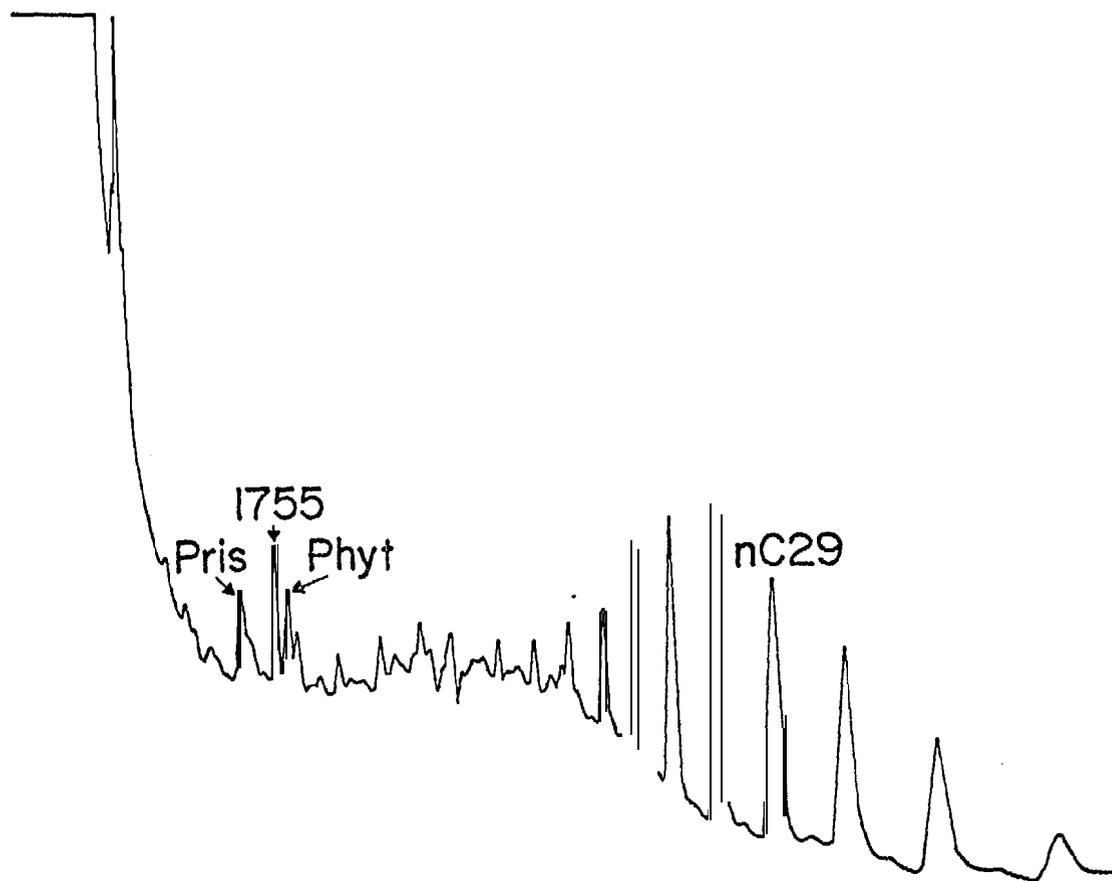


Figure 4c

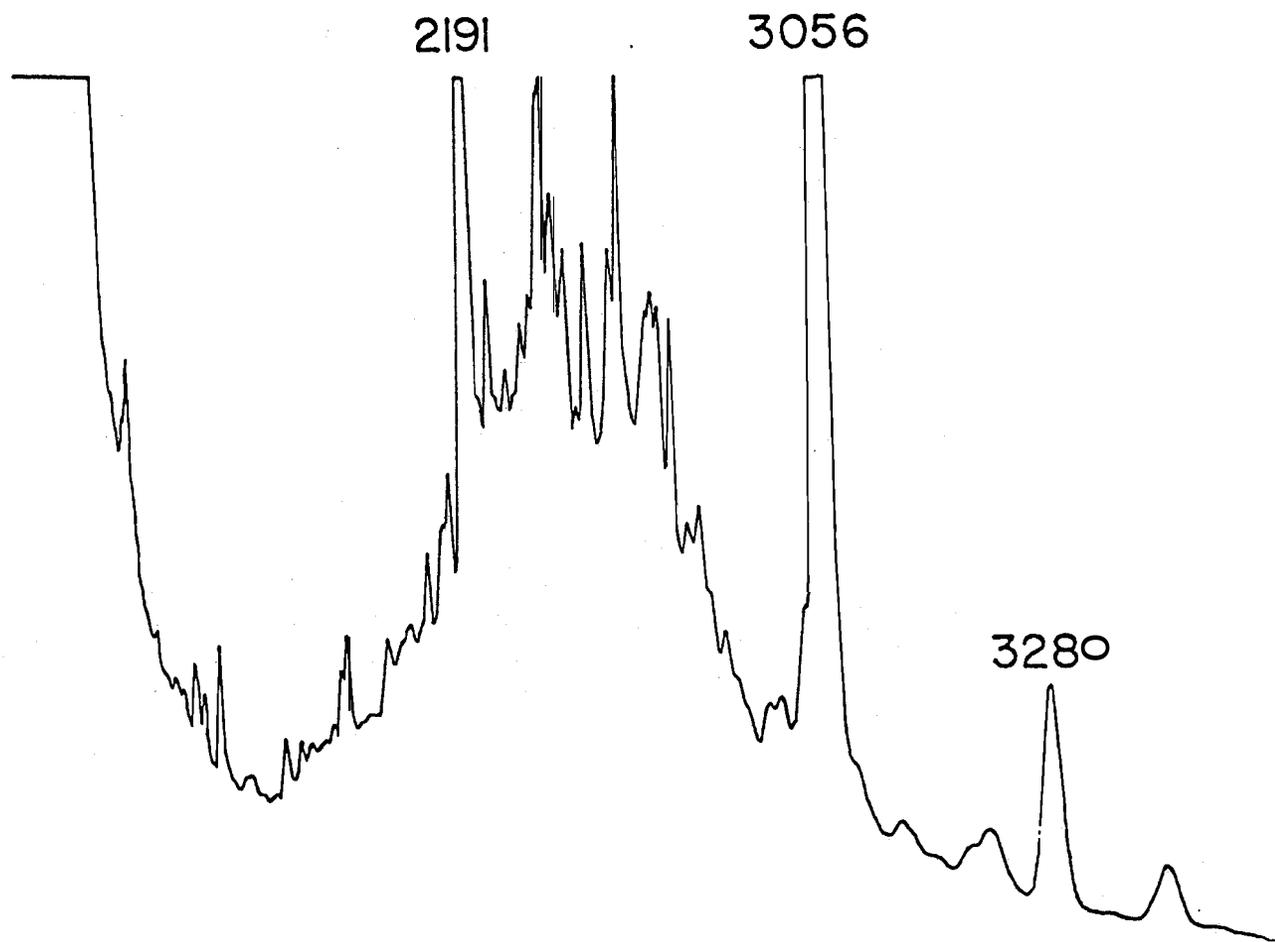


Figure 4d

- Figure 5a. Particulate hydrocarbon distribution, summer 1975.
- 5b. Station 4, aliphatic fraction, particulate hydrocarbons, summer 1975.
- 5c. Station 7, unsaturated/aromatic fraction, particulate hydrocarbons, summer 1975.

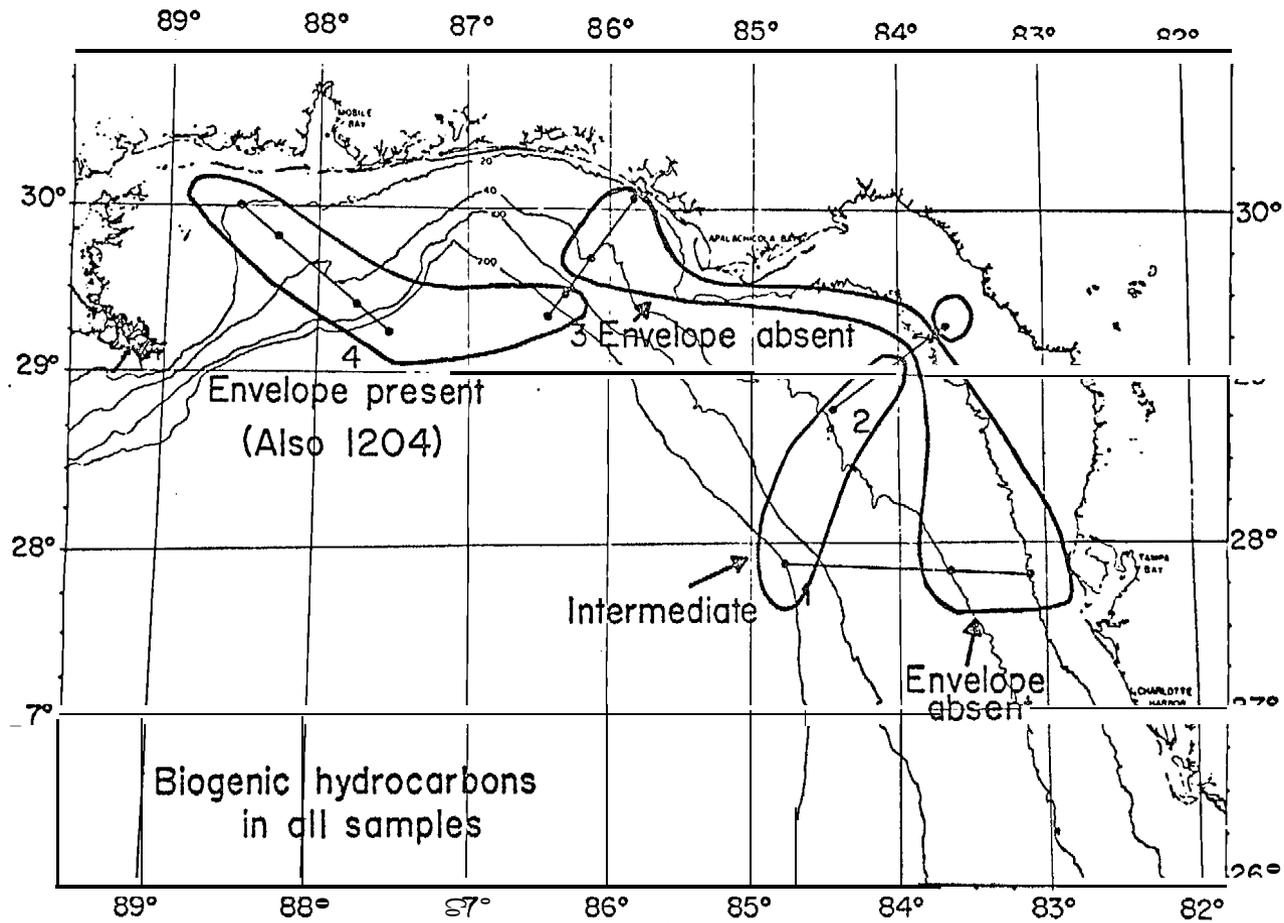
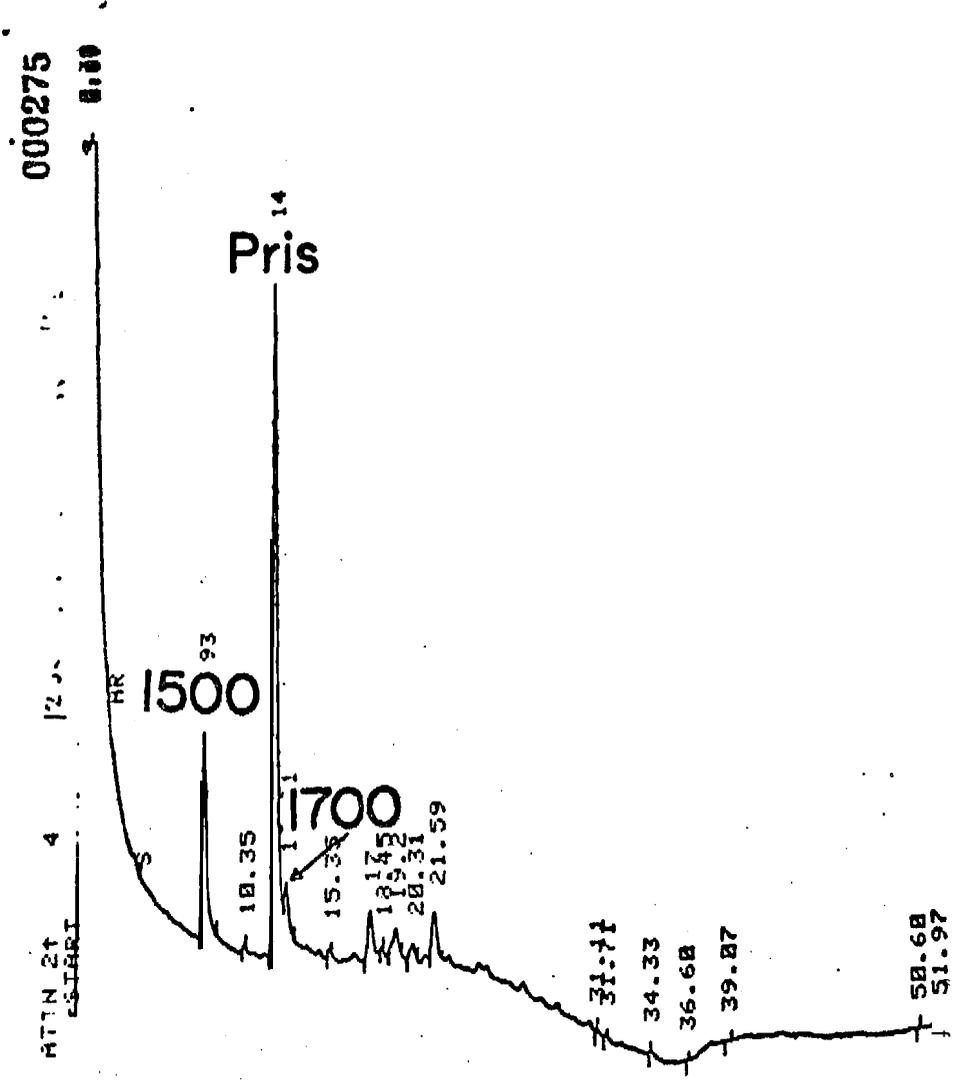


Figure 5a

Fig 3A



0.26

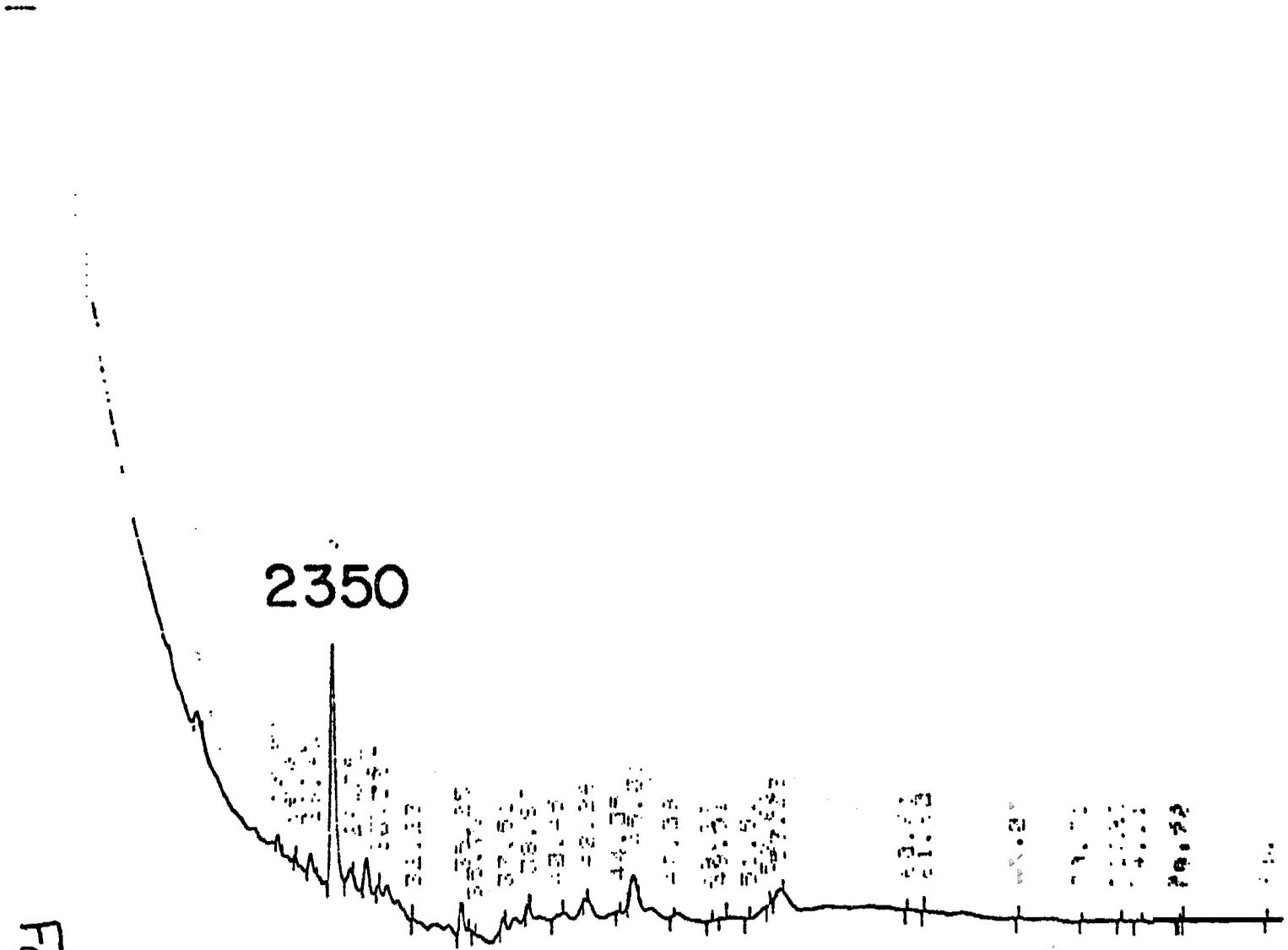
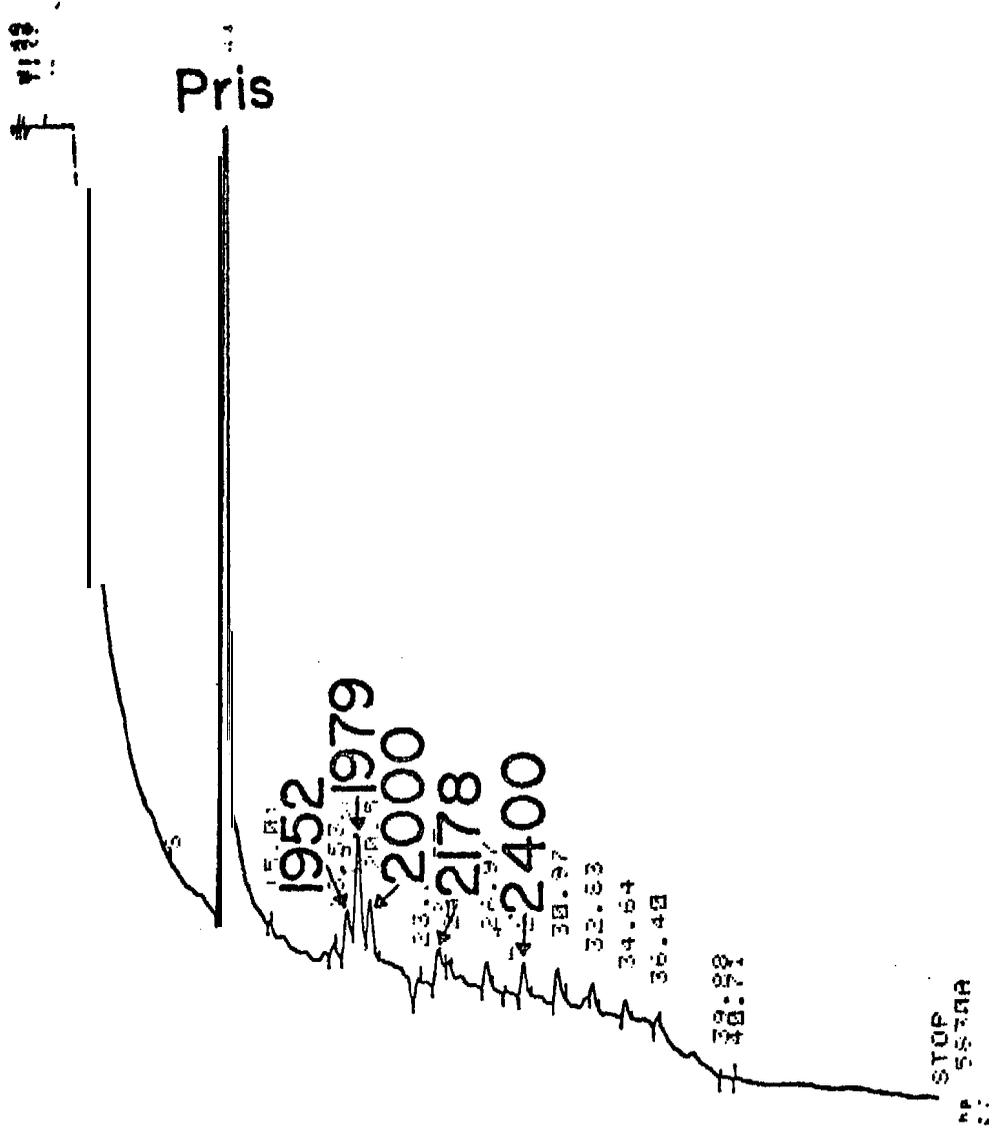


Fig. 3 B

- Fig. 4 Zooplankton hydrocarbons, Group C
- A. Station 1309, hexane fraction, winter 1976
 - B. Station 1415, benzene fraction, summer 1975
 - C. Station 1309, benzene fraction, fall 1975
 - D. Station 1309, benzene fraction, winter 1976

Fig 4 A



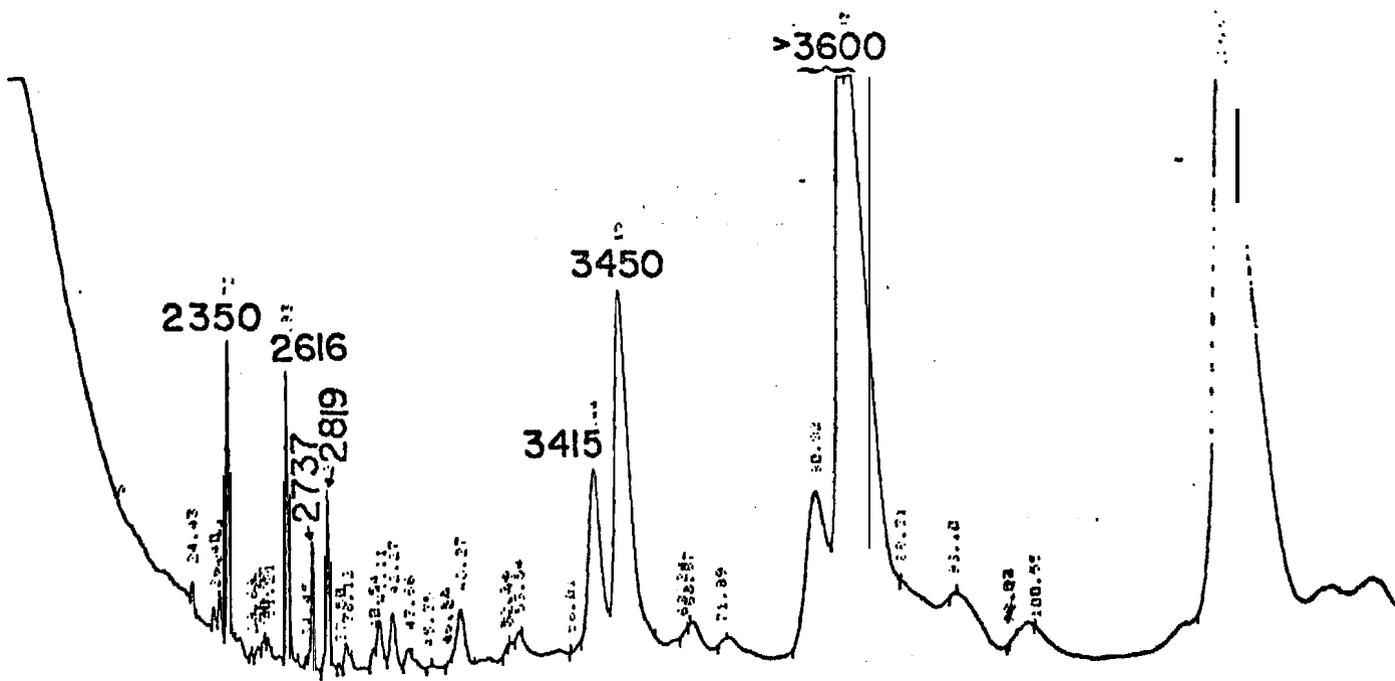


Fig. 4B

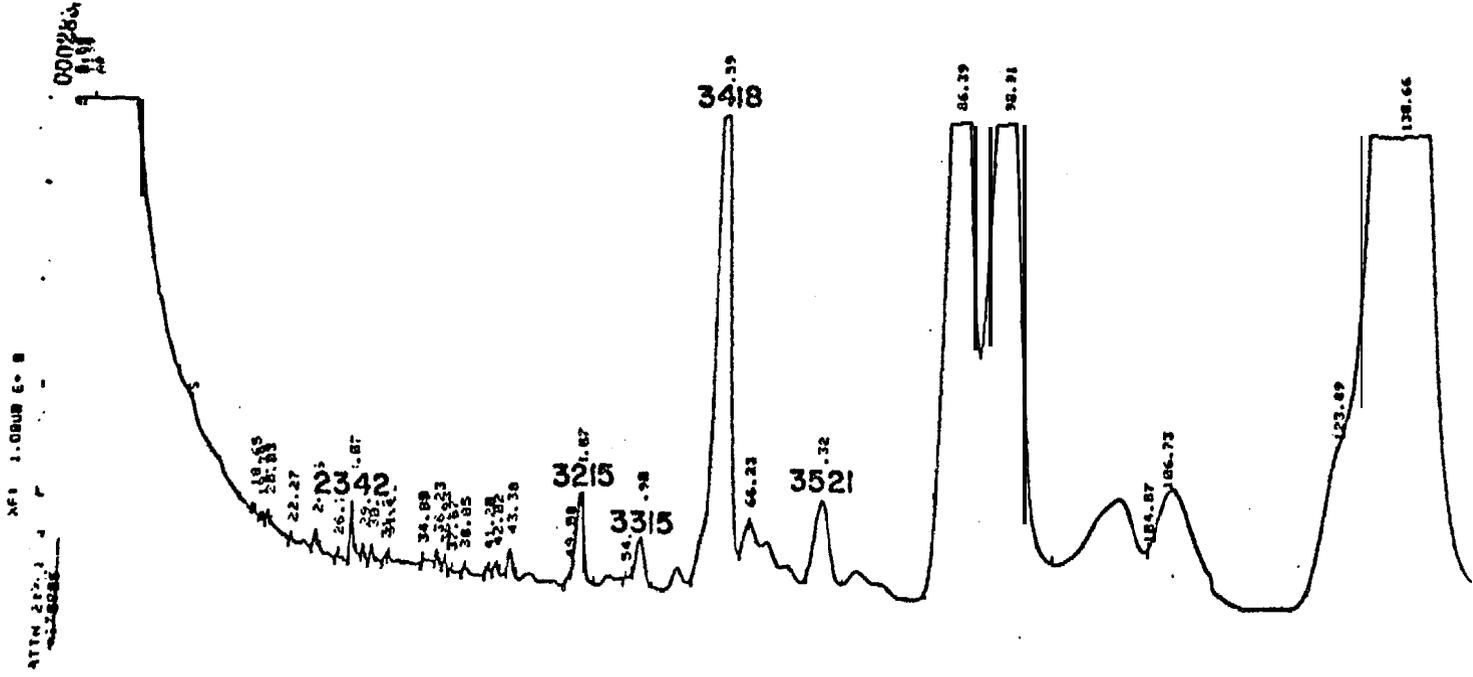


Fig. 4C

110

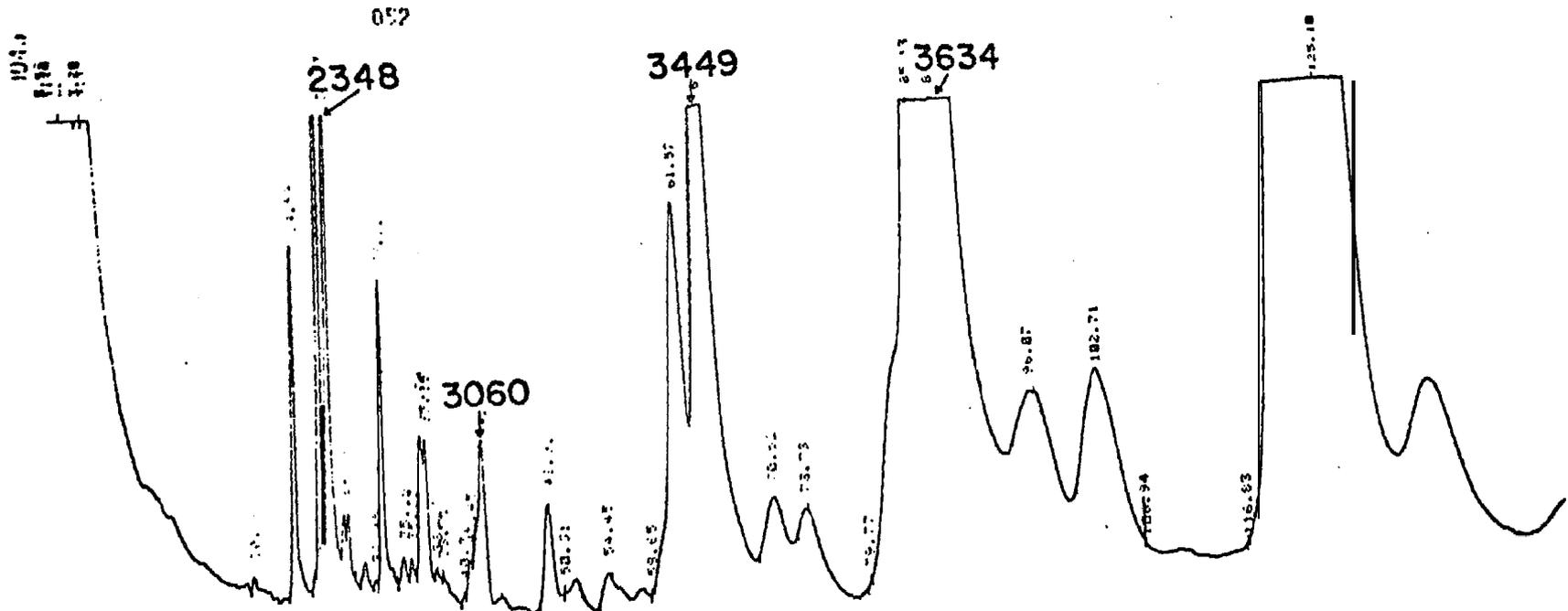


Fig. 4 D

Fig. 5 Zooplankton hydrocarbon group distribution, summer 1975

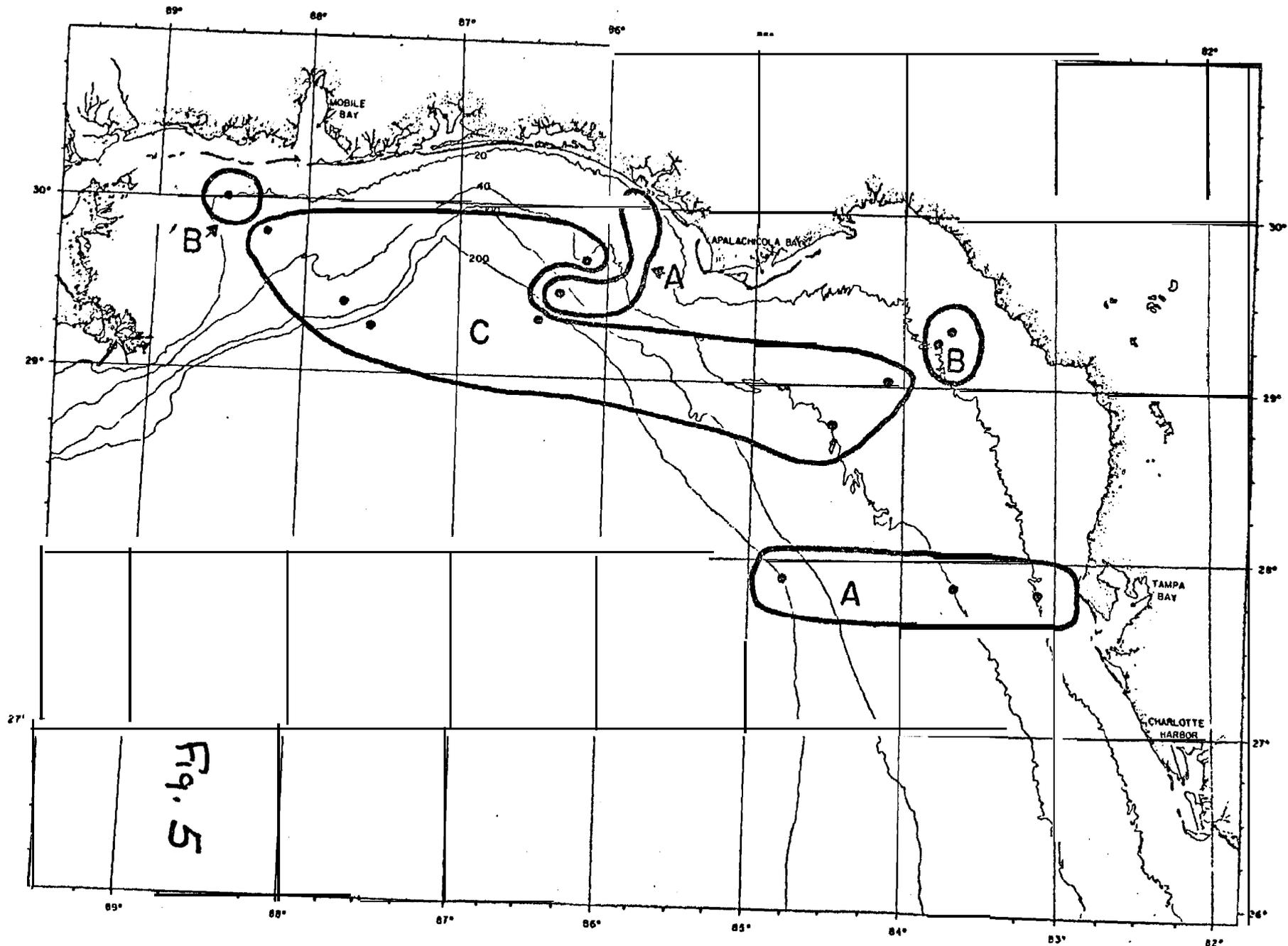


Fig. 6 Zooplankton hydrocarbon group distribution, fall 1975

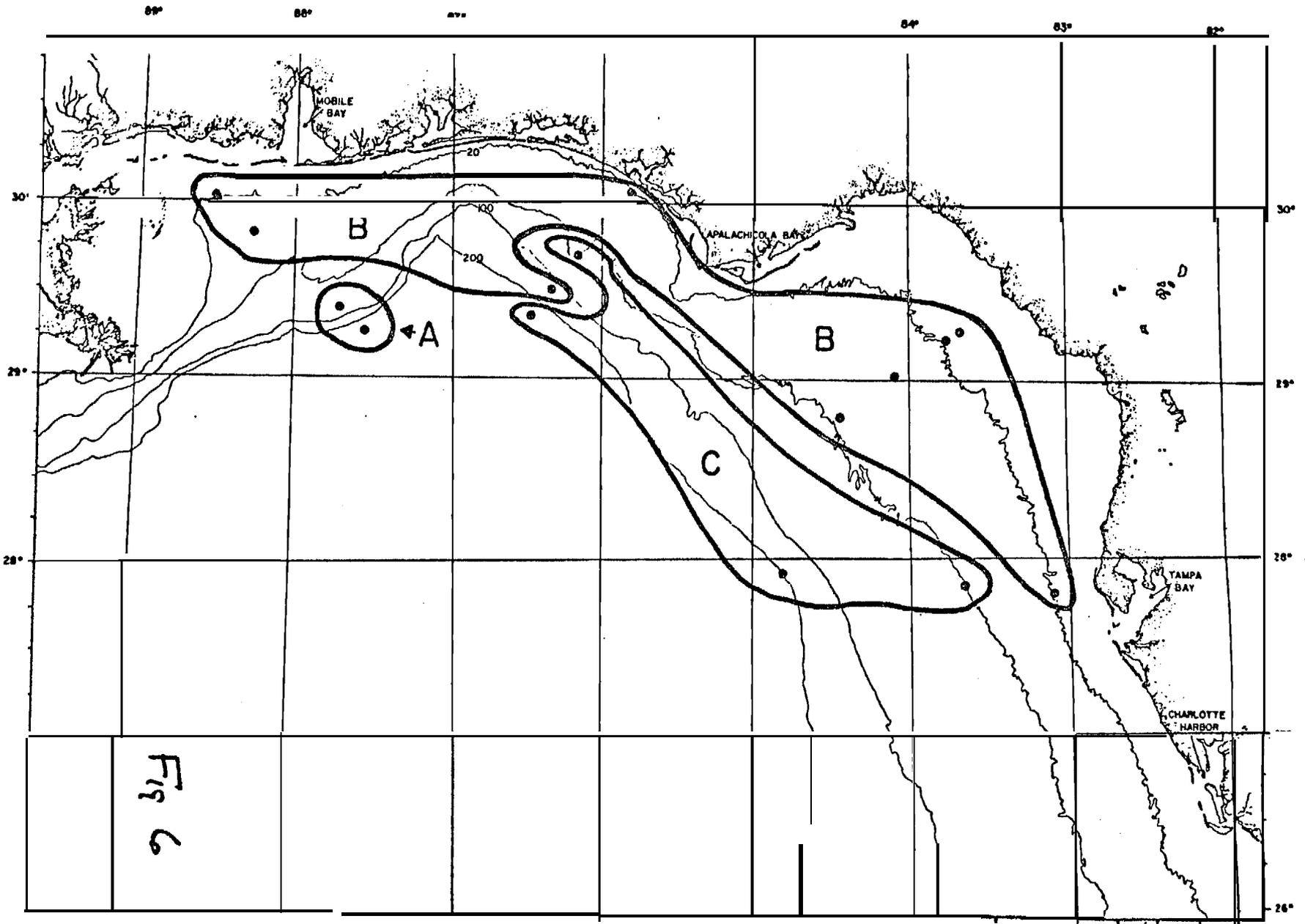
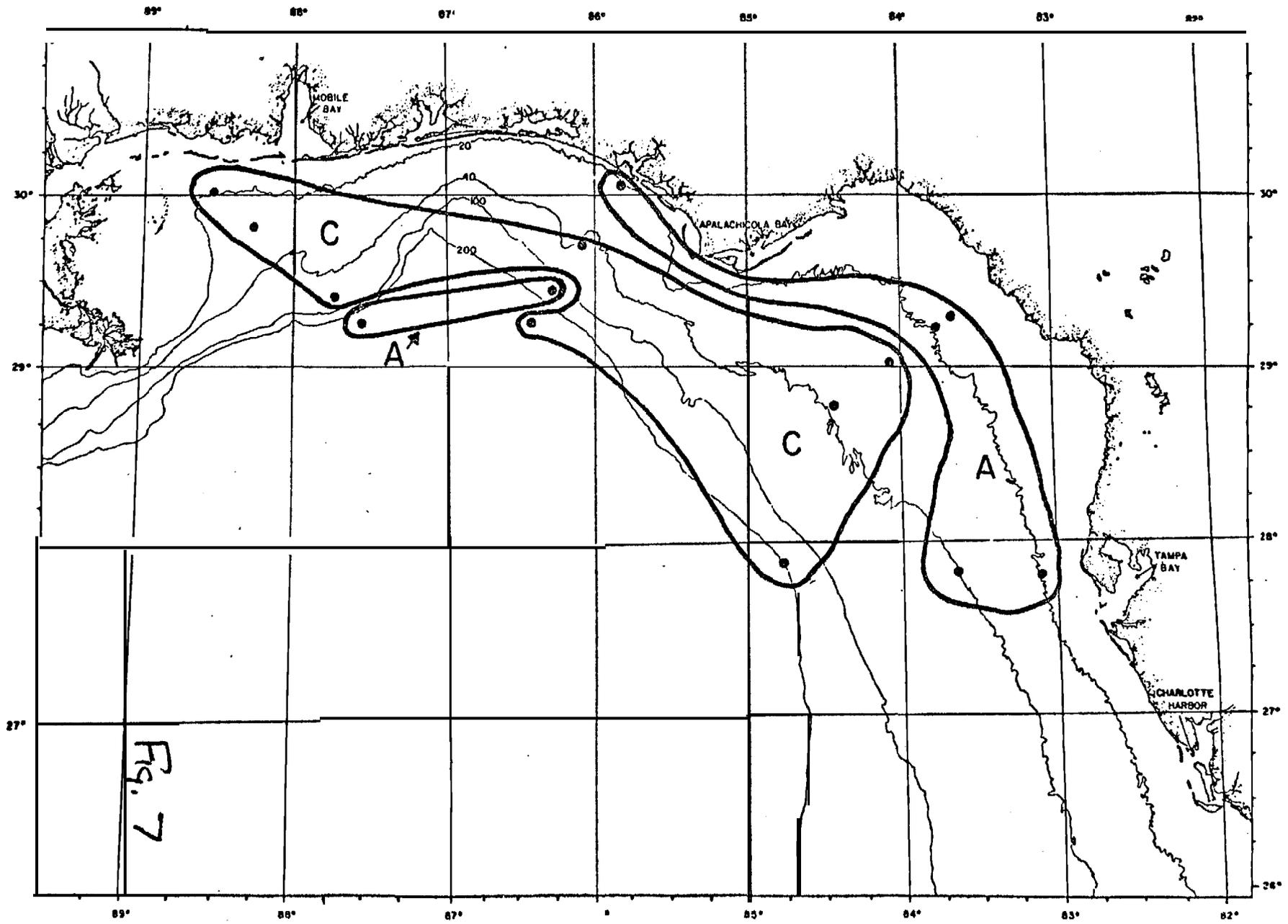


Fig. 7 Zooplankton hydrocarbon group distribution, winter 1976



present and the A group occupied the nearshore stations of Transects I, II and III as well as one offshore station on each of Transects III and IV. The C group occupied the nearshore stations of Transect IV, but was in its usual offshore spot on the other transects.

The three hydrocarbon compositions could be the result of three factors:

- a) different biosynthetic hydrocarbons from different zooplankton species
- b) different hydrocarbons taken up from different food sources or water masses
- c) different biosynthetic hydrocarbons resulting from environmental variation (e. g. temperature)

The taxonomy of the zooplankton was determined by Maturo and Caldwell (1976). A first level examination showed that the major zooplankton groupings occurred in nearly every sample at all seasons. Thus the hydrocarbons in the A and C group must be due to very lipid rich minor components of the zooplankton if taxonomic variation is responsible for observed hydrocarbon variations. This may be more likely than it first seems because the hydrocarbon extraction was done on a bulk zooplankton sample, while taxonomy was performed on a sample that had been split from seven to eleven times. The splitting could have diluted a minor yet lipid rich component.

Neither dissolved hydrocarbons nor those on suspended particulate bear any relation to the zooplankton hydrocarbons (Calder, 1976) and thus the zooplankton hydrocarbons do not appear to have been taken up from different external sources.

Because the C group was, generally found offshore it came from waters

generally deeper, colder and more saline. Yet the inshore stations in winter were just as cold and saline as the offshore stations in summer (Rinkel, 1976) and contained the A, not the C group. Temperature and salinity variations do not seem to cause the zooplankton to alter their biosynthetic hydrocarbon content

Because the hydrocarbon groups do display spatial patterns, rather than random distribution, they must be the result of general circulation phenomena. Hydrocarbon analysis of the major zooplankton groups (e. g. copepods, jellies, etc) might be the best way of clarifying these observations.

Tar balls were ubiquitous in neuston samples and on rare occasion were found in a zooplankton sample. When seen they were removed before analysis. None of the zooplankton analyzed showed any evidence of either fresh or weathered petroleum. For comparison, Figure 8 shows the chromatogram of a contaminated neuston sample.

CONCLUSIONS

1. Zooplankton biomass in the MAFLA area is high in summer, low in fall and winter.
- 2* Total lipid did not vary with season, but total hydrocarbon was much higher in winter. Because of greater biomass the standing crop of zooplankton total hydrocarbons was greatest in summer.
3. The hydrocarbon composition fell into three groups, most definitively characterized by the benzene fraction. The same three groups recurred in each sampling season in spatial configurations which appear to be controlled by general circulation phenomena.

4. There was no evidence for fresh or weathered petroleum
in zooplankton.

Fig. 8 Tar ball contaminated neuston sample
A. hexane fraction
B. benzene fraction

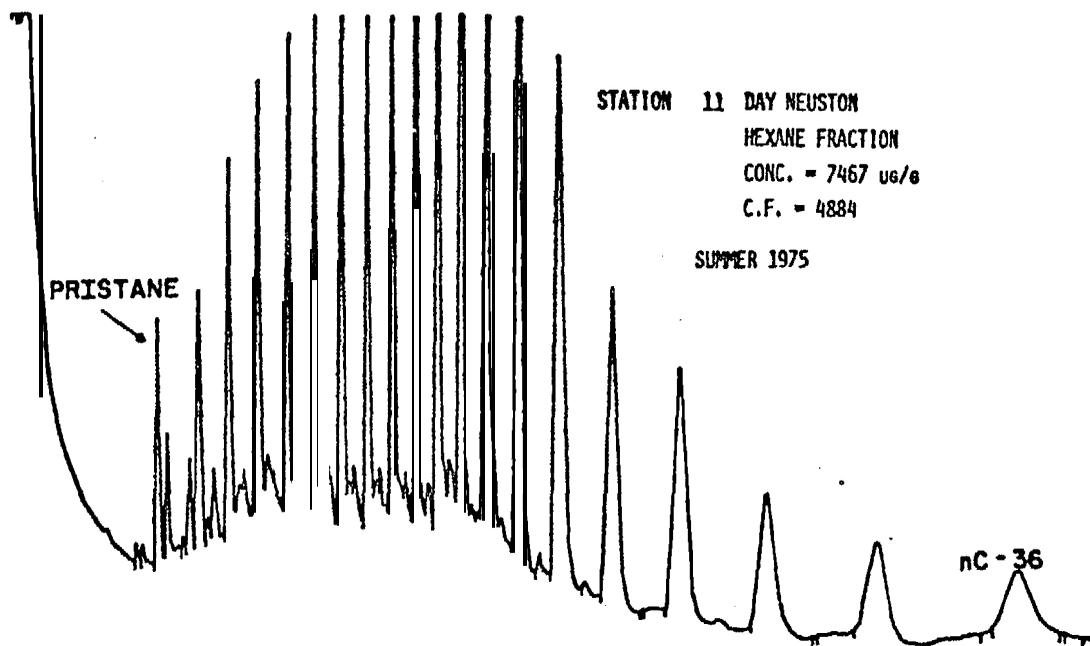


Fig 8A

B

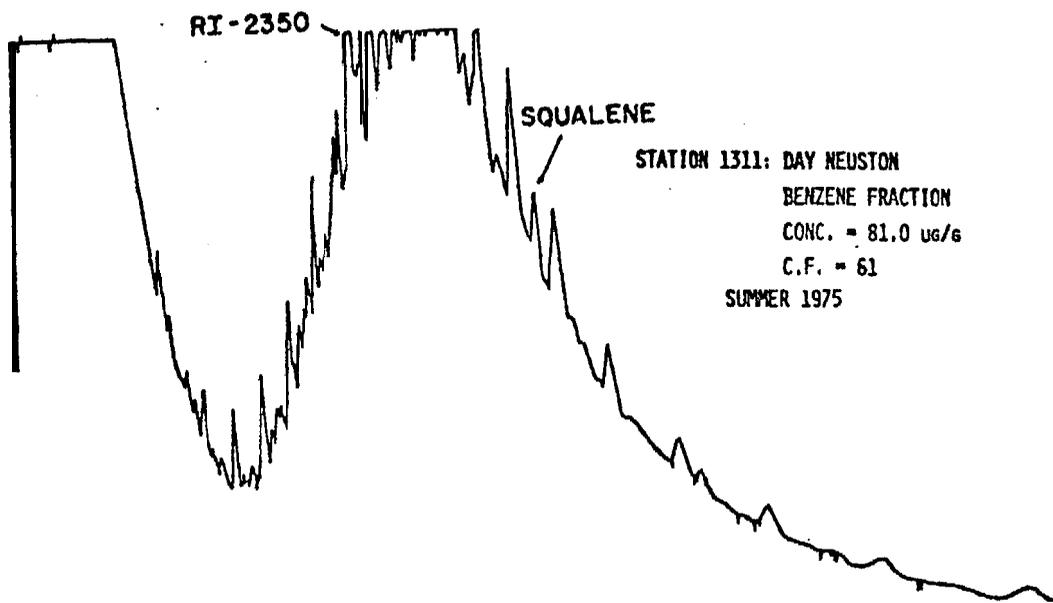


Fig 8B

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B
P

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