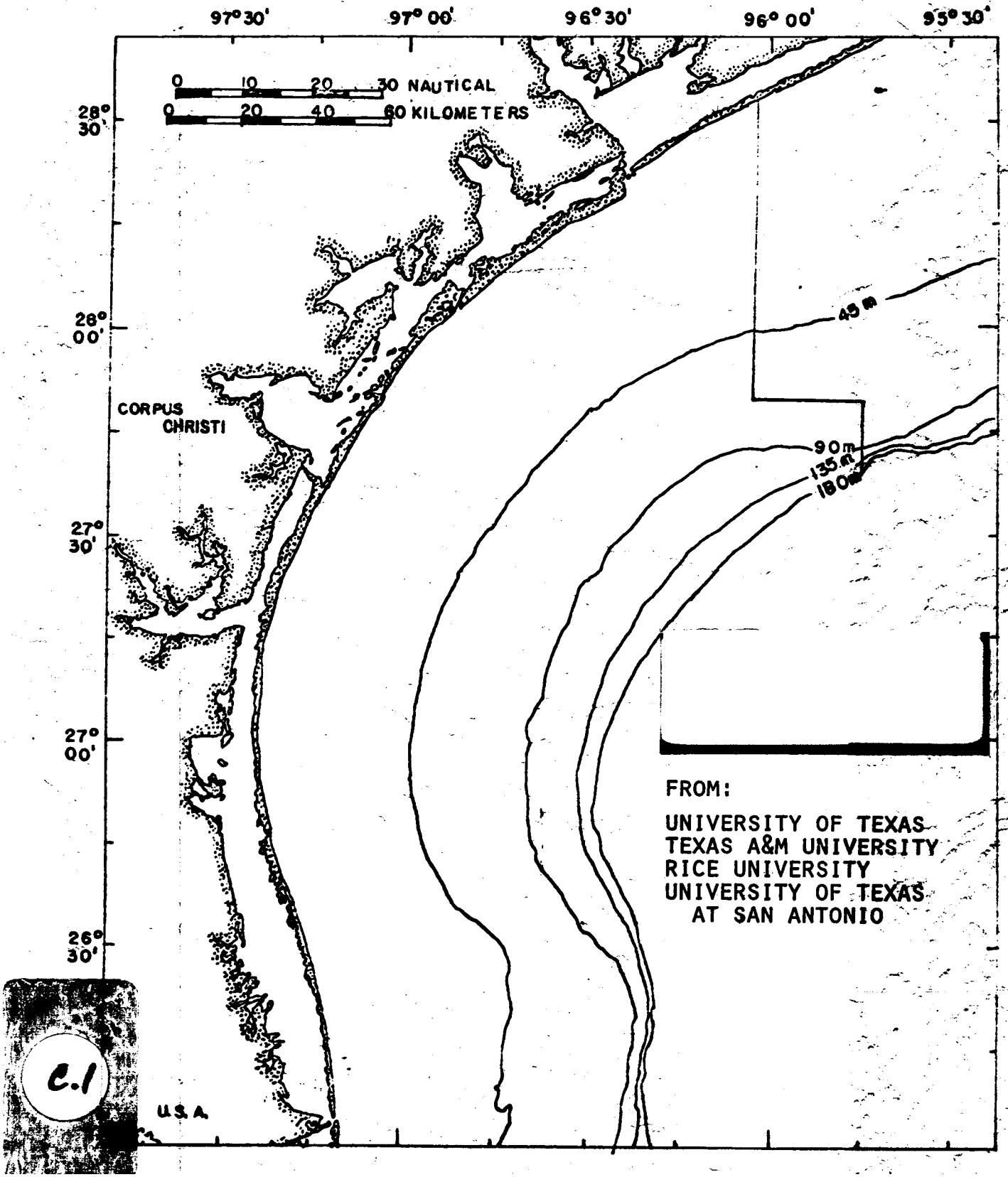
ENVIRONMENTAL STUDIES, SOUTH TEXAS OUTER CONTINENTAL SHELF, BIOLOGY AND CHEMISTRY



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C.1

ENVIRONMENTAL STUDIES,

SOUTH TEXAS OUTER CONTINENTAL SHELF,

BIOLOGY AND CHEMISTRY

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CHAPTER NINE

INVERTEBRATE EPIFAUNA AND MACROINFAUNA

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ABSTRACT

Eight-hundred macroinfauna samples and 222 invertebrate epifauna samples were collected in 1976. Six replicate macroinfauna samples were collected seasonally at each station with a 0.025 m^3 Smith-MacIntyre bottom grab. Epifauna samples were collected with a 10.7-m Texas box otter trawl; one daytime and one nighttime trawl were taken per station.

Based on cluster analyses, macroinfauna collections were divided into three station groups, shallow, mid-depth, and deep. (Station 3/IV and 6/IV were separated as a subgroup of the deep-station group.)

Three habitat types were identified in the STOCS study area based on location and sediment: shallow muddy-sands and mid-depth transitional sediments; deep silty-clays; and, deep muddy sands. Infaunal densities (number of species and individuals) were highest in shallow and deep muddy-sand sediments.

Cluster analyses of invertebrate epifauna grouped stations by depth with a major separation between inner-shelf stations (10-49 m) and outer-shelf stations (65-134 m). Number of species was generally greater in the outer-shelf area than in the inner-shelf, but number of individuals was much greater in the inner-shelf.

An assessment of small-scale distribution patterns revealed that macroinfauna populations were more aggregated inshore than at deeper, offshore stations. Inshore communities were distributed on a smaller scale, *i.e.*, the same sampling effort obtained a greater percentage of the inshore species than of the offshore species.

INTRODUCTION

The benthic invertebrate epifauna and macroinfauna study conducted as an element of the BLM-STOCS project is designed to provide information on the current status of benthic invertebrate populations of the South Texas Outer Continental Shelf. The focus of the first two years of this study has been: 1) the identification and enumeration of the macroinfauna and invertebrate epifauna inhabiting the STOCS study area; 2) delineation of faunistically similar geographical regions and identification of faunal assemblages characteristic of these regions; and 3) correlation of the observed invertebrate epifaunal and macroinfaunal distributions and abundances with other biological, chemical, and physical factors investigated in the study.

Invertebrate epifauna, particularly those of commercial importance, have been relatively well studied along the inner-shelf of the Gulf of Mexico. Results of early biological investigations in the Gulf of Mexico using the USGS steamer BLAKE (1877-1879) under the supervision of A. Agassiz are found in the first 50 volumes of the Bulletin of Comparative Zoology, Harvard University. More recent general surveys were conducted aboard the USFWS exploratory fishing vessel OREGON in the Gulf of Mexico (Springer, 1951; Springer and Bullis, 1956), and by the Texas Parks and Wildlife Department along the Texas coast (Compton and Bradley, 1964; Compton, 1965). These surveys were concerned primarily with commercial species and little attempt was made to quantitatively assess the temporal or spatial distribution of invertebrate populations. The most extensive survey of benthic epifaunal populations of the continental shelf was that of Defenbaugh (1976); however, this study covered only the northern portion of the STOCS study area.

Hedgpeth (1953) presented a general discussion of the zoogeography of the Gulf of Mexico, stating: "There is preliminary evidence that there are several communities [on the continental shelf], each characterized by a somewhat different assemblage of organisms, but their areal limits are yet to be determined." Hildebrand (1954; 1955) delineated some of these "communities" in his surveys of the brown and pink shrimp grounds of the western Gulf. Felder (1973) provided a key and distributional notes to Reptantia in the northwestern Gulf. These surveys were generally conducted in depths less than 50 m. Defenbaugh (1976) presented the only comprehensive survey of the continental shelf from 50 to 150 m. Pequegnat *et al.* (1976) surveyed the continental slope at depths greater than 200 m and presented evidence that the outer continental shelf and upper slope share numerous species.

Less work has been done on the macroinfaunal populations on the continental shelf of the western Gulf of Mexico. Most infaunal surveys of the continental shelf have dealt with a specific group of organisms rather than a broad-based study of all macroinfauna. Molluscs have received the most attention. Parker (1960) surveyed the marine molluscs to depths of 100 m and included a few decapods and echinoderms in his report. Other surveys of the Texas continental shelf molluscan fauna were conducted by Hulings (1955) and Kennedy (1959).

Polychaete distribution was studied by Hartman (1951) who presented a good taxonomic key to littoral marine annelids, and by Harper (1970). However, these studies were restricted to inner-shelf waters. Rowe (1966) discussed polychaete distribution of the Gulf of Mexico shelf and slope. Rowe *et al.* (1974) compared the benthic biomass of the continental shelf and abyssal Gulf samples to benthic biomass estimates from the Atlantic.

The smaller macrocrustacea (*i.e.*, amphipods, ostracods, etc.) and the minor phyla such as Sipuncula and Echiuridea have received little attention in previous benthic community structure studies of the Texas continental shelf.

The delineation of the structure and function of "communities" within the benthos is addressed by many benthic invertebrate studies. Early work on marine communities by Peterson and Thorson stressed recurrence of dominant species as the criteria for identifying communities (Stephenson *et al.*, 1972). More recent work by McIntosh (1967), Pielou (1969), Boesch (1973), Holland *et al.* (1973) and others used information theory to quantitatively assess the "species diversity" of marine benthic communities. Following the "time-stability" hypothesis of Sanders (1968), it was theorized that environments which are stable over long periods of time allow for finer-scaled niche diversification and therefore greater diversity (complexity) of the community. The "diversity" of a collection is composed of two parts, species richness and evenness of individuals among species. Collections which are structurally quite different can have similar diversities. For this and other reasons, information theory "diversity" measures have received much criticism (Hurlbert, 1971; Clifford and Stephenson, 1975).

Numerical analysis methods are currently being tested and used as a means of identifying species assemblages and delineating the spatial or temporal distribution of each assemblage (Field and McFarlane, 1968; Stephenson and Williams, 1971; Eagle, 1975). These methods have proved successful in delineating geographical boundaries of benthic communities on the continental shelf off North Carolina (Day *et al.*, 1971), Virginia (Boesch, 1973), and South Africa (Field, 1971). Performing both normal

(Q mode) and inverse (R mode) cluster analyses allows construction of a two-way coincidence table [see Kikkawa (1968) for good example] to facilitate identification of species assemblages which are characteristic of the geographical zones.

Hill (1975) reported benthic invertebrate work done by the USGS within the STOCS study area. He identified five geographical regions based on similarity of species composition. These regions were best correlated with sand/mud ratios and depth, but he could not designate species which were characteristic of any geographical zone.

Our report of the first year's (1975) work concentrated on spatial patterns in number of species, individuals, and diversity measures. Epifaunal abundance patterns were considered largely a function of depth, whereas infaunal abundance was a function of sediment type as well as depth. This study is one element in a large-scale survey of biological, chemical and physical parameters of the northwest Gulf of Mexico. This report concerns the second year (1976) of the study. This study has expanded upon the community structure investigation to include cluster analysis and correlations with sedimentary, biological and physical data.

METHODS

Sampling and laboratory methods were little changed from those of the first year. During the second year, sampling effort for both infauna and epifauna was intensified. Those animals taken by trawl are operationally defined as epifaunal organisms and those in the grab samples as infauna. Although these are artificial divisions, they are convenient in light of our dual collecting procedures. For the infauna, the increase in sampling effort was spatial, temporal and in number of replicates. Twenty-five transect stations were sampled as opposed to twelve during

year I. Monthly sampling along Transect II was implemented during the present reporting period and the number of replicate infaunal samples was increased from four to six. Eight new sampling stations were added around two topographic features. Epifaunal sampling increased spatially and temporally as did the infauna, but the number of samples (one day, one night) at each station remained the same. Two additional replicates at 10 stations were taken randomly throughout the year. A major improvement in station location and maintaining position was implemented this year by using LORAC navigation.

Macroinfauna was sampled with a Smith-MacIntyre bottom sampler (Smith-MacIntyre, 1954). This device samples 0.1 m^2 surface area to a maximum depth of approximately 17 cm. The volume of sediment sampled is approximately 0.025 m^3. Six replicate samples were taken at each station. Since all specimens from every sample were archived, the extra sample taken during year I for archiving was omitted this year. A single subsample was extracted from each of the six replicate samples for sediment particle size analysis. This sample was a core approximately 2 by 6 cm. Additional sediment samples were taken concurrently with the Smith-MacIntyre sampler for analysis of hydrocarbons and trace metals by other investigators.

Infaunal samples were placed in numbered plastic trays until washed through a 0.5-mm mesh saran bag on board ship. All organisms and debris collected in the bag were placed in a plastic container (normally a 1-l jar), anesthetized with magnesium sulfate, and preserved with 10 percent seawater formalin containing rose bengal to stain the organisms. Each sample was numbered and coded.

In the laboratory, each infaunal sample was washed through a series of three screens (minimum mesh 0.47 mm) to separate the sample into

three size fractions to facilitate separating the organisms from the debris. As the organisms were picked from the debris with the aid of a dissecting microscope, they were separated to taxa, usually family or genus, and preserved in 50 percent isopropyl alcohol. The sorted samples were taken by taxonomists, identified to species (or lowest possible taxon) and counted. All completed samples were archived, awaiting deposition in a reference collection or museum. Data for each sample were recorded by the taxonomist on computer code sheets and given to the data management personnel for keypunching. The original data sheets were returned and maintained on file.

Epifaunal samples were collected with a 35-ft (10.7-m) Texas box otter trawl at all transect stations. The cod end of the trawl had 25-mm stretched mesh. A change in the otter trawl was implemented between the winter and spring sampling periods. During the first year of study and the winter of the second year, the cod end of the otter trawl was lined with a bag which had a stretched-mesh size of 4.8 mm. Due to the severe sampling problems on soft sediments which often resulted in the loss of one or more trawls on a single cruise, the decision was made to sample without the small mesh liner. Each trawl, day or night, was a 15-minute timed trawl. Speed of the R/V LONGHORN during trawling was the minimum, generally less than two knots, to maintain headway with prevailing seas. The invertebrates of each trawl sample were kept for this study element and the vertebrates were studied by Dr. D. E. Wohlschlag. A second trawl was taken at Stations 1, 2, and 3 on each transect for the hydrocarbon and trace metal analysis studies.

All invertebrates collected in each taxonomic trawl were preserved on board in 10 percent seawater formalin. Each sample was numbered, coded

and returned to the laboratory where the organisms were sorted and identified to the lowest possible taxon. Samples were preserved in 50 percent isopropyl alcohol and stored by sample, awaiting final deposition in a reference collection or museum. A list of taxa identified through 1976 is given in Table 1, Appendix G.

Analyses of data involved several methods. Some analyses are completed whereas others are being processed by the data management group. Basically four types of analysis were applied to all or portions of the benthic invertebrate data.

Diversity

The diversity of invertebrate communities at each station was examined by calculation of a modified Shannon-Wiener diversity index (H") (Pielou, 1966), Equitability (E) (Lloyd and Ghelardi, 1964), and Hurlbert's Probability of Interspecific Encounter (P.I.E.) (Hurlbert, 1971).

Diversity is important in that it is commonly considered an attribute of a natural or organized community (Hairston, 1964) or is related to important ecological processes (McIntosh, 1967). McIntosh (*op. cit.*) stated that diversity has been said to relate to community productivity, integration evaluation, niche structure and competition, to be maximal at the climax of a successional sequence and to enhance community stability. Hurlbert (1971) dismisses diversity as an invalid ecological concept and fills the gap with a biologically explicable concept of community structure, P.I.E. Although species diversity indicies may not be as useful as many earlier ecologists maintained, neither are they as useless as portrayed by Hurlbert. It is extremely naive to believe that natural communities can be compared on a single number basis. However, by combining a diversity index (H") with a measure of equitability, showing species

richness and total abundance and providing P.I.E. values on the same data, a reasonable measure of comparison of diversity between communities can be afforded.

Small Scale Distribution

Since the nature of marine benthic sampling habitats (depth and turbidity of water and the burrowing and secretive nature of many of the populations) precludes observing the communities directly, several assumptions are often made concerning marine benthos distribution. First, the number of replicate samples taken is assumed sufficient to adequately sample the community. Often little thought is given to what portion of the community is actually being sampled. The corollary to this assumption is that since a given number of replicates is "adequate" at one portion of the study area, it will be adequate at all others. A technique described by Gaufin *et al.* (1956) was applied to special sets of 12 replicate samples from three stations on Transect II to ascertain what percentage of the "total" number of species might be collected at differing levels of sample replication and to see if the same percentage of the "total" species at various stations would be obtained from similar replicate numbers. Also inherent in many sampling programs and many analysis techniques is the assumption that populations within a community are similarly distributed. Several recent authors, including Gage and Geekie (1973), Alley and Anderson (1968), Kosler (1968), Rosenberg (1974) and Jumars (1975), were interested in dispersion patterns of populations within communities. In this study, several analysis techniques presented by these authors were used to ascertain whether or not species at the several stations on Transect II were aggregating. These techniques included the ratio of the variance to the mean (coefficient of dispersion

of some authors) as used by Gage and Geekie (1973) and several methods for testing the significance of this coefficient (described by Gage and Geekie). Jumar's (1975) Dispersion Chi-Square analysis was also applied to the species data from the suites of 12 samples from Stations 1, 2 and 3, Transect II. This test is a somewhat more sophisticated test for species dispersal patterns than the Coefficient of Dispersion, but tests essentially the same thing.

Multivariate Analysis

One of the opportunities afforded a research element in a research program such as the BLM-STOCS is that of seeking similar patterns or correlations with data from other study elements. Data from other research elements were examined, including bottom temperatures, bottom salinities, sediment particle size and fish distribution. A multi-correlational analysis is being conducted using the benthic data (number of species and number of individuals per station, H", P.I.E., and individual species distribution) as dependent variables with data from other elements within the STOCS project as independent variables. Suites of independent variables are being analyzed as to total correlation with each of the dependent variables, and those single independent variables most closely correlated with the dependent values, will be elicited.

Cluster Analysis

The methods of similarity analysis, developed primarily by taxonomists and plant ecologists, have shown great potential in many fields of ecology. They have the advantage of using all the species abundance information available and are free of distributional assumptions. There are many possible methods for use with a given set of data, including correla-

tion coefficients, information content measures, Euclidian distance and similarity (dissimilarity) coefficients. Comparisons of various methods on a single set of data have yielded some recommendations as to the "best" methods for various types of data. Excellent reviews and discussions of the various methods are given by Anderberg (1973), Sneath and Sokal (1973) and Clifford and Stephenson (1975).

The cluster analysis computations were done on an Amdal 470V/6 at the Texas A&M University Data Processing Center using the program CLASS developed by Dr. Robert W. Smith of the Department of Biology, University of Southern California.

The analysis used in this study involves four steps. The first step is the computation of the dissimilarity between all possible pairs of collections or stations based on the species present. [The term stations used here is synonymous with sites of Stephenson *et al.* (1972) and Operational Taxonomic Units (OTU's) of Sneath and Sokal (1973).] The resulting coefficients were tabulated in matrix form with one coefficient for every pair of entities to be classified. This measure of dissimilarity between pairs of entities can be expressed as the ecological distance between the two and the matrix is commonly called the "distance matrix".

The dissimilarity measure used in this study is the Canberra-Metric, which is:

$$\frac{1}{j} \sum_{1}^{j} \frac{\left| X_1 j - X_2 j \right|}{X_1 j + X_2 j} .$$

Since this is the mean of a series of fractions, an outstandingly large value will contribute to only one of the fractions; however, it is strongly influenced by presence/absence data. That is, if $X_1 j$ is 0 and $X_2 j$ is any whole number, the resulting fraction is unity. Therefore, differences of

0 and 100 and of 0 and 1 carry the same weight, which does not make for good ecological interpretation. The solution to this is to replace the zero values in the matrix with a positive number smaller than any of the recorded values (Stephenson, 1972). A good rule of thumb is to replace the zero values with a number 1/5 of the smallest value recorded (Stephenson *et al.*, 1972).

The second step in the process is clustering of individual sites into groups which have the greatest inter-group affinities. The clustering strategies considered were "group average" [equivalent to the unweighted pair group method of Sneath and Sokal (1973)] and "flexible" (Lance and Williams, 1967). Comparisons of various sorting strategies are given by Field and McFarlane (1968), and Prichard and Anderson (1971).

Group average sorting is a space conserving strategy which clusters only weakly and is little prone to misclassification (Stephenson *et al.*, 1972). Flexible sorting is a space dilating strategy which results in sharper clusters than group average. This sorting strategy is based on a generalized formula for hierarchical clustering methods given by Lance and Williams (1967). A good discussion of its properties are given in Sneath and Sokal (1973) who showed the results of using various values of β. Flexible sorting, using the now conventional value of $\beta = -.25$ (Boesch, 1973; Clifford and Stephenson, 1975), was adopted for this analysis.

The next step was to display the results of the clustering in the form of a dendrogram. This is a "tree" diagram in which the tips of the branches represent individual stations and the successive nodes indicate fusion of individual stations or groups of stations into larger and larger clusters. The vertical distance between nodes represents the amount of dissimilarity or the relative difference between successive groupings.

The analyses described thus far were used to classify stations in terms of species composition to produce "station groups". It is also desirable to classify the species in terms of the stations at which they occur. The former is called a "normal analysis," the latter an "inverse analysis" (Field and McFarlane, 1968). These are referred to as "Q" and "R" analysis, respectively, in some literature. The procedure for inverse analysis is the same as normal analysis except that species were used as entities and station distribution as attributes.

The final step is to display the original data in a two-way coincidence table in which the stations and species are rearranged to conform to the sequence generated by the normal and inverse analysis. In such a table it is readily apparent which species-groups characterize which station-groups. The two-way table is often helpful in deciding whether or not to subdivide ill-defined clusters in the dendrogram. Careful examination of this table may also reveal misclassifications of stations (or species) in the analysis and these can be allocated to a more suitable group.

Taxa which were incompletely or inconsistently identified and those species which occurred so seldom as to contribute little or no additional order to the analysis, were eliminated. Elimination of these species also reduced computing time. For further discussion of data reduction see Stephenson *et al.* (1970), Stephenson *et al.* (1972), Field (1971), and Day *et al.* (1971). In the epifauna, those species collected less than three times in the three seasonal samples were eliminated from analysis. This reduced the number of species by 34 percent, but reduced the number of individuals by only .04 percent. Many more infaunal than epifaunal taxa were taken in each season but many of the infaunal taxa were taken in low abundance. Data reduction in the infaunal analysis was more severe.

Each seasonal infaunal analysis utilized approximately 40 percent of the taxa and 95 to 97 percent of the individuals.

For normal analysis, the data were used with no transformations since the Canberra-Metric coefficient is not sensitive to dominance and no distributional assumptions are made.

For the classification of species groups, it was more important to consider the relative differences of each species at each station rather than the magnitude of the differences between species. To accomplish this, the species abundance data were standardized by norm $\sqrt{\Sigma(n)^2}$ (Noy-Meir, 1973) prior to inverse classification.

The measure of constancy and fidelity of species are good measures of the extent to which a species (or species-group) is related to a particular station-group. Stephenson *et al.* (1970) and Stephenson (1972) give good discussions of these concepts. Briefly, a species has very high constancy if found at all stations within a station-group, although it need not be restricted to only one station-group. A species is highly faithful (high fidelity) if it occurs in only one station-group, although it need not occur at every station within that station-group. Generally, those species which are highly faithful to a station-group are those which have narrow habitat requirements or low tolerance to certain environmental parameters. These highly faithful species can often be used as indicators of particular environmental conditions. Constancy is strongly influenced by relative abundance of individual species. These species which have relatively high abundance are more likely to be captured at many stations (and have high constancy) than are those species with relatively low abundance. The concepts of constancy and fidelity are useful in interpreting the results of cluster analysis.

RESULTS

Epifauna

A total of 140 epifaunal taxa were identified from the 1976 trawl collections. Composition of the epifauna by major taxonomic groups was: Coelenterata - 4 percent; Mollusca - 20 percent; Crustacea - 66 percent; and, Echinodermata - 6 percent. *Trachypenaeus similis*, *Sicyonia dorsalis* (rock shrimp), *Callinectes similis* (Gulf crab), and *Penaeus aztecus* (brown shrimp) were the four most abundant species. A list of species and abundance for each trawl are given in Table 2, Appendix G. A number of new records and probable new species from the northwestern Gulf have resulted from more detailed taxonomic determinations. These findings are interesting from a systematic and zoogeographic viewpoint and will undoubtedly contribute to understanding the ecology of the area. A revised list of benthic invertebrates from the STOCS is presented in Table 1, Appendix G.

Cluster Analysis

Classification analysis of epifaunal seasonal data included a separate analysis of each season and an analysis of the winter, spring and fall data combined. Information on temporal and spatial differences within the study area were much the same in both types of cluster analysis. To avoid redundancy, only the combined seasonal data are presented here.

Normal analysis of the epifaunal data (Figure 1) grouped stations by depth with little tendency toward seasonal or latitudinal groupings. The major separation was between inner-shelf stations (10-49 m) and outer-shelf stations (65-134 m). Subdivisions within these groups formed four station-groups based on depth: Group A -- composed of shallow-intermediate depth stations; B--shallow stations; D--deep stations; and F--deep-inter-

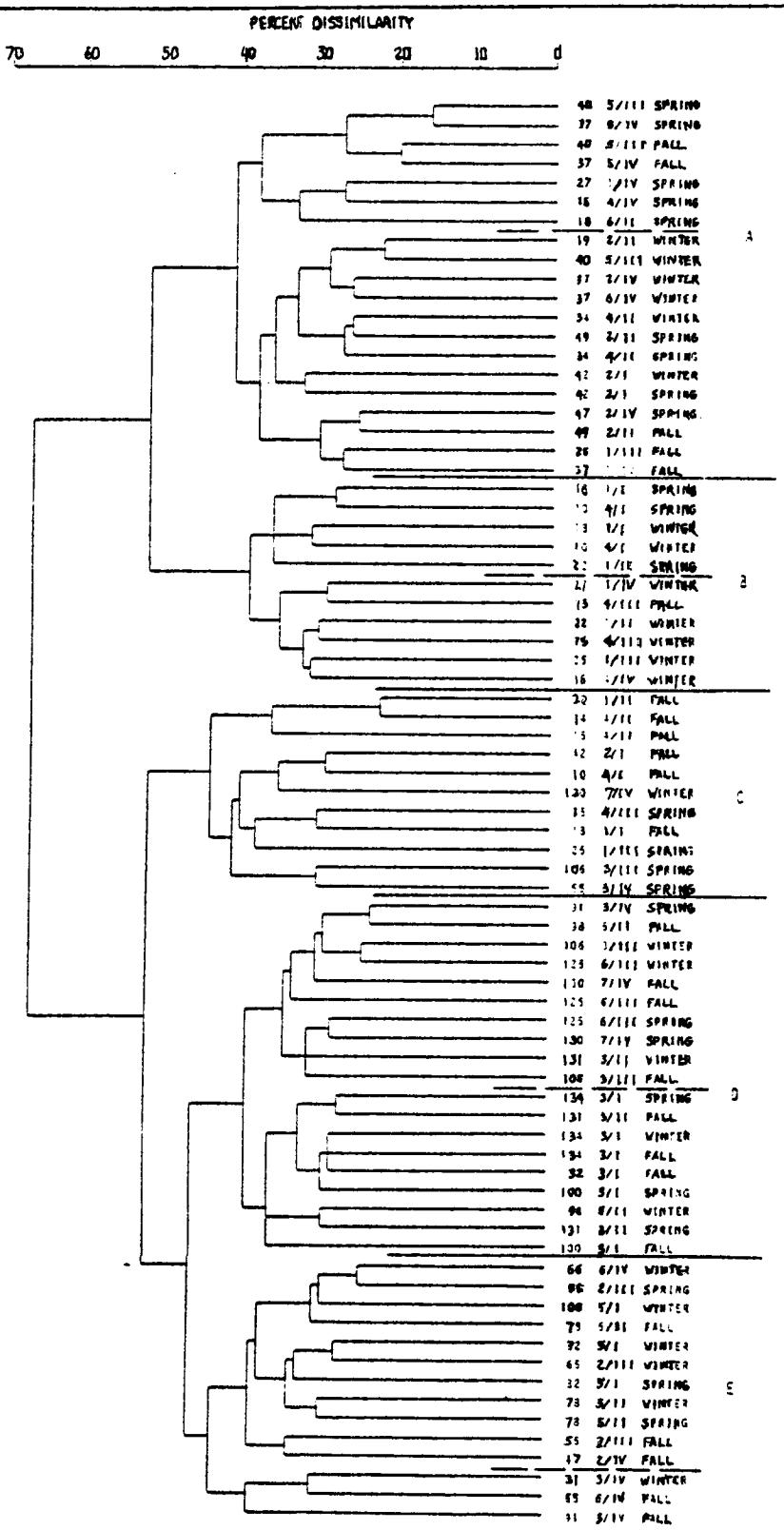

Figure 1. Normal Dendrogram From Cluster Analysis of Seasonal Epifaunal Data. Numbers Preceding the Station Designations Refer to Depth (in meters). Letters Designate Station-Groups.

mediate stations. An exception to this pattern was group C, which contained fall and spring data from stations with a wide range of depths. A map of the study area (Figure 2) shows stations which were clustered in the same station-group in at least two of the three seasons. Station 1/III did not fit into a combined seasonal station-group because Station 1/III (winter) grouped with shallow stations (B), Station 1/III (fall) grouped with the shallow-intermediate stations (A), and in the spring, Station 1/III grouped with the other depauparate stations (C) since it had only one species. In fact, all stations less than 35-m deep showed less fidelity to a station-group than did deeper stations.

Species-groups formed by inverse analysis (Figure 3) are shown in a two-way constancy table (Figure 4). The relationships of species-groups to station-groups are clearly apparent. Collections from shallow stations (group B) had high constancy of species in species-groups 2, 3, and 4. Species in group 2 were more characteristic of Stations 4/I, 1/I and 1/II (group B) than of other shallow stations, but group 4 species were highly constant in all shallow collections. The two-way constancy table allows resolution of the problem of why station-group C did not fit a depth pattern. The paucity of the collections from stations in group C did not provide enough information to group these stations with others. Many shallow stations, collected during the fall season were in group C, implying some seasonal change in the shallow-shelf fauna. Shallow-intermediate depth stations (group A) were characterized by large numbers and very high constancy of most species in group 3, which includes the top four numerically dominant species *Trachypenaeus similis*, *Sicyonia dorsalis*, *Callinectes similis* and *Penaeus aztecus*. Most of the group 3 species were collected at stations in all depths but were rare in deep collections (D).

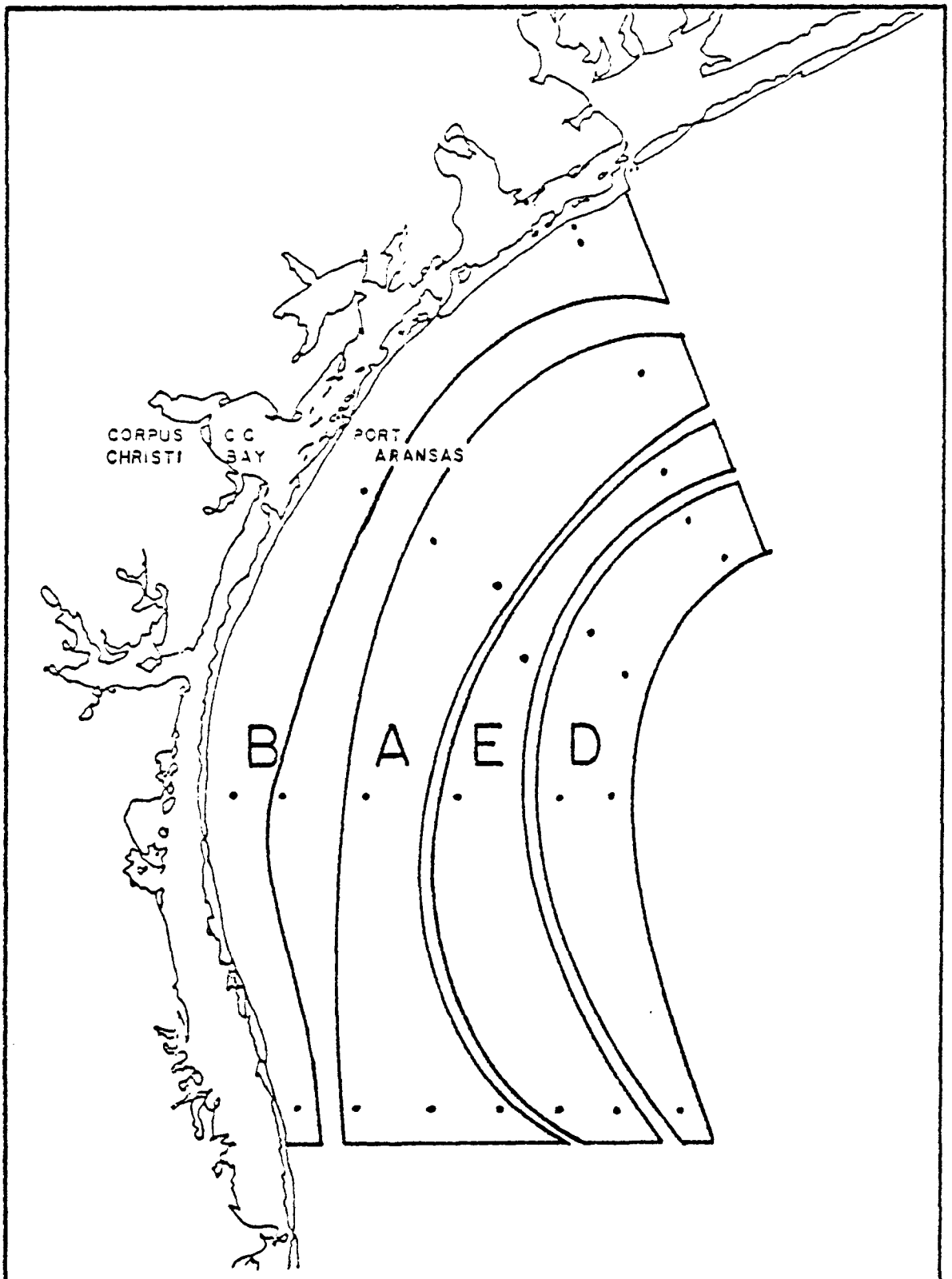

Figure 2. Location of Station-Groups (A-E) from Cluster Analysis of Seasonal Epifaunal Data.

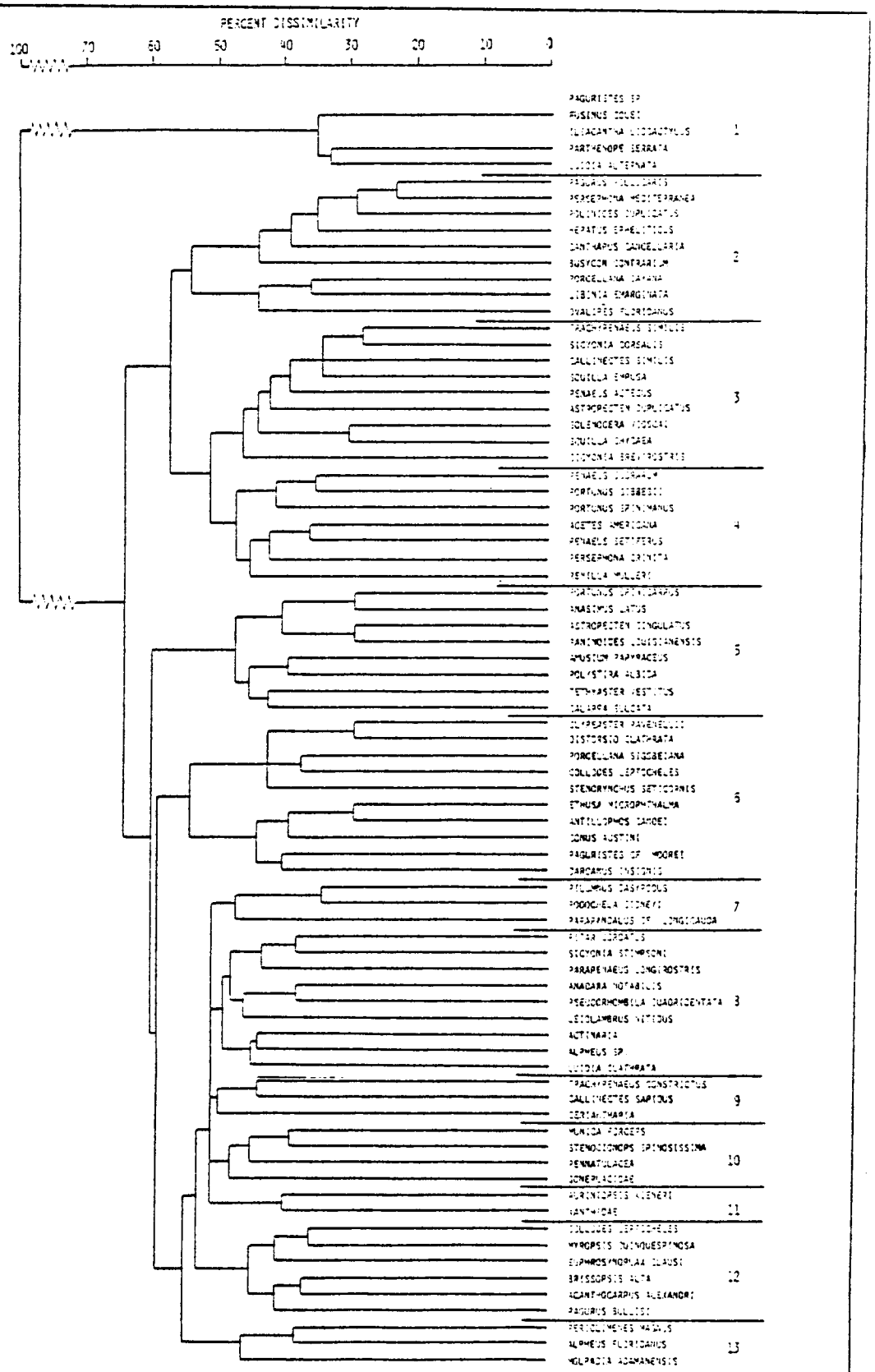

Figure 3. Species Dendrogram from Inverse Analysis of Seasonal Epifaunal Data. Numbers Refer to Species-Groups.

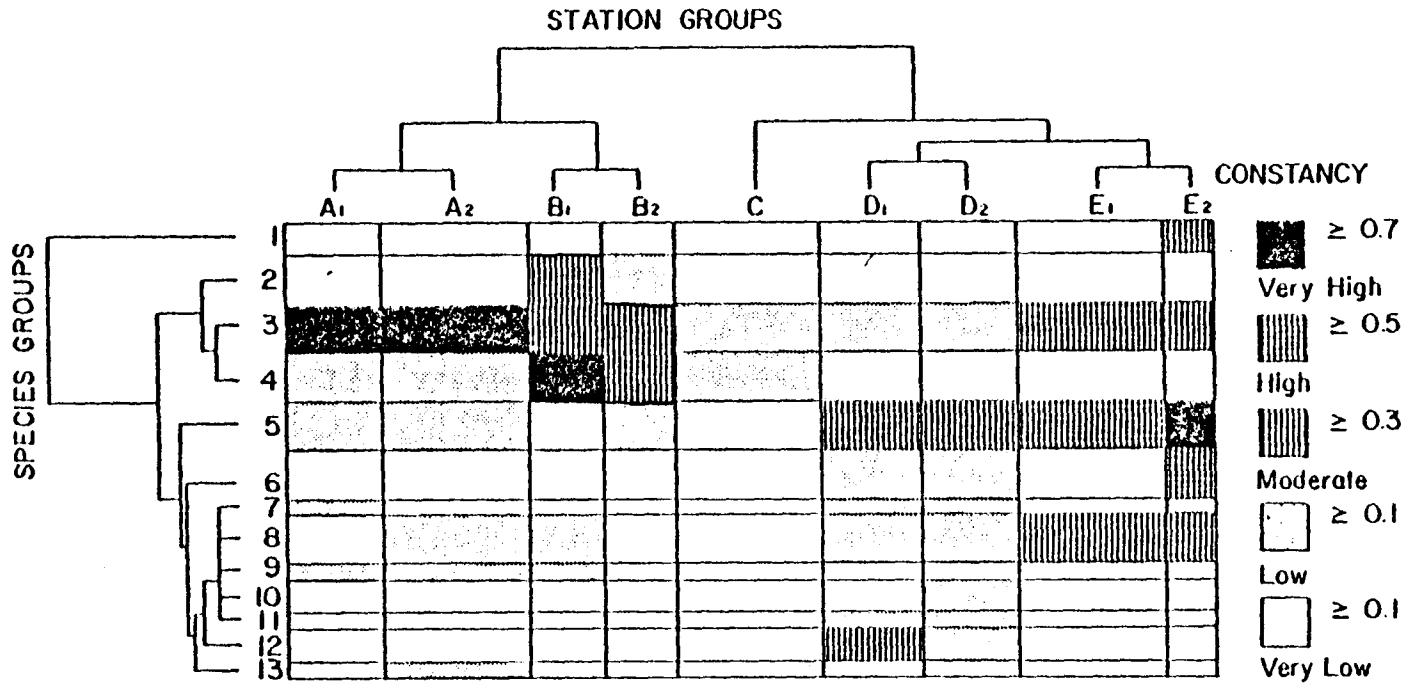

Figure 4. Nodal Constancy in a Two-Way Table of Species Groups in Station Groups from Seasonal Epifaunal Data.

Penaeus aztecus was the only species collected regularly throughout the study area. Species most abundant and constantly found in each depth zone are listed in Table 1. Most shallow shelf species had their widest distribution in winter, were most abundant in winter or spring and were very reduced in distribution and abundance in the study area in fall. Examples of this distribution are *Penaeus setiferus* (white shrimp) and *Renilla mulleri* (sea pansy) (Figures 5 and 6).

Shallow-intermediate zone species were generally most numerous in spring and this high abundance in both the shallow-intermediate and shallow zones contributed to seasonal variation in inner-shelf station patterns. Intermediate-depth and deep-shelf species numbers and distribution were relatively constant through the seasons. Many deep-shelf species were collected in low numbers and were not highly constant to the deep station-group.

There was a minor division of Transect IV winter and fall collections from other intermediate-deep stations (E_2) due to a small group of species taken only at these southern stations. Sediment composition at Stations 6/IV and 3/IV in winter and fall samples was 50 percent or more sand as compared to generally less than 10 percent sand at the other outer shelf stations. There seemed to be an influence of latitude and bottom type contributing to the differences found at the southern-most stations.

Results of cluster analysis of monthly collections on Transect II (Figures 7 and 8) reaffirm the depth zonation seen in seasonal analysis and show strong seasonal variation in shallow-intermediate Stations 1/II, 4/II and 2/II. Station-groups D and E, containing these 22-49 m stations, are well separated from the deeper stations. Group D contains most of the July through December collections while group E contains November through spring. All collections from Station 3/II clustered together

TABLE 1

SPECIES MOST ABUNDANT AND CONSTANT AT COMBINED-SEASONAL STATIONS FOR EPIFAUNAL CLUSTER ANALYSIS

Depth Zone	Abundance Rank	Species	Range of Distribution (m)[a]
Shallow		Coelenterates	
	9	*Renilla mulleri*	10-40 (+1 at 98 m)
		Molluscs	
	25	*Cantharus cancellaria*	10-22
		Stomatopods	
	7	*Squilla empusa*	11-98
		Natantia	
	4	*Penaeus aztecus*	10-131
	12	*Penaeus duorarum*	10-27
	22	*Penaeus setiferus*	10-65
	1	*Trachypenaeus similis*	10-98
	14	*Acetes americana*	10-34
		Reptantia	
	45	*Hepatus epheliticus*	10-25
	44	*Persephona mediterranea*	10-18
	3	*Callinectes similis*	10-98
	13	*Portunus gibbesii*	10-27
Shallow-Intermediate		Stomatopods	
	10	*Squilla chydaea*	22-134
	7	*Squilla empusa*	10-98
		Natantia	
	4	*Penaeus aztecus*	10-131
	2	*Sicyonia dorsalis*	10-98
	6	*Solenocera vioscai*	34-134
	1	*Trachypenaeus similis*	10-98
		Reptantia	
	3	*Callinectes similis*	10-98
		Echinoderms	
	11	*Astropecten duplicatus*	10-131
Deep-Intermediate		Molluscs	
	16	*Polystira albida*	47-130
	24	*Anadara notabilis*	65-131 (+2 at 10 m)
	5	*Amusium papyraceus*	27-131
	23	*Pitar cordatus*	34-131
		Stomatopods	
	10	*Squilla chydaea*	22-134
		Natantia	
	30	*Parapenaeus longirostris*	40-134
	4	*Penaeus aztecus*	10-131
	20	*Sicyonia brevirostris*	10-98
	6	*Solenocera vioscai*	34-134

TABLE 1. CONT.'D

Depth Zone	Abundance Rank	Species	Range of Distribution (m)[a]
Deep-Intermediate		Reptantia	
	26	*Raninoides louisianensis*	47-134
	8	*Portunus spinicarpus*	27-134
		Echinoderms	
	11	*Astropecten duplicatus*	10-131
	19	*Astropecten cingulatus*	49-134
	21	*Tethyaster vestitus*	40-134
Deep		Natantia	
	6	*Solenocera vioscai*	34-134
		Reptantia	
	38	*Pagurus bullisi*	91-134
	55	*Ethusa microphthalma*	91-134
	37	*Acanthocarpus alexandri*	98-134
	54	*Myropsis quinquespinosa*	65-134
	15	*Anasimus latus*	25-134
	49	*Collodes leptocheles*	82-131
	8	*Portunus spinicarpus*	27-134
		Echinoderms	
	18	*Brissopsis alta*	65-131
Transect IV		Molluscs	
	76	*Fusinus couei*	65
		Reptantia	
	77	*Iliacantha liodactylus*	65
	40	*Parthenope serrata*	15-65
		Echinoderms	
	53	*Luidia alternata*	27-65
	42	*Clypeaster ravenellii*	65-91

[a]Depth of water column.

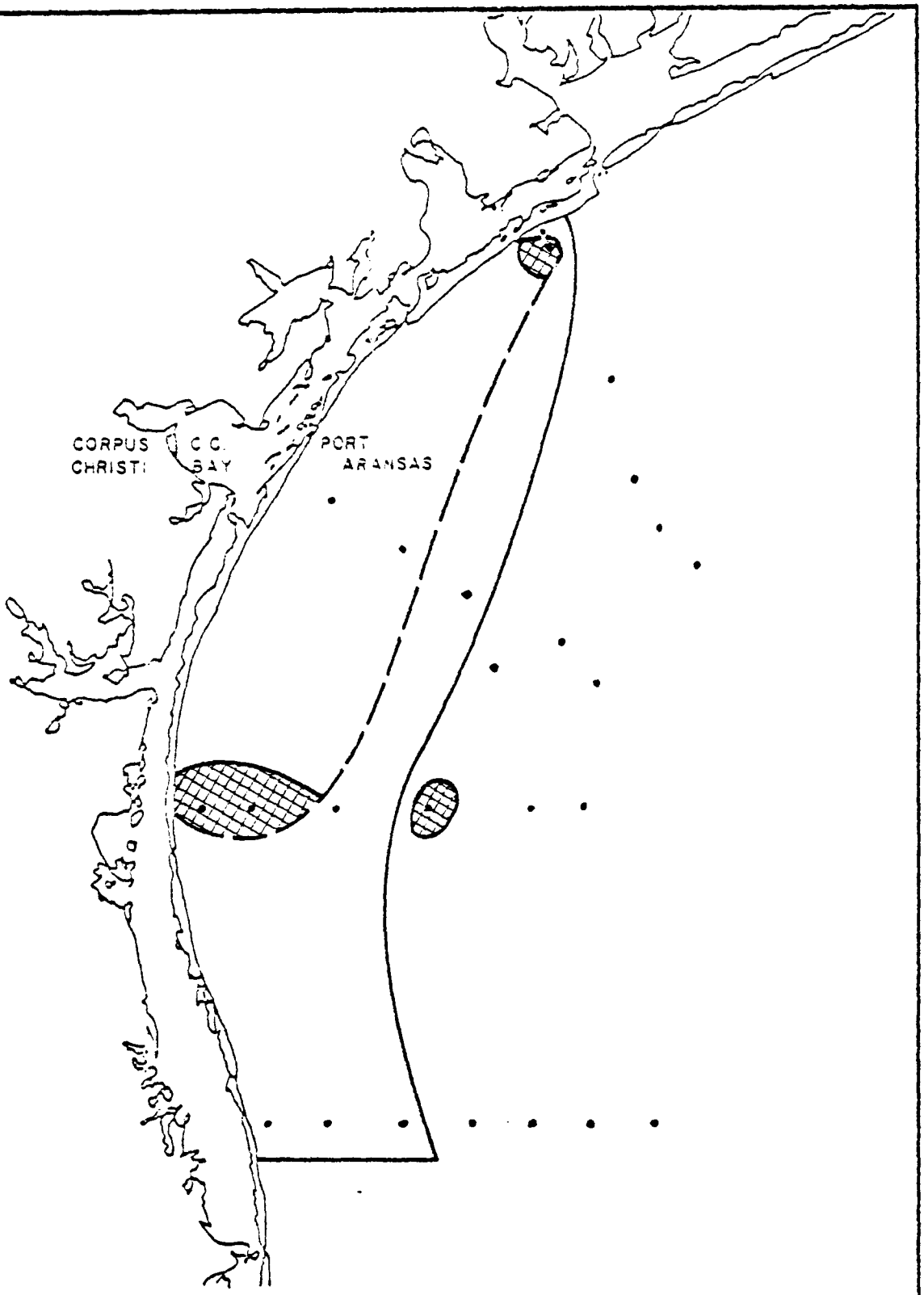

Figure 5. Seasonal Distribution of *Penaeus setiferus*. Winter Distribution-Solid Line, Spring-Dashed Line and Fall-Cross Hatched.

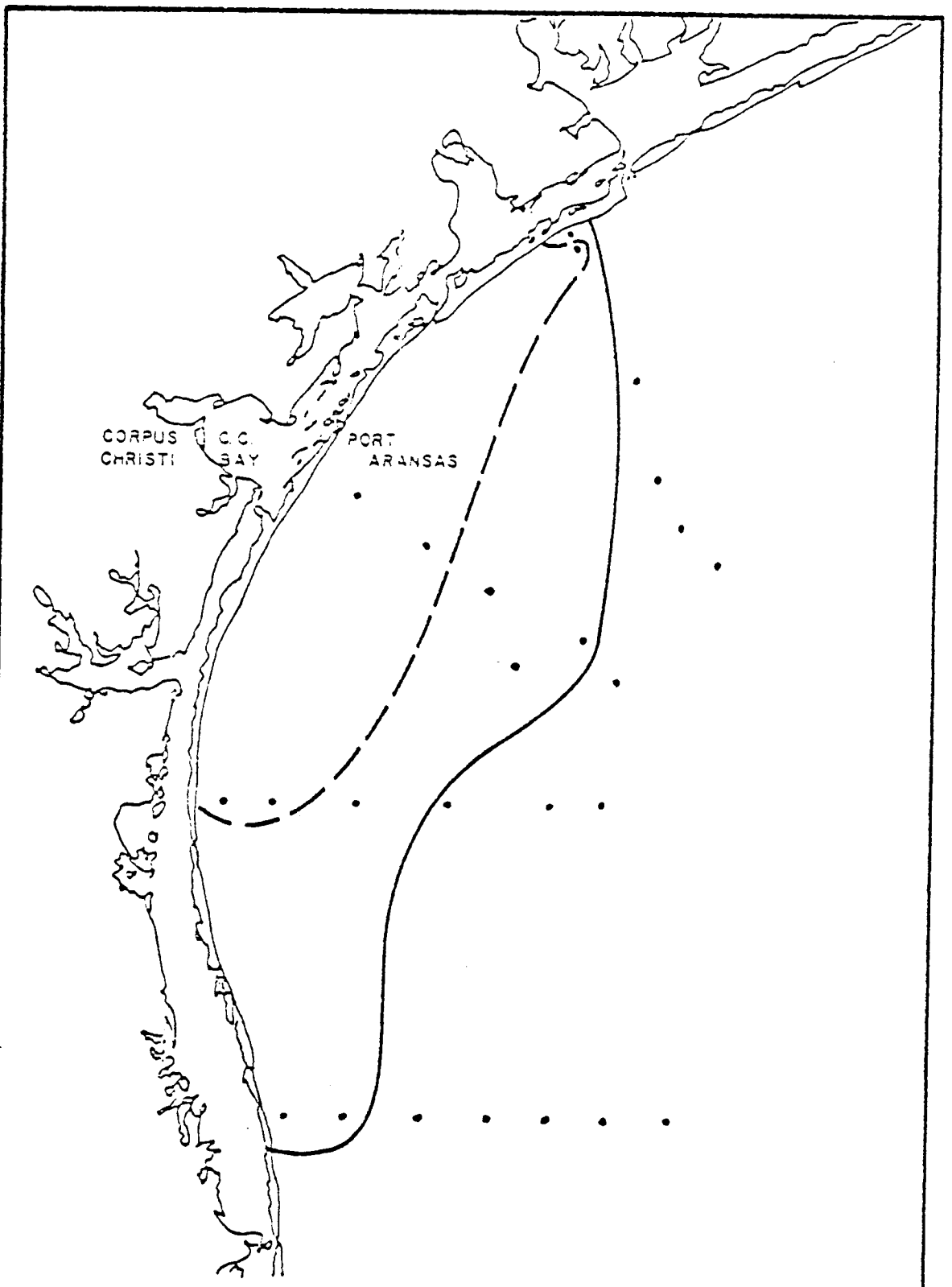

Figure 6. Seasonal Distribution of *Rentilla mulleri*. Winter-Solid Line, Spring-Dashed Line and Fall-Cross Hatched.

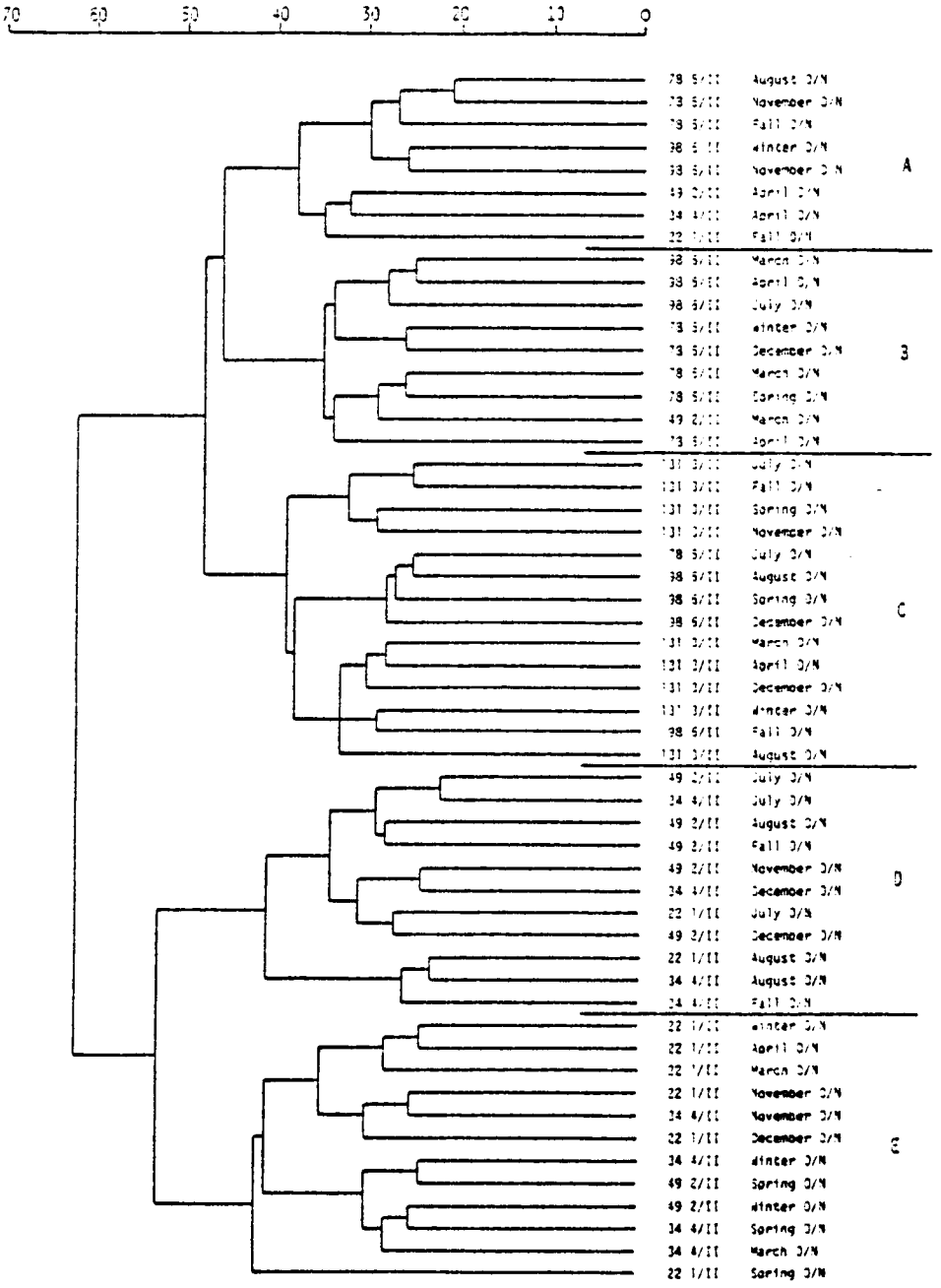

Figure 7. Normal Dendrogram from Cluster Analysis of Monthly Epifaunal Data. Numbers Preceding the Station Designation Refer to Depth (in meters). Letters Deisgnate Station-Groups.

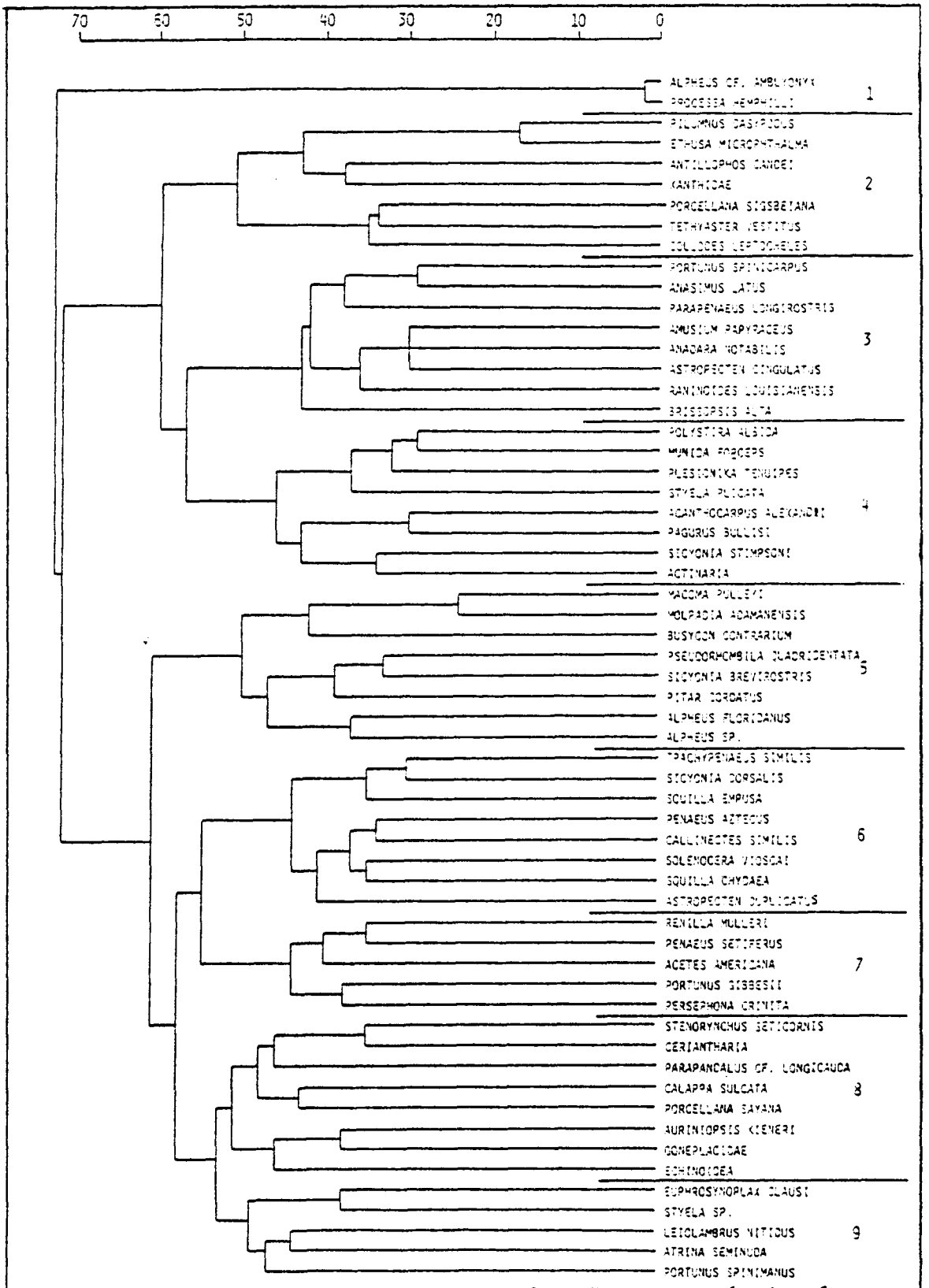

Figure 8. Species Dendrogram Resulting from Inverse Analysis of Monthly Epifaunal Data. Numbers Refer to Species-Groups.

in group C, implying a temporally stable epifauna. The two-way constancy table (Figure 9) shows that many species-groups are moderately to highly constant at Stations 5/II and 6/II (group B). The largest numbers of species were collected at these stations. Group A is composed of stations with sparse collections and contains some monthly collections from all stations except 3/II.

Species characteristic of the depth zones on Transect II are much the same as those listed for the seasonal analysis with the exception of *Penaeus duorarum* (pink shrimp) which was not collected on Transect II.

Diversity

Spatial relationships of number of species, number of individuals, H", and equitability at each station are shown in Figures 10 through 12. Number of species was generally greater in the outer shelf area (65-134 m) than in the inner shelf (10-49 m), but number of individuals was much greater in the inner shelf. One striking exception was the large number of individuals in the outer shelf area of Transects III and IV in the fall. The large number of individuals at Station 6/IV were mainly of one species (*Amusium papyraceus*, paper scallop), but other stations had an unusually large number of individuals from several species. Equitability and diversity were generally higher in the outer shelf in all seasons and on all transects.

All parameters in the monthly samples (Figure 13) followed the same spatial pattern as seen in the seasonal samples; that is, generally higher numbers of species, equitability, and diversity at the deeper stations and generally higher numbers of individuals at the shallower stations. Number of species and number of individuals were generally higher in winter through July than in August through November at both

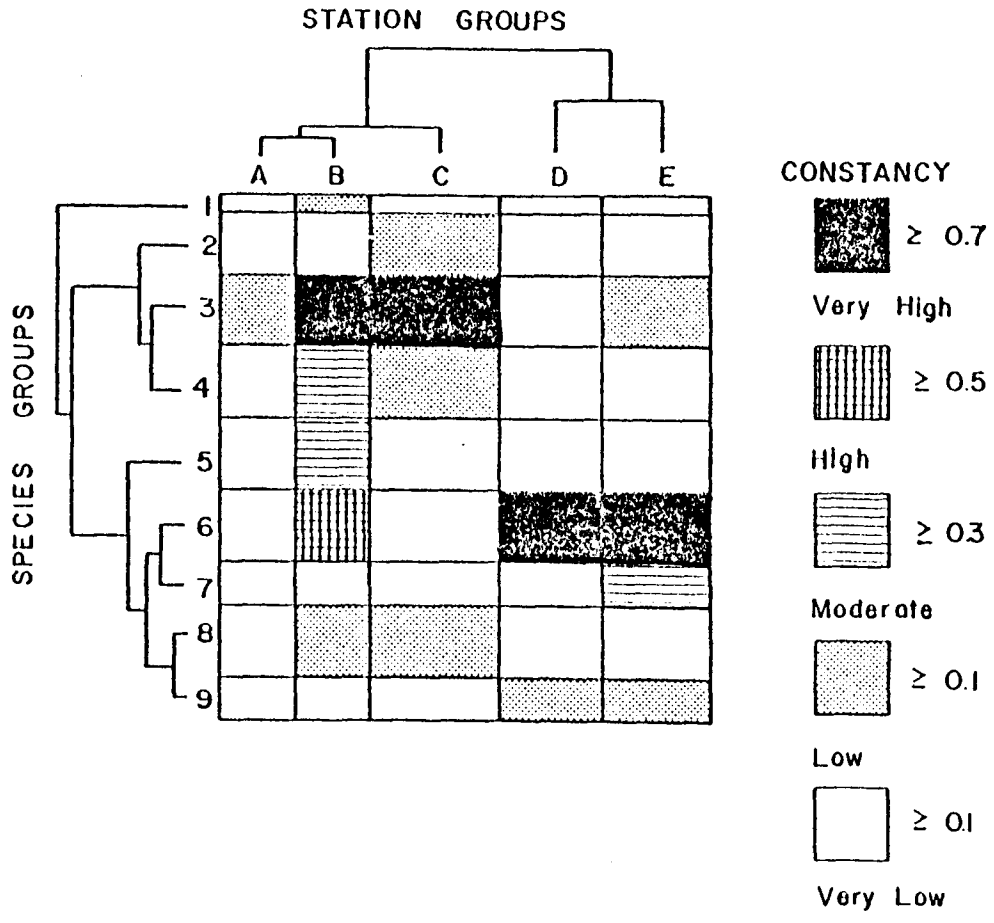

Figure 9. Nodal Constancy in a Two-Way Table of Species Groups in Station Groups from Monthly Epifaunal Data.

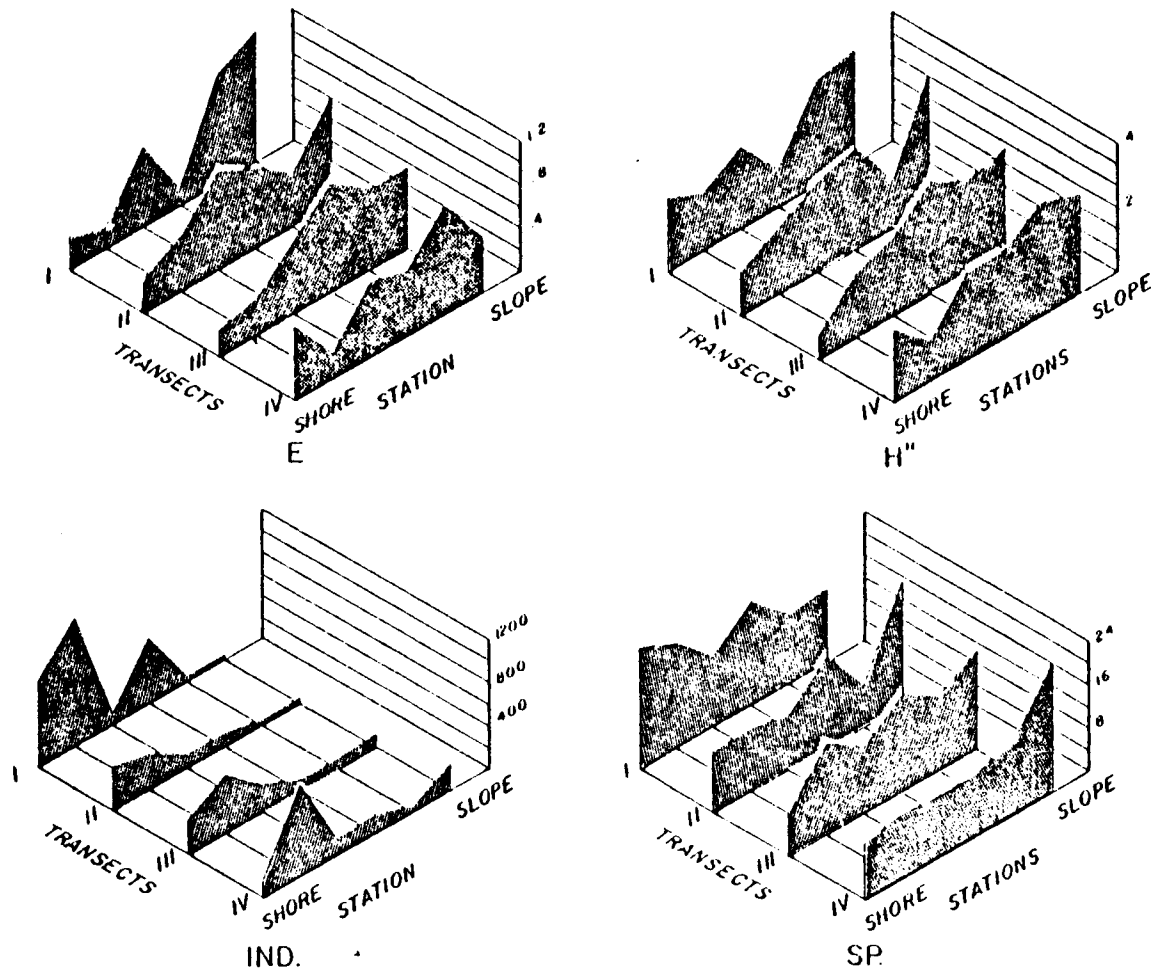

Figure 10. Shannon Diversity Values - H", Equitability - E, Number of Species and Number of Individuals for Winter Epifaunal Data.

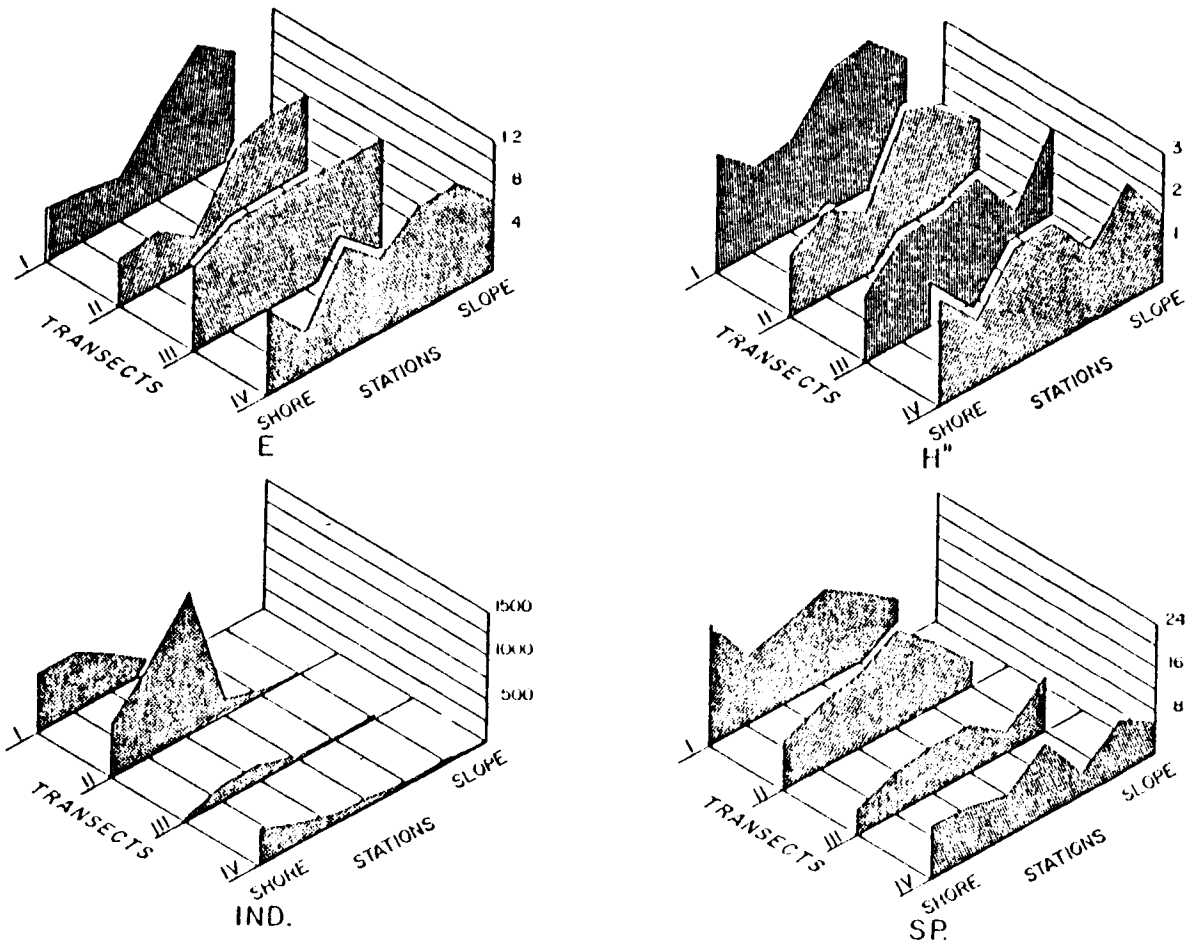

Figure 11. Shannon Diversity Values - H", Equitability - E, Number of Species and Number of Individuals for Spring Epifaunal Data.

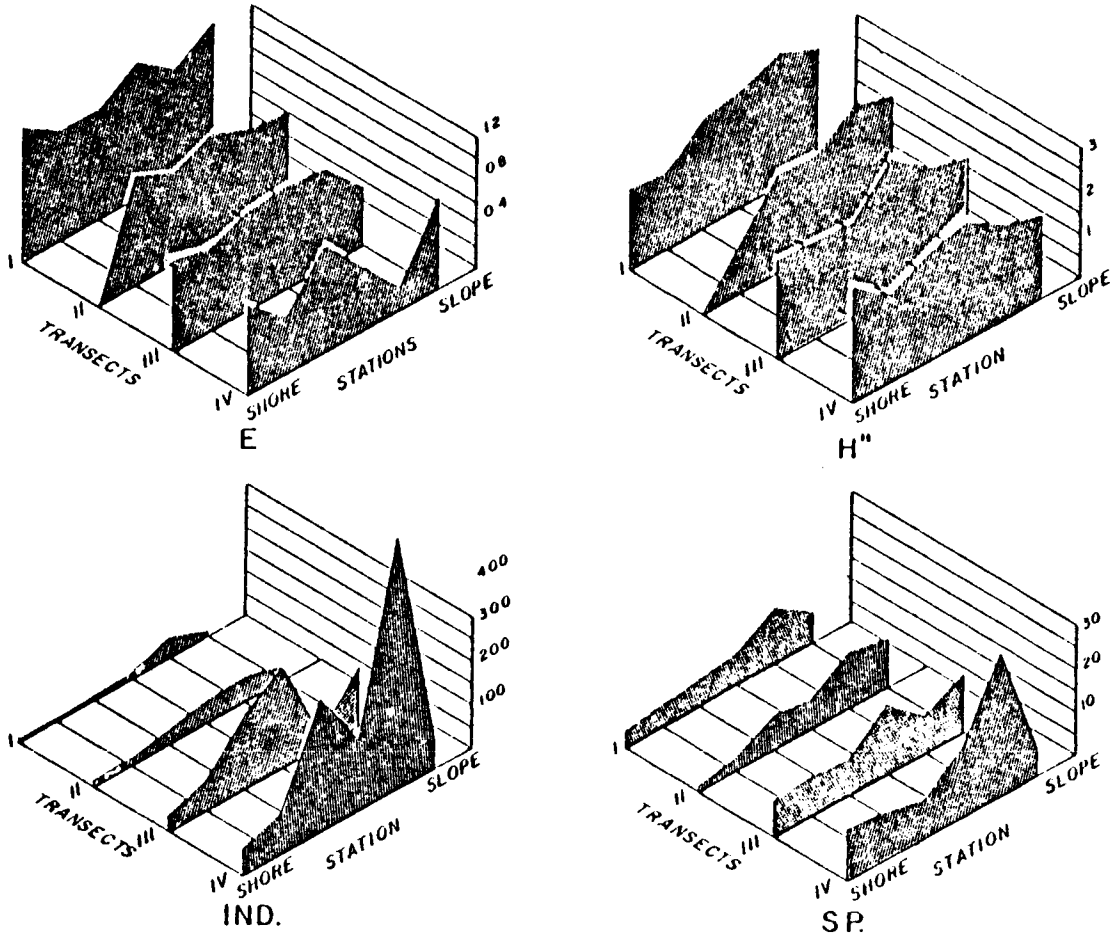

Figure 12. Shannon Diversity Values - H", Equitability - E, Numbers of Species and Numbers of Individuals for Fall Epifaunal Data.

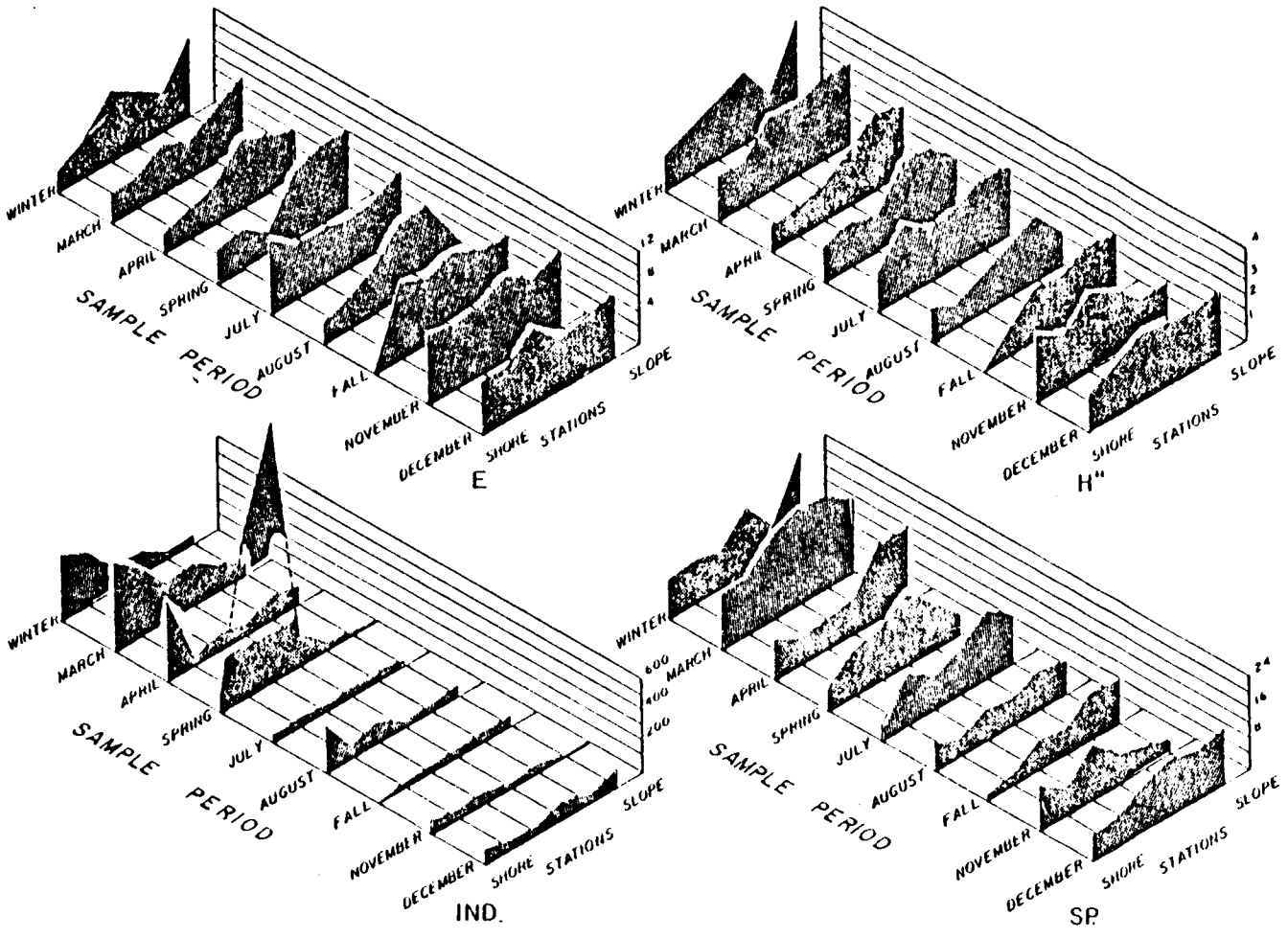

Figure 13. Shannon Diversity Values - H", Equitability - E, Number of Species and Number of Individuals for Monthly Epifauna Data.

inner and outer shelf stations.

Infauna

Many more species were collected in grab samples than in trawl collections. A total of 79,949 individuals representing 715 infaunal taxa were identified and are listed with abundance per sample in Table 3, Appendix G. The polychaete, *Magelona phyllisae*, was the numerically dominant organism in all seasons; four of the five most abundant species in each season were polychaetes. The amphipod *Ampelisca agassizi* was in the top five most abundant species in winter, and *Abra aequalis* was the second most abundant in spring.

Cluster Analysis

The large number of infaunal taxa were, and remain, difficult to analyze but the use of cluster analysis enabled definition of broad outlines in this mass of data. Due to the volume of data, cluster analysis of the combined seasonal data was impossible at the time of this report. Therefore, only separate seasonal analyses are presented.

Normal analysis of infaunal data for each season resulted in three clusters of stations (Figures 14, 15 and 16) which were amazingly similar temporally. These clusters divide the study area into shallow, mid-depth and deep station zones. In winter, the shallow zone included Stations 1/I and 1/II (Figure 17) which were classified in the mid-depth zone during other seasons. Station 2/IV clustered with deep stations in winter and in spring (Figure 18), but grouped with the mid-depth stations in fall (Figure 19). Stations 6/IV and 3/IV formed a separate cluster in fall, were divided from other deep stations in winter, but in spring, followed the normal pattern of division by depth.

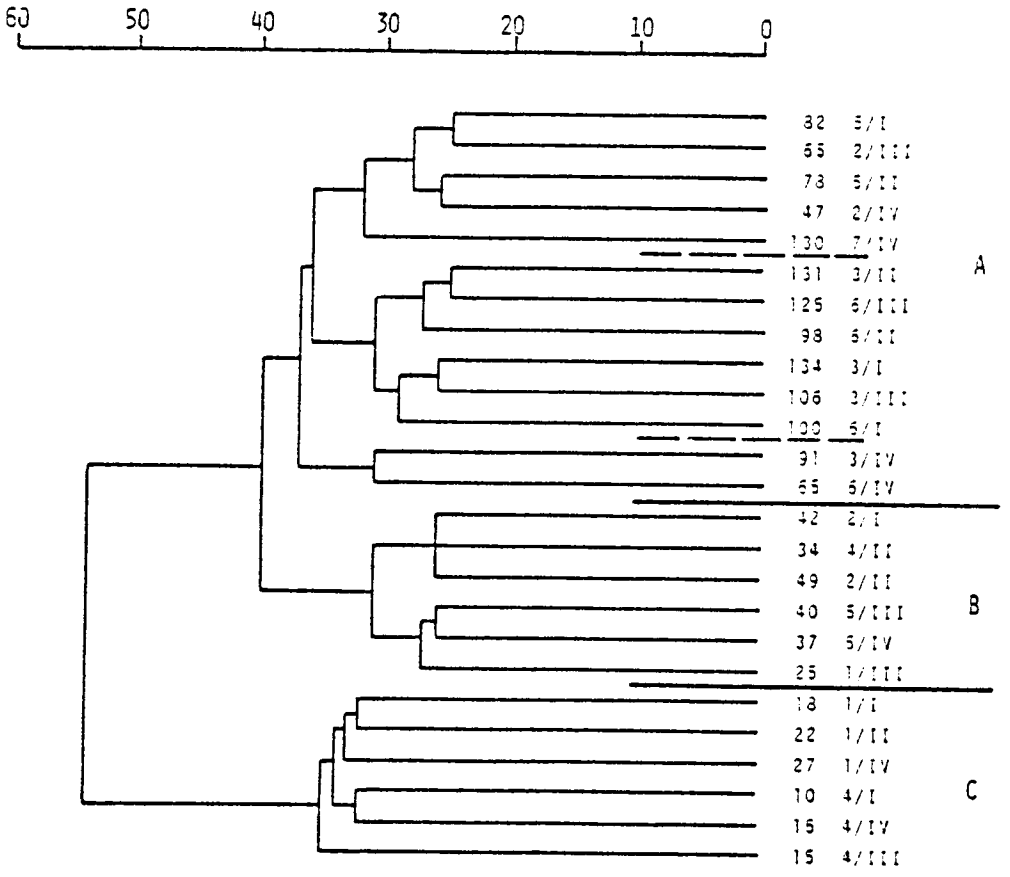

Figure 14. Normal Dendrogram from Cluster Analysis of Winter Infaunal Data. Numbers Preceding the Station Designations Refer to Depth (in meters). Letters Refer to Station-Groups.

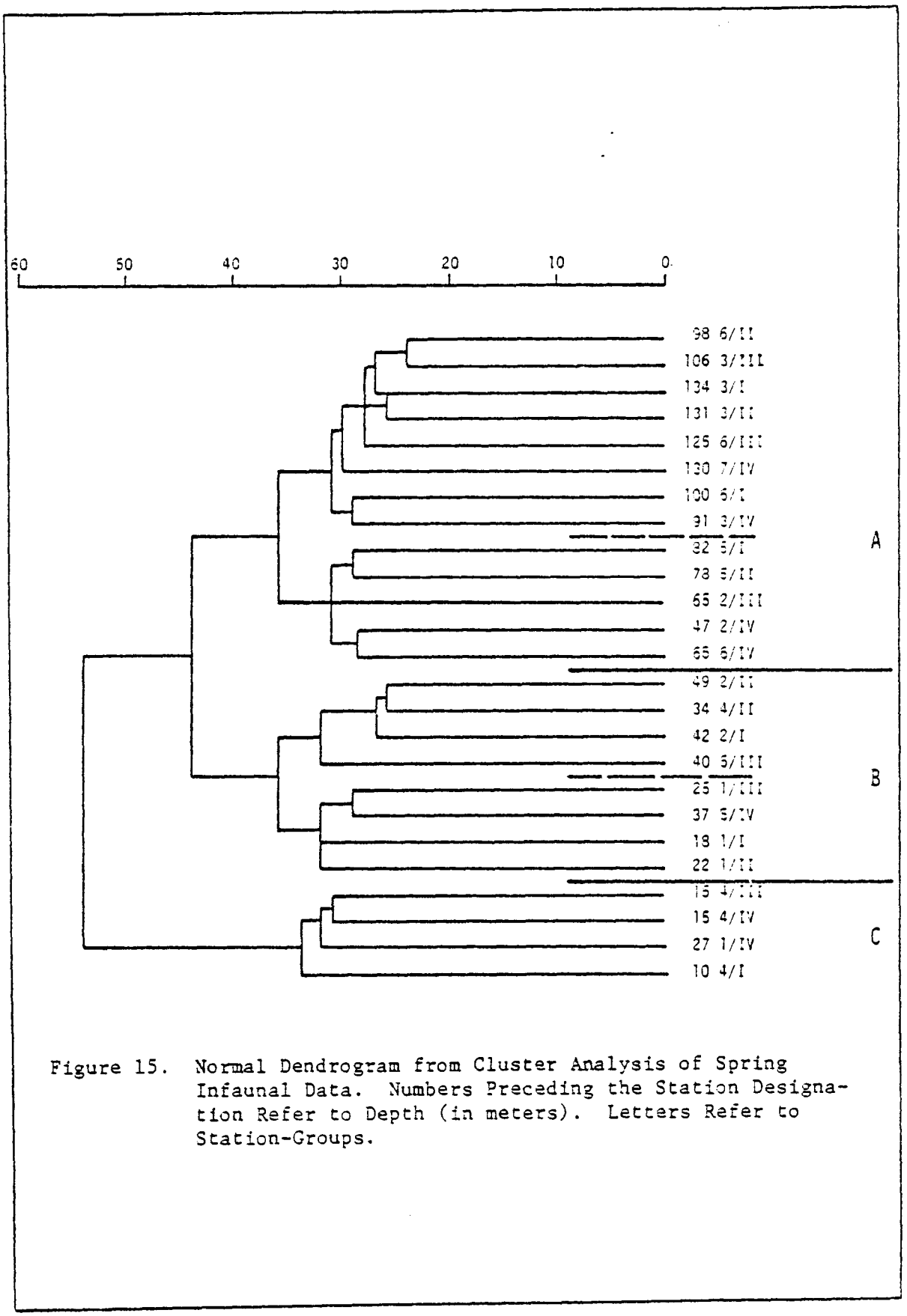

Figure 15. Normal Dendrogram from Cluster Analysis of Spring Infaunal Data. Numbers Preceding the Station Designation Refer to Depth (in meters). Letters Refer to Station-Groups.

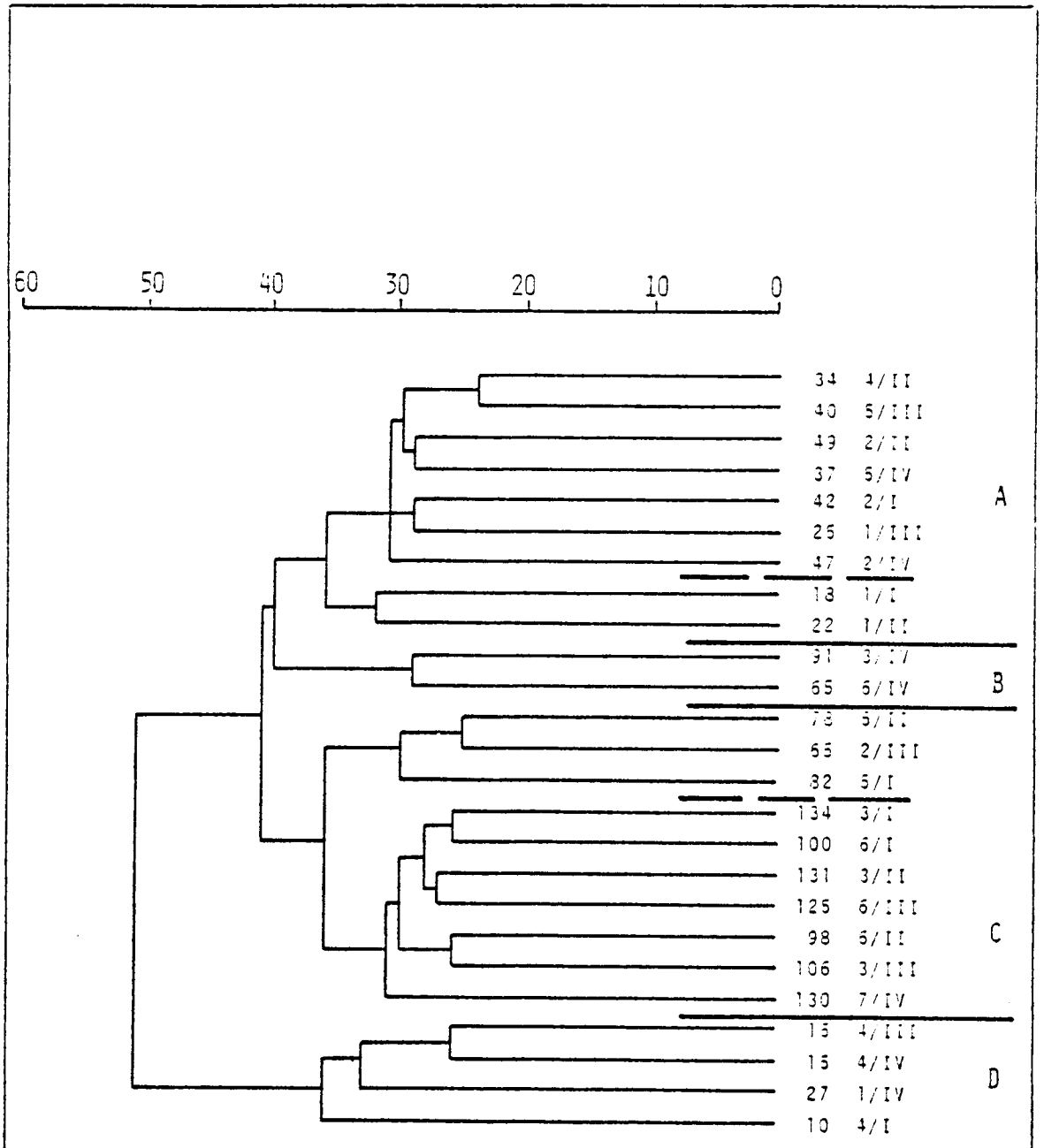

Figure 16. Normal Dendrogram from Cluster Analysis of Fall Infaunal Data. Numbers Preceding the Station Designation Refer to Depth (in meters). Letters Refer to Station-Groups.

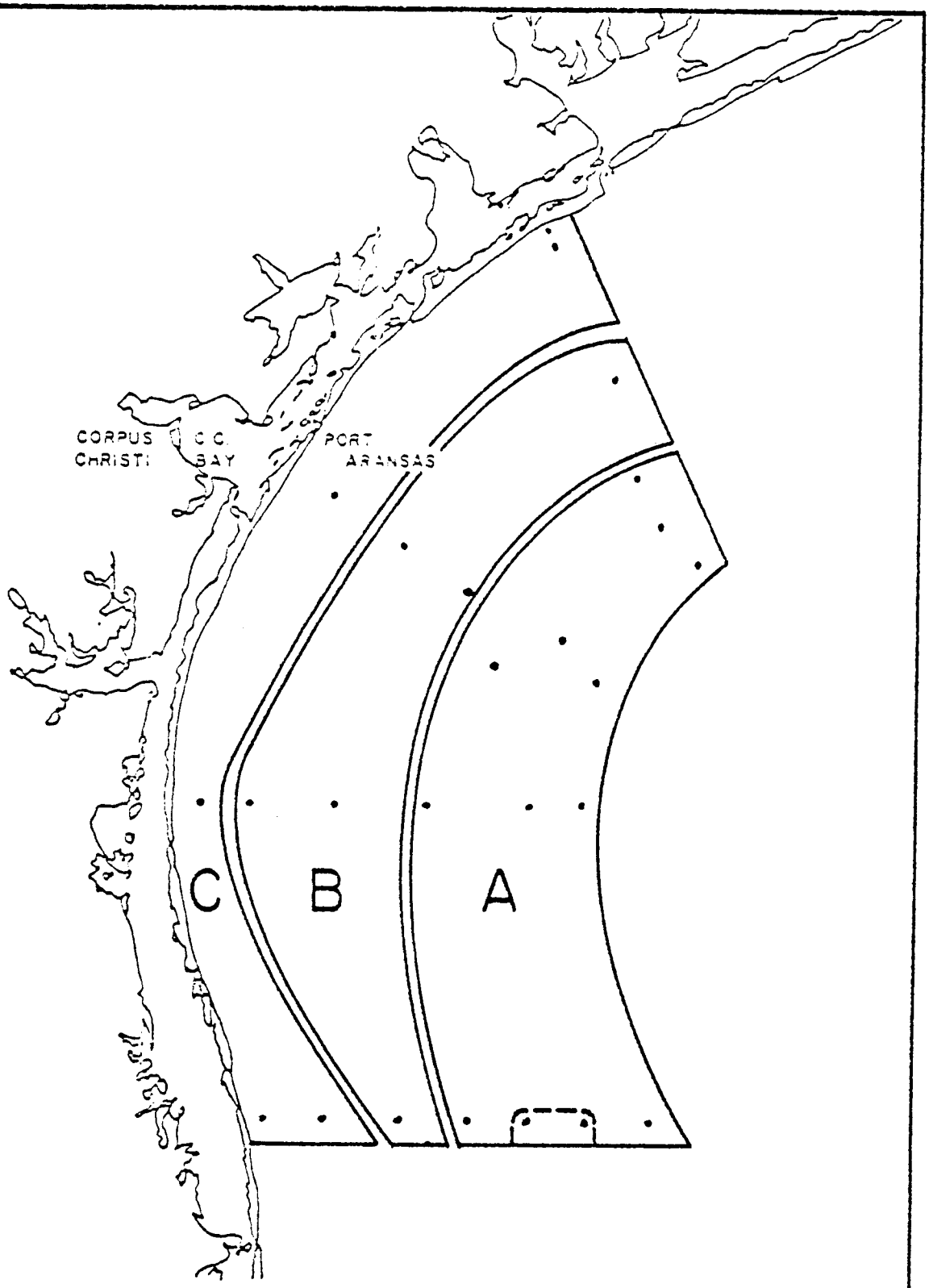

Figure 17. Location of Station-Group (A-C) from Cluster Analysis of Winter Infaunal Data.

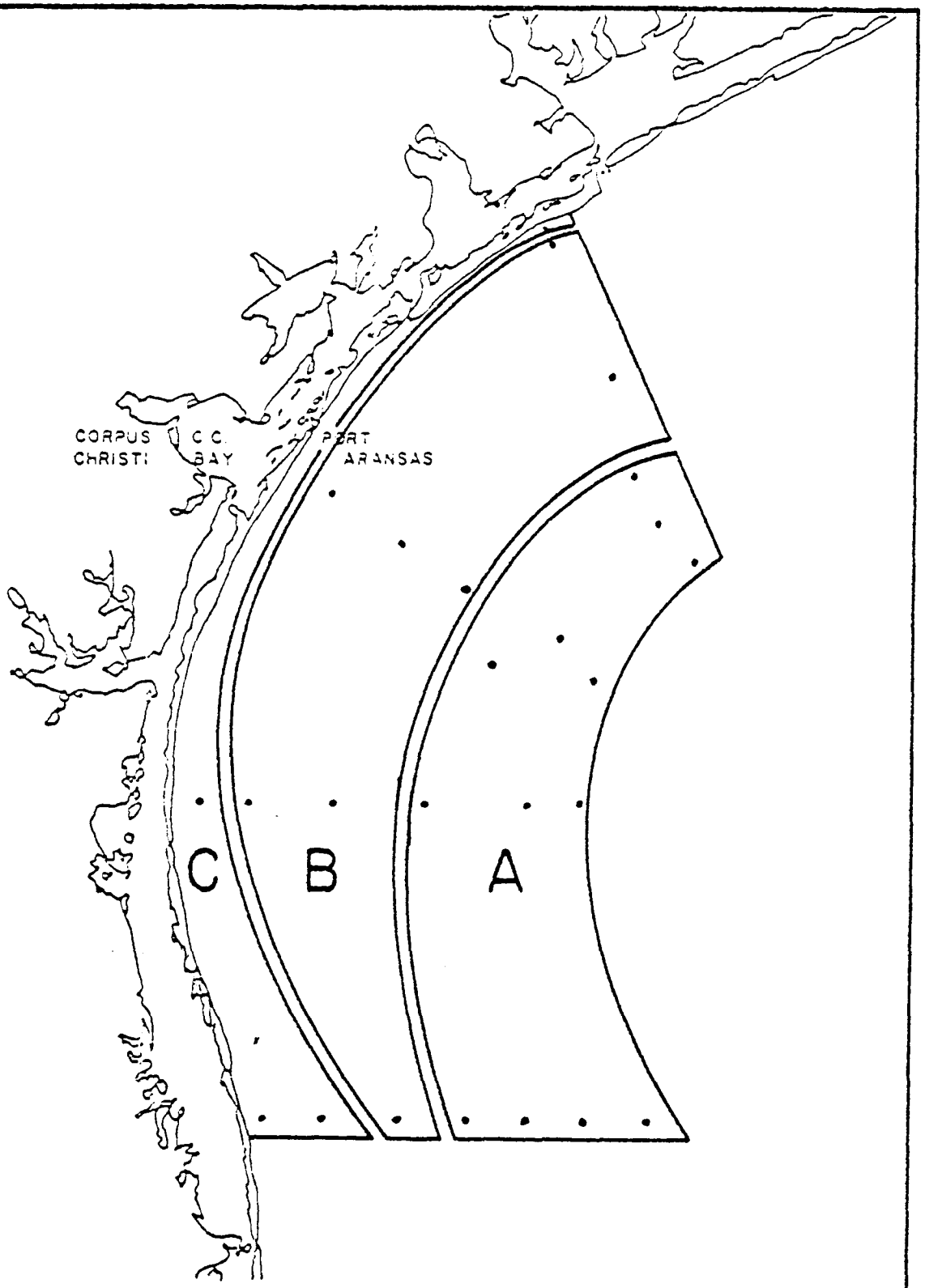

Figure 18. Location of Station-Groups (A-C) from Cluster Analysis of Spring Infaunal Data.

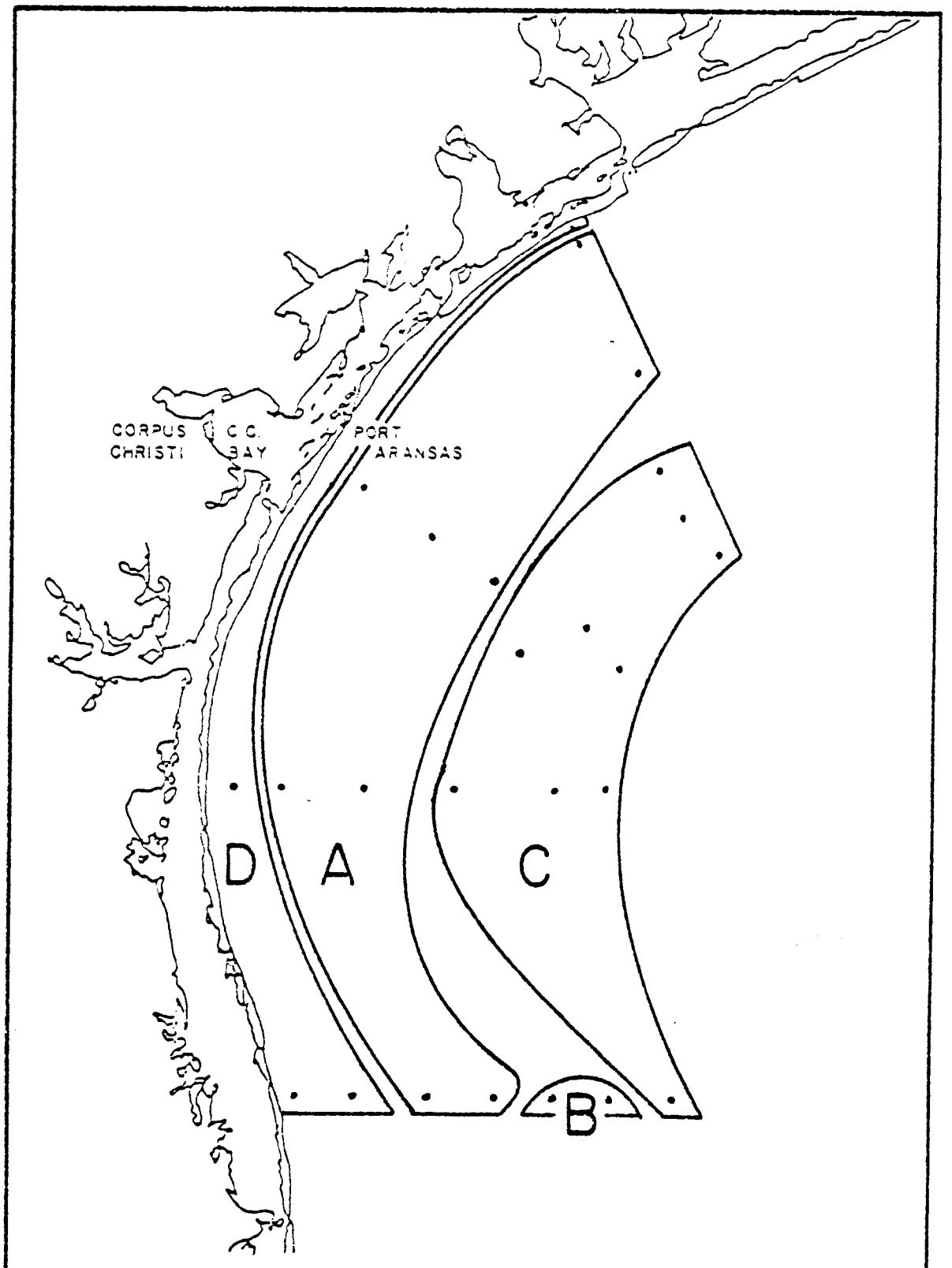

Figure 19. Location of Station-Groups (A-D) from Cluster Analysis of Fall Infaunal Data.

Station-groups formed from cluster analyses were compared with sediment data. Sediments at shallow stations were muddy-sand and at deep stations were silty-clay. Mid-depth station sediments represented a transition zone that ranged from sandy-mud to silty-clay. Sediment composition of Stations 2/IV, 3/IV and 6/IV were variable between seasons, ranging from muddy-sand through silty-clay, implying an extremely heterogenous substrate in that area.

Species typical of the station-groups are presented in Table 2. Many infaunal taxa were collected at sandy stations which included Stations 4/I, 4/III, 4/IV and 1/IV in all seasons and Stations 6/IV and 3/IV in most seasons. Polychaetes dominated the sandy assemblage. Mid-depth collections did not contain any species unique to that area but shared species with shallow stations, and, of course, contained the ubiquitous species. Species found only in deep-water collections were dominated by small crustaceans (ostracods and amphipods) and molluscs. Only two species of polychaetes were confined to deep-water stations but the majority of ubiquitous species at deep stations (as well as elsewhere) were polychaetes.

Comparisons were made at each station of the percentage of sedentary deposit-feeding polychaetes, such as *Magelona phyllisae* and *Paraprionospio pinnata*, to the actively burrowing detritous feeders, such as *Mediomastus californiensis* and *Paraonis gracilis*. Some of the sedentary forms such as *Owenia fusiformis* may be partially or wholly suspension feeders. Sedentary deposit-feeding polychaetes generally dominated the shallowest stations, percentages converged at mid-depths, and active burrowers were dominant at deeper stations.

TABLE 2

SPECIES TYPICAL OF SEASONAL INFAUNAL
CLUSTER ANALYSIS STATION-GROUP

Station-Group	Species
Sandy Sediments	
Shallow (10-27 m)	Coelenterates
	Zoantharian sp. A
	Molluscs
	Cadulus carolinensis
	Lucina multilineata
	Eryxina sp. A
	Polychaetes
	Aglaophamus verrilli
	Spiophanes bombyx
	Armandia agilis
	Owenia fusiformis
	Isolda pulchella
	Amphipods
	Ampelisca sp. B
Shallow and some of the deeper Transect IV stations	Molluscs
	Lucina amiantus
	Tellina versicolor
	Polychaetes
	Paleanotus heteroseta
	Apoprionospio pygmaea
	Prionospio cristata
	Magelona pettiboneae
	Clymenella torquata
	Cumaceans
	Oxyurostylis sp. A
	Amphipods
	Ampelisca cf. *cristata*
Stations 6/IV, 3/IV only	Molluscs
	Crassinella martinicensis
	Polychaetes
	Onuphis sp. B
	Ostracods
	Ostracod sp. GG
	Amphipods
	Cerapus tubularis
Shallow to mid-depth stations (10-49 m and 6/IV, 3/IV)	Polychaetes
	Sthenelais boa
	Nereid (*Nicon*) sp. A
	Diopatra cuprea
	Lumbrineris tenuis
	Drilonereis magna

TABLE 2. CONT.'D

Station-Group	Species
Shallow to mid-depth stations (10-49 m and 6/IV, 3/IV) cont.'d	*Magelona phyllisae* *Magelona rosea* *Aricidea taylori* *Aricidea wassi* *Notomastus hemipodus* *Notomastus* cf. *latericeus* Amphipods *Ampelisca abdita* *Ampelisca verrilli* *Listriella barnardi*
Deep stations (65-134 m)	Molluscs *Scutopus* sp. A *Dentalium sowerbyi* *Solemya* sp. A Pelecypod sp. A Polychaetes *Paralacydonia paradoxa* *Sternaspis scutata* Ostracods Ostracod sp. D Ostracod sp. AA *Philomedes* sp. A *Altermochelata* sp. A *Sarsiella* sp. B Amphipods *Byblis* cf. *gaimardi* *Eriopisa incisa* *Heterophoxus* cf. *oculatus*
Ubiquitous	Molluscs *Pitar cordatus* Polychaetes *Sigambra tentaculata* *Nephtys incisa* *Lumbrineris parvapedata* *Minuspio cirrifera* *Paraprionospio pinnata* *Tharyx marioni* *Cossura delta* *Paraonis gracilis* *Mediomastus californiensis* Amphipods *Ampelisca agassizi*

Diversity

Spatial variation in number of species, number of individuals, equitability and diversity (Figures 20-22) showed similar patterns in all seasons. On Transects I, III and IV, the greatest number of species was taken at the shallow stations (4 and 1) and at deeper stations (6 and 3). On Transect II, however, number of species was relatively consistent at all depths. Total number of individuals was much higher at the shallowest stations (Station 4, Transects I, III and IV) than at deeper stations. The lower number of individuals at the shallowest station on Transect II was consistent with other transects since Station 1/II was 7-m deeper than the shallowest stations on the other transects. There was a secondary peak in number of individuals at Stations 6 and 3, Transect IV, in winter and fall.

Infaunal equitability values showed a general increase with depth on all transects, reflecting the relatively even distribution of individuals among species at the deep stations. High equitability at deep stations, combined with high numbers of species at inshore stations, produced a pattern of relatively even diversity over the study area. Transect IV averaged slightly higher diversity than the others.

Number of species, individuals, equitability and diversity were plotted for the monthly infaunal collections on Transect II, along with the Transect II data from each seasonal cruise (Figure 23). Seasonal infaunal data (Figures 20-22) showed that abundance and distribution patterns on Transect II were not typical of the other transects. Number of species was generally higher at the mid-depth stations in winter through spring and higher at the deeper stations in July through December. Equitability and diversity generally increased with depth. The change in number of

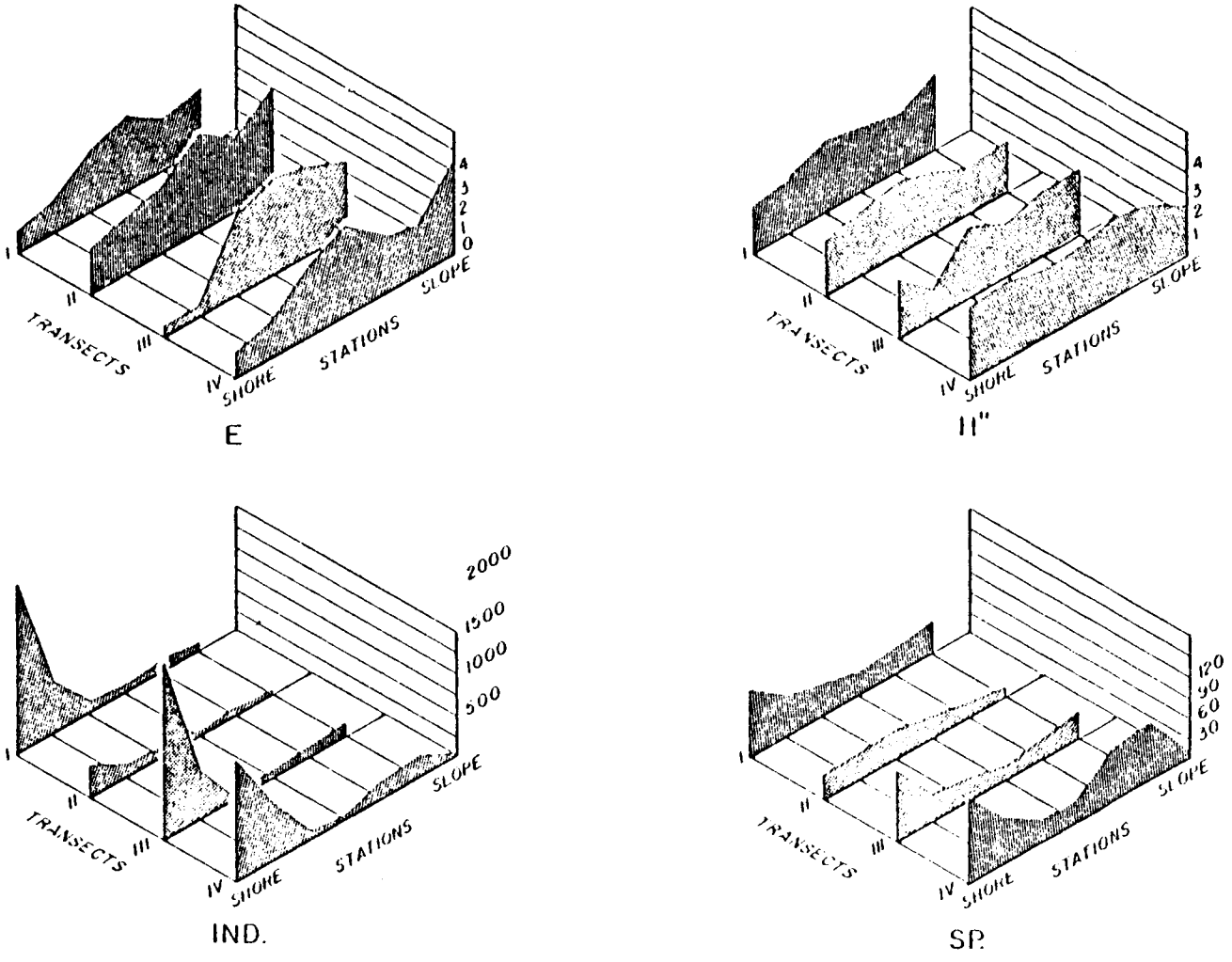

Figure 20. Shannon Diversity Values - H", Equitability - E, Number of Species and Number of Individuals for Winter Infaunal Data.

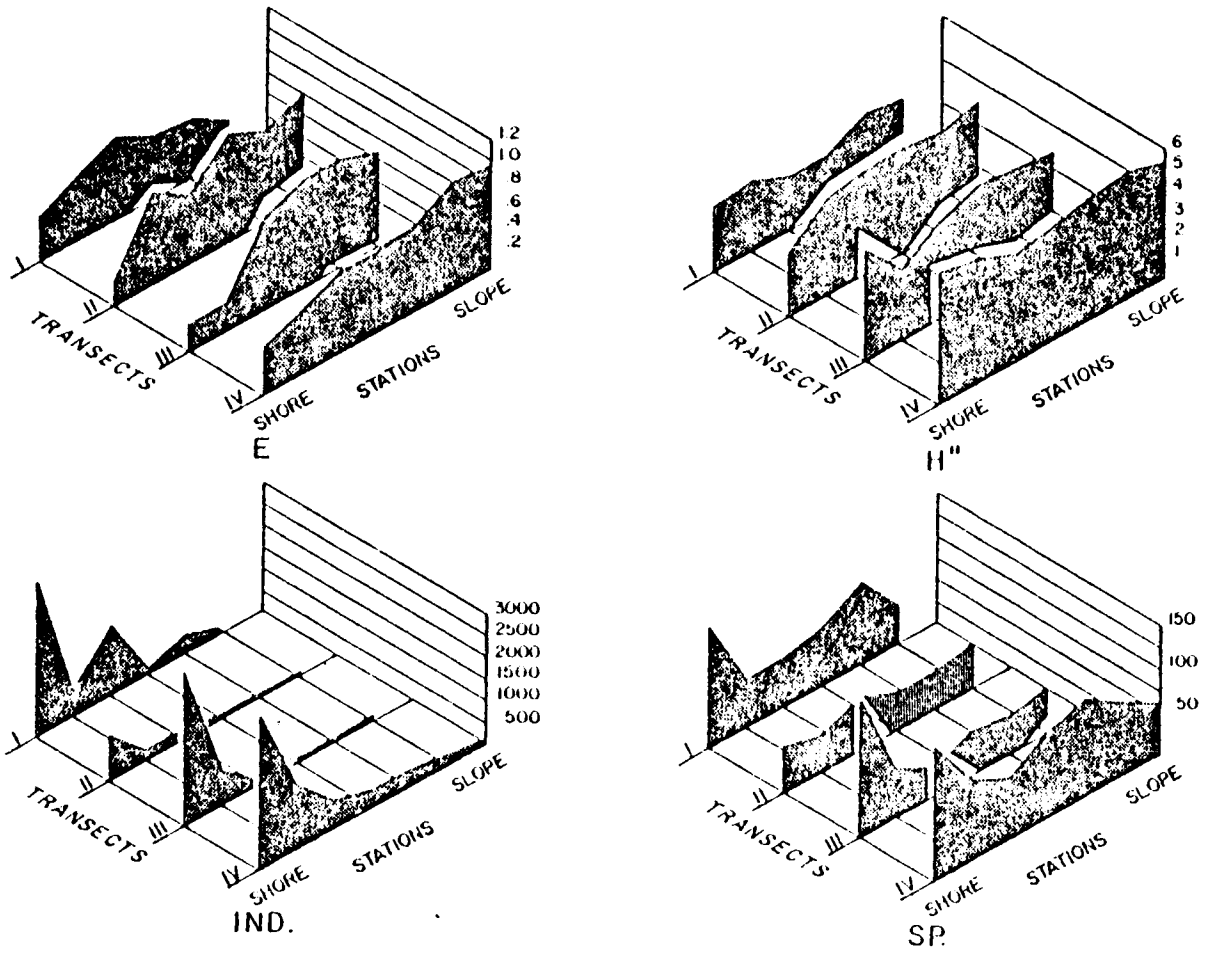

Figure 21. Shannon Diversity Values - H", Equitability - E, Number of Species and Number of Individuals for Spring Infaunal Data.

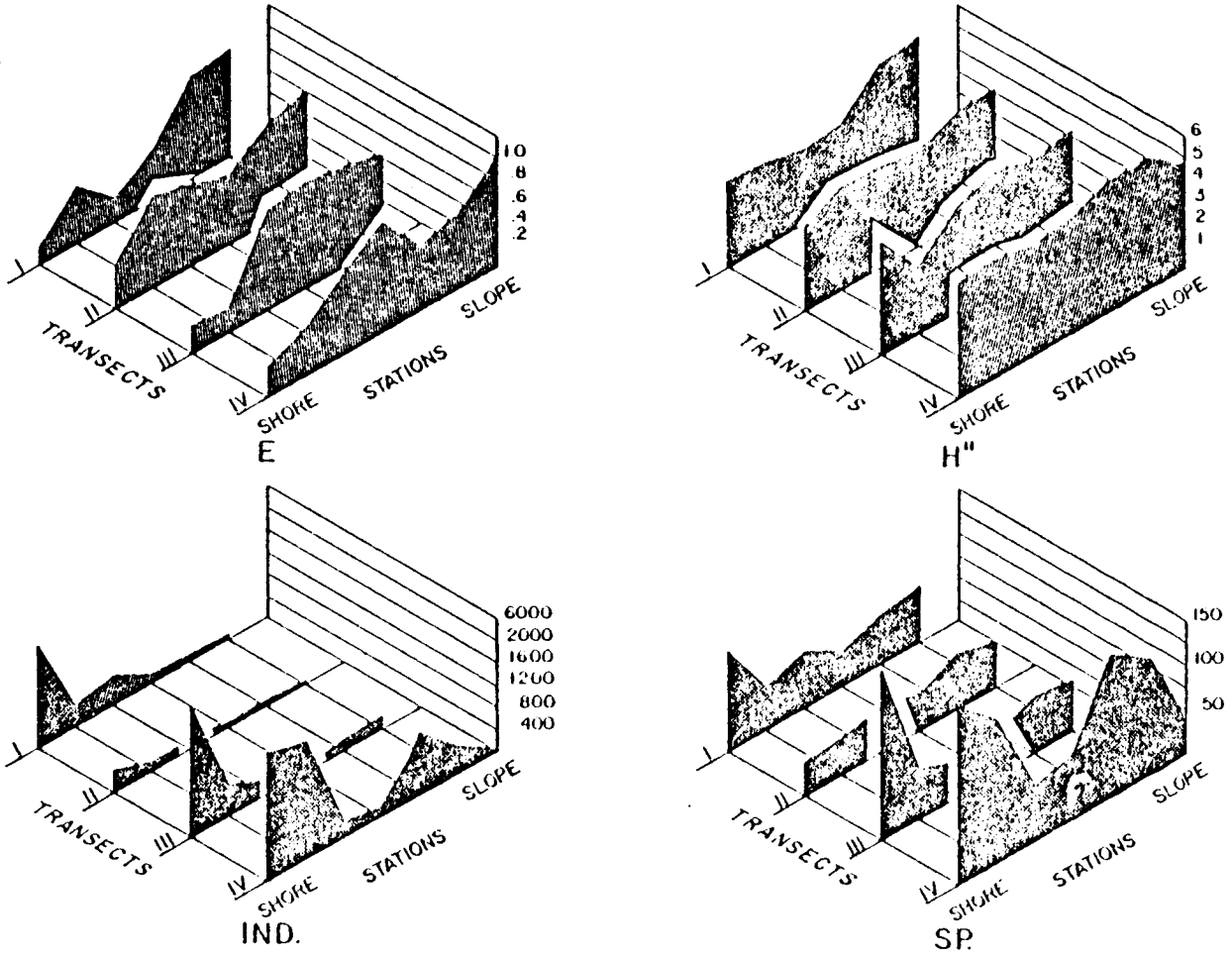

Figure 22. Shannon Diversity Values - H", E - Equitability, Number of Species and Number of Individuals for Fall Infaunal Data.

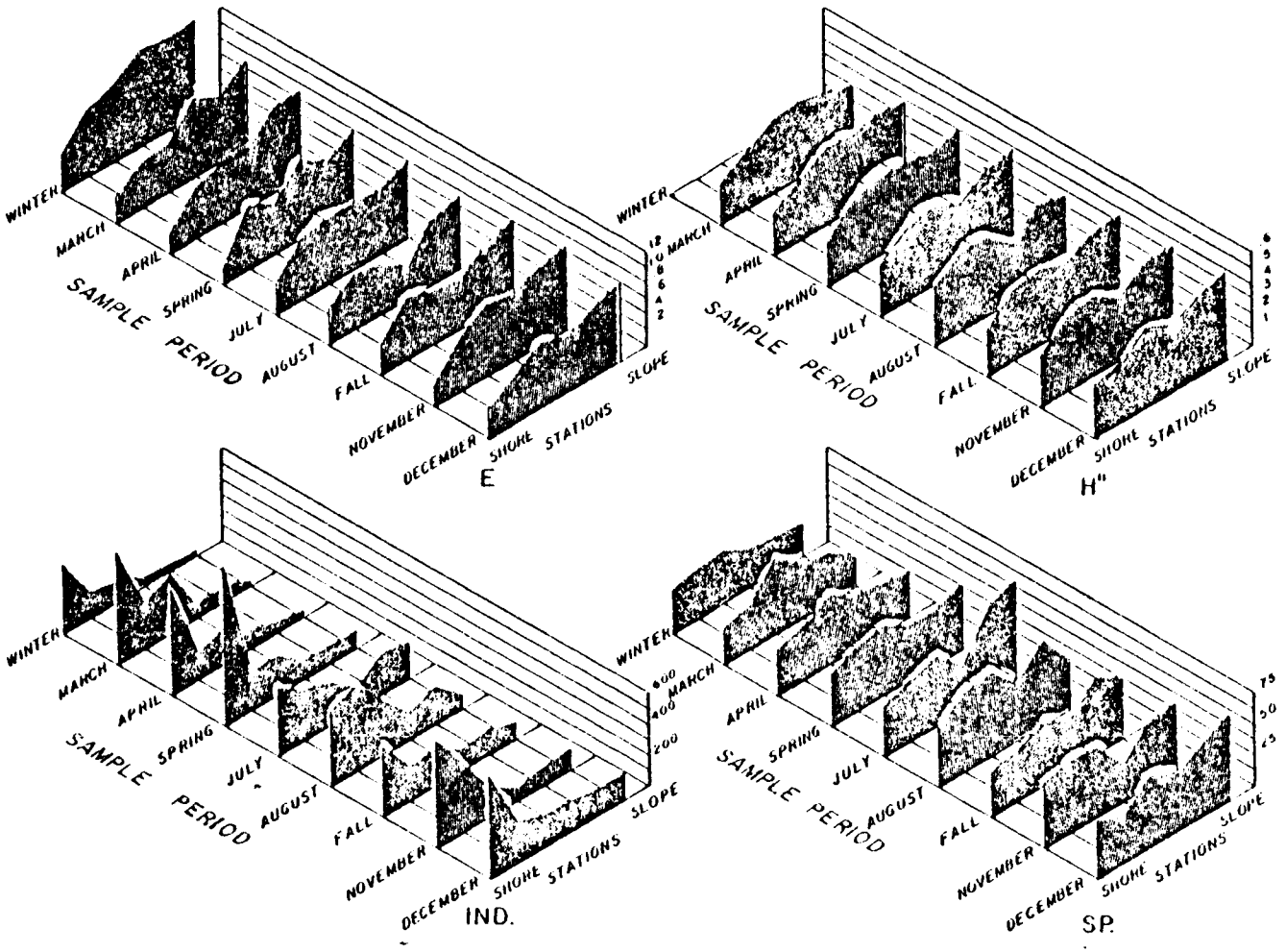

Figure 23. Shannon Diversity Values - H", Equitability - E, Number of Species and Number of Individuals for Monthly Infaunal Data.

species and individuals at the deeper stations might reflect seasonal variation in the infaunal samples on Transect II. This apparent seasonal cycle was somewhat disrupted by the increase in number of species and individuals in December, which did not correspond well with the low number of species the previous winter (February).

Microhabitat Distribution

While some analytical techniques used with the data primarily ascertain the structure of benthic communities on a temporal or broad spatial scale, other techniques were used to assess the small-scale (within-station) distribution patterns of benthic populations. Knowledge of these distribution patterns is necessary to ascertain the effectiveness of the present sampling regime.

Data from three suites of 12 replicate samples from Stations 1, 2 and 3, Transect II, during the winter of 1976 were analyzed using a technique (P_K) presented by Gaufin *et al.* (1956). The portion of the community (percent of species) sampled at different stations with various sample replication was the information sought. Results indicated that one sample would provide less than 30 percent of the species at any inshore or mid-depth station and less than 20 percent of the species at deep stations (Figure 24). A second data analysis, omitting those species found in only one of 12 samples (operationally defined as rare species), indicated that a single sample would collect approximately 45 percent of the non-rare species at Station 1, 38 percent of non-rare species at Station 2 and 28 percent of non-rare species at Station 3, Transect II (Figure 25). Percentage of species composition sampled by four and six replicates are given for both rare and non-rare species in Figures 24-25 as these were the levels of sampling effort for this study in year I (1975) and year II

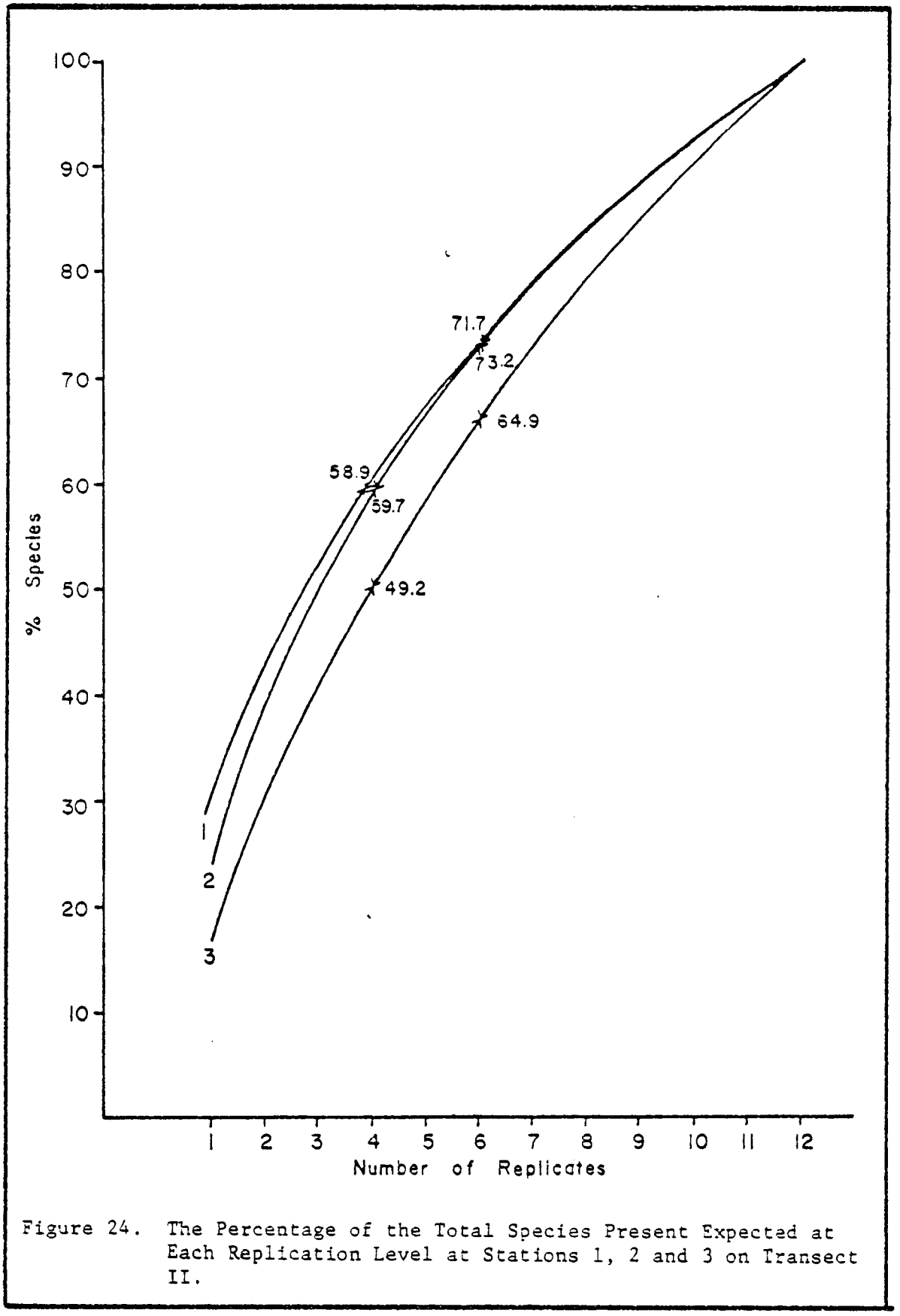

Figure 24. The Percentage of the Total Species Present Expected at Each Replication Level at Stations 1, 2 and 3 on Transect II.

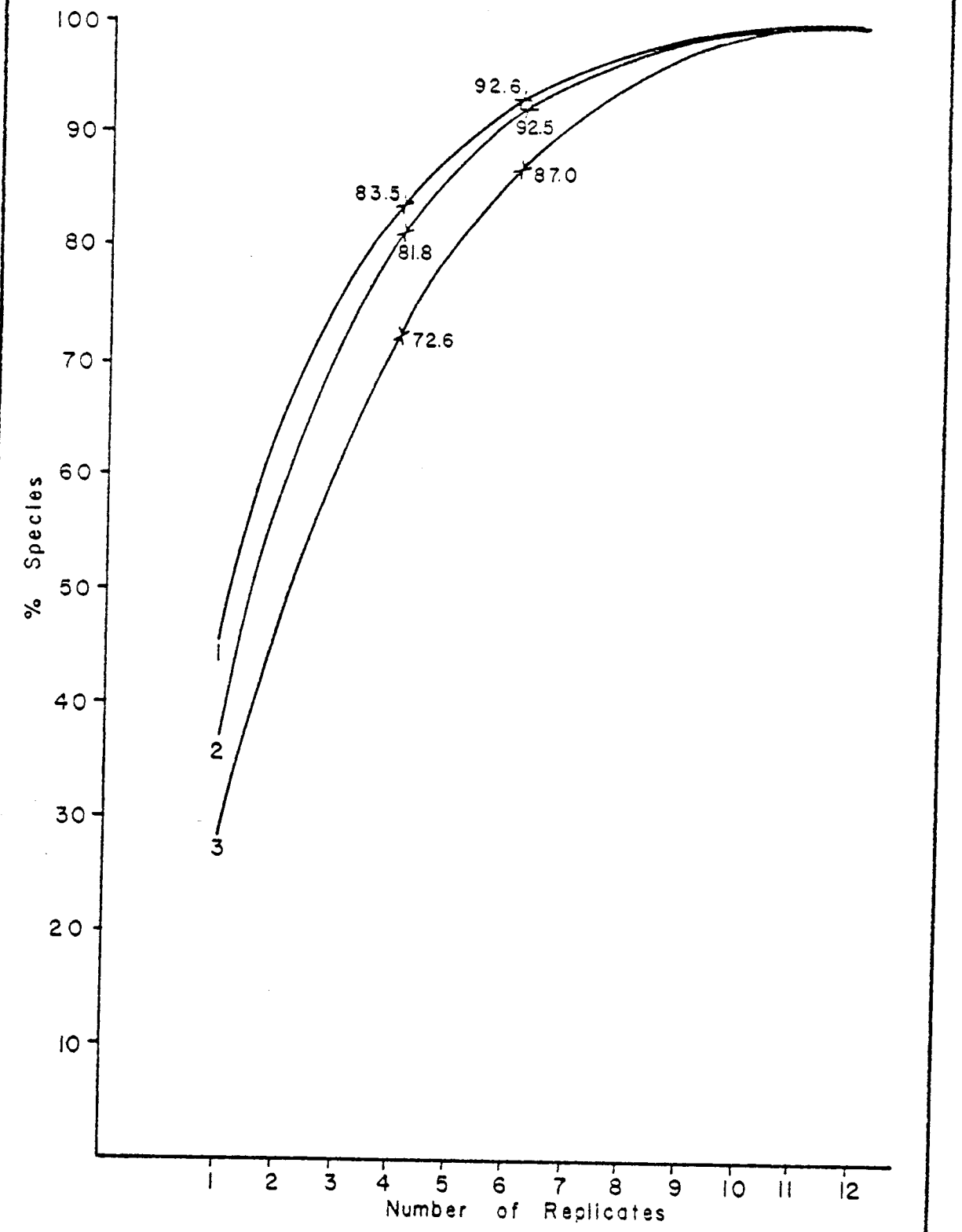

Figure 25. The Percentage of the Non-Rare Species Present Expected at Each Replication Level at Stations 1, 2 and 3 on Transect II.

(1976), respectively.

Data from the three suites of 12 replicate samples were analyzed for dispersion patterns of benthic communities at each station and to assess the distribution pattern of certain invertebrate species. An index of dispersion ($\frac{S^2}{\bar{X}}$) was calculated for each included species from each suite of replicates. Inclusion was on a criterion similar to that set forth by Gage and Geekie (1973). All species occurring at each station in excess of one singleton were included in the analysis. The log of the variance (log S^2) was plotted against the log of the mean number of individuals (log $\bar{X}$) to indicate dispersion patterns of infaunal invertebrates at each of the three stations (Figure 26). Index of dispersion values for each included species were checked for significance of departure from the expected (Poisson) distribution. The percentage of included species showing significant departure from the Poisson distribution is given below:

Station 1/II	Station 2/II	Station 3/II
34.5	46.5	24.0

The same analysis ($\frac{S^2}{\bar{X}}$) was applied to the normal suites (6 replicates) from all stations on Transect II from the March and April collections and on data from all samples from the winter (1976) cruises. The winter collection percentages of species showing significant departure from the Poisson toward aggregation are given in Table 3. There was apparently a definite trend in community dispersion patterns toward greater numbers of species aggregating at the nearshore stations. Offshore stations in general showed lower percentages of the species comprising the community having clumped distributions. Several stations (5/IV, 2/IV and 3/II) were without species showing clumped distribution patterns.

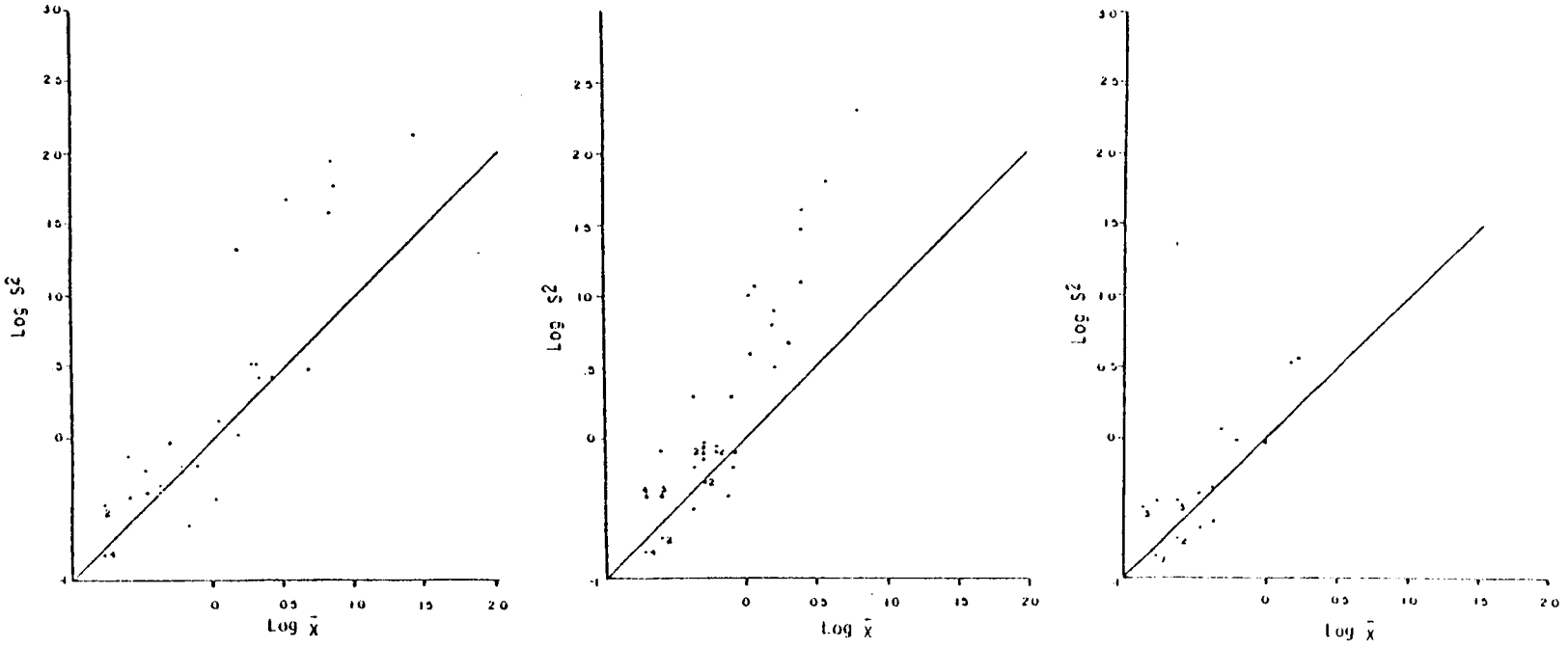

Figure 26. Plot of Log Variance (S^2) Against Log Mean ($\bar{X}$) of the Numerical Occurrence of the Species in 12 Replicate Samples Obtained from Stations 1, 2, and 3, Transect II. Where Values Plotted were Identical, the Numbers of Species Occurring is Given Beside the Point.

TABLE 3

PERCENTAGE OF SPECIES INCLUDED IN DISPERSION ANALYSIS WHICH SHOWED SIGNIFICANT TENDENCY TOWARD AGGREGATION AT EACH STATION DURING WINTER COLLECTIONS. STATIONS ARE ARRANGED IN ORDER OF INCREASING DEPTH

Transect							
	Station	4	1	2	5	6	3
I		35.0	28.9	29.6	22.2	17.4	10.0
	Station	1	4	2	5	6	3
II		33.3	19.0	13.0	12.5	25.0	0
	Station	4	1	5	2	3	6
III		32.4	3.7	6.3	18.2	6.7	14.3
	Station	4	1	5	2	6	3
IV		26.3	31.3	0	0	17.9	19.0

A final analysis technique, Dispersion Chi-Square (Jumars, 1975), was applied to data from the three suites of 12 samples taken on Transect II during the winter cruises. Results corroborated those previously found with the index of dispersion.

Topographic Features Stations

Macroinfaunal data from four stations around Hospital Rock (HR) and four stations around Southern Bank (SB) are presented in Table 4, Appendix G. Numbers of species and individuals, diversity, P.I.E. and equitability values varied both temporally and spatially (Tables 4 and 5). Differences in community structure parameters among the four stations around each bank station were accompanied by changes in dominant species. Dominance at each station was analyzed by ranking species in each collection and assigning a value to each rank (10 points for the first ranking species, nine points for the second, etc.) and summing these values over the number of collections at each station. The species having the largest summed values were considered dominants (Table 6).

The eight stations divided into two major groupings. Two stations (SB 3 and HR 1) had decidedly different benthic invertebrate communities when compared to the other six stations. These two stations (Group A) were characterized by large numbers of species and individuals (Tables 4, 5 and 7) averaging three to five times the number of species found at stations of the second group (Group B) and approximately an order of magnitude greater number of individuals. Two families of polychaetes (Syllidae and Spionidae) were highly represented in the Group-A stations (Table 7). The overwhelming dominant organism at these two stations was the polychaete, *Sphaerosyllis* cf. *sublaevis*. Several other polychaetes,

TABLE 4

NUMBERS OF SPECIES AND INDIVIDUALS AND DIVERSITY (H''), P.I.E. AND EQUITABILITY (E) VALUES FOR STATIONS AROUND SOUTHERN BANK

		Species	Ind.	H''	P.I.E.	E
Station 1						
Winter		43	131	4.8092	.9548	1.000
March		40	183	3.4806	.7584	.4250
April						
Spring						
July		73	334	5.0754	.9489	.6986
August						
Fall		64	151	5.5175	.9765	1.000
November		46	92	5.0613	.9670	1.000
December						
	$\bar{X}$	53.20	178.20	4.7888	.9211	.8247
	S	14.48	93.13	0.7745	.0916	.2588
Station 2						
Winter		25	59	3.9676	.9117	.9600
March						
April		31	75	4.2379	.9182	.9355
Spring						
July		39	105	4.6486	.9489	.9744
August						
Fall		50	148	4.8441	.9442	.8800
November		43	120	4.9492	.9648	1.000
December						
	$\bar{X}$	37.60	101.40	4.5295	.9403	.9500
	S	9.84	35.44	0.4154	.0480	.0456
Station 3						
Winter						
March						
April		124	817	5.1079	.9360	.4194
Spring		189	1443	5.9412	.9671	.4974
July						
August		161	1509	5.4235	.9452	.4037
Fall						
November						
December		158	1494	5.1571	.9241	.3416
	$\bar{X}$	158	1315.75	5.4074	.9431	.4153
	S	26.62	333.69	0.3819	.0182	.0641

TABLE 4. CONT.'D

Station 4		Species	Ind.	H"	P.I.E.	E
Winter						
March		49	116	5.1766	.9712	1.000
April						
Spring		56	130	5.1659	.9641	.9821
July						
August		52	130	5.1619	.9665	1.000
Fall						
November						
December		51	94	5.2875	.9755	1.000
	$\bar{X}$	52	117.50	5.1980	.9693	.9955
	S	2.94	17.00	0.0600	.0051	.0089

TABLE 5

NUMBERS OF SPECIES AND INDIVIDUALS AND DIVERSITY (H''), P.I.E. AND EQUITABILITY (E) VALUES FOR STATIONS AROUND HOSPITAL ROCK

		Species	Ind.	H''	P.I.E.	E
Station 1						
Winter		117	884	4.6914	.9011	.3333
March		180	1404	5.3984	.9389	.3556
April						
Spring						
July		159	1222	5.7534	.9551	.5157
August						
Fall		172	1539	5.4625	.9437	.3895
November		167	1273	5.7460	.9620	.4910
December						
	$\bar{X}$	159	1264.4	5.4103	.9402	.4170
	S	24.69	245.70	0.4330	.0237	.0818
Station 2						
Winter		23	51	4.0233	.9333	1.000
March		29	97	4.3125	.9418	1.000
April						
Spring						
July		47	170	4.6883	.9469	.8298
August						
Fall		50	215	4.4185	.8942	.6400
November		43	115	4.8680	.9602	1.000
December						
	$\bar{X}$	38.4	129.60	4.4621	.9353	.8940
	S	11.78	63.99	0.3289	.0250	.1600
Station 3						
Winter						
March						
April		42	73	4.9874	.9692	1.000
Spring		50	124	5.0260	.9543	1.000
July						
August		66	307	5.2229	.9624	.8636
Fall						
November						
December		45	117	4.9755	.9607	1.000
	$\bar{X}$	50.75	155.25	5.0530	.9629	.9659
	S	10.69	103.65	0.1153	.0044	.0682

TABLE 5. CONT.'D

		Species	Ind.	H"	P.I.E.	E
Station 4						
Winter						
March						
April		39	84	4.5739	.9375	.9231
Spring		64	296	4.8245	.9374	.6719
July						
August		53	132	5.1276	.9644	1.000
Fall						
November						
December		56	147	5.1905	.9632	1.000
	$\bar{X}$	53	164.75	4.9291	.9506	.8988
	S	10.42	91.53	0.2857	.0152	.1555

TABLE 6

DOMINANCE INDEX FOR THE TOP FIVE INFAUNAL SPECIES
IN HOSPITAL ROCK AND SOUTHERN BANK MACROINVERTEBRATE COMMUNITIES

SB 1 (50 max)*		SB 2 (50 max)	
Heterophoxis cf. *oculatus*	(34)	*Cossura delta*	(37)
Gnathia sp.	(29)	Nemertinea	(26)
Nemertinea	(23)	*Sigambra tentaculata*	(21)
Cossura delta	(23)	*Paraprionospio pinnata*	(19)
Sipuncula	(21)	*Corbula contracta*	(18)

SB 3 (40 max)		SB 4 (40 max)	
Sphaerosyllis cf. *sublaevis*	(39)	*Cossura delta*	(40)
Paleonotus heteroseta	(30)	Nemertinea	(26)
Sipuncula	(28)	*Sigambra tentaculata*	(20)
Nemertinea	(23)	*Pitar cordatus*	(18)
Pitar cordatus	(23)	*Paraonis* sp. A	(15)

HR 1 (50 max)		HR 2 (50 max)	
Sphaerosyllis cf. *sublaevis*	(49)	Nemertinea	(35)
Sipuncula	(42)	*Magelona longicornis*	(33)
Nemertinea	(39)	*Pitar cordatus*	(33)
Paleonotus heteroseta	(32)	*Cossura delta*	(31)
Laonice cirrata	(16)	*Paraonis* sp. A	(31)

HR 3 (40 max)		HR 4 (40 max)	
Rissoina cancellata	(34)	*Magelona longicornis*	(36)
Nephtys incisa	(27)	*Rissoina cancellata*	(31)
Nemertinea	(24)	Sipuncula	(26)
Abra aequalis	(15)	*Thyasira* sp.	(17)
Magelona longicornis	(12)	*Cossura delta*	(15)

*Number of collections times 10.

TABLE 7

MEANS OF COMMUNITY STRUCTURE PARAMETERS
FROM THE EIGHT TOPOGRAPHIC FEATURES STATIONS
ARRANGED ACCORDING TO PROPOSED STATION GROUPINGS.

		SPECIES	IND.	H"	P.I.E.	E	SYLLIDS	SPIONIDS
A	SB 3	158	1315.75	5.4074	0.9431	0.4153	11.0	10.75
	HR 1	159	1264.4	5.4103	0.9402	0.4170	12.6	10.60
B1	SB 2	37.6	101.4	4.5295	0.9403	0.9500	0.0	2.4
	SB 4	52.0	117.5	5.1980	0.9693	0.9955	0.0	3.0
	HR 2	38.4	129.60	4.4621	0.9353	0.8940	0.4	2.0
B2	SB 1	53.20	178.20	4.7888	0.9211	0.8247	0.8	3.8
	HR 3	50.75	155.25	5.0530	0.9629	0.9659	0.25	3.75
	HR 4	53.60	164.75	4.9291	0.9506	0.8988	0.5	2.25

HR = Hospital Rock
SB = Southern Bank

including *Paleanotus heteroseta* and *Laonice cirrata*, were highly characteristic of Group-A stations. Community structure at these stations had much less variability in the dominance pattern than at Group-B stations. Only two species (*S.* cf. *sublaevis* and *Pitar cordatus*) occupied the top ranking position at SB 3, with the former being the numerical dominant in three of four collections. Essentially the same dominance pattern was observed at HR 1 with *S.* cf. *sublaevis* numerically dominant in four of five collections and the taxa Sipuncula the top numerical dominant in the November collection.

Group-B stations were characterized by having far fewer species and individuals than Group-A stations. Basically these stations were characterized by species considered to be ubiquitous to the STOCS area. However, differences in dominant species indicated a separation of Group-B into Groups B1 and B2. Group B1 included Stations SB 2, SB 4 and HR 2. Group B1 was characterized by a loose pattern of dominance in that different species were dominant with each collection. The majority of the dominant species were found in the ubiquitous group of the STOCS study (Table 2). Dominant species at the B1 stations included the polychaetes *Cossura delta*, *Sigambra tentaculata*, *Paraprionospio pinnata*, *Paraonia* sp. A and *Magelona longicornis* and the molluscs *Pitar cordatus* and *Corbula contracta* (Table 7). Nemertinea and Sipuncula were also found among the dominants of Group B1. Group B2, including Stations SB 1, HR 3 and HR 4, shared many of the ubiquitous species with Group B1 but had decidedly different dominant species. Station SB 1 was characterized by having large populations of the amphipod *Heterophoxus* cf. *oculatus* and to a lesser extent, the isopod *Gnathia* sp. While these two species were found sporadically in small numbers at other stations in the Topographic Features Study, they never reached the dominance

level apparent at SB 1. Stations HR 3 and HR 4 had several molluscan species that were characteristic of the two stations. The gastropod *Rissoina cancellata* was a predominant species at these two stations but was found only sporadically at other Topographic Features stations. The pelecypods *Abra aequalis* and *Thyasira* sp. showed limited dominance at HR 3 and HR 4, respectively. Other dominent organisms at Group B2 stations included the ubiquitous polychaetes *Cossura delta*, *Magelona longicornis* and *Nephtys incisa* which were shared with Group B1 stations.

DISCUSSION

Epifauna

Northern Gulf of Mexico epifauna is considered by many investigators as an extension of the Carolinian province with faunal divisions at the Mexican border and just east of the Mississippi delta (Hedgpeth, 1953; Defenbaugh, 1976). The STOCS study area falls within Defenbaugh's Texas to Mississippi delta region, but by virtue of the southernmost stations, is influenced by Caribbean fauna of the Mexican coast.

Distribution of any species is based on a complex of environmental factors. Temperature and salinity control the range of benthic species, but within that range, more subtle factors determine faunal distribution. Depth was the most apparent factor controlling epifaunal distribution in this study. Defenbaugh (1976) found depth to be of major importance in his work and suggested that distance from shore, or pressure restrictions, could be involved. The pattern of station-groups based on depth, or some factor associated with depth, was remarkably constant throughout the year in the study.

The inner-shelf, including both the very shallow and shallow-intermediate stations, had large numbers of individuals and low equitability and species diversity. Many of the species had their widest distribution

in winter, occurred in large numbers in spring (May-June), and by fall (September-October), were limited to only a few stations. Many of the species most characteristic of the shallow shelf are motile decapods found in inlets, bays and shoal areas in summer and early fall. Copeland (1965) collected large numbers of *Trachypenaeus similis* and *Squilla empusa* in Aransas Pass Inlet in late summer and early fall. Large numbers of *Penaeus setiferus* are found in the bays in fall and support a sizable bay fishery. Seasonal changes in population may be related to the annual temperature (14-29°C in 1976) and salinity (31-36 ppt in 1976) extremes at inner-shelf stations.

Large numbers of species with low abundance characterize the outer-shelf assemblage. High equitability and species richness of this area reflect the relatively stable environmental conditions characteristic of the area.

Cluster analysis of seasonal demersal fishes data delineated shallow and deep-station groups. Intermediate depth stations occurred as sub-groups in one or both of the major divisions. The diversity of demersal fishes was low at shallow stations and increased with depth to about 85 m. As with the epifauna, seasonal changes in fish populations appeared to be related to depth, temperature, and movements into and out of estuaries.

Infauna

Hartman (1951) characterized the littoral polychaete communities of the Gulf of Mexico as little known, diverse and with components of the Caribbean, Eastern Pacific and Atlantic faunas. Parker (1960) recognized inner-shelf, intermediate-shelf and outer-shelf faunal assemblages in the Gulf. He concluded that distribution of these assemblages followed temperature ranges, and within these, separated according to major sediment type. Hill (1975) presented the only other study which attempted

to define benthic infaunal assemblages in the northwestern Gulf. He identified five zones which he generally correlated with depth and sediment type.

Zonation of infaunal assemblages with depth on continental shelves is a commonly reported pattern (Day *et al.*, 1971; Field, 1971). This study showed the same general pattern of distribution by depth, modified by the influence of sandy sediments. There were apparently three habitat types in the STOCS study area: shallow-muddy sand, deep-silty clay and deep-muddy sand. The mid-depth station group, which showed a transition from shallow-sandy sediments to deep-silty clays, may be considered a depauperate extension of the shallow faunal assemblage. This region, which comprises a large part of the study area, had no unique species but shared many species with the shallow stations, and ubiquitous species with deep and shallow stations. The shallow-muddy sand and deep-muddy sand stations were similar in that they shared many species and both were rich in number of species and individuals. Despite these similarities, the deep-muddy sand stations were grouped by cluster analysis with deep stations, implying that depth or the stable physical environment associated with depth was more important than substrate in regulating infaunal distribution.

Seasonal changes in temperature and salinity, and turbulence due to wave action in the shallow muddy-sand habitat, make it a highly variable environment. The shallow infaunal assemblage was rich in number of species and individuals but numerical dominance by some species resulted in low equitability. The deep-silty clay habitat was a relatively stable environment, little affected by seasonal temperature and salinity changes but possibly influenced by deep Gulf water. The deep benthic assemblage was characterized by high diversity and equitability with fewer species

than shallow stations and with little or no numerical dominance. The deep-sandy habitat had an environmental regime similar to that of the deep-silty clay habitat but had more species and individuals. The high equitability at deep-sandy, as compared to shallow-sandy stations, can be attributed to a more stable environment at the deep stations.

Attempts have been made to delineate faunal assemblages based on feeding types (Sanders, 1968; Young and Rhodes, 1971). In the STOCS study area, sedentary deposit feeders comprised the bulk of polychaete populations inshore, but were generally replaced by actively burrowing detrital feeders offshore. High productivity and water turbulence in nearshore areas allowed sedentary deposit feeders to be successful. As distance from shore increased, there was a reduction of food in the water column and at the substrate surface. Species which actively burrow through sediments in search of food dominated deep station communities. This type of analysis suffers from lack of life history information for many species and families. This is particularly true for macroinfauna found in the northwestern Gulf of Mexico.

Both macroinfauna and invertebrate epifauna had similar patterns of distribution by depth. A major difference between the two groups was a generally greater number of epifaunal species offshore but a greater number of infaunal species inshore. Highly variable physical conditions at shallow stations produced populations characterized by numerical dominance of some species. Large numbers of infaunal species in both shallow and deep muddy-sand habitats are due to the increased "spatial diversity" (Pianka, 1966) afforded by the greater interstitial space in muddy sands as compared to silty clays. A rich food supply both in the water column (due to the influx of continental runoff on the inner shelf)

and in the substrate allows greater abundance in the shallow muddy-sand habitat.

The STOCS shallow-muddy sand assemblage appeared to fit Sanders' (1968) physically-controlled community which is governed mainly by physical conditions which vary widely. Hill (1975) proposed that the entire South Texas Continental Shelf is a physically controlled environment. However, observations of high equitability and diversity of the outer-shelf assemblage, coupled with the stable environmental conditions there, lead us to believe that the benthic fauna of the deeper water may represent biologically-accommodated communities.

Within these broad habitat zones, niche diversity may be important in the small-scale distribution of individual species.

The use of P_k analysis to understand the distribution of benthic infaunal macroinvertebrates and how different sampling replication schemes may be sampling the various communities, must be preceded by an understanding of some basic limitations of the technique. In essence, the P_k analysis, as described by Gaufin *et al.* (1956), was used to compare the relative sampling efficiency of different types of bottom samplers in a stream bed.

A large suite of samples (12 in the present study) was taken. The total number of species in the suite and the number of species at each replication level (one sample, two samples, etc.) were tabulated. Using this information, the P_k technique was used to calculate the number of species (percentage of the total found in the original suite) not found previously that would be expected in each replicate. By accumulating these percentages and plotting against replicate number, a curve representing sampling efficiency at each replication number was constructed. Several assumptions are made in the use of the P_k. The first assumption

is that the total number of species in the original suite of samples is the total number of species in the area. This, in some cases, may approach reality, in other cases, it does not. With the benthic macroinvertebrates of the STOCS study area, the latter is probably true. Although 12 samples were taken at each station, in the original comparisons the total number of species actually in the area was not closely approximated. Thus, the P_k analysis was used with the realization that the total number of species was low, and consequently the percentage of the total community sampled by a given replicate number was an over-estimate. The justification for using this technique is based on the fact that those species not found in 12 replicate samples would be extremely rare and that by modifying the technique, *i.e.*, not using those species operationally defined as rare, a comparison of the distribution of the non-rare species at various stations can be made. Also, the efficiency of replicate sampling within a site can be estimated. It is readily apparent that the species populations of infaunal macroinvertebrates are physically much more interrelated in the nearshore Gulf areas than at the deeper stations. With similar numbers of species in the original suite, both rare and non-rare species are more efficiently sampled at nearshore stations by a given number of replicates than at the offshore stations. This apparently is a function of the numbers of individuals present with the inshore stations being more densely populated. Thus, a hypothesis for a changing scale of macroinfaunal community distribution pattern can be postulated. The inshore communities are distributed on a smaller scale, *i.e.*, the same sampling effort will obtain a greater percentage of the community than for offshore communities. This is probably best explained by the greater food available as evidenced by the phytoplankton and zooplankton populations present; greater phys-

ical variability in the sediment grain sizes allows for greater density on a physical basis and, the possibility of niche fractionization, particularly with reference to trophic type, is affecting the distribution scale in the two areas. That is, benthic species dependent on the water column for food (filter feeders, suspension feeders) may require less bottom surface space per individual than deposit feeders, whether selective or non-selective. Selective sedentary deposit feeders similarly would require less space than active burrowing, non-selective types.

The second major analytical technique used in within-station distribution analysis was the index of dispersion $(\frac{S^2}{\bar{X}})$ which led to similar conclusions. This technique, as used by Gage and Geekie (1973), is basically a sample statistic to infer community traits. They found that a basic difference existed in the relative amounts of population aggregation between muddy-sand areas and silty-clay areas. Our results on both the initial suites of 12 samples from Transect II and subsequent analyses involving the normal suites of six samples from all winter collections followed a similar pattern. The inshore stations, particularly very sandy stations (4/I, 4/III and 4/IV), showed more tendency toward aggregation of various populations comprising the community than did deeper, more silty-clay stations. Again, as noted by Gage and Geekie (1973), several considerations must be noted in the interpretation of this analysis. There is a decided tendency for the index of dispersion $(\frac{S^2}{\bar{X}})$ to show significant aggregation in species where it may not be occurring. This is particularly true in species with high mean numbers per sample. The variance, as noted by Gage and Geekie of species distribution, tends to increase at a more rapid rate than the mean, although with a true Poisson distribution the two should change together. This factor

among others tends to show a type I error, predicting significance when it actually does not occur. Even though this bias exists, the relative numbers of populations aggregating are thought to be indicative of the true situation. A further test using Jumars Chi-Square dispersion technique further corroborated the aggregation of species at the Transect II stations.

Topographic Features Stations

The Topographic Features (Hospital Rock and Southern Bank) on the STOCS had some effects on the level-bottom infaunal invertebrate communities surrounding them. These effects became apparent through an adjunct study of the areas surrounding certain topographic features of the BLM-STOCS study area. This study was carried out under a separate contract (AA550-CT7-15) but will be reported herein. The major factor affecting these communities was the amount of gravel-sized rubble apparently washed from the banks by prevailing currents or storms. Two stations, of the eight sampled, had significant amounts of shell rubble in the sediments. These stations (HR 1 and SB 3) were located on the southwestern side of Hospital Rock and Southern Bank, respectively. The benthic invertebrate communities at these two stations were similar to each other but different from the other six topographic features stations and from most STOCS primary transect stations of similar depth. These communities were different in species composition and most community structure parameters. They were much more diverse, had far greater numbers of individuals and species, and a very stable pattern of dominance.

Although the macroinfauna of the banks proper was not sampled, it is assumed that the "shelly" stations had a fauna that was probably similar to that of the slopes of the banks. Generally, the dominant species at the shelly stations were filter feeders, or errant, probably predacious species. When the macroinfauna data were compared to the

meiofauna data from other investigators in the Topographic Features study, very low macroinfauna to meiofauna ratios, ranging from one to one-half to around one to ten, were found. One collection showed a ratio of one to forty-five but the meiofauna from that station had an abnormally high number of nematodes. These low ratios probably precluded the use of meiofauna as food items for the majority of the macroinfauna.

The general explanation of the high macroinfaunal diversity of shelly areas such as HR 1 and SB 3 is the physical space provided by the greater interstitial areas among the shell rubble as compared to those of silty-clay dominated sediments of the surrounding Gulf bottom. Also, the gravel-sized fragments provide substrate for those species that require a firm surface for settling on or boring into.

The remaining six stations were basically similar in species composition and community structure. The various community structure parameters (H', P.I.E., E, number of species and number of individuals) were similar to those found at the STOCS primary transect stations of similar depth. Macroinfauna/meiofauna ratios were much higher than at the shelly stations. These stations were separated into two groups of three [SB 2, SB 4 and HR 2 (B1), and SB 1, HR 3, HR 4 (B2)] on the basis of different numerical dominants. The dominant species at the non-shelly stations varied greatly through time (with the exception of SB 4 where *Cossura delta* was consistently numerically dominant) so that the dominance index indicated a much less consistent pattern of dominance than at the shelly stations. The dominant species at the B1 station group were comprised of the more ubiquitous species found in the STOCS area and the general community structure indicated no significant difference from that expected of normal soft-bottom communities of the Gulf. The other group of three

stations (B2) shared many of the species, but differed in having unusual numerically dominant species including *Heterophoxus* cf. *oculatus* at SB 1 and *Rhepoxynius epistomus* at HR 3 and HR 4. These species were found elsewhere in the study area but not with the same consistency or numbers.

Since sediment type, *e.g.* gravel, obviously affected the differentiation between the A and B stations, the sedimentary differences among the B group stations was examined to see if the different dominants could further be explained by sediment particle size. Three stations (SB 2, HR 3, and HR 4) had sediments consisting entirely of silt and clay. The remaining three had varying percentages of sand (usually less than 20%). Since sediment patterns (all silt/clay vs silt/clay with some sand) cut across the biological groupings, no obvious sediment effects were observed. Correlation coefficients (Pearson r) were computed for number of species and number of individuals with percent sand, silt and clay for stations within each sediment group. No significant patterns of correlation were evident.

Distance and direction from the Topographic Features were examined as possible factors in the dominance patterns of the B2 stations. There is a possibility that the strictly silt/clay stations are associated with troughs around the Topographic Features. These troughs (areas that are deeper than the normal bottom surrounding the topographic features) are readily seen in bathymetric maps prepared by the Texas A&M project studying the biology and geology of the STOCS topographic features (Bright and Rezak, 1976). While this may explain the lack of sand at these stations, it does not fit the biological pattern. No effect of distance or direction could be ascertained which corresponded to the biological data.

The data indicate that two of the eight stations are directly affected by the topographic highs, primarily as a result of the gravel-sized debris found on the southwest side of the banks. The other six stations are divided into two groups, one, in which no effect of the banks was

seen, and one which had some differences in dominant species which could not be definitely attributed to the presence of the banks.

CONCLUSIONS

1. A total of 887 taxa were identified from trawl and grab collections in the STOCS study area in 1976.

2. *Trachypenaeus similis*, *Sicyonia dorsalis*, *Callinectes similis* and *Penaeus aztecus* were the four most abundant invertebrate epifaunal species collected.

3. The polychaetes *Magelona phyllisae*, *Paraprionospio pinnata* and *Mediomastus californiensis* dominated macroinfaunal collections.

4. Invertebrate epifaunal distributions indicated an inner-shelf (10-47 m) and an outer-shelf (65-134 m) assemblage in the STOCS study area.

5. The invertebrate epifaunal assemblage of the inner-shelf was characterized by large numbers of individuals, low diversity and low equitability. Typical inner-shelf species, such as *Trachypenaeus similis*, *Squilla empusa* and *Penaeus setiferus* have strong affinities for estuaries and showed seasonal variability.

6. The outer-shelf invertebrate epifaunal assemblage was characterized by temporal stability, high diversity and high equitability. Typical species included the mollusc *Amusium papyraceus*, the decapod *Anasimus latus* and the echinoderm *Astropecten cingulatus*.

7. Macroinfaunal distribution data indicated three habitat-defined assemblages: shallow-muddy-sand (10-27 m), deep silty-clay (65-134 m) and deep muddy-sand (65 and 91 m). Intermediate depth stations were transitional in sediment and infaunal species composition.

8. Macroinfaunal equitability values showed a general increase with depth but total number of species and individuals decreased with depth.

9. Macroinfauna were most abundant along the inner-shelf and in the deep muddy-sand habitats due to increased "spatial diversity" afforded by greater interstitial space in muddy sands as compared to silty clays.

10. The major difference between macroinfauna and invertebrate epifauna was a generally greater number of invertebrate epifaunal species offshore, but a greater number of macroinfaunal species inshore.

11. Populations comprising the macroinfaunal communities showed more tendency toward aggregation at the shallow muddy-sand stations than at deeper silty-clay stations.

LITERATURE CITED

Alley, W. P., and R. F. Anderson. 1968. Small-scale patterns of spatial distribution of the Lake Michigan macrobenthos. Proc. 11th Conf. Great Lakes Res.:1-10.

Anderberg, M. R. 1973. Cluster analysis for applications. Academic Press, New York. 359 pp.

Boesch, D. F. 1973. Classification and community structure of macrobenthos in the Hampton Roads area, Virginia. Mar. Biol. 21:226-244.

Bright, T. J., and R. Rezak. 1976. A biological and geological reconnaissance of selected topographic features on the Texas continental shelf. A final report to the U.S. Dept. of the Interior, Bureau of Land Management Outer continental shelf office, New Orleans, Louisiana. Contract No. AA550-CT5-4. Texas A&M University Research Foundation and TAMU Dept. of Oceanography.

Clifford, H. T., and W. Stephenson. 1975. An introduction to numerical classification. Academic Press, New York. 229 pp.

Compton, H. 1965. A study of Texas shrimp populations; biological survey of the commercial shrimp and associated organisms in the inshore Gulf of Mexico. Tex. Parks Wildl. Dept., Coastal Fish Proj. Rept. 1964, Proj. No. MS-R-6:145-147.

_____, and E. Bradley. 1964. A study of Texas shrimp populations; biological survey of the commercial shrimp and associated organisms of area 20 in the Gulf of Mexico. Tex. Parks Wildl. Dept., Coastal Fish Proj. Rept. 1963. Proj. No. MS-R-5:143-162.

Copeland, B. J. 1965. Fauna of the Aransas Pass Inlet, Texas. I. Emigration as shown by tide trap collections. Publs. Inst. Mar. Sci. Univ. Tex. 10:9-21.

Day, J. S., J. G. Field, and M. Montgomery. 1971. Use of numerical methods to determine the distribution of benthic fauna across the continental shelf of North Carolina. J. Animal Ecol. 40:93-126.

Defenbaugh, R. E. 1976. A study of the benthic macroinvertebrates of the continental shelf of the northern Gulf of Mexico. Ph.D. Dissertation, Texas A&M Univ. 476 pp.

Eagle, R. A. 1975. Natural fluctuations in a soft bottom benthic community. J. mar. biol. Ass. U. K. 55:865-878.

Felder, D. L. 1973. An annotated key to crabs and lobsters (Decapoda, Reptantia) from coastal waters of the northwestern Gulf of Mexico. Center for Wetland Resources, Louisiana St. Univ. Publ. No. LSU-SG-73-02. 103 pp.

Field, J. G. 1971. A numerical analysis of the changes in the soft bottom fauna along a transect across False Bay, South Africa. J. Exp. Mar. Biol. Ecol. 7:215-253.

_____, and G. McFarlane. 1968. Numerical methods in marine ecology. I. A quantitative "similarity" analysis of rock shore samples in False Bay, South Africa. Zool. Afr. 3:119-137.

Gage, J., and A. D. Geekie. 1973. Community structure of the benthos in Scottish sea-lochs. II. Spatial pattern. Mar. Biol. 19:41-53.

Gaufin, A. R., E. K. Harris, and H. J. Walter. 1956. A statistical evaluation of stream bottom sampling data obtained from three standard samplers. Ecology 37:643-648.

Hairston, N. G. 1964. Studies on the organization of animal communities. Jubilee Symposium Supplement, J. Ecol. 52:229-239.

Harper, D. E. Jr. 1970. Ecological studies of selected level-bottom macroinvertebrates off Galveston, Texas. Ph.D. Dissertation, Texas A&M Univ. 70 pp.

Hartman, O. 1951. The littoral marine annelids of the Gulf of Mexico. Publs. Inst. Mar. Sci. Univ. Tex. 2:7-124.

Hedgpeth, J. W. 1953. An introduction to the zoogeography of the northwestern Gulf of Mexico with reference to the invertebrate fauna. Publs. Inst. Mar. Sci. Univ. Tex. 3:107-224.

Hildebrand, H. H. 1954 . A study of the brown shrimp (*Penaeus aztecus* Ives) grounds in the western Gulf of Mexico. Publs. Inst. Mar. Sci. Univ. Tex. 3:233-366.

_____. 1955. A study of the fauna of the pink shrimp (*Penaeus duorarum* Burkenroad) grounds in the Gulf of Campeche. Publs. Inst. Mar. Sci. Univ. Tex. 4:167-232.

Hill, G. 1975. Animal sediment relationships. Pages 133-187 *in* Environmental assessment of the south Texas outer continental shelf-Geologic investigations. U.S.G.S. report to Bureau of Land Management Contract No. 08550-MU5-20.

Holland, J. S. Jr., N. J. Maciolek, and C. H. Oppenheimer. 1973. Galveston Bay benthic community structure as an indicator of water quality. Contr. Mar. Sci. 17:169-188.

Hulings, N. C. 1955. An investigation of the benthic invertebrate fauna from the shallow waters of the Texas coast. Masters Thesis, Tex. Christian Univ. 88 pp.

Hurlbert, S. H. 1971. The non-concept of species diversity: A critique and alternative parameters. Ecology 52:557-586.

Jumars, P. A. 1975. Methods for measurement of community structure in deep-sea macrobenthos. Mar. Biol. 30:245-252.

Kennedy, E. A. 1959. A comparison of the molluscan fauna along a transect extending from the shoreline to a point near the edge of the Texas coast. Masters Thesis, Tex. Christian Univ. 136 pp.

Kikkawa, J. 1968. Ecological associations of bird species and habitat in eastern Australia: Similarity analysis. J. Anim. Ecol. 37:143-165.

Kosler, A. 1968. Distributional patterns of the eulittoral fauna near the Isle of Hiddensee (Baltic Sea, Rugia). Mar. Biol. 1:266-268.

Lance, G. N., and W. T. Williams. 1967. A general theory of classificatory sorting strategies. I. Hierarchical systems. Comput. J. 9: 373-380.

Lloyd, M., and R. J. Ghelardi. 1964. A table for calculating the "equitability" component of species diversity. J. Anim. Ecol. 33:217-225.

McIntosh, R. P. 1967. An index of diversity and the relation of certain concepts to diversity. Ecology 48:392-404.

Noy-Meir, I. 1973. Data transformations in ecological ordination. I. Some advantages of non-centering. J. Ecol. 61:329-341.

Parker, R. H. 1960. Ecology and distributional patterns of marine macroinvertebrates, northern Gulf of Mexico. Pages 302-337 *in* F. P. Shepard, F. B. Phleger, and T. H. van Andel (eds.), Recent sediments, northwest Gulf of Mexico. Amer. Assoc. Petrol. Geol., Tulsa, Okla.

Pequegnat, W. E., R. M. Darnell, B. M. James, E. A. Kennedy, L. H. Pequegnat, and J. T. Turner. 1976. Ecological aspects of the upper continental slope of the Gulf of Mexico. Final report to U. S. Dept. Interior, Bur. Land Mgmt., Div. Min. Env. Ass., Contract No. 08550-CT4-12. 360 pp.

Pianka, E. R. 1966. Latitudinal gradients in species diversity: A review of concepts. Am. Nat. 100:33-46.

Pielou, E. C. 1966. Shannon's formula as a measure of specific diversity: Its use and misuse. Am. Nat. 100:463-465.

_____. 1969. An introduction to mathematical ecology. Wiley Interscience, New York. 286 pp.

Prichard, N. M., and A. J. B. Anderson. 1971. Observations on the use of cluster analysis in botany with ecological examples. J. Ecol. 59:727-747.

Rosenberg, R. 1974. Spatial dispersion of an estuarine benthic faunal community. J. Exp. Mar. Biol. Ecol. 15:69-80.

Rowe, G. T. 1966. A study of the deep water benthos of the northwestern Gulf of Mexico. Masters Thesis, Texas A&M Univ. 89 pp.

_____, P. T. Polloni, and S. G. Horner. 1974. Benthic biomass estimates from the northwestern Atlantic Ocean and the northern Gulf of Mexico. Deep-Sea Research 21:641-650.

Sanders, H. L. 1968. Benthic community diversity: A comparative study. Amer. Nat. 102:243-283.

Smith, W., and A. D. McIntyre. 1954. A spring-loaded bottom sampler. J. mar. biol. Ass. U. K. 33:257-264.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco, Calif. 573 pp.

Springer, S. 1951. The OREGON's fishery exploration in the Gulf of Mexico. Comm. Fish. Rev. 13:1-8.

_____, and H. R. Bullis. 1956. Collections for the OREGON in the Gulf of Mexico; list of crustaceans, mollusks, and fishes identified from collections made by the exploratory fishing vessel OREGON in the Gulf of Mexico and adjacent seas 1950 through 1955. U. S. Fish Wildl. Serv., Spec. Sci. Rept. Fish. No. 196. 134 pp.

Stephenson, W. 1972. The use of computers in classifying marine bottom communities. Ecol. Monogr. 42:387-415.

_____, and W. T. Williams. 1971. A study of the benthos of soft bottoms, Sek Harbour, New Guinea, using numerical analysis. Aust. J. Mar. Freshwat. Res. 22:11-34.

_____, and G. N. Lance. 1970. The macrobenthos of Moreton Bay. Ecol. Monogr. 40:459-494.

_____, and S. Cook. 1972. Computer analysis of Petersen's original data on bottom communities. Ecol. Monogr. 42:387-415.

Young, D. K., and D. C. Rhoads. 1971. Animal sediment relations in Cape Cod, Massachusetts. I. A transect study. Mar. Biol. 11:242-254.

CHAPTER TEN

DEMERSAL FISHES

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ABSTRACT

A series of standardized, trawled fish samples were collected from six stations on each of four transects to provide information on South Texas Outer Continental Shelf (STOCS) fish distribution and abundance. Day and night collections were made at each station during winter, late spring and late summer-autumn; supplementary day-night collections were made in intervening months at stations on Transect II.

For each sample, numbers of individual species, weights, numbers of individuals per species, and individual weights and lengths provided the basic data, all of which except lengths and weights provided primary data for this study. For each sample, calculations of Shannon diversity indices (both numerical and ponderal), the Hurlbert probability of interspecific encounter (P.I.E.) and the Lloyd and Ghelardi equitability value (E) provided derived data for various comparisons among the 1976 collections and between 1976 and the equivalent 1975 collections.

For equivalent times and stations, the 1976 numbers of species, individuals, and biomasses were less than in 1975. Whether the slight change in sampling nets caused the declines or whether there were actually fewer fish in 1976 could not be demonstrated with completely tenable explanations. (A proposed analysis of length-weight-frequency analysis of individual species should clarify the viability of alternative explanations.) In general, both numerical and ponderal diversity indices were lower in 1976. Isopleth plots for day-night and seasonal data pertinent to observed and derived data indicated that there were pronounced day-night differences in most cases throughout the year. In the winter and spring, gradients for the various data tended to be depth-related with some indication of north-south transect differentiation by autumn. Analysis of variance for the various data categories revealed that few individual effects (depths, transects, day-night, seasons) were consistently and statistically significant, but interactions involving seasons and individual effects were more so.

A pooled yearly comparison of day-night catches by species indicated that day species were ordinarily those that had schooling propensities; predominantly nocturnal species tended to be solitary. Comparisons by the Wilcoxon rank sum showed statistically significant diurnal prominence for 10 species and nocturnal prominence for 36. Of the fishes not showing significant day-night prevalence in numbers or weights for pooled data, a breakdown of catches into seasons yielded statistically significant maxima in day-night differences in the spring and minima in the autumn. From published data, the day-night differences were related to activity and aggregational associations, to food habits, and to feeding tactics.

The Bray-Curtis cluster analysis formulation was chosen over the Canberra-metric system for ascertaining station and species distributional characteristics. With species as attributes of the individual stations, the Bray-Curtis technique with flexible sorting by normal analysis showed clearly that there were depth related groupings, three in winter and four in spring and autumn. With stations as attributes of the species, inverse analysis showed seven species-groups in winter, eight in spring and six in autumn. The two-way relationships of station-groups and species-groups showed species-environmental relationships rather clearly for most of the

species, 67 of 96 in winter, 68 of 89 in spring and 62 of 82 in autumn.

In addition to species associations determined by clustering, the cluster analyses indicated that zonation was depth-related, with temperature and seasonal migration patterns as major associative features of the groups through the seasons. There was little evidence that zonation was directly related to sediment type or salinity. The shallowest station groupings had high numbers of individuals, especially in winter and spring, and generally lower species diversities through the year. When temperatures were highest in late summer and autumn, nearshore species associations tended to dissipate; midwater and deepwater associations were somewhat more stable throughout the year; midshelf groups had the highest species diversity throughout. There was a weak indication of species associations breaking into north-south groupings in autumn only, which implied that north or south movements to or from areas outside STOCS was relatively unimportant for the great majority of species. However, within the STOCS area there was considerable species "shuffling" during the year to the extent that clearcut species-domination by one or a few species was not suggested.

The 1975 and 1976 data, except for length-weight measurements of individual fish, are now incorporated into the data management system. Recommendations are suggested to: (a) further clarify relationships among the fish data *inter alia*, and (b) relate the benthic fish data to other abiotic and biotic (especially invertebrate forage organisms) data, and (c) to explore selected population processes identified by the above analyses. Also recommended are acquisitions of data on feeding growth-metabolism rate properties of individual species. These recommendations are based on the premise that such functional characteristics and processes provide baselines that are immediately much more sensitive to environmental perturbations, both natural and man-induced, than are the generalized attributive "inventories" of raw counts and biomasses, changes in which can accrue only as long-term results of such functional characteristics and processes at the species and population levels.

INTRODUCTION

The purpose of this study was the continuation of the development of a baseline pertinent to the abundance and distribution of benthic fishes of the South Texas Outer Continental Shelf (STOCS).

The major aims and rationale of the 1975 benthic fish sampling operations (Wohlschlag, 1976) continued during 1976. However, the 1976 sampling was expanded considerably to allow for more detailed analyses of the distribution and abundance of fishes, as well as for appropriate comparisons between years.

Analyses of the 1976 data proceeded along the following three lines:

1. Analysis of variance comparisons of bulk data from the separate trawl samples provided a system for overall evaluation of differences for various attributes among seasons, transects, stations (depths) and times of day, along with all possible interactions among these variables. The attributes included number of species, numbers of individuals, biomasses, numerical and ponderal Shannon diversity indices and Hurlbert's probability of interspecific encounter. By plotting the various fish distribution-abundance attributes as isopleths over the STOCS study area, visual comparisons can be made with other biological, chemical or geological conditions.

2. The difference between day and night collections during 1975 was pronounced only in winter and spring; collections in summer often did not show day-night differences among derived indices, although the individual species differences were pronounced between day and night. Accordingly, the 1976 data provided an abundant series of samples for day-night comparisons at the species level.

3. Attempts at "clustering" species groups were indicated by the

1975 study, both to evaluate characteristics of individual stations and species associations themselves. Particularly, it was deemed essential to evaluate seasonal changes in terms of collection localities and species affinities insofar as depths and north-south influences might be involved.

These three types of assessments, collectively, should be far superior in determining baselines for adjudicating any changes in overall long-term abundance and distribution of fishes. Additionally, these assessments in the various derived forms are compatible for analysis and synthesis operations with other biotic and abiotic segments of the STOCS study or with similar studies in other localities.

In a separate section of the report, recommendations are made for the synthesis of the three major components of the demersal fish studies and for the eventual interpretation of the fish distribution and abundance patterns in relation to their biotic and abiotic components.

General Distribution and Abundance

The purpose of the Results section entitled General Distribution and Abundances is to continue and expand an analysis of the type of data utilized in the 1975 study. Without regard to the nature of individual species, the comparisons on numbers, biomass, diversity indices, equitability, and probability of interspecific encounter were set up for comparisons of stations (depths), transects, seasons, day-night abundance and distributional changes, and for comparisons among these spatial and temporal characteristics. Preliminary comparisons of 1975 and 1976 collections were also desired.

The fishes of the STOCS study area currently being intensively studied are well-known taxonomically. Particularly notable are the studies of Gunter (1941; 1945; 1958). Many additional studies

have concerned the estuarine fishes and the fishes taken by the large-scale shrimp trawling industry of the area. There are numerous studies dealing with individual species of the STOCS study area. Among the studies dealing with the overall distribution and abundance characteristics of the area are those of Moore *et al.* (1970), Chittenden and Moore (1976) and Chittenden and McEachran (1976); these also contain fairly complete bibliographies for the northwestern portion of the Gulf of Mexico.

Because this study is a part of the overall BLM program to establish baselines, there is a necessity to present data in forms useful for both theoretical and practical purposes. Time-honored measures of abundance, along with the more recently derived indices that characterize distributions, are both of interest for data reduction and synthesis and for comparative purposes. The recent usages of diversity indices, presumably firmly based on information theory, have come to be criticized in terms of usage for assessments of species diversity, environmental stability, ecological optimization (evolution), community structure, etc. Hurlbert (1971) considers the notion of species diversity based on information theory a non-concept. Goodman (1975) summarizes much of the criticism of diveristy-stability relationships in ecology and concludes that there are no simple relationships. Attempts to define quantitatively the nature of community structure have continued to yield controversies and contradictions. Recent reviews on the subject, *e.g.* Caswell (1976), May (1975), among others, generally imply that community structure models themselves are deficient with regard not only to inclusion of all biotic and abiotic interactions, but to the degree of such interactions, as well. Because of these deficiencies and the reductionist approach of some

models, there is a tendency for intuitive interpretations, and sometimes for mathematical-statistical analyses of community information, to contradict the control-theoretic and cybernetic theories of diversity regulation (Caswell, 1976). Yet, the usage of diversity measurements, however empirical, has provided practical measures of community characterization, although subject to the constraints of sampling.

Day-Night Variability

Differences in species composition between day and night trawls are not necessarily reflected in measures of abundances, distribution or diversity calculated for each trawl sample from data for all species combined. However, experience with day and night trawled samples from this and earlier studies indicates that day-night differences in species composition do exist. The investigation of the diel differences in the trawl catches has become a master's thesis problem for Elizabeth F. Vetter and this portion of the report presents her preliminary findings.

While a large amount of information on distributional differences in trawled and seined fish from the northwest coastal and bay Gulf of Mexico waters (0-50 m) has been published, relatively little attention has been paid to diel variability in catch data for fish, and reports of diel variability have been largely incidental to distributional studies. A two-year sampling survey by Gunter (1945) covered the bay and inshore waters, but no mention was made of sampling times nor is any reference made to considerations of diel variability. Hildebrand's (1954) study distinguished two penaeid faunal areas on deeper areas of the continental shelf; the nearshore diurnal white shrimp grounds extending to depths of about 27 m, and the nocturnal brown shrimp grounds from 27 to at least 45 m. However, his survey was conducted aboard commercial shrimp trawlers and was subject

to the sampling strategies best suited for shrimp capture, *i.e.* nocturnal trawling on brown shrimp grounds, diurnal trawling on white shrimp grounds, so that strict day-night comparisons in each area were not feasible. Springer and Bullis (1956) and Moore *et al.* (1970) have reported distributional data for depths greater than 45 m, but again, diel differences in catch data received brief consideration. Chittenden and Moore (1976) and Chittenden and McEachran (1976) discussed in great detail the results of other collections, again, from a distributional rather than a diel viewpoint.

While previous studies in this area were not primarily concerned with day-night comparisons, in other areas of the world diel variations in benthic fish habits are well known and often of great importance to fisheries (DeGroot 1971; Stickney *et al.*, 1974; Robins, 1971; among others). Direct observations in natural habitats have revealed major diel changes in behavior of coral reef fishes (Hobson, 1965, 1968, 1972, 1973, 1974, 1975; Hobson and Chess, 1976; among others) and of fishes near oil rigs (Hastings *et al.*, 1976). The behavioral changes that occur daily along with variations in light intensity, turbidity and life cycle stages often result in reduced or enhanced vulnerability of fishes to trawling (Hoese *et al.*, 1968; Hobson and Chess, 1976). Thus, it would seem that diel variability in the occurrence of fishes taken by a given sampling method, such as trawling in this study, depends upon many factors that must include behavioral characteristics, plus a large number of other biotic and abiotic considerations.

Cluster Analysis

The nature of species associations is not clearly elucidated by analyses that utilize numbers, biomasses or diversity (and similar) indices

as shown for the 1975 data summary in the STOCS study area. As a consequence, the requirement for additional information on how the various stations and their species complements differed among themselves became a separate requirement for better analysis. The cluster analysis techniques have been accordingly applied for a master's thesis topic by Mr. James Cole whose thesis materials are applied to this report with slight modification.

It is well known that the benthic fauna changes with depth between the shore and the edge of the continental shelf, but beyond this there are many doubts and uncertainties (Day, Field and Montgomery, 1971; Field, 1971; Haedrich, Rowe and Polloni, 1975; Stephenson, Williams and Cook, 1972a; Boesch, 1973). It is not known how many faunistic zones are represented, at what depths the divisions occur or whether the changes are abrupt or gradual. Above all, the nature of the fauna is related in some way to the nature of the substrate and there is increasing evidence that the whole pattern of distribution on rocks differs from that on soft sediments (Day *et al.*, 1971).

The pioneering work of Petersen (1918) in Denmark on the shallow-water benthos of soft bottom involved quantitative data upon the numbers and weights of species present and resulted in descriptions of different benthic communities associated with various types of substratum, with each community named after, and characterized by, a few dominant species. As more work was done, Petersen-type bottom communities were recognized by almost all workers in the field. These findings led Thorson (1957) to present the concept of parallel soft-bottom communities. In the late 1950's and in the 1960's increasing numbers of investigations were conducted in warmer waters where some workers found Petersen-type communities, for example, in Southern California (Hartman and Barnard,

1958, 1960; Barnard and Ziesenhenne, 1961), West Africa (Longhurst, 1957, 1958; Buchanan, 1958), India (Seshappa, 1953) and Madagascar (Plante, 1967). Day (1963) challenged the concept of parallel soft-bottom communities and pointed out that in South African waters dredging yielded many different large species characteristic of different areas, many of which did not fit in with Thorson's communities, while grab samples yielded diverse assemblages containing large numbers of small animals belonging to many species and not dominated by just a few. Others also failed to find communities with a few dominant species, for example, in Southern California (Hartman, 1955), in the Gulf of Mexico (Parker, 1956, 1960) and off Thailand (Thorson, 1966). As Mills (1969) mentioned, these diverse assemblages resist classification by means of dominant species in the classical Petersen manner and methods of analysis are needed which can simultaneously take into account all or most of the species in comparing samples.

Plant ecologists have developed numerical clustering methods aimed at revealing structural and causal relationships in survey data and some of these methods have recently been used by marine ecologists. Lie and Kelley (1970) and Hughes and Thomas (1971) outlined the use of clustering methods in animal and marine ecology. Contemporary numerical classification techniques were used in studies of marine benthic communities by Stephenson, Williams and Lance (1970), Day, Field and Montgomery (1971), Field (1971), Stephenson, Williams and Cook (1972a), Boesch (1973), Haedrich, Rowe and Polloni (1975) and Holt (1976). Reviews and discussions of the various methods are given by Sneath and Sokal (1973), Clifford and Stephenson (1975) and Williams (1971).

Computer clustering methods similar to those used in the above-

mentioned marine benthic studies were employed to better understand the community structure of benthic ichthyofauna in the STOCS study area.

METHODS AND MATERIALS

General Collecting, Data Processing and Analysis

Except for the addition of the data to the computerized data system, the 1976 collections were made and processed quite similarly to the 1975 collections. The same four transects were sampled in 1976. All stations were sampled by day and night by 15-minute standardized trawling for both epibenthic macroinvertebrates and demersal fish.

The trawl was a conventional Gulf coast 10.7-m "flat trawl" with a 12.2-m lead line and a 9.1-m head line, each made of 12.7-mm steel impregnated rope. There was a 0.9-m separation between the net wings and the 0.76- by 1.52-m doors (otter boards) fitted with steel runners. Net materials were of white, untreated nylon twine. Wings and the main body of the net had 44.5-mm stretched mesh of No. 18 nylon twine. The 3.0-m bag was made of 44.5-mm No. 36 nylon stretched mesh. The bag liner used for the 1975 collections was not used in 1976. Conventional chafing gear surrounded the bag. All trawls were from the twin-screw R/V LONGHORN at 900 rpm which, with net drag, was equivalent to a dragging speed of approximately 2 knots.

For primary stations at all transects, winter collections (January-February), spring collections (May-June) and fall collections (September-October) were made in both day and night. Monthly collections were made only at primary stations of Transect II in March, April, July, August, November and December. Identifications of each fish, their numbers and weights were made shortly after the iced specimens were returned to

the laboratory. Information for each collection was incorporated into the computerized data base.

For all collections, the Shannon species diversity index was calculated. This index is widely used and also known as the Shannon-Wiener or Shannon-Weaver index and is described in Shannon (1948), Wiener (1948) and Shannon and Weaver (1963). Essentially the index H" is estimated as

$$H'' = -\Sigma (n_i/N) \log_e (n_i/N),$$

where n_i is the number of individuals of the i^{th} species and N is the total number of individuals. The H" values are in natural bels per individual, and in numerical form, indicated as H_n".

Likewise, for all samples, the ponderal equivalent, H_w", as suggested by Wilhm (1968), was calculated by using n_i as the weights (biomass in grams) of the i^{th} species and N as the weight of individuals in the sample.

Equitability was calculated for each of the individual collections by the use of tabulated values in Lloyd and Ghelardi (1964, Table 1).

Probability of interspecific encounter (P.I.E.) was also calculated for each station sample. This value may have merit from the standpoint that species diversity may be a non-concept in the sense of Hurlbert (1971). The value is calculated as:

$$P.I.E. = \left(\frac{N}{N-1}\right)\left(1 - \sum_{i=1}^{S} \pi_i^2\right),$$

where N_i = number of individuals of the i^{th} species in the collection;

$N = \Sigma_i N_i$ = the total number of individuals in the sample;

$\pi_i = N_i/N$, and;

S = number of species in the sample.

Because the 1975 collections differed from those of 1976 in respect to the elimination of the trawl bag liner in 1976, the total numbers of species, individuals weights and the various diversity values were compared for the two years. Also, the analyses of variance of these various numerical values were carried out for each year and both years were combined for all transects and Stations 1, 2 and 3, which were common to both years.

Standard statistical methods were utilized and the various transformations were used in accordance with standard practices, namely, square root of numbers of individuals, arcsin of the square root of equitability (E), arcsin of the square root of PIE and the logarithm of the weights. Since H_n" and H_w" are more or less normally distributed, they were not transformed. It should be noted that partitioning of the degrees of freedom without replicates could be handled in several different ways other than those tabulated under Results. Rather than separate yearly comparisons (with 1 degree of freedom) and consider the seasons as "seasons-within-years", it was decided that since seasons during 1976 were about one month later than during 1975, it would be feasible to consider all six seasons individually for a total of 5 degrees of freedom. The analysis was based on the principle of comparing mean squares to single factors (transects, stations depths, time of day, and season) with double interactions, to double interactions with triple interactions and to triple interactions with the quadruple interaction. Since the higher order interactions have relatively high mean squares, only a few of the single effects and several of the interactions are of interest, at least statistically.

Of special interest for visual comparisons were isopleths of the various measures of abundance and diversity, particularly with reference

to time of day and seasons. These were drawn for a visual means of comparing biotic and abiotic data from other BLM studies of the STOCS study area.

Day-Night Comparisons

To compare day-night sample pairs in terms of numbers or biomasses of the speices, the nonparametric Wilcoxon rank sum test was utilized. The test rationale is explained in Bradley (1968). Verdooren (1963) tabulated critical values for the rank sum test and the IMSL subroutine for performing the Wilcoxon Rank Sums Test was used to determine the statistical significance of differences between the day-night paired trawls. The use of ranks is a useful method for handling the non-normal distributions, particularly when one member of a day-night pair has no occurrence of a given species. The tests were used for comparisons at $P \leq 0.05$ for statistical "significance" and at $P \leq 0.20$ for consideration of possible differences. Confidence limits of 95 percent and 80 percent were also utilized in making some comparisons.

Comparisons were made for each species for both the number of individuals per trawl sample and the biomass of individuals per trawl sample. The data by species were pooled over the entire year for one set of analyses. For another set of analyses, the year was separated into the three seasonal collections with the monthly collections on Transect II between seasons combined to make a total of six periods for the purpose of assessing any seasonal diel changes in abundance; the periods were thus, winter (January-February); March plus April; spring (May-June); July plus August; fall (September-October); and November plus December.

Cluster Analysis

The methods of similarity analysis developed originally by plant

ecologists have had widespread use in many fields of ecology. They have the advantage of using all the species abundance information available and are free of distributional assumptions. Since there are several methods for use with any set of given data, discussion here will be limited to those methods found to be best-suited for this study.

Computations were done on an IBM 360 computer in the Texas A&M Data Processing Center using the program CLASS developed by Dr. Robert W. Smith, Department of Biology, University of Southern California. Analyses were performed for each of the three seasonal collection periods. For each set of data, day and night collections were combined to give a better idea of the total species at each station.

The first step in the analysis was computation of the dissimilarity between all possible pairs of collections or stations based on the species present. The resulting coefficients were tabulated in matrix form with one coefficient for every pair of entities to be classified. This measure of dissimilarity can be expressed as the ecological distance between pairs of entities and the matrix is commonly called the "distance matrix".

Two dissimilarity measures were used in the analyses. One is known as the Bray-Curtis measure and "is the complement of that given by Bray and Curtis (1957)" (Stephenson, 1972). If X_{1j} and X_{2j} are numbers of the j^{th} species at two sites, then the expression is:

$$\frac{\sum_1^n |X_{1j} - X_{2j}|}{\sum_1^n (X_{1j} + X_{2j})}$$

In this expression, $|X_{1j} - X_{2j}|$ indicates the absolute value of the difference. The denominator is the sum of the values for all species at

both stations. It is, therefore, strongly influenced by outstandingly large values and minimally influenced by small values. Transformation of the raw data (discussed later) minimized this problem; however, the Bray-Curtis coefficient is still sensitive to dominance. Joint absences have no effect on this coefficient, therefore it is suitable for analysis of heterogeneous sets of ecological data, *i.e.* where many species are absent from most of the samples (Day *et al.*, 1971). This coefficient has been used in recent marine studies by Stephenson, Williams and Lance (1970) and Stephenson and Williams (1971) and in a slightly different form by Field and McFarlane (1968), Field (1969, 1970, 1971) and Day, Field and Montgomery (1971) who refer to it as the Czekanowski coefficient.

The other dissimilarity measure is the Canberra-Metric which is:

$$\frac{1}{j} \sum_{1}^{j} \frac{|X_{1j} - X_{2j}|}{(X_{1j} + X_{2j})}$$

Since it is a mean of a series of fractions, an outstandingly large value only contributes to one of the fractions; however, it possesses a troublesome singularity at zero. If X_{1j} is zero, the contribution of that species takes its maximum possible value of 1, irrespective of the value X_{2j}. Therefore, differences of 0 and 1000 and of 0 and 1 carry the same weight, which does not make good ecological sense. It is usual to counter this by replacing X_{1j} with a value somewhat similar that the smallest value appearing in the data matrix (Stephenson *et al.*, 1972a). A good rule of thumb is to replace the zero values with a number 1/5 of the smallest value recorded (Stephenson *et al.*, 1972b).

From the distance matrices, individuals may be clustered into related groups. This can be done most effeciently by one of the agglomerative hierarchical clustering strategies (Williams, 1971) in which indivi-

duals are successfully clustered into groups which have the greatest inter-group affinities. Following Stephenson *et al.* (1972a), two different clustering strategies were used: "group average" [equivalent to the unweighted pair group method of Sneath and Sokal (1973)] and "flexible" (Lance and Williams, 1967). Comparisons of various sorting strategies are given by Field and McFarlane (1968), Stephenson, Williams and Cook (1972b) and Prichard and Anderson (1971).

Flexible sorting clusters intensely, giving sharp clusters and emphasizing weak boundaries, while group average sorting clusters only weakly and tends to give large groups of poorly differentiated stations or species. These two types of sorting strategies are termed space-dilating and space-conserving, respectively (Lance and Williams, 1967).

In group average sorting, the mean distance to a given site from every other site is computed and the site is fused to that group or individual site with the smallest mean distance. This strategy clusters only weakly and is little prone to misclassification (Stephenson *et al.*, 1972a). It has been used with success in marine benthic studies by Field and McFarlane (1968), Field (1971), Day, Field and Montgomery (1971) and Boesch (1973).

Flexible sorting is based on a generalized formula for hierarchical clustering methods given by Lance and Williams (1967). A good discussion of its properties is given by Sneath and Sokal (1973) who show the results of using various values of the cluster intensity coefficient (β). The now conventional value of β=-0.25 (Stephenson and Williams, 1971; Clifford and Stephenson, 1975) was used in this analysis. This strategy was used and discussed by Boesch (1973), Stephenson, Williams and Lance (1970), Stephenson and Williams (1971) and Stephenson, Williams and Cook (1972b).

After individuals are successfully grouped, their relationships are optimized in a dendrogram which Mayr, Linsley and Usinger (1953) define as ". . . a diagramatic illustration of relationships based on degree of similarity . . . ". It is a rooted tree diagram with the branches representing individual stations. The successive nodes represent the clusters resulting from fusion of prior clusters and the interval between nodes represents the degree of dissimilarity or the relative difference between successive groupings.

Thus far, the analysis used stations as individuals and species as attributes to produce "station groups". This is known as "normal analysis". It is also desirable to classify the species in terms of the stations at which they occur. This is known as "inverse analysis" and the stations become the attributes of the species.

The final step is to present the results of both normal and inverse analysis as a full two-way coincidence table. This is the original matrix resorted into groups as indicated by the classification. Use of the two-way table is desirable for two reasons: first, to make decisions on where to subdivide ill-defined clusters; and second, the location of "misclassifications". These can occur in agglomerative programs because fusions begin where group affinities are weakest (Clifford and Stephenson, 1975). Careful examination of the table will allow these "misclassifications" to be reallocated to a more suitable group.

Reduction, Transformation and Standardization of the Data

There are two reasons why data reduction is desirable in this study. The first is to reduce the computation time, and hence, the resultant expense. The second is that if data show little or nothing of biological meaning there is no point in including them. For this study, those

species which were represented by less than three individuals during a seasonal period were eliminated from the data. For further rationale see Clifford and Stephenson (1975), Stephenson *et al.* (1970), Field (1971), Stephenson *et al.* (1972a) and Day *et al.* (1971). The resultant reduction of species was from 96 to 67 in the winter, from 89 to 68 in the spring and from 82 to 62 in the fall.

Since the Bray-Curtis coefficient is sensitive to dominance, with high values overly influencing the measure, the raw data were transformed using square roots. This reduced the largest numbers in the data set to about 50.

Prior to inverse classification, species abundance was standardized by norm ($\sqrt{\sum (n)^2}$) to emphasize the relative differences of each species at each station rather than the magnitude of the differences between species (Noy-Meir, 1973).

Interpretation of Analysis

Constancy and fidelity are good measures of species (or species-groups) and station-group associations. These concepts have been extensively discussed by Stephenson *et al.* (1970), Stephenson *et al.* (1972a) and Stephenson (1972). Constancy is a measure of the extent to which a given species may be expected to occur in similar stations, and fidelity measures the extent to which a species is confined to a set of stations. Constancy is defined as the number of stations in a site-group in which a given species occurs, expressed as a percentage of the total number of stations available. Thus, a species is highly constant if found at all stations within a site-group, although it need not be restricted to only one site-group. The ratio of the frequency of occurrence of a species within a site-group to the overall frequency of occurrence in the whole

experimental area, again expressed as a percentage, is an adequate measure of fidelity. A species is highly faithful if it occurs in only one site-group, although it need not occur at every station within that site-group. A species which showed only one occurrence within the whole experimental area was not considered to be a faithful species to the site-group in which it occurred. It was felt that two or more occurrences within a site-group were necessary before a species could be considered as possessing the quality of fidelity.

Stephenson's (1972) method included the use of four reference grades for both constancy and fidelity: very high (VH) 95-100%, high (H) 66-94%, medium (M) 65-33%, and low (L) 32-0%. For this analysis, the grades of VH and H were used, since percentages less than 66% for some of the smaller site-groups (four stations) were rather meaningless.

RESULTS

General Distribution and Abundance

Although nearly four times as many samples were taken in 1976 as in 1975, only 131 species were taken in 1976 as compared to 117 species in 1975. Over both years a total of 150 species was taken. Many of these species were single occurrences. Table 1, Appendix H contains a list of all trawls taken during 1976 and the species composition of the trawls, the number of individuals of each species, and the total weight of all individuals of each species. Table 2, Appendix H, contains a list of species by order and family for 1975 and 1976, along with total numbers of individuals and weights in grams.

The collecting stations were divided into three station groups, based on depth (see Table 1). Previous workers (Chittenden and Moore, 1976; Chittenden and McEachran, 1976) have recognized changes with depth

TABLE 1

DIVISION OF COLLECTING STATIONS INTO STATION GROUPS
BASED ON DEPTH
(ROMAN NUMERALS INDICATE TRANSECTS)

	Stations			
Station Group 1	4/I	1/II	4/III	4/IV
(≤ 30 meters)	1/I		1/III	1/IV
Station Group 2	2/I	4/II	5/III	5/IV
(31-90 meters)	5/I	2/II	2/III	2/IV
		5/II		6/IV
Station Group 3	3/I	3/II	3/III	3/IV
(≥ 91 meters)	6/I	6/II	6/III	7/IV

in the ichthyofauna of the continental shelf in the Gulf of Mexico. In addition, our own analysis of the data using clustering techniques (presented later in this report) justify this division of stations with respect to depth.

The depths delimiting station groups were arbitrarily chosen, although the 30 m boundary between station groups 1 and 2 corresponds roughly to the transition zone between the so-called white shrimp and brown shrimp grounds (Chittenden and McEachran, 1976).

Summary data for the 1976 sampling program are presented in Tables 2-9. Data for monthly samples are included in these tables; however, because of the limited number of trawls taken during the monthly sampling periods, the following discussion will be restricted to data from the seasonal samples (winter, spring and fall).

Inspection of Tables 4 and 5 reveals no obvious trends over seasons in the numbers of species captured. It can be seen, however, that for both day and night sampling, trawls in Station Group 3 yielded lower numbers of species caught than did trawls taken in other station groups.

Tables 6 and 7 show that for both day and night trawls, the greatest catch (expressed in numbers of individuals caught per trawl) occurred during the spring in station group 1, while the lowest catch also occurred during the spring but in station group 3. There is a general tendency among both day and night trawls for the lowest catches to occur in station group 3. The only instance where the catch for station group 3 exceeded the catch in another station group occurred during the fall night trawls (where station group 3 > station group 1).

No regular patterns are evident among the station groups or through seasons for data on total biomass taken per trawl (Tables 8-9).

TABLE 2

TOTAL NUMBER OF DAY TRAWLS MADE IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	7	7	7	1	3	1	1	1	1
Station Group 2	10	12	10	3	5	5	3	3	3
Station Group 3	7	8	8	2	2	2	2	2	2

TABLE 3

TOTAL NUMBER OF NIGHT TRAWLS MADE IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	7	9	9	1	1	1	3	3	3
Station Group 2	10	10	12	3	3	3	3	3	3
Station Group 3	7	8	8	2	2	2	2	2	2

TABLE 4

TOTAL NUMBER OF SPECIES CAUGHT IN DAY TRAWLS IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	40	41	26	14	13	8	5	9	9
Station Group 2	43	33	43	29	20	25	18	19	18
Station Group 3	28	24	22	27	21	12	12	16	20

TABLE 5

TOTAL NUMBER OF SPECIES CAUGHT IN NIGHT TRAWLS IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	42	55	38	16	9	9	17	14	11
Station Group 2	51	50	52	45	34	23	21	19	22
Station Group 3	34	30	34	33	33	19	16	19	18

TABLE 6

TOTAL NUMBER OF INDIVIDUALS (OF ALL SPECIES) CAUGHT (PER TRAWL)
IN DAY TRAWLS IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	404	819	90	307	193	72	29	25	19
Station Group 2	162	141	124	121	34	31	27	44	28
Station Group 3	31	20	40	159	117	25	15	72	42

TABLE 7

TOTAL NUMBER OF INDIVIDUALS (OF ALL SPECIES) CAUGHT (PER TRAWL)
IN NIGHT TRAWLS IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	254	509	85	566	64	25	29	25	38
Station Group 2	187	207	100	209	139	39	56	38	60
Station Group 3	71	48	95	197	214	42	41	78	72

TABLE 8

TOTAL BIOMASS (IN GRAMS) OF ALL SPECIES CAUGHT (PER TRAWL)
IN DAY TRAWLS IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	3849	10576	1062	6900	9495	1070	249	209	115
Station Group 2	2949	3719	2900	3133	2291	2051	1009	1390	1350
Station Group 3	1387	1326	1482	4879	3065	971	794	4155	1182

TABLE 9

TOTAL BIOMASS (IN GRAMS) OF ALL SPECIES CAUGHT (PER TRAWL)
IN NIGHT TRAWLS IN EACH STATION GROUP

	Winter	Spring	Fall	March	April	July	August	November	December
Station Group 1	2967	5641	1545	4250	986	273	443	390	249
Station Group 2	3425	2270	2270	4398	2450	755	1520	1372	1521
Station Group 3	2671	2248	3142	4307	6368	2443	1607	4207	2970

Tables 10 and 11 give values for various diversity indices computed for each station group and for each season. There are no obvious patterns shown by H_n" and PIE values. Equitability (E) however, shows an increase proceeding from station group 1 to station group 3 for both day and night data in all seasons except spring (day).

Listings of the ten most abundant species in each station group during each season are given in Tables 12 (based on day trawls) and 13 (based on night trawls). The frequencies with which the more prominent species occurred in the "top ten" listings are shown in Table 3, Appendix H. This table gives an indication of the seasons and station groups in which given species are predominant components of the benthic ichthyofauna. For example, it can be seen that *Micropogon undulatus* is caught predominantly in station group 1 (shallow stations) during both day and night sampling. Similarly, *Anchoa hepsetus* is predominant in station group 1 during both day and night. *Peprilus burti*, however, is caught in abundance over all three station groups, but only during the day.

The abundances of each of the major species (expressed as numbers of individuals caught per trawl) in the three station groups during different seasons are presented in Table 4, Appendix H. These data indicate roughly the station groups and seasons in which each of the species show their greatest and least concentrations. With the exception of three species, all of the major species listed in Table 4, Appendix H, occurred at least to some extent in each of the three station groups. The three exceptions are listed in Table 14.

Table 5, Appendix H gives abundances (as number of individuals caught per trawl) on each of the four transects during the three different seasons. Again, these data give a rough indication of the areas (represented by

TABLE 10

VALUES FOR THE SHANNON-WEAVER DIVERSITY INDEX (H_n"), PROBABILITY OF INTERSPECIFIC ENCOUNTER (PIE) AND EQUITABILITY (E) FOR EACH STATION GROUP. (BASED ON NUMERICAL ABUNDANCES OF SPECIES IN DAY TRAWLS)

		Winter	Spring	Fall
Station Group 1	H_n"	2.110	2.309	1.289
	PIE	.824	.869	.459
	E	.125	.171	.115
Station Group 2	H_n"	2.114	1.374	2.710
	PIE	.868	.496	.874
	E	.139	.091	.209
Station Group 3	H_n"	2.613	2.267	2.415
	PIE	.900	.817	.880
	E	.286	.271	.341

TABLE 11

VALUES FOR THE SHANNON-WEAVER DIVERSITY INDEX (H_n"), PROBABILITY OF INTERSPECIFIC ENCOUNTER (PIE), AND EQUITABILITY (E) FOR EACH STATION GROUP. (BASED ON NUMERICAL ABUNDANCES OF SPECIES IN NIGHT TRAWLS)

		Winter	Spring	Fall
Station Group 1	H_n"	1.996	2.792	2.144
	PIE	.727	.916	.802
	E	.119	.173	.158
Station Group 2	H_n"	2.653	2.788	2.739
	PIE	.858	.893	.865
	E	.176	.180	.173
Station Group 3	H_n"	2.523	2.401	2.105
	PIE	.871	.869	.796
	E	.235	.250	.176

TABLE 12

TEN MOST ABUNDANT SPECIES CAPTURED IN DAY TRAWLS IN EACH STATION GROUP DURING EACH SEASON.
SPECIES LISTED ALSO HAVE ABUNDANCE ≥ 10 INDIVIDUALS.
NUMBERS OF INDIVIDUALS CAPTURED ARE GIVEN FOR EACH SPECIES

Station Group 1

Winter		Spring		Fall	
Species	No.	Species	No.	Species	No.
Anchoa hepsetus	749	*Micropogon undulatus*	1216	*Chloroscombrus chrysurus*	462
Cynoscion nothus	646	*Polydactylus octonemus*	1012	*Lutjanus campechanus*	34
Micropogon undulatus	570	*Trachurus lathami*	891	*Stenotomus caprinus*	21
Peprilus burti	221	*Cynoscion arenarius*	577	*Micropogon undulatus*	18
Anchoa micthilli	177	*Cynoscion nothus*	465	*Eucinostomus gula*	16
Syacium gunteri	72	*Chloroscombrus chrysurus*	449	*Syacium gunteri*	16
Cynoscion arenarius	66	*Anchoa hepsetus*	449	*Sphoeroides parvus*	12
Sphoeroides parvus	57	*Trichiurus lepturus*	170	*Diplectrum bivittatum*	10
Trachurus lathami	55	*Peprilus burti*	105		
Trichiurus lepturus	36	*Upeneus parvus*	76		

TABLE 12 CONT.'D

Station Group 2

Winter		Spring		Fall	
Species	No.	Species	No.	Species	No.
Saurida brasiliensis	552	*Trachurus lathami*	1189	*Prionotus stearnsi*	366
Serranus atrobranchus	342	*Lagocephalus laevigatus*	76	*Peprilus burti*	131
Synodus poeyi	99	*Saurida brasiliensis*	66	*Serranus atrobranchus*	129
Syacium gunteri	98	*Upeneus parvus*	59	*Trachurus lathami*	111
Diplectrum bivittatum	70	*Peprilus burti*	57	*Saurida brasiliensis*	47
Synodus foetens	63	*Synodus foetens*	51	*Polydactylus octonemus*	47
Prionotus stearnsi	56	*Pristipomoides aquilonaris*	42	*Upeneus parvus*	39
Upeneus parvus	45	*Serranus atrobranchus*	36	*Chloroscombrus chrysurus*	38
Stenotomus caprinus	43	*Prionotus stearnsi*	30	*Syacium gunteri*	34
Pristipomoides aquilonaris	37	*Stenotomus caprinus*	22	*Porichthys porosissimus*	25
				Stenotomus caprinus	25

TABLE 12 CONT.'D

Station Group 3

Winter

Species	No.
Serranus atrobranchus	40
Prionotus paralatus	34
Trichopsetta ventralis	30
Pristipomoides aquilonaris	23
Upeneus parvus	17
Saurida brasiliensis	11
Halieutichthys aculeatus	11
Stenotomus caprinus	10

Spring

Species	No.
Pristipomoides aquilonaris	62
Serranus atrobranchus	25
Peprilus burti	12
Trichopsetta ventralis	11
Prionotus paralatus	10

Fall

Species	No.
Serranus atrobranchus	77
Trachurus lathami	49
Pristipomoides aquilonaris	38
Trichopsetta ventralis	28
Stenotomus caprinus	27
Upeneus parvus	19
Prionotus paralatus	17
Saurida brasiliensis	14
Prionotus stearnsi	11
Pontinus longispinis	10

TABLE 13

TEN MOST ABUNDANT SPECIES CAPTURED IN NIGHT TRAWLS IN EACH STATION GROUP DURING EACH SEASON.
SPECIES LISTED ALSO HAVE ABUNDANCE ≥ 10 INDIVIDUALS.
NUMBERS OF INDIVIDUALS CAPTURED ARE GIVEN FOR EACH SPECIES

Station Group 1

Winter		Spring		Fall	
Species	No.	Species	No.	Species	No.
Cynoscion nothus	880	*Micropogon undulatus*	776	*Sphoeroides parvus*	259
Syacium gunteri	191	*Prionotus rubio*	508	*Syacium gunteri*	180
Micropogon undulatus	131	*Polydactylus octonemus*	506	*Micropogon undulatus*	104
Symphurus plagiusa	101	*Stenotomus caprinus*	418	*Lutjanus campechanus*	51
Anchoa hepsetus	96	*Sphoeroides parvus*	326	*Diplectrum bivittatum*	23
Larimus fasciatus	95	*Larimus fasciatus*	284	*Eucinostomus gula*	17
Sphoeroides parvus	59	*Anchoa hepsetus*	258	*Prionotus rubio*	17
Etropus crossotus	36	*Centropristis philadelphica*	233	*Centropristis philadelphica*	16
Cynoscion arenarius	28	*Conodon nobilis*	191	*Chloroscombrus chrysurus*	15
Prionotus rubio	27	*Cynoscion arenarius*	190	*Polydactylus octonemus*	13

TABLE 13 CONT.'D

Station Group 2

Winter

Species	No.
Serranus atrobranchus	590
Syacium gunteri	295
Porichthys porosissimus	152
Bollmannia communis	80
Trichopsetta ventralis	77
Engyophrys senta	76
Prionotus rubio	73
Centropristis philadelphica	64
Cyclopsetta chittendeni	52
Stenotomus caprinus	43

Spring

Species	No.
Stenotomus caprinus	497
Serranus atrobranchus	334
Syacium gunteri	169
Trachurus lathami	126
Sphoeroides parvus	116
Prionotus stearnsi	111
Centropristis philadelphica	94
Lagocephalus laevigatus	80
Bollmannia communis	48
Synodus poeyi	42

Fall

Species	No.
Serranus atrobranchus	389
Stenotomus caprinus	108
Prionotus stearnsi	94
Syacium gunteri	87
Halieutichthys aculeatus	64
Centropristis philadelphica	53
Polydactylus octonemus	43
Sphoeroides parvus	36
Pristipomoides aquilonaris	35
Porichthys porosissimus	25
Prionotus rubio	25

TABLE 13 CONT.'D

Station Group 3

Winter		Spring		Fall	
Species	No.	Species	No.	Species	No.
Serranus atrobranchus	132	*Serranus atrobranchus*	87	*Serranus atrobranchus*	291
Pristipomoides aquilonaris	85	*Pristipomoides aquilonaris*	73	*Pristipomoides aquilonaris*	115
Trichopsetta ventralis	63	*Prionotus paralatus*	50	*Prionotus paralatus*	99
Prionotus paralatus	46	*Trichopsetta ventralis*	43	*Trichopsetta ventralis*	86
Stenotomus caprinus	32	*Stenotomus caprinus*	37	*Halieutichthys aculeatus*	35
Halieutichthys aculeatus	23	*Halieutichthys aculeatus*	22	*Stenotomus caprinus*	31
Prionotus rubio	22	*Pontinus longispinis*	11	*Pontinus longispinis*	25
Porichthys porosissimus	13				

TABLE 14

ABSENCE OF MAJOR SPECIES IN THE STATION GROUPS

Species	Never found in station group: I	II	III
Sphaeroides parvus			X
Syacium gunteri			X
Trichopsetta ventralis	X		

transects) and seasons in which the major species are concentrated.

When the data are ranked in order by the number of occurrences for each species, along with total numbers and weights, the 1976 data appear as in Table 6, Appendix H. Also in this table are the statistical confidence limits within which some of the species are either predominantly taken during the day (D) or night (N).

Table 15 includes the winter day-night series for all stations on the four transects. Day Station 3/I was not sampled and substitute values based on averages of Day Station 6/I and Day Station 3/II, the nearest stations and those most likely to have similar species compositions, were used. This procedure seemed more biologically justifiable than the usual statistically justifiable system of calculating missing values based on all numerical or weight data, regardless of species composition. Table 16 is a summary of the observed and calculated values for the March 1976 series on Transect II. Table 17 is similar, but for the April monthly series with replicates as indicated. Table 17 is for the series during April for Transect II with both observed and calculated values.

Table 18 represents the observed and calculated values for the spring series over all stations and transects, with replicates where indicated. The Transect II, July monthly data are given in Table 19, and the August monthly data in Table 20.

The fall 1976 data series (roughly comparable to the late summer 1975 series) is included in Table 21. The ensuing November and December monthly Transect II series are in Tables 22 and 23, respectively.

For each of the individual collections from each trawl sample, the Shannon diversity index for numbers, the index for weights, the number of species and their numbers and weights, the PIE value, and the equitability E value were tabulated. These tabulations are also included in Tables 15-23.

TABLE 15

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE WINTER 1976 COLLECTIONS. ASTERISK DENOTES SUBSTITUTE VALUE.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/I Day	HBK		13	655	3124.9	0.308	0.754	1.575	1.694
1/I Night	HBU		16	427	3820.3	0.188	0.472	1.186	1.604
2/I Day	HCX		20	186	3257.7	0.250	0.735	1.932	2.124
2/I Night	HER		16	76	1033.1	0.375	0.825	2.107	2.036
3/I Day	HEG	*	12	40	1296.6*	0.521*	0.859*	2.068*	1.965*
3/I Night	HEO		15	91	4216.3	0.333	0.846	2.050	2.100
4/I Day	HFM		17	674	2606.2	0.235	0.688	1.536	1.912
4/I Night	HFS		16	505	3068.5	0.250	0.660	1.483	1.736
5/I Day	HGN		24	321	4660.0	0.250	0.832	2.256	2.539
5/I Night	HGR		23	162	4697.5	0.348	0.878	2.531	2.453
6/I Day	HHN		17	59	1851.6	0.471	0.909	2.476	2.150
6/I Night	HHR		23	159	4872.4	0.304	0.856	2.339	2.476
1/II Day	HJC		13	373	13556.2	0.231	0.601	1.358	1.280
1/II Night	HJL		20	292	6075.9	0.150	0.566	1.353	1.215
2/II Day	HKP		19	310	6492.8	0.263	0.797	1.978	2.018
2/II Night	HKX		30	383	5595.2	0.200	0.769	2.214	2.489
3/II Day	HMA		7	21	741.6	0.571	0.809	1.660	1.780
3/II Night	HMI		11	60	2355.8	0.455	0.785	1.834	1.798
4/II Day	HNG		14	210	4151.5	0.214	0.575	1.439	1.931
4/II Night	HNK		20	195	2687.8	0.400	0.893	2.504	2.208
5/II Day	HOP		14	84	4477.6	0.500	0.881	2.315	1.781
5/II Night	HOT		22	231	8142.7	0.273	0.839	2.238	2.321
6/II Day	HQE		10	30	1788.7	0.500	0.809	1.810	1.664
6/II Night	HQI		10	38	1639.0	0.500	0.873	2.041	1.803
1/III Day	HUQ		13	74	3175.6	0.308	0.700	1.729	2.026
1/III Night	HUY		16	105	2872.1	0.313	0.789	2.017	1.815
2/III Day	IAR		9	49	2117.0	0.333	0.651	1.472	1.411
2/III Night	IAZ		22	175	2510.2	0.182	0.608	1.677	1.972
3/III Day	ICN		13	63	1938.7	0.385	0.836	2.033	2.261
3/III Night	ICV		9	37	1413.0	0.444	0.734	1.651	1.647

TABLE 15 CONT.'D

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
4/III Day	IEI		12	764	1730.4	0.083	0.041	0.149	1.049
4/III Night	IEO		11	119	672.0	0.273	0.442	1.064	1.830
5/III Day	IGB		18	110	1684.6	0.333	0.862	2.210	2.197
5/III Night	IGF		23	219	3386.6	0.261	0.813	2.081	2.444
6/III Day	IHT		6	20	2740.0	0.500	0.726	1.400	1.215
6/III Night	IHX		13	73	3221.5	0.462	0.849	2.063	2.139
1/IV Day	IJU		10	98	1120.8	0.300	0.652	1.412	1.774
1/IV Night	IKC		16	202	2622.8	0.250	0.732	1.758	1.502
2/IV Day	ILO		7	21	312.5	0.571	0.819	1.682	1.536
2/IV Night	ILS		20	246	3176.6	0.250	0.804	2.041	2.331
3/IV Day	INI		10	26	650.7	0.500	0.821	1.871	1.842
3/IV Night	INQ		10	42	939.1	0.500	0.831	1.896	2.023
4/IV Day	IPF		7	189	1602.8	0.429	0.499	1.097	1.270
4/IV Night	IPJ		13	151	1640.3	0.154	0.376	1.000	1.239
5/IV Day	IQX		13	79	1561.0	0.385	0.835	2.046	2.000
5/IV Night	IRA		20	156	2519.7	0.250	0.669	1.845	1.848
6/IV Day	ISX		14	54	2189.8	0.500	0.889	2.299	2.025
6/IV Night	ITB		14	28	495.1	0.571	0.936	2.474	2.257

TABLE 16

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE MARCH 1976 MONTHLY COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/II Day	JUI		13	306	6899.5	0.307	0.642	1.516	1.237
1/II Night	JUK		16	566	4248.7	0.187	0.655	1.467	1.293
2/II Day	JWF		24	201	4668.3	0.291	0.883	2.437	2.230
2/II Night	JWH		32	260	5398.9	0.218	0.815	2.356	2.463
3/II Day	JYZ		22	148	4875.7	0.318	0.884	2.426	2.387
3/II Night	JZB		25	279	4617.5	0.240	0.783	2.147	2.490
4/II Day	JVE		13	101	1717.9	0.384	0.818	2.020	1.737
4/II Night	JVG		23	210	2750.4	0.347	0.889	2.507	2.375
5/II Day	JXB		18	60	3010.3	0.444	0.918	2.570	2.051
5/II Night	JXD		17	158	5040.2	0.235	0.802	1.764	1.992
6/II Day	JYA		19	170	4879.1	0.315	0.880	2.169	1.861
6/II Night	JYC		23	114	3995.1	0.347	0.879	2.473	2.438

TABLE 17

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE APRIL 1976 MONTHLY COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/II Day	KMT		10	188	2805.9	0.400	0.737	1.500	1.572
1/II Day	KUT	1	9	161	2245.4	0.444	0.735	1.512	1.492
1/II Day	KUV	2	6	231	4441.9	0.500	0.725	1.374	1.481
1/II Night	KMV		9	64	984.7	0.222	0.433	1.034	1.177
2/II Day	KOQ		14	71	2371.3	0.429	0.871	2.174	1.623
2/II Day	KUX	1	9	21	1391.6	0.555	0.828	1.856	1.234
2/II Day	KUZ	2	7	23	1207.5	0.571	0.794	1.639	1.461
2/II Night	KOS		27	172	2588.9	0.259	0.815	2.404	2.494
3/II Day	KRM		15	90	2980.1	0.467	0.880	2.303	1.922
3/II Night	KRO		25	222	7551.3	0.280	0.856	2.356	2.139
4/II Day	KNS		9	44	1083.3	0.444	0.790	1.794	1.603
4/II Night	KNU		18	52	989.5	0.444	0.929	2.624	2.180
5/II Day	KPP		6	11	326.8	0.667	0.800	1.540	1.604
5/II Night	KPR		19	202	3769.6	0.315	0.817	2.137	2.420
6/II Day	KQN		17	163	3150.2	0.353	0.892	2.094	2.516
6/II Night	KWP		22	205	5182.6	0.272	0.829	2.178	2.473

TABLE 18

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE SPRING 1976 COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/I Day	LXZ		20	1558	26090.2	0.250	0.806	1.886	2.101
1/I Night	LYC		22	1383	22490.9	0.182	0.739	1.740	1.422
2/I Day	MAA		17	571	9542.7	0.118	0.270	0.766	0.846
2/I Night	MAD		14	64	935.2	0.357	0.746	1.903	1.795
3/I Day	MBW		2	2	44.7	1.000	1.000	0.693	0.692
3/I Night	MBZ		10	59	2758.8	0.500	0.815	1.869	1.451
4/I Day	MDR		19	2223	36958.1	0.211	0.671	1.479	1.626
4/I Night	MDT		22	541	7296.9	0.273	0.856	2.159	2.050
5/I Day	MFL		14	44	1657.6	0.429	0.885	2.264	2.027
5/I Night	MFN		20	198	5262.1	0.250	0.779	2.042	2.434
6/I Day	MHF		2	5	317.7	1.000	0.400	0.500	0.324
6/I Night	MHH		12	47	2596.3	0.500	0.874	2.145	1.922
1/II Day	MIZ		7	38	549.0	0.571	0.813	1.704	1.257
1/II Night	MJC		9	52	505.2	0.444	0.768	1.712	1.594
1/II Night	MJX	1	11	157	1636.0	0.455	0.814	1.844	1.650
1/II Night	MJZ	2	16	148	1804.9	0.313	0.831	2.048	1.737
2/II Day	MLA		10	50	2026.0	0.500	0.867	2.040	1.315
2/II Day	MMC	1	9	94	2965.3	0.333	0.493	1.137	1.449
2/II Day	MME	2	11	107	2417.7	0.455	0.825	1.964	1.948
2/II Night	MLD		26	257	4112.9	0.308	0.866	2.444	2.330
3/II Day	MNB		8	44	2938.1	0.375	0.669	1.452	1.174
3/II Night	MNE		15	59	3555.0	0.400	0.839	2.080	2.071
4/II Day	MOW		10	204	3600.7	0.200	0.329	0.778	0.998
4/II Night	MOY		30	779	2618.9	0.167	0.708	1.858	2.154
5/II Day	MQO		12	36	2189.8	0.500	0.887	2.177	2.072
5/II Night	MQQ		21	69	2145.3	0.381	0.909	2.594	2.464
6/II Day	MSG		7	15	522.2	0.571	0.866	1.783	1.825
6/II Night	MSI		11	53	3125.7	0.455	0.849	2.015	1.839
1/III Day	MXB		11	745	9939.2	0.182	0.299	0.745	0.573
1/III Night	MXE		22	581	1227.1	0.182	0.701	1.638	2.480

TABLE 18 CONT.'D

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
2/III Day	MZB		9	80	2427.6	0.222	0.317	0.786	1.462
2/III Night	MZE		11	30	683.2	0.545	0.908	2.231	2.128
3/III Day	NAY		5	7	350.0	0.600	0.857	1.475	1.214
3/III Night	NBB		9	28	1675.0	0.556	0.859	1.926	1.622
4/III Day	NCT		10	398	5294.2	0.200	0.539	0.915	0.623
4/III Night	NCY		16	428	4974.3	0.188	0.663	1.391	1.106
5/III Day	NEN		9	107	2585.3	0.222	0.335	0.822	1.271
5/III Night	NEP		20	110	1143.3	0.400	0.915	2.610	2.496
6/III Day	NGF		9	17	2167.1	0.556	0.875	1.952	1.315
6/III Night	NGH		11	67	2336.2	0.364	0.823	1.758	1.963
1/IV Day	NHY		13	335	4606.7	0.154	0.451	1.010	1.018
1/IV Night	NIB		16	303	1357.0	0.250	0.701	1.591	1.851
2/IV Day	NJY		7	41	898.8	0.429	0.704	1.433	1.488
2/IV Night	NKB		20	95	1638.6	0.400	0.868	2.462	2.448
3/IV Day	NLV		6	16	521.3	0.667	0.841	1.663	0.932
3/IV Night	NLY		12	34	1005.0	0.500	0.869	2.139	2.279
4/IV Day	NNQ		18	409	11777.7	0.222	0.618	1.640	1.164
4/IV Night	NNS		24	398	3514.9	0.208	0.739	1.929	2.134
5/IV Day	NPI		10	330	5102.4	0.200	0.336	0.737	0.718
5/IV Night	NPK		28	466	3835.8	0.214	0.833	2.130	1.946
6/IV Day	NRA		7	25	1708.6	0.571	0.826	1.700	1.654
6/IV Night	NRC		3	4	163.0	0.667	0.883	1.039	0.178
7/IV Day	NSS		8	56	3744.2	0.375	0.618	1.318	0.905
7/IV Night	NSU		12	38	926.9	0.417	0.769	1.851	1.955

TABLE 19

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE JULY 1976 COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/II Day	OIP		8	72	1069.0	0.500	0.710	1.485	1.584
1/II Night	OIS		9	25	270.9	0.556	0.893	2.044	1.962
2/II Day	OJQ		12	27	1074.0	0.417	0.769	1.906	1.310
2/II Day	OJV	1	15	31	1577.7	0.467	0.881	2.333	1.430
2/II Day	OJX	2	12	27	778.9	0.600	0.897	2.236	1.815
2/II Night	OJT		14	42	685.2	0.367	0.765	1.910	1.996
3/II Day	OKV		9	18	783.2	0.444	0.758	1.714	1.690
3/II Night	OKY		12	29	1585.1	0.500	0.852	2.086	2.082
4/II Day	OLV		9	28	360.2	0.444	0.780	1.732	1.482
4/II Night	OLX		15	44	439.7	0.467	0.891	2.317	2.229
5/II Day	OMU		6	42	2361.5	0.333	0.380	0.845	0.551
5/II Night	OMW		10	30	1139.3	0.500	0.857	1.976	1.845
6/II Day	ONT		9	31	1157.0	0.444	0.850	1.697	2.053
6/II Night	ONV		13	55	1577.7	0.462	0.859	2.088	1.858

TABLE 20

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE AUGUST 1976 MONTHLY COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/II Day	OUT		8	29	745.5	0.800	0.645	1.171	0.870
1/II Night	OUW		5	18	383.9	0.625	0.869	1.875	1.648
1/II Night	RJS	1	7	23	509.7	0.571	0.771	1.492	1.151
1/II Night	RJU	2	11	46	443.8	0.455	0.809	1.945	1.990
2/II Day	OVU		11	28	1218.6	0.545	0.915	2.246	1.873
2/II Night	OVX		18	85	3030.4	0.444	0.915	2.565	2.251
3/II Day	OWV		6	13	730.5	0.667	0.820	1.585	1.244
3/II Night	OWY		12	35	1158.1	0.500	0.887	2.179	2.047
4/II Day	OXV		8	25	406.0	0.625	0.843	1.808	1.669
4/II Night	OXX		13	62	809.6	0.462	0.878	2.223	2.263
5/II Day	OYU		5	28	1409.0	0.800	0.751	1.478	1.265
5/II Night	OYW		7	20	737.6	0.571	0.821	1.704	1.754
6/II Day	OZT		8	16	855.3	0.625	0.899	1.960	1.302
6/II Night	OZV		11	47	2064.5	0.545	0.856	2.056	1.891

TABLE 21

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE FALL 1976 COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/I Day	QYL		5	406	2985.6	.200	.024	0.082	0.132
1/I Night	QYO		13	42	515.0	.385	.806	1.925	2.238
2/I Day	RAC		9	52	3180.9	.444	.687	1.511	1.493
2/I Night	RAF		12	38	500.9	.500	.877	2.160	1.939
3/I Day	RCF		4	16	763.9	.500	.616	1.033	0.772
3/I Night	RCI		11	36	1709.0	.455	.861	2.031	1.990
4/I Day	RDW		3	60	573.5	.667	.287	0.504	0.768
4/I Night	RDY		9	26	530.1	.444	.760	1.695	1.377
5/I Day	RFN		7	17	1058.4	.714	.889	1.874	1.493
5/I Night	RFP		10	46	3514.6	.400	.806	1.781	1.716
6/I Day	RHE		8	27	975.4	.625	.849	1.838	1.731
6/I Night	RHG		12	46	1491.7	.500	.839	2.059	2.099
1/II Day	RIZ		7	15	449.5	.571	.819	1.679	1.386
1/II Night	RJC		11	17	316.9	.545	.911	2.196	2.052
1/II Night	RJE	1	11	26	622.2	.545	.889	2.147	1.721
1/II Night	RJG	2	9	12	297.1	.667	.939	2.094	1.950
2/II Day	RLA		6	31	1324.6	.333	.402	0.884	0.498
2/II Night	RLD		13	39	1125.2	.462	.818	2.191	1.649
2/II Night	RLF	1	10	36	670.4	.500	.823	1.855	1.822
2/II Night	RLH	2	11	45	837.7	.455	.853	2.006	1.905
3/II Day	RND		9	36	1667.9	.444	.787	1.707	1.560
3/II Night	RNG		9	25	1158.0	.556	.873	1.987	1.731
4/II Day	ROU		3	12	342.5	.666	.318	0.566	0.384
4/II Night	ROW		8	28	434.3	.500	.801	1.737	1.839
5/II Day	RQD		9	13	1101.3	.666	.935	2.098	1.847
5/II Night	RQF		13	117	3172.1	.385	.801	1.929	2.115
6/II Day	RRM		8	31	1815.6	.500	.804	1.701	1.336
6/II Night	RRP		12	36	1751.0	.500	.887	2.182	2.001

TABLE 21 CONT.'D

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/III Day	RSX		12	50	1355.3	.333	.738	1.699	1.435
1/III Night	RTA		17	187	5925.0	.235	.675	1.562	1.113
2/III Day	RUV		20	457	9207.7	.150	.588	1.467	2.163
2/III Night	RUY		17	122	3568.1	.353	.779	2.075	2.210
3/III Day	RWX		9	29	1339.9	.555	.857	1.953	1.425
3/III Night	RXA		14	181	5514.8	.286	.713	1.620	1.871
4/III Day	RYO		4	30	998.3	.500	.526	0.950	1.184
4/III Night	RYQ		11	130	2971.3	.273	.630	1.419	1.446
5/III Day	RZX		16	144	3850.7	.250	.608	1.492	1.912
5/III Night	RZZ		20	207	2386.8	.250	.758	2.022	2.297
6/III Day	SBG		11	80	1908.5	.455	.842	1.978	2.016
6/III Night	SBI		16	131	3098.9	.313	.797	1.948	2.163
1/IV Day	SCQ		8	35	437.1	.625	.828	1.826	1.941
1/IV Night	SCT		13	248	1421.1	.154	.376	0.866	1.479
2/IV Day	SEN		22	213	4902.7	.364	.906	2.583	2.452
2/IV Night	SEQ		25	147	2279.1	.320	.902	2.605	2.751
3/IV Day	SGM		8	70	3002.6	.500	.797	1.671	1.643
3/IV Night	SGP		14	76	1173.0	.357	.784	2.019	2.321
4/IV Day	SIE		12	36	648.9	.500	.863	2.121	2.147
4/IV Night	SIG		13	73	1320.9	.385	.772	1.848	1.629
5/IV Day	SJO		24	172	6235.4	.333	.877	2.505	2.172
5/IV Night	SJQ		23	242	5897.8	.261	.811	2.176	2.268
6/IV Day	SKY		22	128	3588.9	.318	.823	2.318	2.517
6/IV Night	SLA		16	135	2759.0	.313	.727	1.799	2.403
7/IV Day	SMI		7	31	519.2	.571	.799	1.661	1.768
7/IV Night	SMK		9	227	7191.5	.333	.616	1.216	1.130

TABLE 22

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE NOVEMBER 1976 MONTHLY COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/II Day	VBB		9	25	627.9	0.444	0.783	1.754	1.791
1/II Night	VBF		11	42	667.2	0.364	0.731	1.747	1.696
1/II Night	VLG	1	3	3	92.0	1.000	1.000	1.098	0.551
1/II Night	VLJ	2	11	30	411.9	0.545	0.882	2.127	1.965
2/II Day	VCI		15	37	756.4	0.400	0.819	2.131	1.771
2/II Night	VCM		15	67	2836.4	0.467	0.873	2.273	1.758
3/II Day	VDP		9	66	3837.3	0.444	0.798	1.745	1.515
3/II Night	VDT		10	81	3829.0	0.400	0.772	1.661	1.535
4/II Day	VEV		4	51	765.0	0.250	0.151	0.356	0.607
4/II Night	VEY		11	40	940.6	0.455	0.819	1.949	1.632
5/II Day	VGA		10	44	2646.2	0.500	0.865	2.044	1.510
5/II Night	VGD		6	7	338.3	0.667	0.952	1.747	1.432
6/II Day	VHF		15	77	4469.4	0.467	0.886	2.285	2.158
6/II Night	VHI		16	75	4582.1	0.375	0.838	2.185	2.167

TABLE 23

TOTAL NUMBER OF SPECIES, TOTAL NUMBER OF INDIVIDUALS, TOTAL WEIGHT, H_n" AND H_w" DIVERSITY INDICES, EQUITABILITY (E), AND HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) FOR EACH SAMPLE IN THE DECEMBER 1976 MONTHLY COLLECTIONS.

Station/Transect	Code	Replicate	Species	Individuals	Weight (g)	E	P.I.E.	H_n"	H_w"
1/II Day	VYY		9	19	343.9	0.556	0.853	1.935	1.672
1/II Night	VZC		7	50	228.4	0.428	0.677	1.345	1.429
1/II Night	VZF	1	9	41	231.2	0.444	0.729	1.605	1.769
1/II Night	VZI	2	7	24	287.6	0.571	0.753	1.535	1.788
2/II Day	WAL		11	43	987.8	0.545	0.881	2.128	1.490
2/II Night	WAP		10	47	1360.0	0.400	0.675	1.571	1.877
3/II Day	WBS		15	41	1098.3	0.467	0.898	2.363	2.390
3/II Night	WBV		12	79	3450.3	0.500	0.864	2.116	2.207
4/II Day	WCY		5	15	407.9	0.600	0.724	1.353	1.388
4/II Night	WDB		11	64	848.8	0.273	0.479	1.186	0.795
5/II Day	WED		11	26	2653.8	0.545	0.910	2.215	1.645
5/II Night	WEG		13	68	2352.3	0.461	0.834	2.082	1.902
6/II Day	WFI		12	43	1264.4	0.500	0.856	2.131	2.063
6/II Night	WFL		12	64	2488.5	0.417	0.834	1.961	2.016

The replicated data from the sample Tables 15-23 are summarized separately in Table 24 in calendar sequence. The number of species in the first sample, the additional species in the second, and the additional species in the third replicate are summarized in the columns for "No. of New Species" and for "Cumulative No. of Species", while the column for "No. of Species" pertains to the number of species actually observed in each sample listed in Table 24. This table is included simply to indicate the degree of variability among observed and calculated values from about the best kind of replication possible in open-ocean trawl sampling techniques.

For comparative purposes, yearly summary values from 1975 are repeated in Table 25 pertinent to Stations 1, 2 and 3 on each of the four transects; the 1976 data for the same stations and transects are given in Table 26.

The analysis of variance of the several values, or their transformed values, are in Table 27 for the 1975 data, in Table 28 for the 1976 data, and in Table 29 for the combined 1975-1976 data.

The approaches above on "bulk" characteristics of distribution and abundance quite obviously involve differences both in day and night collections and in seasonal collections, apart from species differences that characterize the samples. To assess these differences in a more graphical way for comparisons with the other biotic and abiotic presentations over the STOCS area, a series of isopleth diagrams were prepared to show how the various numerical, weight and index values are spatially distributed seasonally and during the entire year for combined data. These figures are as follows:

Figure Number	Season	Time	Characteristics
1	Winter	Day	Number of Individuals
2	Winter	Night	Number of Individuals
3	Spring	Day	Number of Individuals
4	Spring	Night	Number of Individuals
5	Fall	Day	Number of Individuals
6	Fall	Night	Number of Individuals
7	Combined	Day	Number of Individuals
8	Combined	Night	Number of Individuals
9	Winter	Day	Number of Species
10	Winter	Night	Number of Species
11	Spring	Day	Number of Species
12	Spring	Night	Number of Species
13	Fall	Day	Number of Species
14	Fall	Night	Number of Species
15	Combined	Day	Number of Species
16	Combined	Night	Number of Species
17	Winter	Day	Biomass (g)
18	Winter	Night	Biomass (g)
19	Spring	Day	Biomass (g)
20	Spring	Night	Biomass (g)
21	Fall	Day	Biomass (g)
22	Fall	Night	Biomass (g)
23	Combined	Day	Biomass (g)
24	Combined	Night	Biomass (g)
25	Winter	Day	H_n"
26	Winter	Night	H_n"
27	Spring	Day	H_n"
28	Spring	Night	H_n"
29	Fall	Day	H_n"
30	Fall	Night	H_n"
31	Winter	Day	H_w"
32	Winter	Night	H_w"
33	Spring	Day	H_w"
34	Spring	Night	H_w"
35	Fall	Day	H_w"
36	Fall	Night	H_w"
37	Winter	Day	PIE
38	Winter	Night	PIE
39	Spring	Day	PIE
40	Spring	Night	PIE
41	Fall	Day	PIE
42	Fall	Night	PIE

Note that the scales for the various isopleth units were simplified; the key on each figure should be consulted for absolute values.

Day-Night Comparisons

The basic data for the Wilcoxon ranked sum testing of day-night differences throughout the 1976 station day-night pairs are presented

TABLE 24

REPLICATE TRAWL DATA INCLUDING ABUNDANCE (H_n") AND BIOMASS (H_w") DIVERSITY INDICES, HURLBERT'S PROBABILITY OF INTERSPECIFIC ENCOUNTER (P.I.E.) AND EQUITABILITY (E) 1976

Station/ Transect	Date		No. New Species	Cumulative No. Species	No. of Species	No. of Fish	Weight (g)	H_n"	H_w"	P.I.E.	E
1/II D	April 8	KMT	10	10	10	188	2805.9	1.500	1.572	.737	.400
		KUT	3	13	9	161	2245.4	1.512	1.492	.735	.444
		KUV	1	14	6	231	441.9	1.374	1.481	.725	.500
1/II N	June 10	MJC	9	9	9	52	505.2	1.712	1.594	.768	.444
		MJX	6	15	11	147	1636.0	1.844	1.650	.814	.455
		MJZ	5	20	16	148	1804.9	2.048	1.737	.831	.313
	Aug. 7	OUW	5	5	5	18	383.9	1.975	1.648	.869	.625
		RJS	4	9	7	23	509.7	1.492	1.151	.771	.571
		RJU	5	14	11	46	443.8	1.945	1.990	.809	.455
	Oct. 6	RJC	11	11	11	17	316.9	2.196	2.052	.911	.545
		RJE	4	11	15	26	622.2	2.147	1.721	.889	.545
		RJG	4	19	9	12	297.1	2.094	1.950	.939	.667
2/II D	June 10	MLA	10	10	10	50	2026.0	2.040	1.315	.867	.500
		MMC	4	14	9	94	2965.3	1.137	1.449	.493	.333
		MME	1	15	11	107	2417.7	1.964	1.948	.825	.455
	April 9	KOQ	14	14	14	71	2371.1	2.174	1.623	.871	.429
		KUX	1	15	9	21	1391.6	1.856	1.234	.828	.555
		KUZ	1	16	7	23	1207.5	1.639	1.461	.794	.571
	July 18	OJQ	12	12	12	27	1074.0	1.096	1.310	.769	.417
		OJV	7	19	15	31	1577.7	2.333	1.430	.881	.467
		OJX	3	21	12	27	778.9	2.236	1.815	.897	.500
2/II N	Oct. 6	RLD	13	13	13	39	1125.2	2.191	1.649	.818	.462
		RLF	3	16	10	36	670.4	1.855	1.822	.823	.500
		RLH	1	17	11	45	837.7	2.006	1.905	.853	.455

TABLE 24 CONT.'D

Station/ Transect	Date		No. New Species	Cumulative No. Species	No. of Species	No. of Fish	Weight (g)	H_n"	H_w"	P.I.E.	E
1/II N	Nov. 15	VBF	11	11	11	42	667.2	1.747	1.696	.731	.364
		VLG	0	11	3	3	92.0	1.098	.551	1.000	1.000
		VLJ	3	14	11	30	411.9	2.127	1.965	.882	.545
	Dec. 8	VZC	7	7	7	50	228.4	1.345	1.429	.667	.428
		VZF	2	9	9	41	231.2	1.605	1.769	.729	.444
		VZI	0	9	7	24	287.6	1.535	1.788	.753	.571

TABLE 25

SELECTED MEANS FOR BENTHIC FISH DATA, STATIONS 1, 2 AND 3, 1975

	No. of Species	No. of Indiv.	H_n"	E	P.I.E.	Weight (g)	H_w"
Day	18.03	322.26	1.95	0.32	0.76	6084.1	1.98
Night	21.08	272.56	2.07	0.29	0.78	6022.4	2.05
Sta. 1	20.13	464.21	1.86	0.27	0.73	6748.7	1.90
Sta. 2	19.75	213.71	2.18	0.34	0.81	3917.7	2.15
Sta. 3	18.79	214.46	2.00	0.30	0.76	7493.3	2.00
Line I	20.67	464.89	1.93	0.26	0.74	7223.0	2.04
Line II	16.33	221.44	1.96	0.36	0.79	4500.7	1.85
Line III	19.39	249.83	2.00	0.29	0.76	6803.2	2.07
Line IV	21.83	253.67	2.16	0.29	0.78	5686.0	2.10
Winter	16.75	245.33	1.77	0.31	0.72	5559.8	1.91
Spring	22.21	403.54	2.12	0.29	0.78	6268.9	2.14
Summer	19.71	243.50	2.14	0.32	0.81	6331.1	1.99

TABLE 26

SELECTED MEANS FOR BENTHIC FISH DATA, STATIONS 1, 2 AND 3, 1976

	No. of Species	No. of Indiv.	H_n"	E	P.I.E.	Weight (g)	H_w"
Day	10.56	189.81	1.52	0.41	0.69	3620.5	1.46
Night	15.44	173.31	1.91	0.36	0.77	2868.9	1.93
Sta. 1	13.46	343.38	1.51	0.30	0.66	2918.3	1.54
Sta. 2	15.88	155.54	1.86	0.34	0.73	3035.4	1.87
Sta. 3	9.67	45.75	1.77	0.55	0.81	1700.4	1.67
Line I	12.83	316.78	1.59	0.38	0.71	5014.8	1.58
Line II	12.78	116.78	1.80	0.41	0.76	3030.3	1.62
Line III	13.22	166.67	1.65	0.34	0.70	3152.2	1.71
Line IV	13.17	126.00	1.81	0.39	0.76	1781.4	1.87
Winter	14.46	168.83	1.77	0.34	0.74	3100.6	1.84
Spring	12.54	268.83	1.64	0.40	0.73	4244.9	1.56
Summer	12.00	107.00	1.72	0.40	0.73	2388.5	1.68

TABLE 27

ANALYSIS OF VARIANCE OF THE VALUES COMPARED, STATIONS 1, 2 AND 3, 1975

Source of Variation	df	Log Species Number	$\sqrt{N}$	H_n"	$\sin^{-1}\sqrt{E}$	$\sin^{-1}\sqrt{PIE}$	Log Weight (g)	H_w"
D, Depths	2	2.2483E-3	2.3105E+2$^+$	6.3556E-1	3.4462E-2	4.7924E-2	6.5377E-1$^+$	3.8561E-1
L, Transects	3	8.0670E-2$^+$	1.3383E+2$^+$	1.8954E-1	8.8476E-2	1.2973E-2	2.4392E-1	2.2661E-1
T, Times	1	8.1430E-2	8.5347E+0	2.4640E-1	1.4568E-2	4.3127E-3	7.7917E-3	9.6141E-2
S, Seasons	2	1.1542E-1$^+$	1.3569E+2	1.0377E+0	6.8047E-2	7.1570E-2$^+$	2.8836E-1	3.3148E-1
D x L	6	1.0317E-3	7.1477E+1	2.7713E-1	1.8003E-2	5.7561E-2	5.0191E-2	3.6955E-2
D x T	2	1.0850E-2	1.2195E+0	2.4866E-1	2.8857E-2	3.3549E-2	2.6070E-2	2.0556E-1
D x S	4	5.1838E-2$^+$	1.8046E+2*	3.7550E-1	3.8142E-2	2.3201E-2	7.6619E-1*	1.3245E-1
L x T	3	1.4059E-2	2.0377E+1	1.2827E-1	2.2653E-2	1.3041E-2	1.6061E-1$^+$	6.7394E-2
L x S	6	2.2643E-2	6.1705E+1$^+$	8.0132E-2	1.1002E-2	4.5258E-3	1.5611E-1	8.7826E-2
T x S	2	6.1960E-3	6.4020E+1	7.5928E-1$^+$	3.6317E-2	8.5088E-2$^+$	5.9489E-2	4.9029E-1$^+$
D x L x T	6	2.5913E-2	2.3285E+1	2.0888E-1	1.1586E-2	3.5069E-2	5.6135E-2	2.6869E-1*
L x T x S	6	7.2062E-3	1.2922E+1	2.6094E-1*	1.0656E-2	2.2223E-2	2.3154E-2	6.6391E-2
D x T x S	4	1.9550E-2	2.7581E+1	1.9919E-1	1.6831E-2	1.7745E-2	3.0042E-2	2.8553E-1*
D x L x S	12	8.6748E-3	3.0973E+1	1.4689E-1	2.4716E-2	2.6672E-2	7.0578E-2	9.7692E-2
D x L x T x S	12	1.5572E-2	2.0669E+1	7.5744E-2	1.6059E-2	1.6353E-2	9.0281E-2	8.9364E-2
Total	71							

* All comparisons at P <0.05.

$^+$ Half, or more, of comparisons at P<0.05; remainder at about P = 0.1.

TABLE 28

ANALYSIS OF VARIANCE OF THE VALUES COMPARED, STATIONS 1, 2 AND 3, 1976

Source of Variation	df	Log Species Number	$\sqrt{N}$	H_n"	$\sin^{-1}\sqrt{E}$	$\sin^{-1}\sqrt{PIE}$	Log Weight (g)	H_w"
D, Depths	2	2.8083E-1*	5.4382E+2+	7.9503E-1+	3.2214E-1+	2.2295E-1+	4.6556E-1	6.5865E-1+
L, Transects	3	7.2581E-3	7.4832E+1	2.1874E-1	1.7730E-2	2.4251E-2	1.8557E-1	2.9479E-1
T, Times	1	6.5889E-1*	4.1180E+0	2.7919E+0+	5.9033E-2	1.2807E-1	6.4508E-3	3.8568E+0+
S, Seasons	2	6.8602E-2	8.1834E+1	1.0687E-1	3.8456E-2	2.7826E-3	1.3224E-1	4.7345E-1
D x L	6	1.9343E-2	9.0457E+1	1.3137E-1	2.7342E-2	3.6960E-2	2.4293E-1	6.1913E-2
D x T	2	2.1331E-3	1.4315E+1	2.5334E-1+	3.8690E-2	6.0546E-2+	5.1486E-1	1.2961E-1
D x S	4	3.1848E-2	1.2653E+2+	3.6467E-2	3.6469E-2	1.2353E-2	3.6497E-1	1.0230E-1
L x T	3	6.6661E-3	3.5819E+1	9.8319E-2	1.3993E-2	1.3994E-2	3.7550E-2	4.1920E-2
L x S	6	5.4703E-2+	8.8097E+1	1.3791E-1	1.4959E-2	1.9746E-2	3.4200E-1	1.9146E-1
T x S	2	1.7418E-2	5.5653E+0	5.2434E-1+	3.4887E-3	5.2974E-2	8.5522E-2	7.9120E-1+
D x L x T	6	3.1113E-2	2.3038E+1	7.2556E-2	2.7709E-2	1.6939E-2	2.8208E-1	1.1618E-1
L x T x S	6	1.0408E-2	4.6872E+0	2.8934E-1	2.1223E-2	7.7199E-2	1.3363E-1	2.9666E-1
D x T x S	4	2.9924E-2	1.2076E+1	4.0057E-2	2.4457E-2	1.5615E-2	1.8354E-1	6.9948E-2
D x L x S	12	3.9468E-2*	4.0001E+1	2.3623E-1	3.5887E-2	4.8399E-2	2.5410E-1	2.4127E-1
D x L x T x S	12	1.3727E-2	1.9650E+1	1.3097E-1	2.2047E-2	3.7633E-2	1.0814E-1	2.1329E-1
Total	71							

* All comparisons at $P<0.05$.

+ Half, or more, of comparisons at $P<0.05$; remainder at about $P = 0.1$.

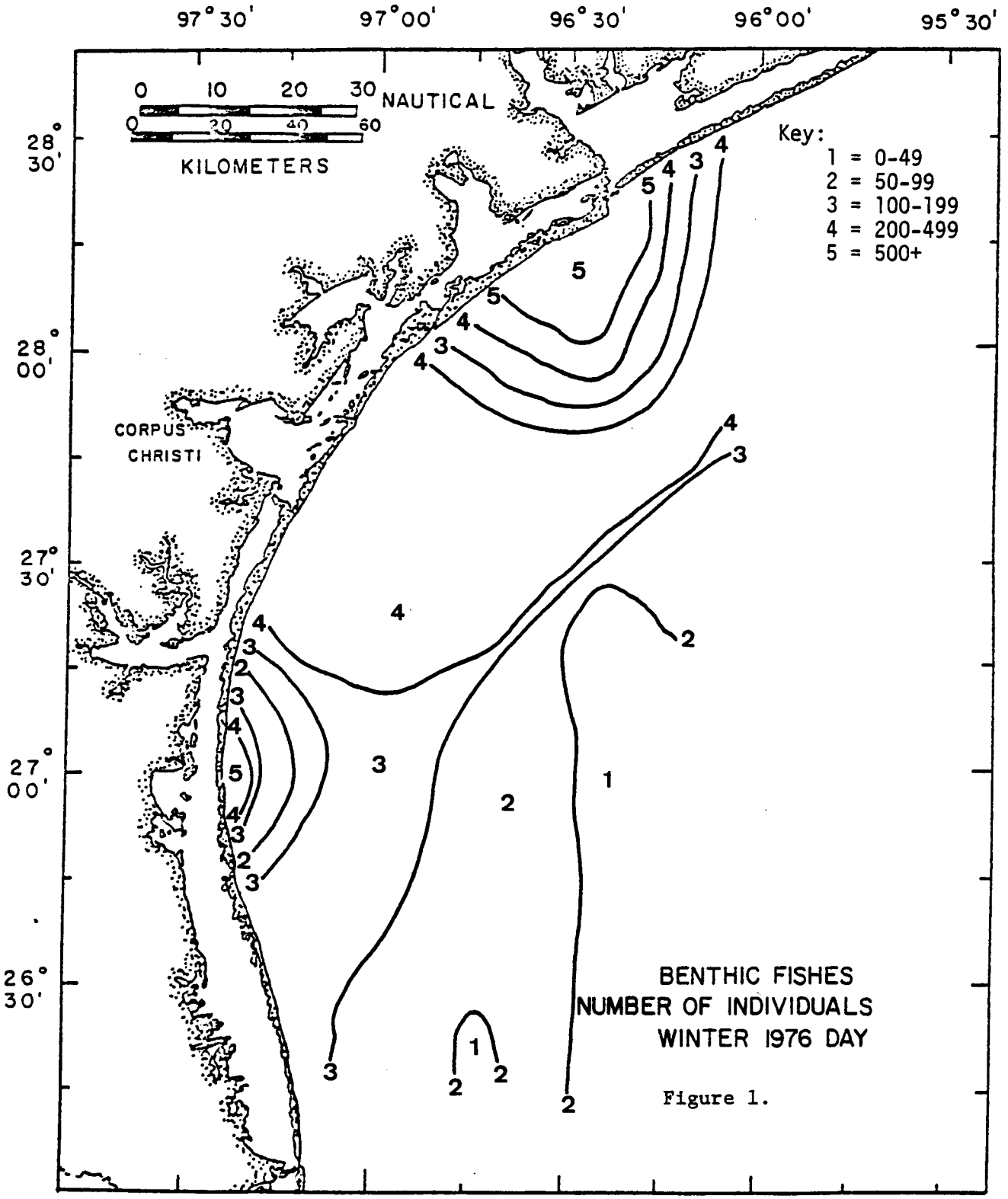
TABLE 29

ANALYSIS OF VARIANCE OF THE VALUES COMPARED, STATIONS 1, 2 AND 3, 1975-1976 COMBINED

Source of Variation	df	Log Species Number	$\sqrt{N}$	H_n"	$\sin^{-1}\sqrt{E}$	$\sin^{-1}\sqrt{PIE}$	Log Weight (g)	H_w"
D, Depths	2	1.5153E-1$^+$	6.7650E+2$^+$	1.3871E+0$^+$	1.8232E-1	1.7482E-1	9.5403E-2	1.0261E+0$^+$
L, Transects	3	5.0080E-2	2.0154E+2	3.2688E-1	3.7062E-2	3.3272E-2	2.5871E-1	4.1155E-1$^+$
T, Times	1	6.0179E-1*	3.9796E-1	2.3486E+0	6.6127E-2	8.9695E-2	3.1631E-5	2.5854E+0
S, Seasons	5	3.5906E-1*	2.3279E+2$^+$	1.0954E+0$^+$	7.3686E-2	4.1896E-2	1.2282E+0$^+$	1.0619E+0$^+$
D x L	6	1.3332E-2	1.5103E+2*	1.9169E-1	3.9411E-2	6.1006E-2	1.9233E-1	3.8870E-2
D x T	2	1.0058E-2	1.0188E+1	3.8935E-1	5.1984E-2	2.8368E-2	2.8006E-1	2.4729E-1
D x S	10	5.9783E-2	1.4247E+2*	1.7350E-1	6.4701E-2*	3.3433E-2	6.5725E-1*	9.7535E-2
L x T	3	5.0191E-3	3.8438E+1	5.4552E-2	8.1134E-4	9.6727E-3	8.8888E-2	3.5235E-2
L x S	15	3.8508E-2$^+$	6.1346E+1*	1.0350E-1	1.4213E-2	1.0499E-2	2.3340E-1$^+$	1.3368E-1
T x S	5	3.7151E-2$^+$	3.0285E+1	6.5140E-1$^+$	1.7417E-2	6.3763E-2	6.0846E-2	7.8610E-1*
D x L x T	6	1.9202E-2	2.1389E+1	7.9181E-2	2.6046E-2	3.8977E-2	1.8752E-1	1.0701E-1
L x T x S	15	1.0187E-2	1.0595E+1	2.5452E-1*	1.9919E-2	4.3241E-2	8.4566E-2	1.6003E-1
D x T x S	10	2.0375E-2	1.6932E+1	1.1823E-1	1.9448E-2	2.6489E-2	1.3761E-1	1.5977E-1
D x L x S	30	2.0666E-2	3.0570E+1	1.9661E-1	2.5428E-2	3.6731E-2	1.5003E-1	1.4758E-1
D x L x T x S	30	1.9284E-2	2.1114E+1	1.2314E-1	1.7892E-2	2.4201E-2	1.0951E-1	1.7663E-1
Total	143							

* All comparisons at $P<0.05$.

$^+$ Half, or more, of comparisons at $P<0.05$; remainder at about $P = 0.1$.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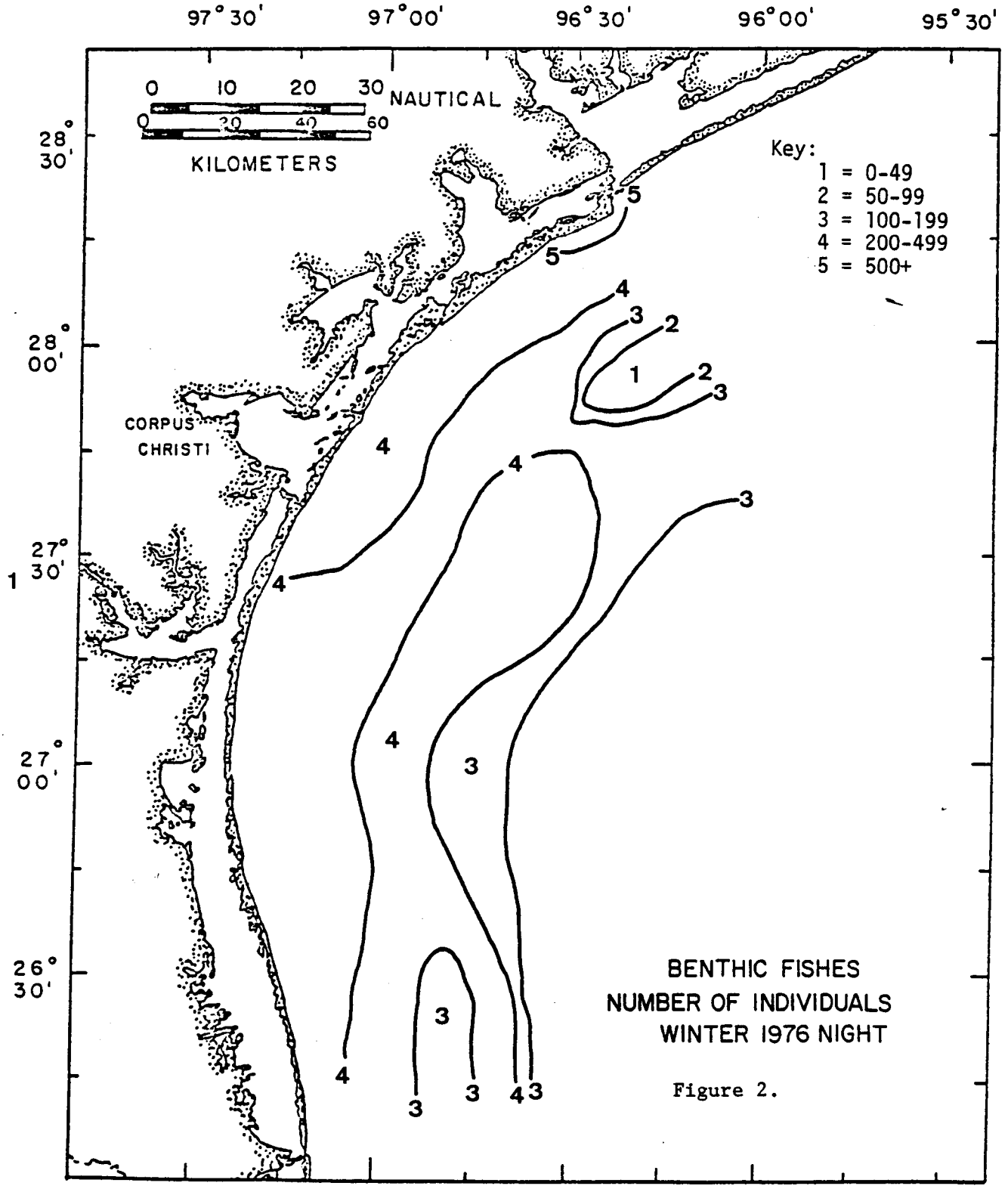
97°00'
96°30'
96°00'
95°30'
28° 30'
28° 00'
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-49
2 = 50-99
3 = 100-199
4 = 200-499
5 = 500+
BENTHIC FISHES
NUMBER OF INDIVIDUALS
WINTER 1976 DAY

Figure 1.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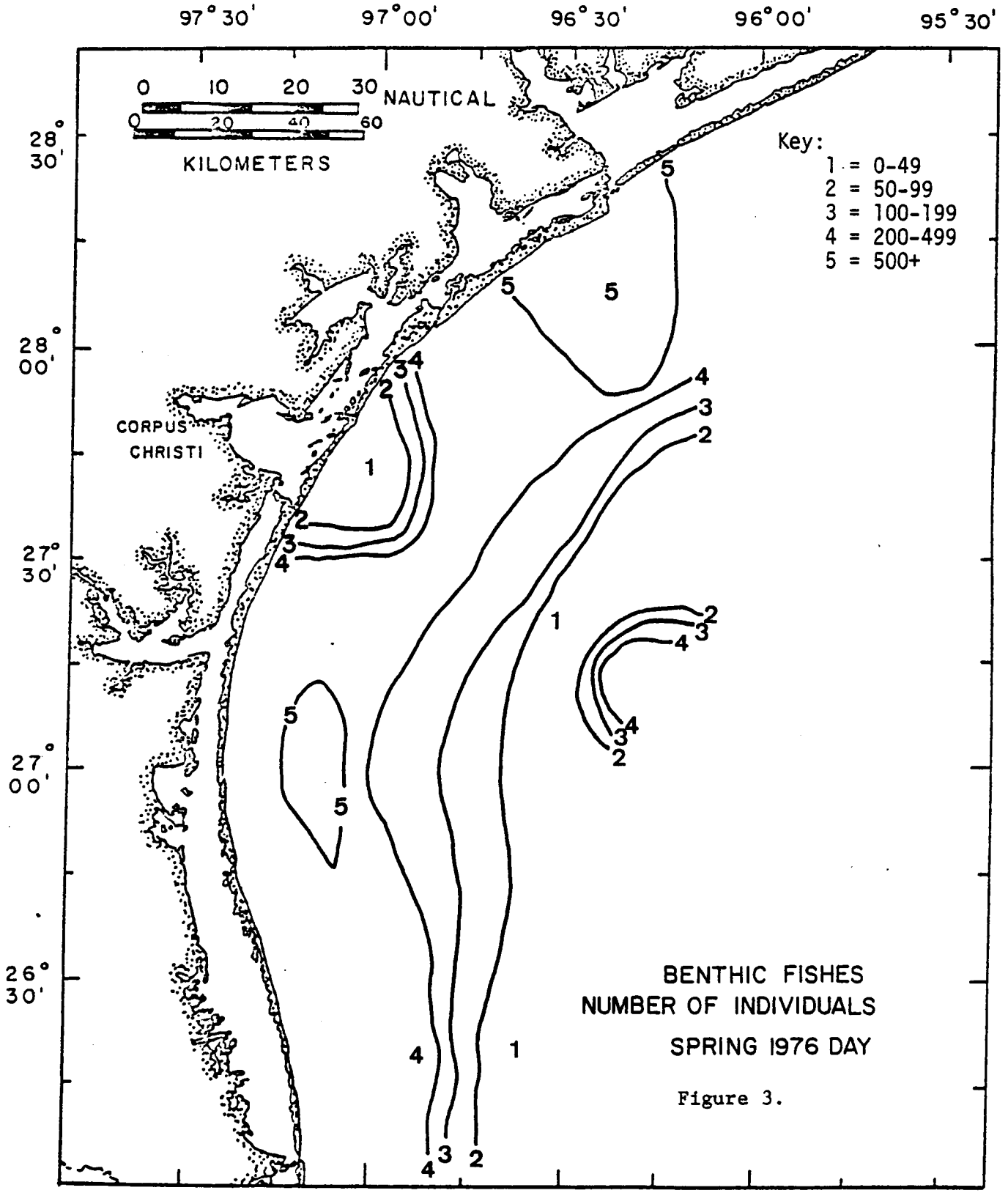
97° 00'
96° 30'
96° 00'
95° 30'
28° 30'
28° 00'
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-49
2 = 50-99
3 = 100-199
4 = 200-499
5 = 500+
BENTHIC FISHES
NUMBER OF INDIVIDUALS
WINTER 1976 NIGHT

Figure 2.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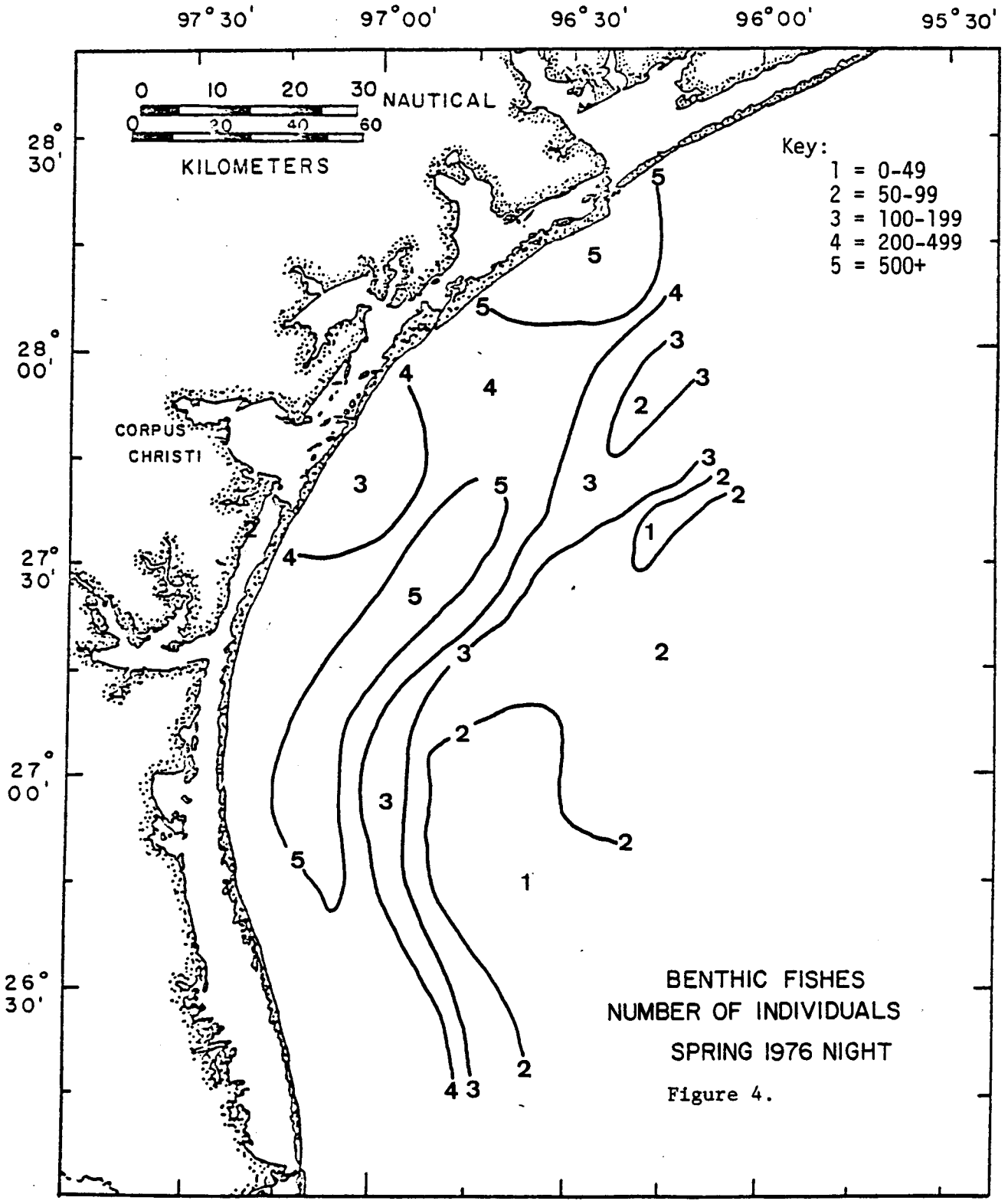
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
1 = 0-49
2 = 50-99
3 = 100-199
4 = 200-499
5 = 500+
CORPUS CHRISTI
BENTHIC FISHES
NUMBER OF INDIVIDUALS
SPRING 1976 DAY

Figure 3.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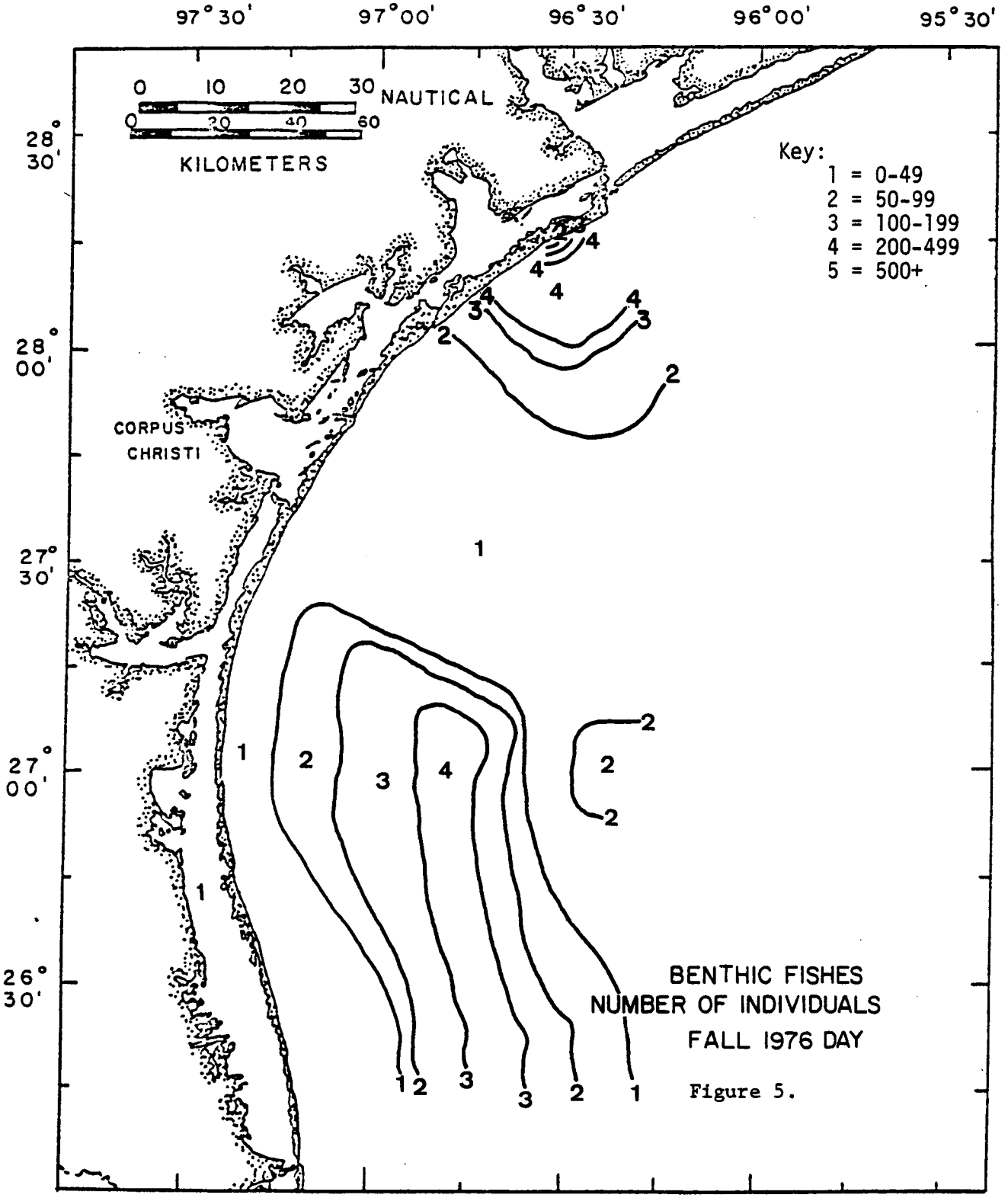
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-49
2 = 50-99
3 = 100-199
4 = 200-499
5 = 500+
BENTHIC FISHES
NUMBER OF INDIVIDUALS
SPRING 1976 NIGHT

Figure 4.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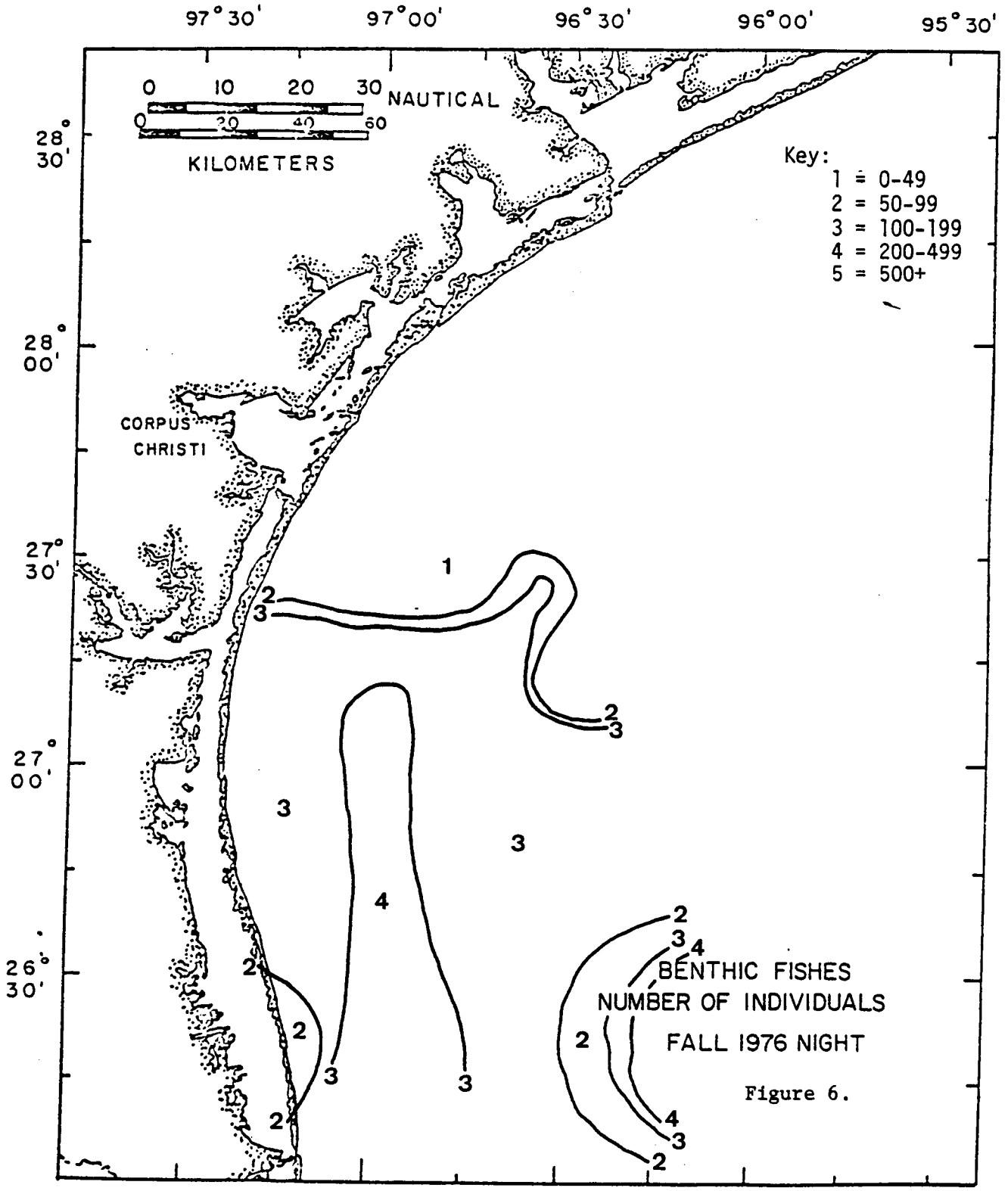
0 20 40 60
KILOMETERS
Key:
1 = 0-49
2 = 50-99
3 = 100-199
4 = 200-499
5 = 500+
CORPUS CHRISTI
BENTHIC FISHES
NUMBER OF INDIVIDUALS
FALL 1976 DAY

Figure 5.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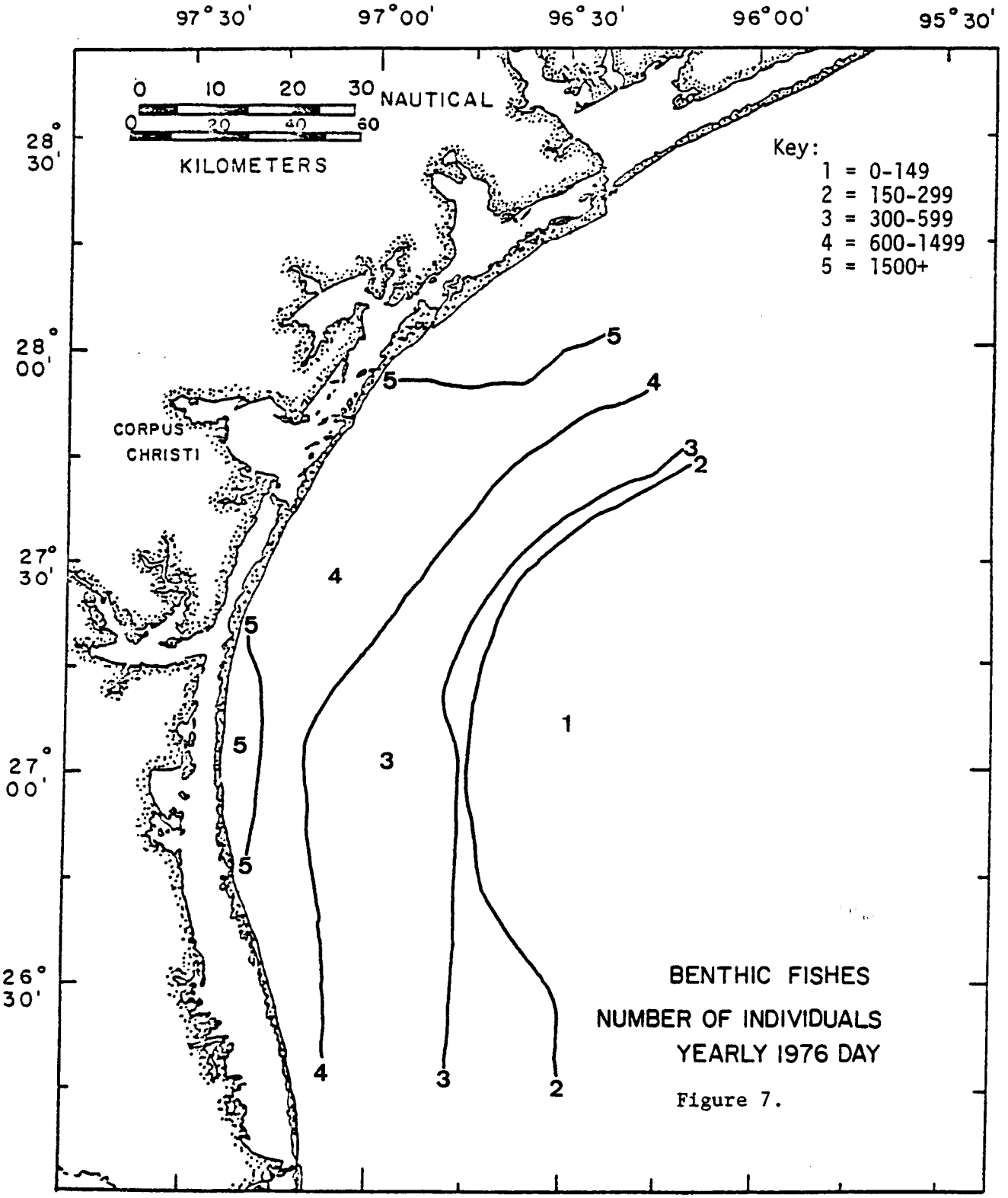
0 20 40 60
KILOMETERS
Key:
1 = 0-49
2 = 50-99
3 = 100-199
4 = 200-499
5 = 500+
CORPUS CHRISTI
BENTHIC FISHES
NUMBER OF INDIVIDUALS
FALL 1976 NIGHT

Figure 6.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27° 00'
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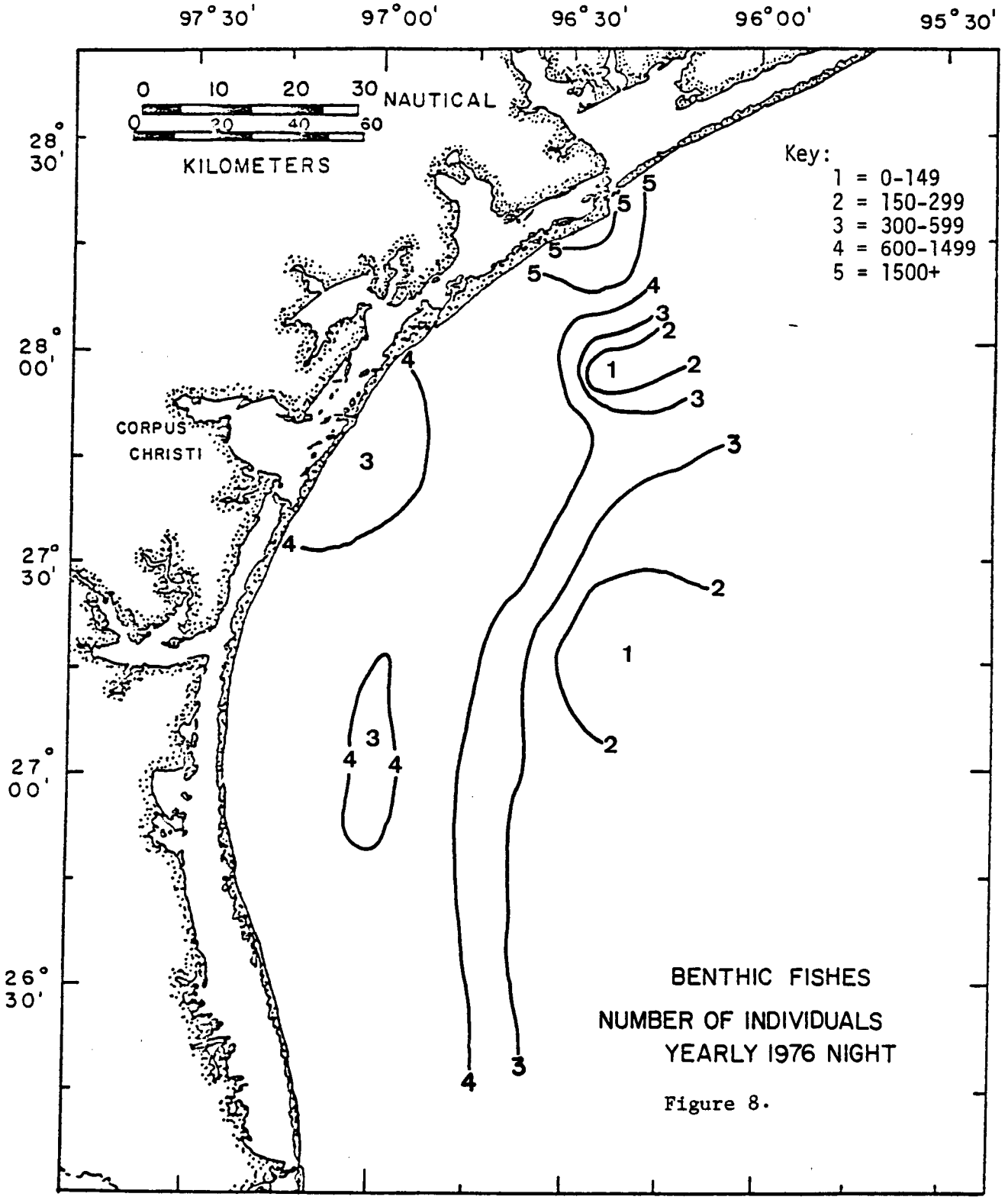
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-149
2 = 150-299
3 = 300-599
4 = 600-1499
5 = 1500+
BENTHIC FISHES
NUMBER OF INDIVIDUALS
YEARLY 1976 DAY

Figure 7.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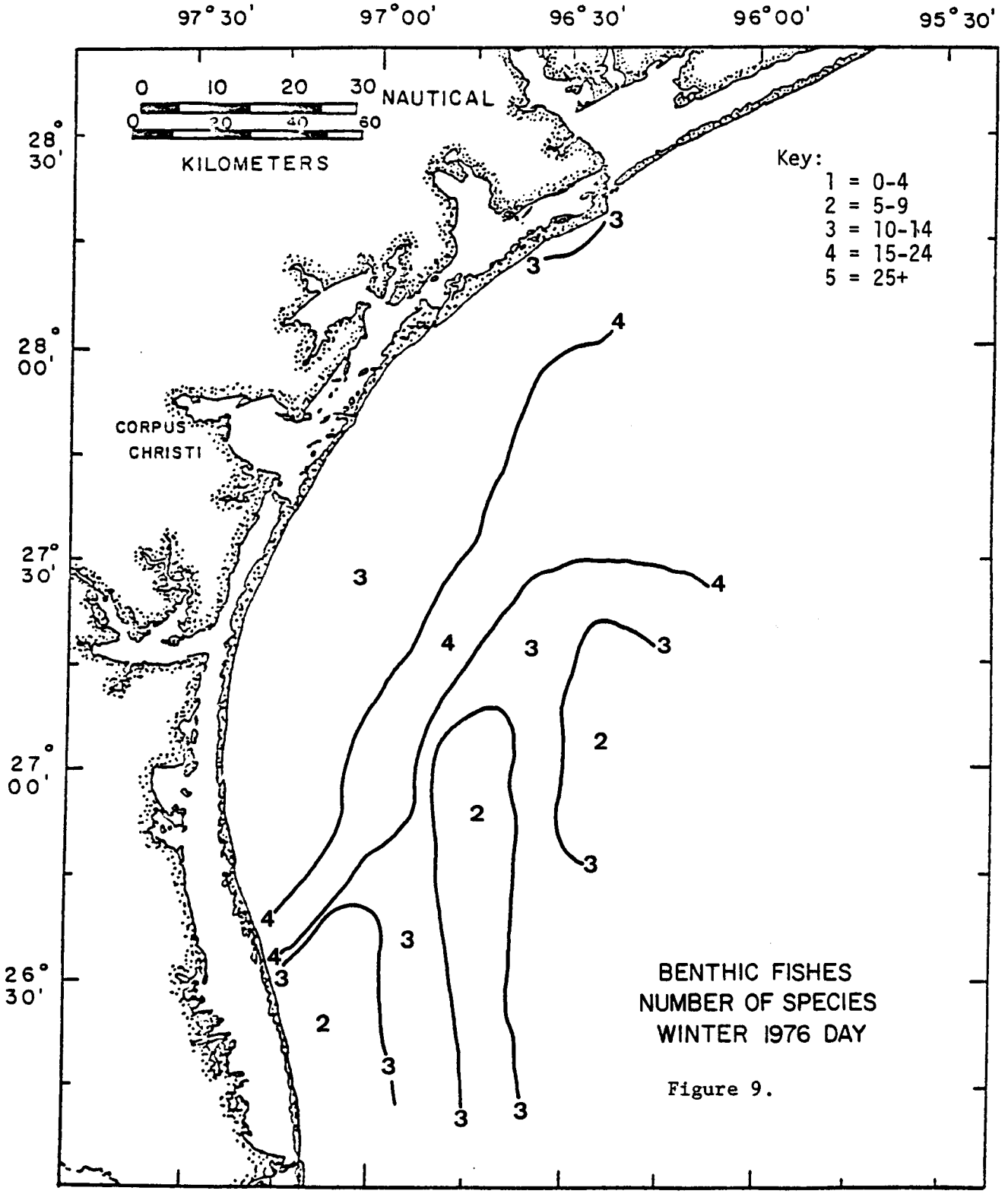
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-149
2 = 150-299
3 = 300-599
4 = 600-1499
5 = 1500+
BENTHIC FISHES
NUMBER OF INDIVIDUALS
YEARLY 1976 NIGHT

Figure 8.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95° 30'
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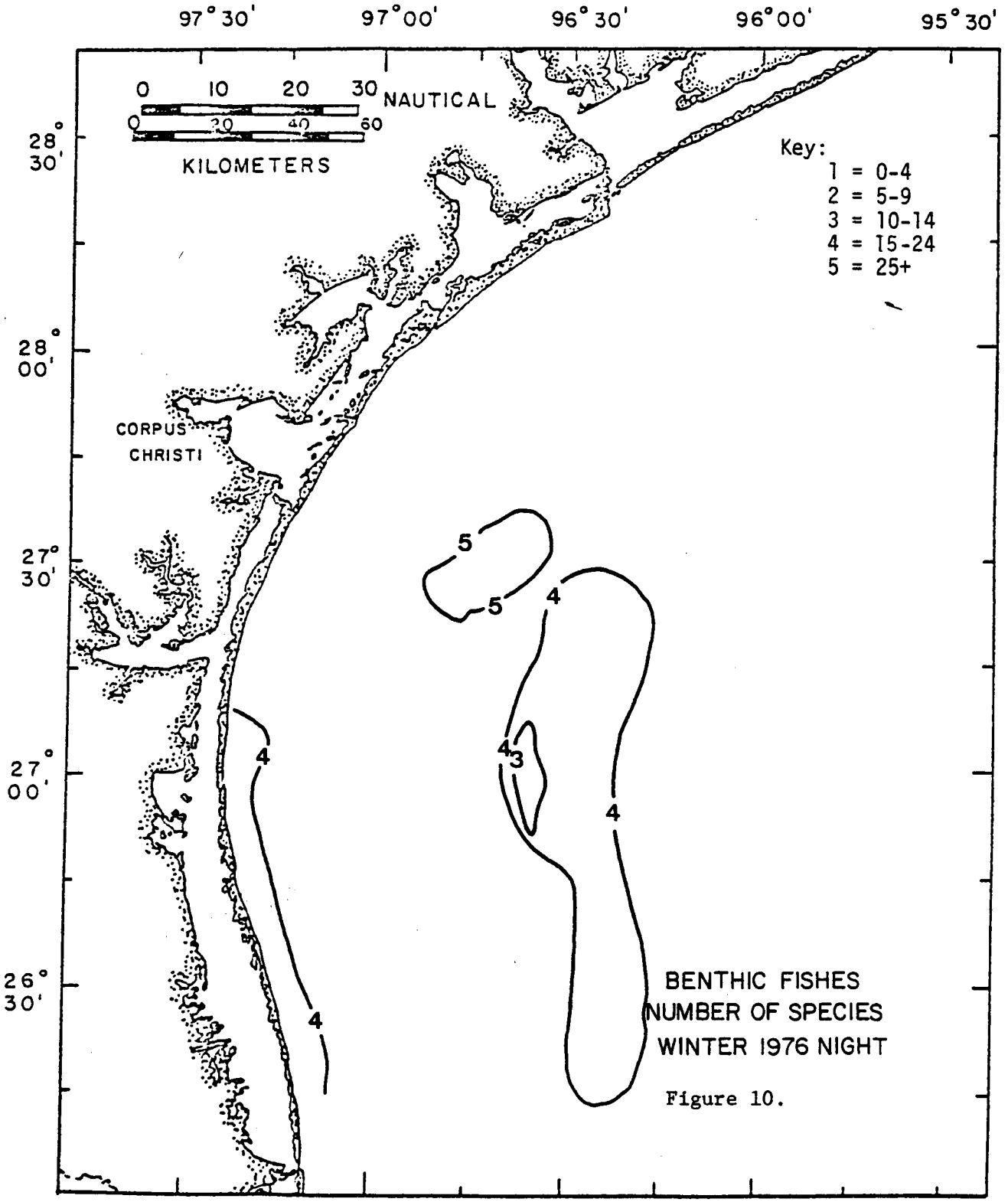
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-4
2 = 5-9
3 = 10-14
4 = 15-24
5 = 25+
BENTHIC FISHES
NUMBER OF SPECIES
WINTER 1976 DAY

Figure 9.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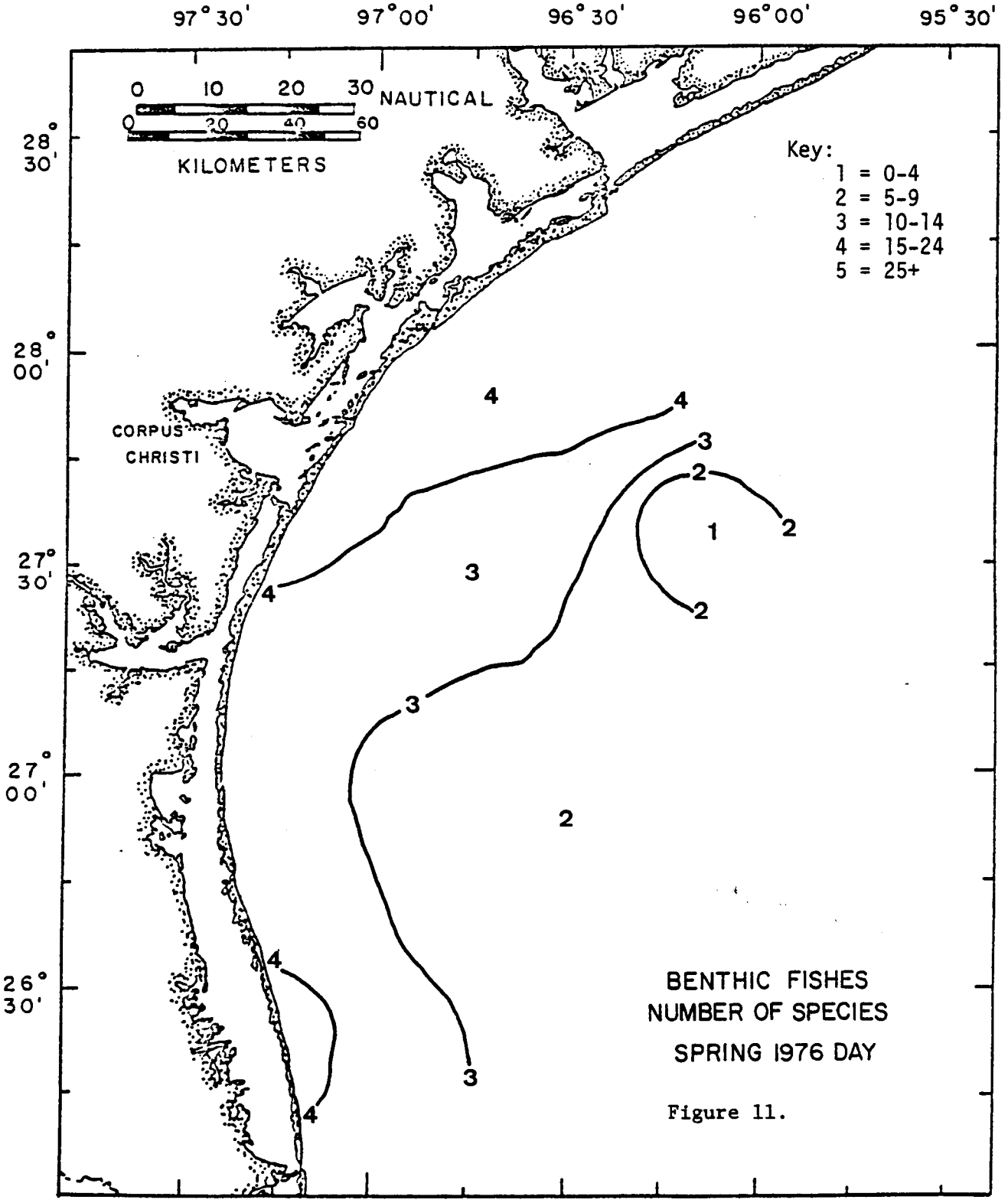
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
1 = 0-4
2 = 5-9
3 = 10-14
4 = 15-24
5 = 25+
CORPUS CHRISTI
5
5
4
4
3
4
4
4
BENTHIC FISHES
NUMBER OF SPECIES
WINTER 1976 NIGHT

Figure 10.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27° 00'
26° 30'
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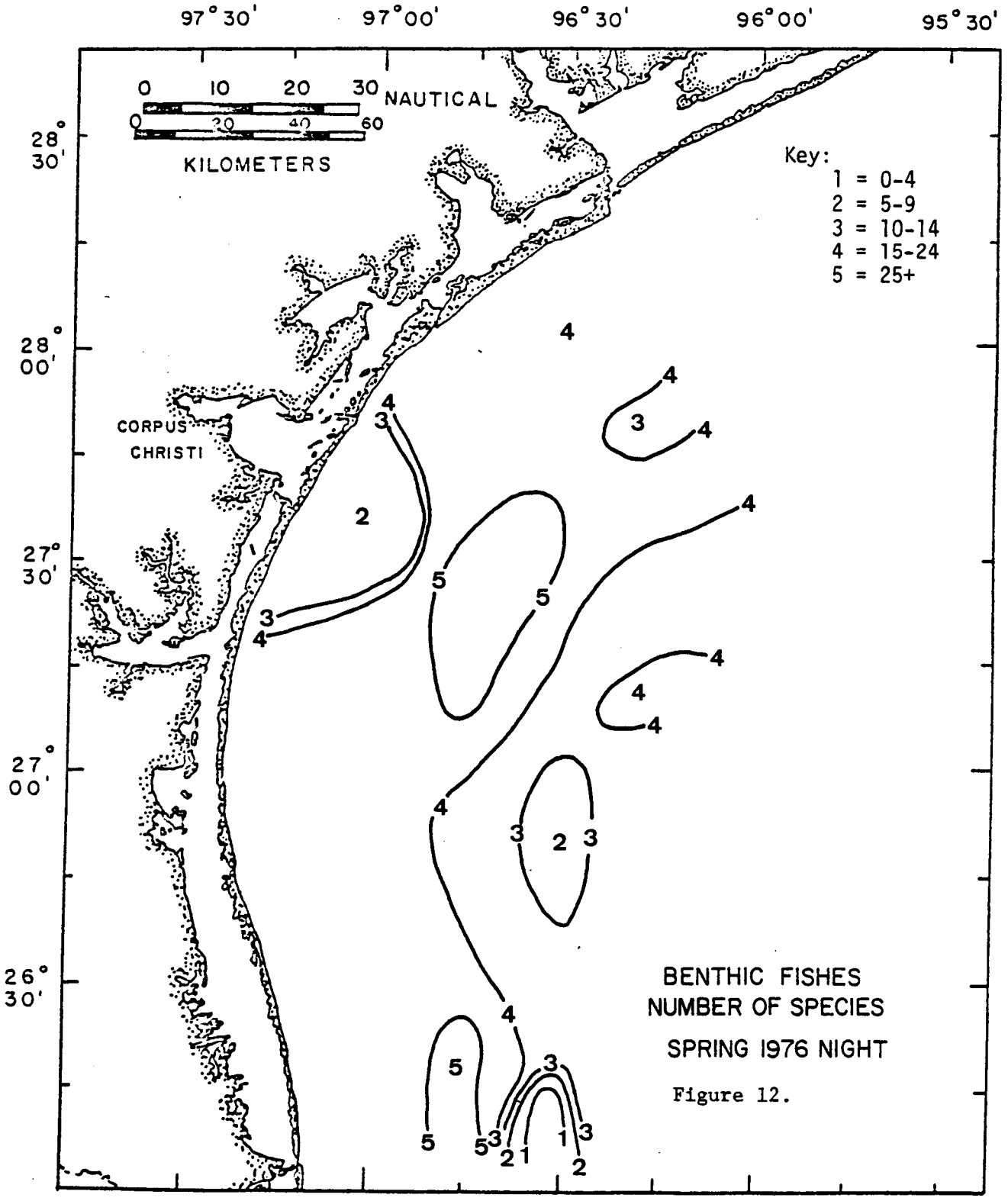
0 20 40 60
KILOMETERS
Key:
1 = 0-4
2 = 5-9
3 = 10-14
4 = 15-24
5 = 25+
CORPUS CHRISTI
BENTHIC FISHES
NUMBER OF SPECIES
SPRING 1976 DAY

Figure 11.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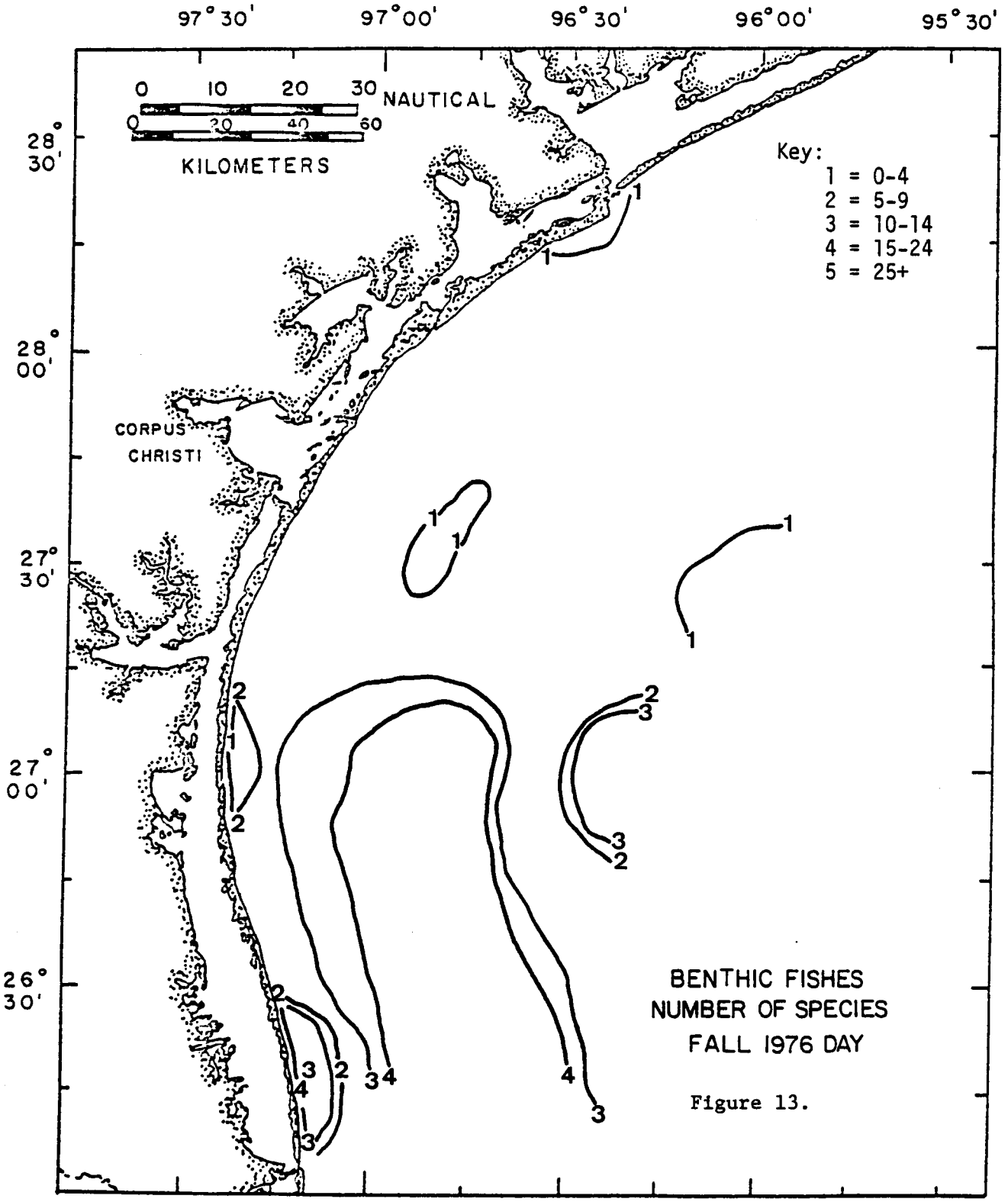
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-4
2 = 5-9
3 = 10-14
4 = 15-24
5 = 25+
BENTHIC FISHES
NUMBER OF SPECIES
SPRING 1976 NIGHT

Figure 12.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27° 00'
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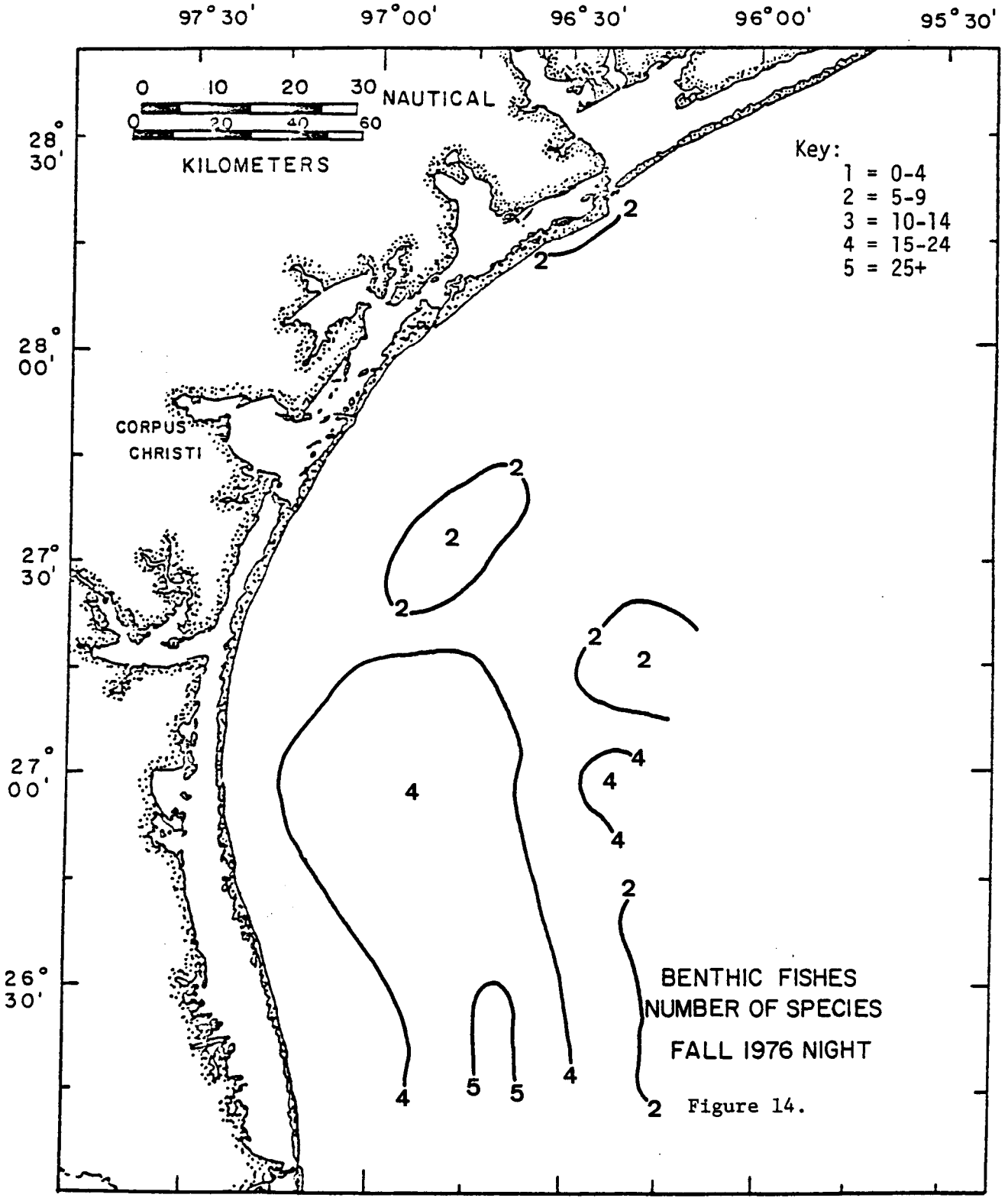
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-4
2 = 5-9
3 = 10-14
4 = 15-24
5 = 25+
BENTHIC FISHES
NUMBER OF SPECIES
FALL 1976 DAY

Figure 13.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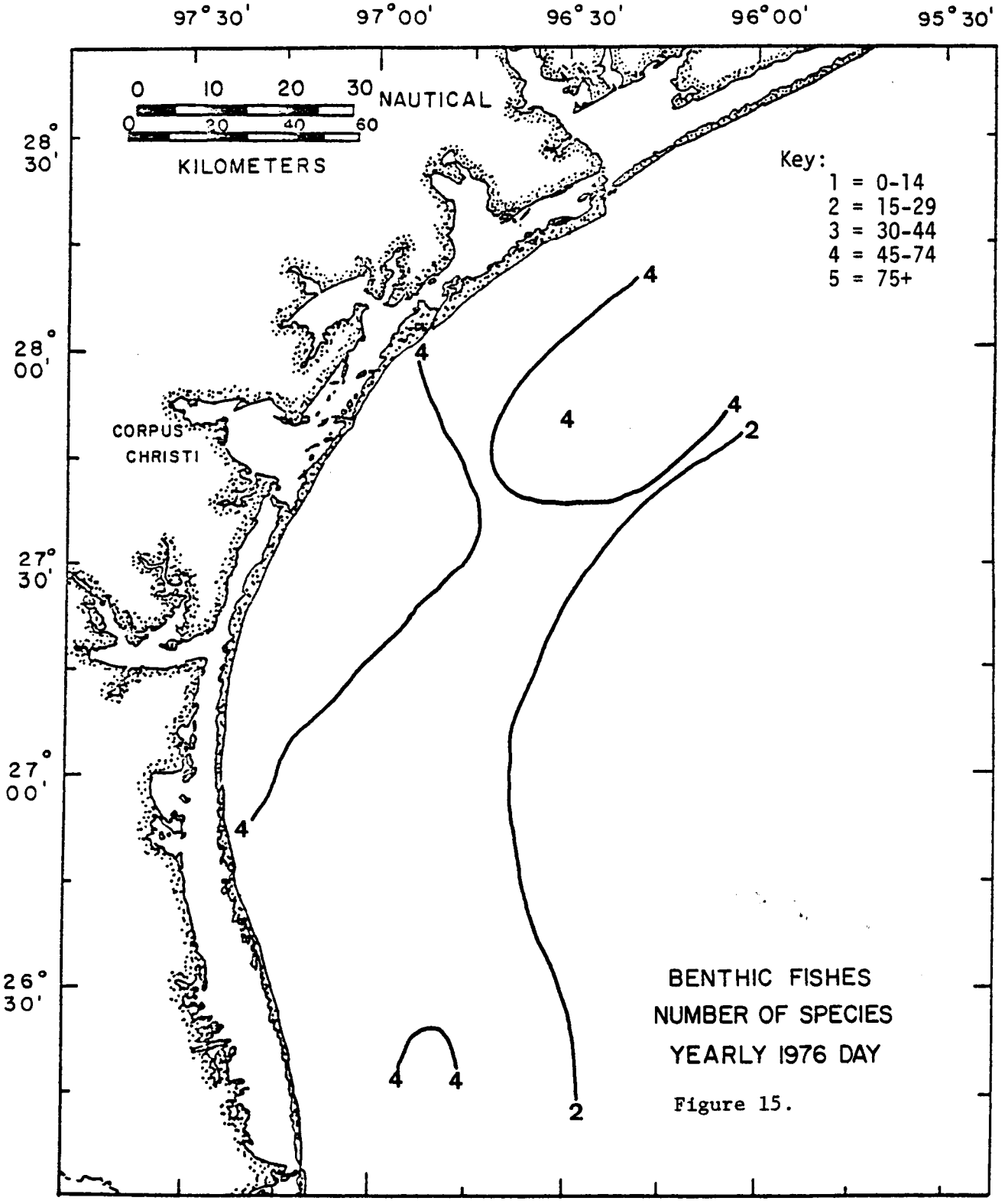
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-4
2 = 5-9
3 = 10-14
4 = 15-24
5 = 25+
BENTHIC FISHES
NUMBER OF SPECIES
FALL 1976 NIGHT

Figure 14.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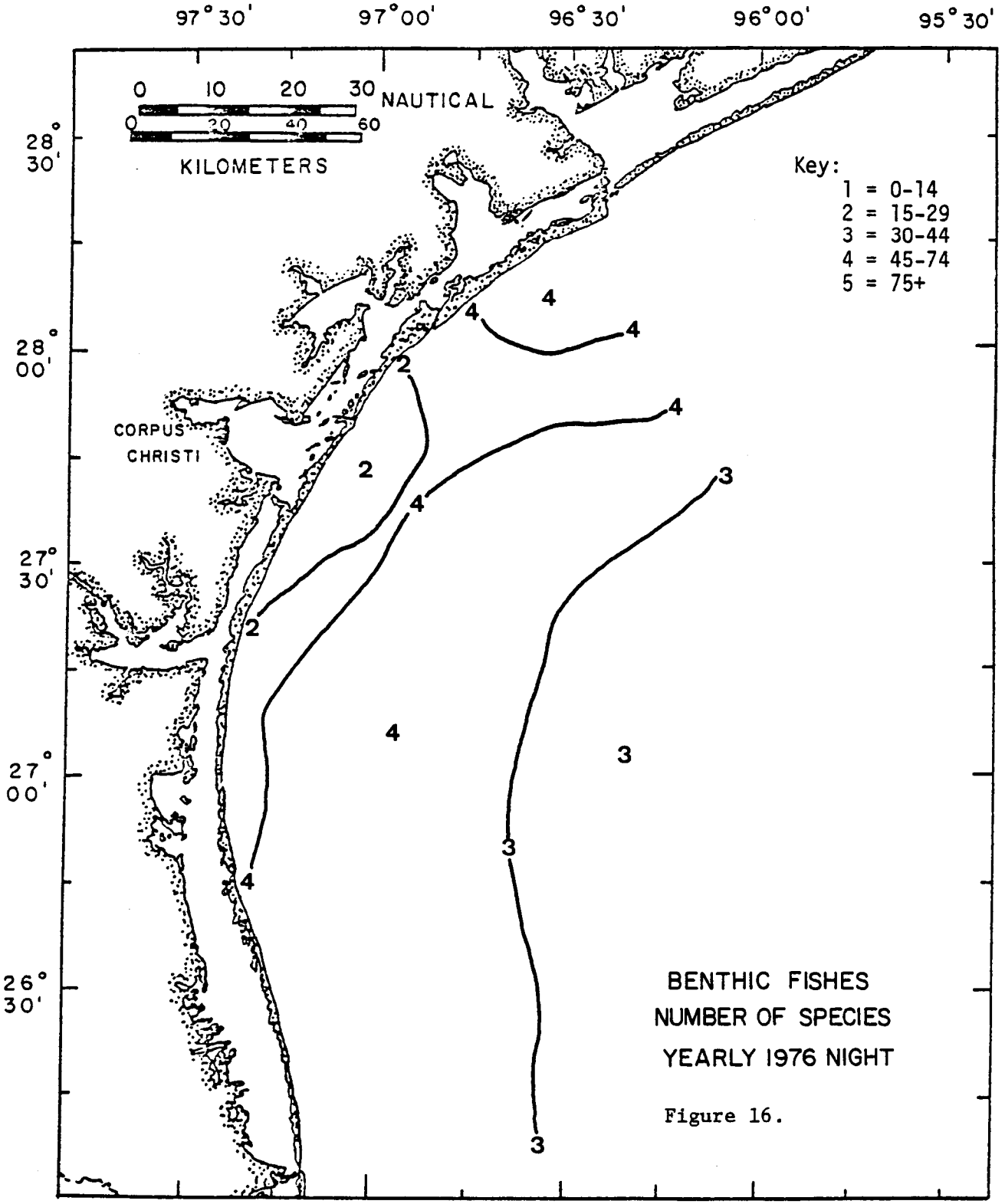
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-14
2 = 15-29
3 = 30-44
4 = 45-74
5 = 75+
BENTHIC FISHES
NUMBER OF SPECIES
YEARLY 1976 DAY

Figure 15.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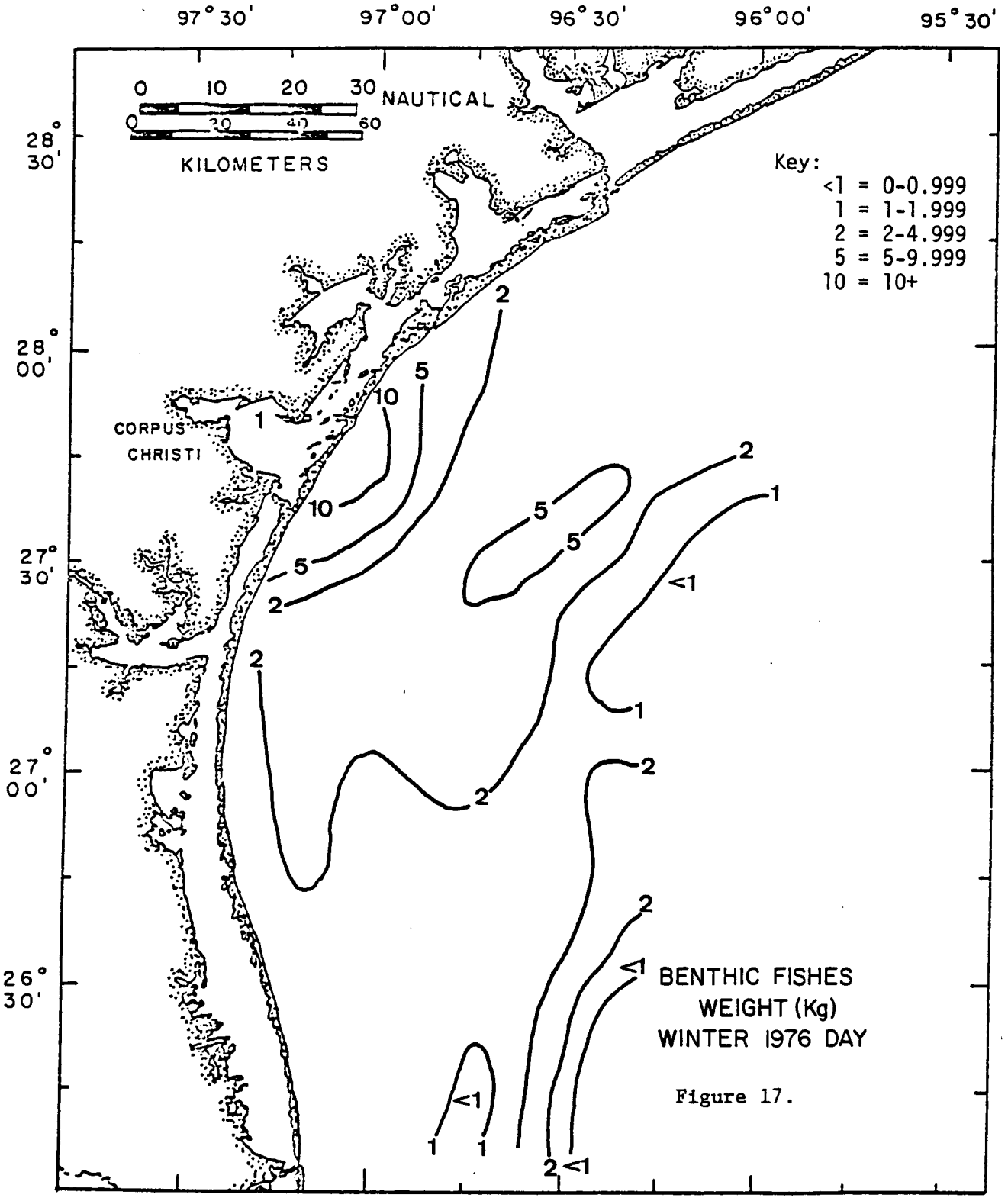
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-14
2 = 15-29
3 = 30-44
4 = 45-74
5 = 75+
BENTHIC FISHES
NUMBER OF SPECIES
YEARLY 1976 NIGHT

Figure 16.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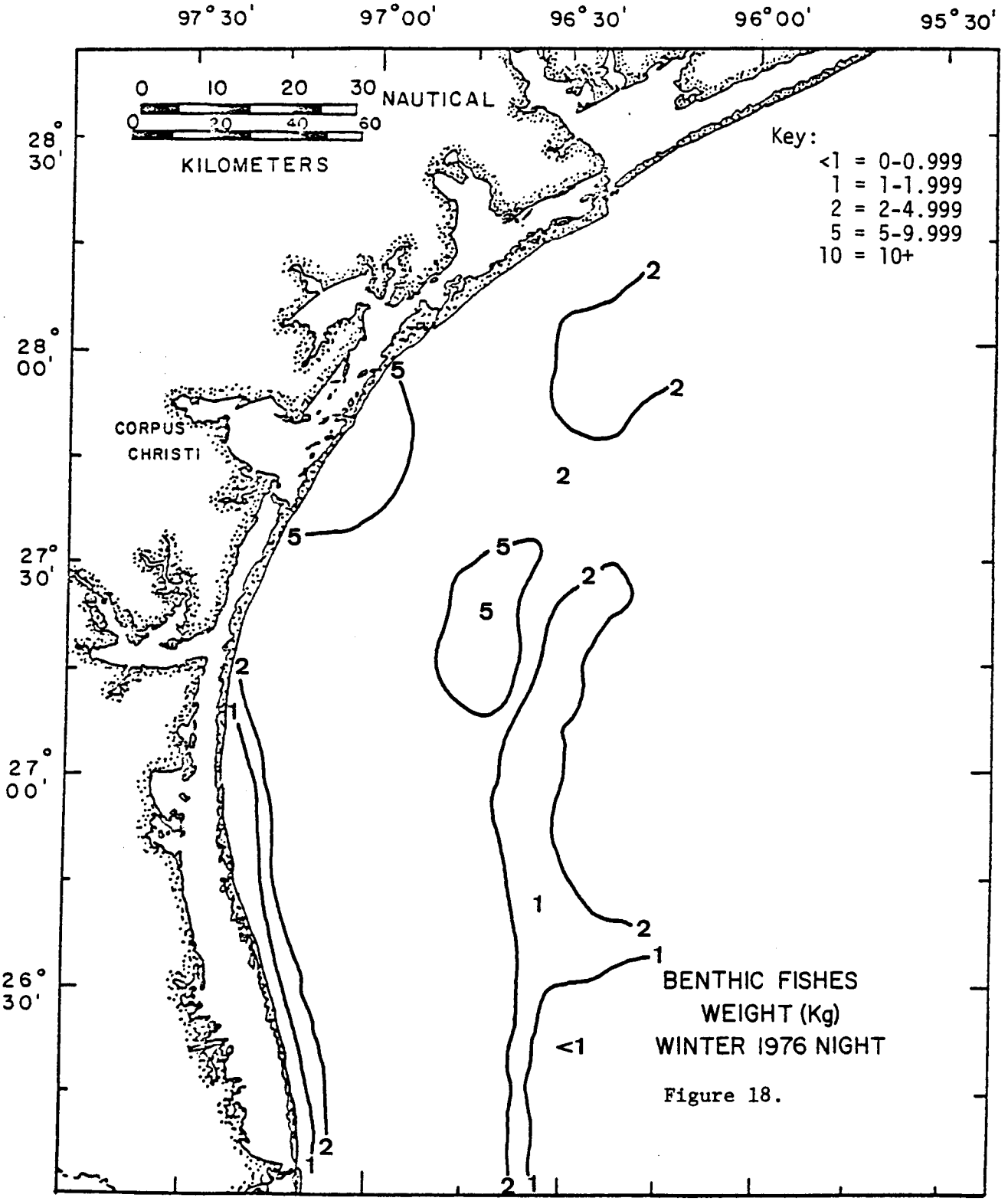
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
<1 = 0-0.999
1 = 1-1.999
2 = 2-4.999
5 = 5-9.999
10 = 10+
CORPUS CHRISTI
BENTHIC FISHES
WEIGHT (Kg)
WINTER 1976 DAY

Figure 17.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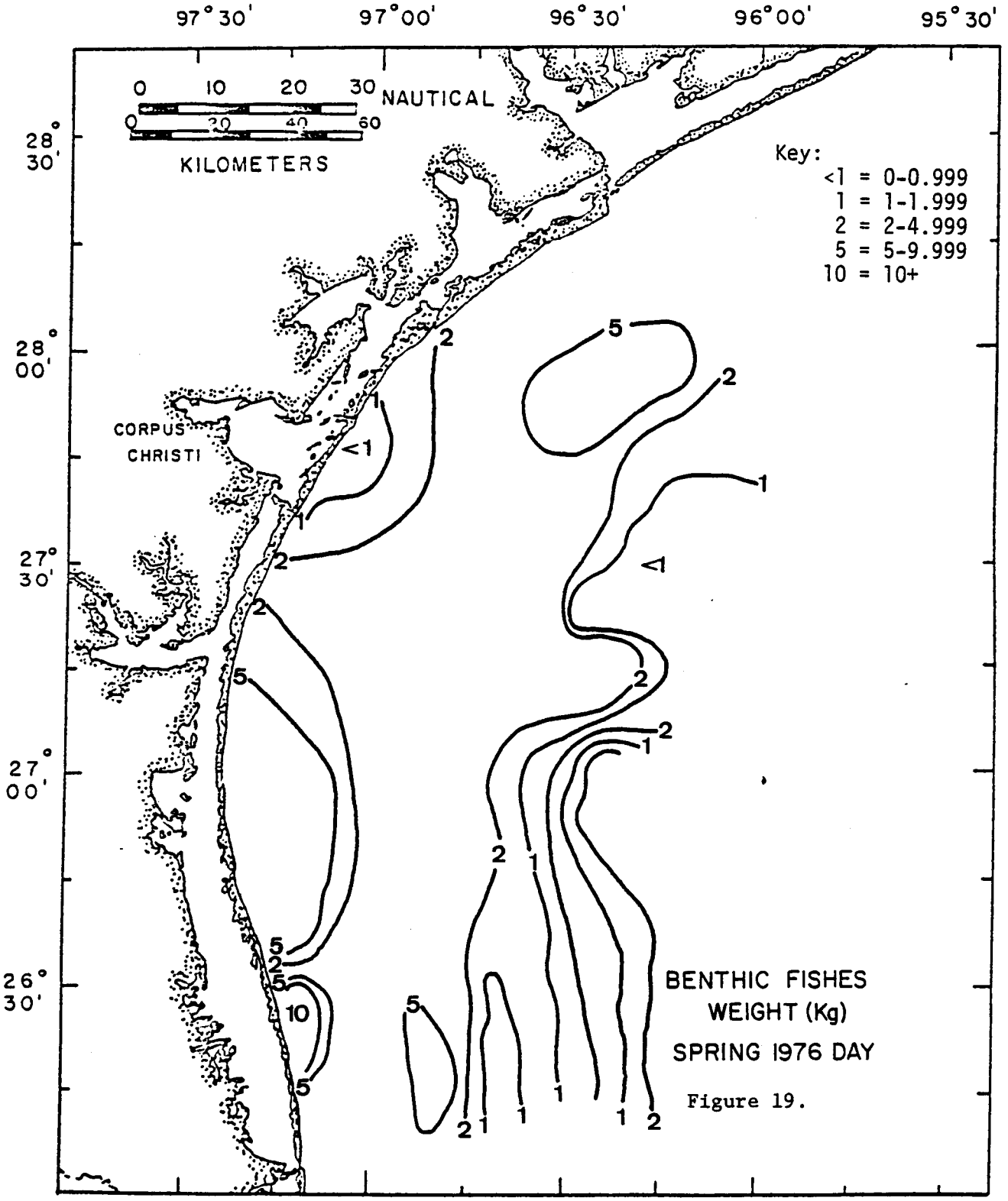
0 20 40 60
KILOMETERS
Key:
<1 = 0-0.999
1 = 1-1.999
2 = 2-4.999
5 = 5-9.999
10 = 10+
CORPUS CHRISTI
BENTHIC FISHES
WEIGHT (Kg)
WINTER 1976 NIGHT

Figure 18.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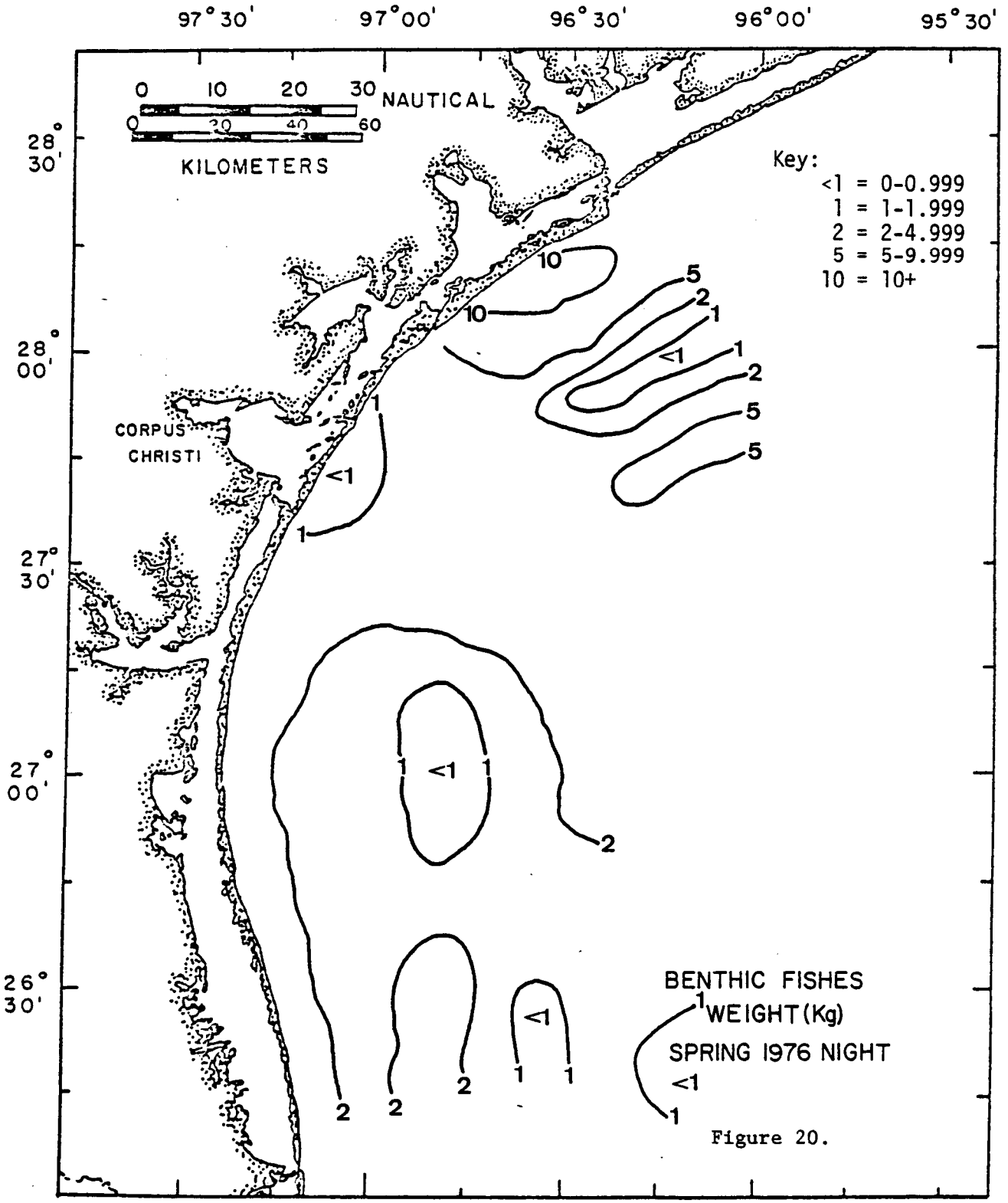
0 20 40 60
KILOMETERS
Key:
<1 = 0-0.999
1 = 1-1.999
2 = 2-4.999
5 = 5-9.999
10 = 10+
CORPUS CHRISTI
BENTHIC FISHES
WEIGHT (Kg)
SPRING 1976 DAY

Figure 19.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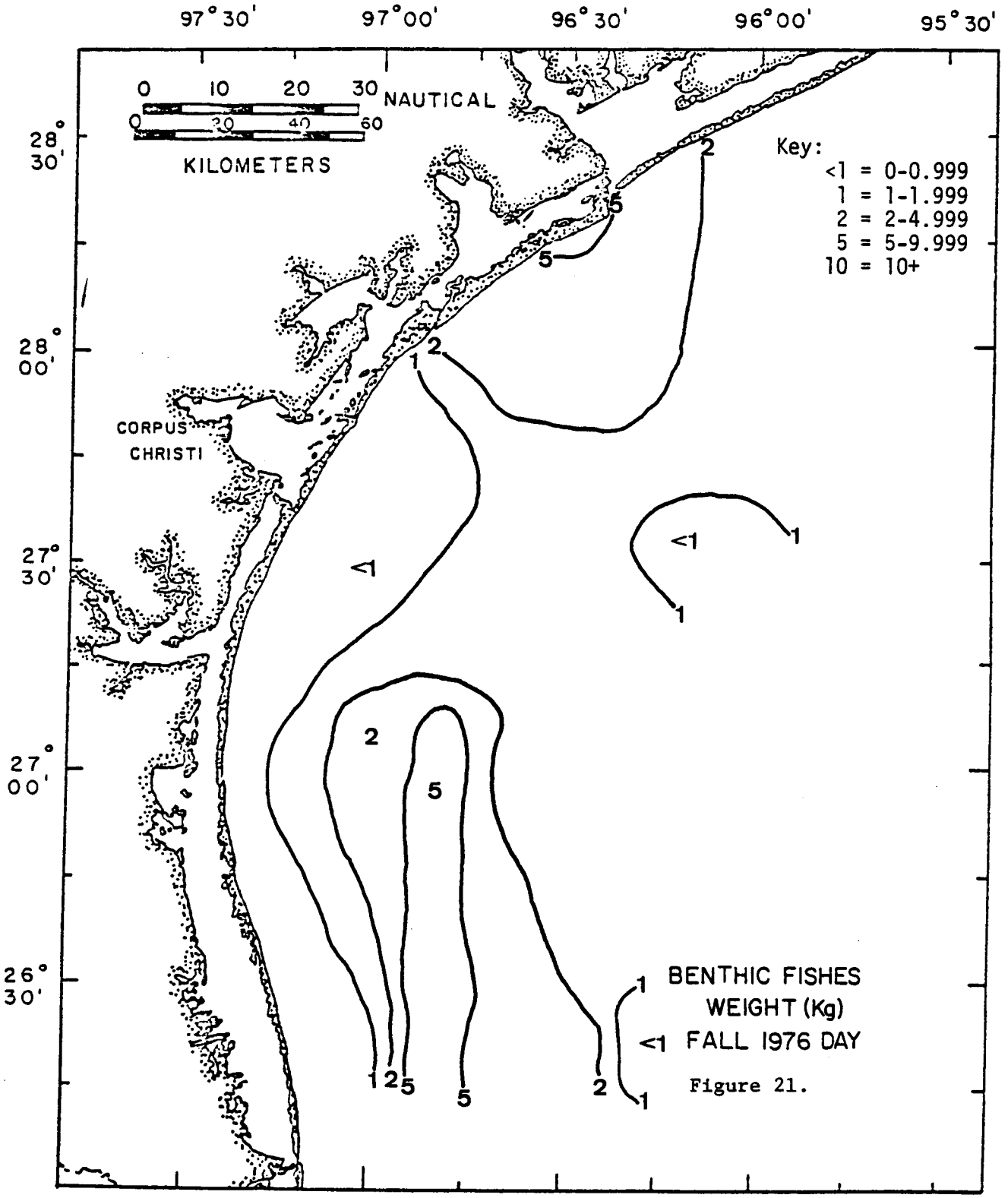
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
<1 = 0-0.999
1 = 1-1.999
2 = 2-4.999
5 = 5-9.999
10 = 10+
CORPUS CHRISTI
BENTHIC FISHES
WEIGHT (Kg)
SPRING 1976 NIGHT

Figure 20.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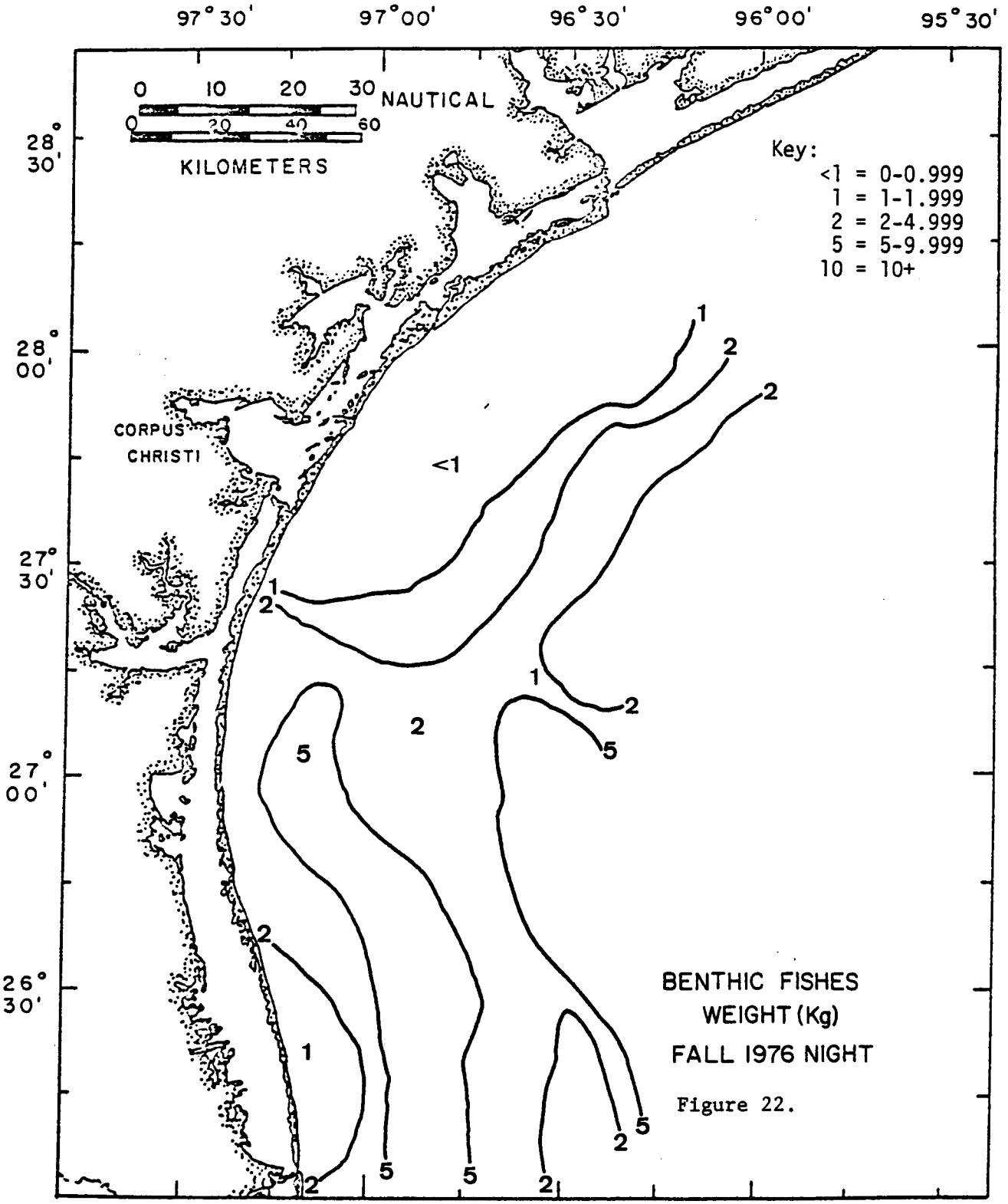
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
<1 = 0-0.999
1 = 1-1.999
2 = 2-4.999
5 = 5-9.999
10 = 10+
BENTHIC FISHES
WEIGHT (Kg)
FALL 1976 DAY

Figure 21.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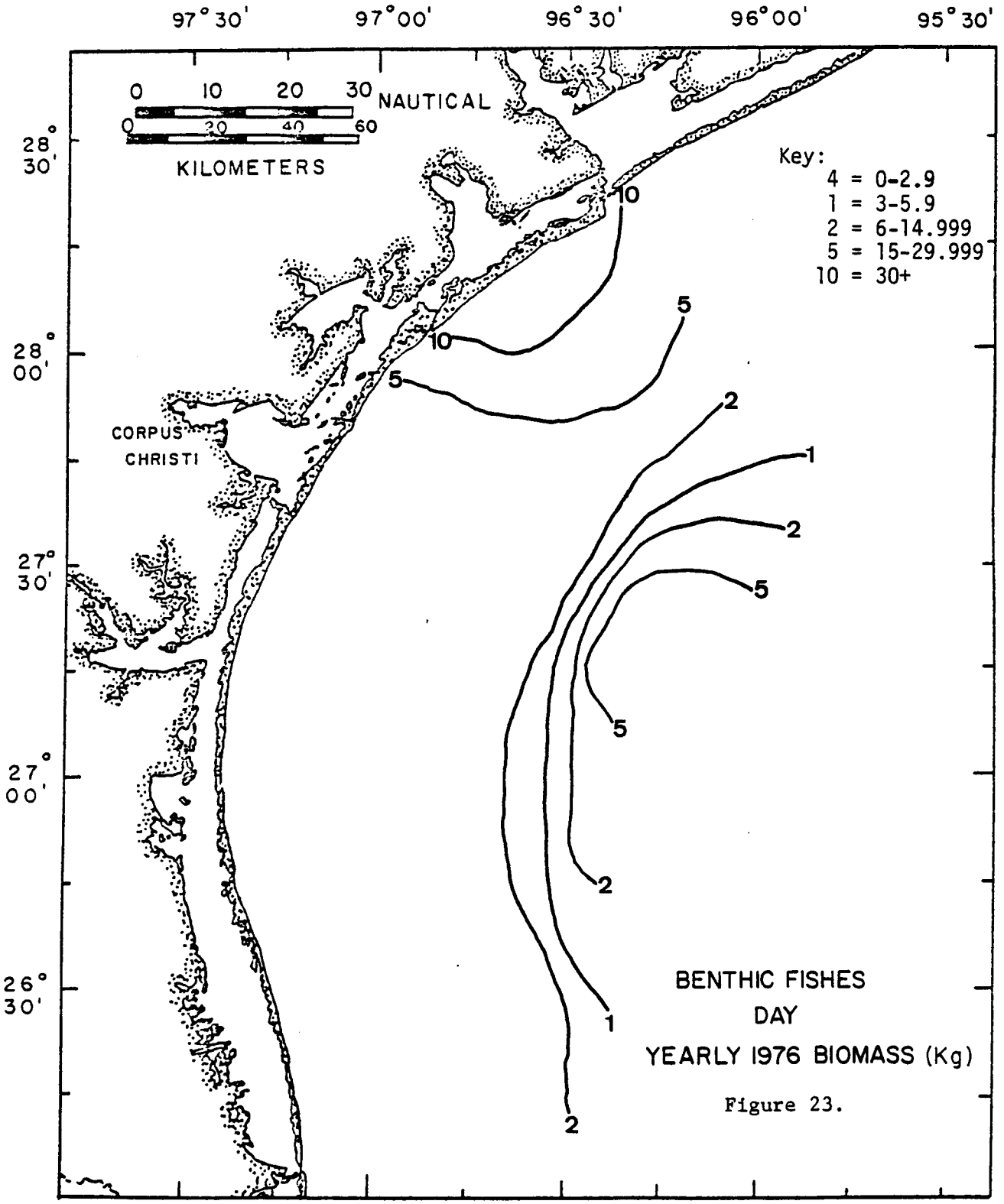
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
<1 = 0-0.999
1 = 1-1.999
2 = 2-4.999
5 = 5-9.999
10 = 10+
BENTHIC FISHES
WEIGHT (Kg)
FALL 1976 NIGHT

Figure 22.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0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
4 = 0-2.9
1 = 3-5.9
2 = 6-14.999
5 = 15-29.999
10 = 30+
10
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10
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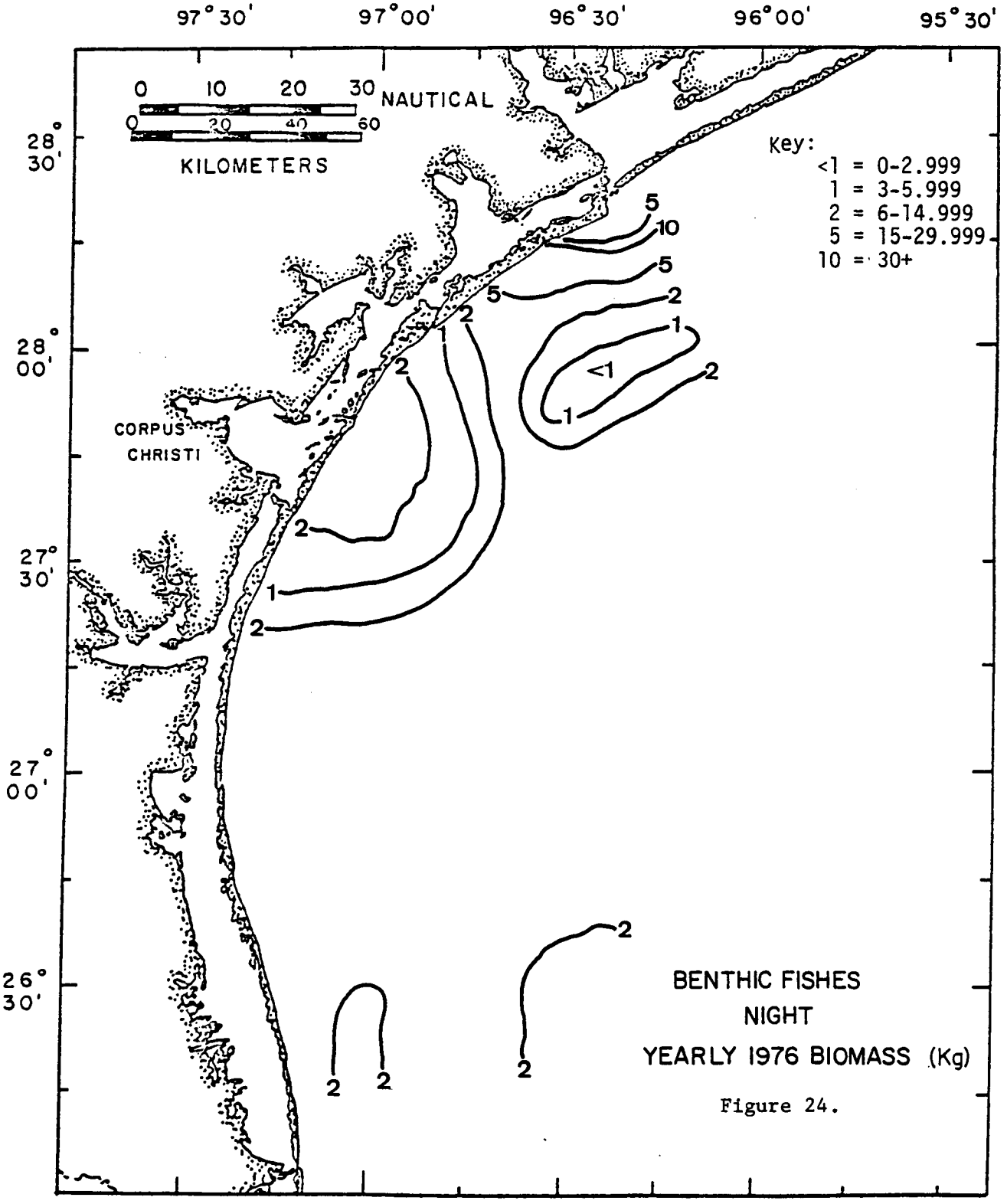
2
BENTHIC FISHES
DAY
YEARLY 1976 BIOMASS (Kg)

Figure 23.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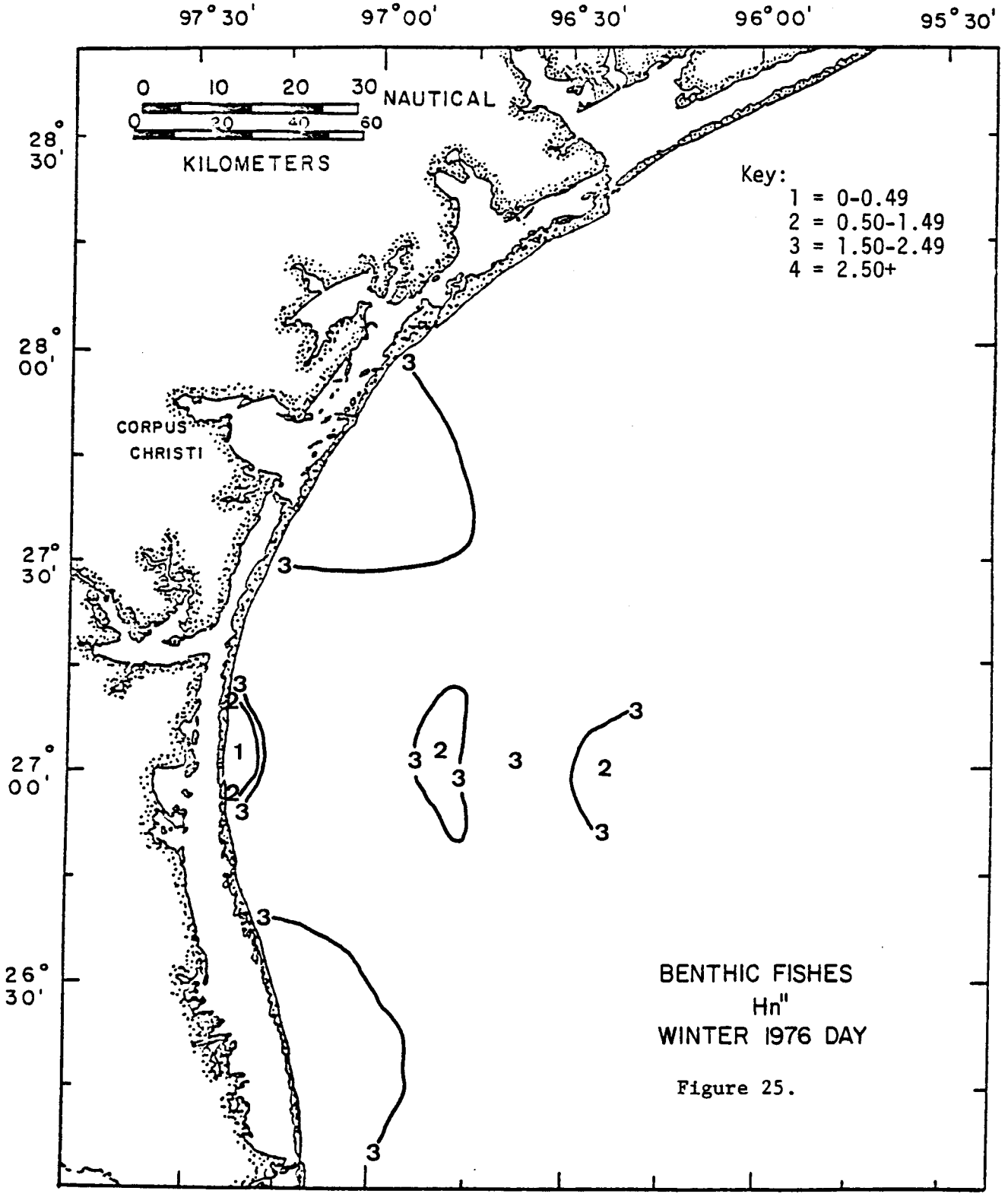
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
<1 = 0-2.999
1 = 3-5.999
2 = 6-14.999
5 = 15-29.999
10 = 30+
BENTHIC FISHES
NIGHT
YEARLY 1976 BIOMASS (Kg)

Figure 24.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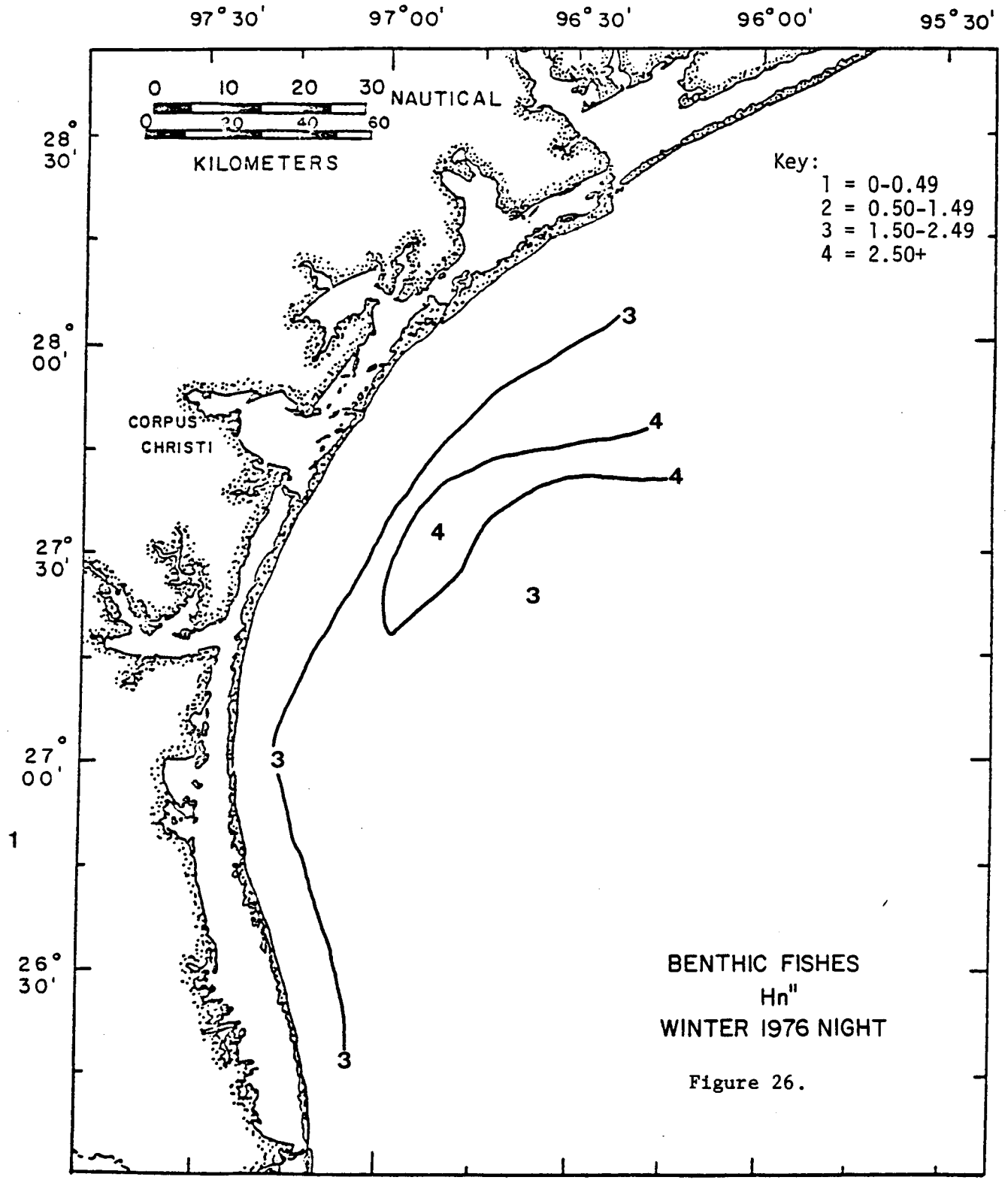
26° 30'
0 10 20 30 NAUTICAL
0 30 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-1.49
3 = 1.50-2.49
4 = 2.50+
BENTHIC FISHES
Hn"
WINTER 1976 DAY

Figure 25.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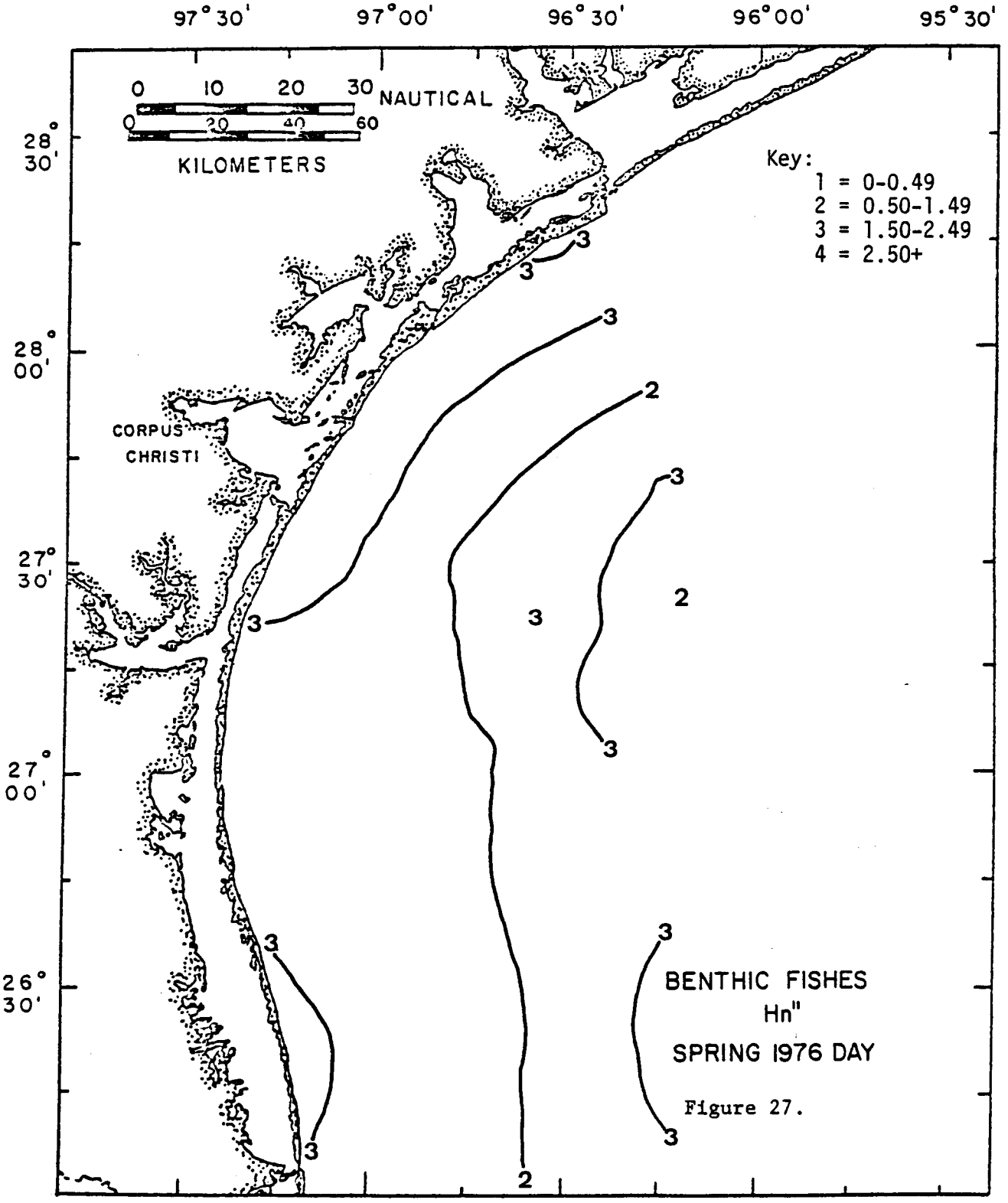
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
1 = 0-0.49
2 = 0.50-1.49
3 = 1.50-2.49
4 = 2.50+
CORPUS CHRISTI
3
4
4
4
3
3
3
1
BENTHIC FISHES
Hn"
WINTER 1976 NIGHT

Figure 26.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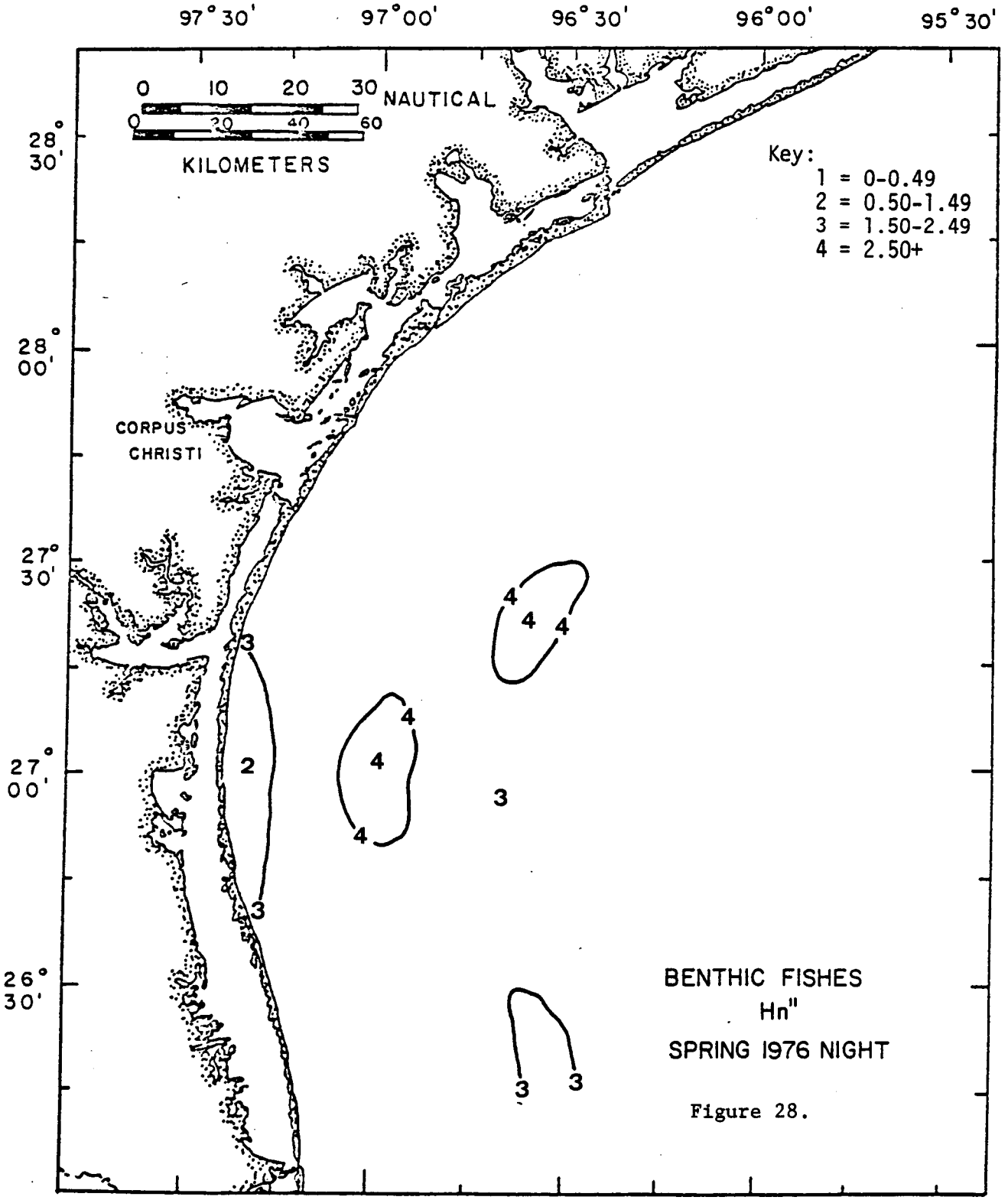
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-1.49
3 = 1.50-2.49
4 = 2.50+
BENTHIC FISHES
Hn"
SPRING 1976 DAY

Figure 27.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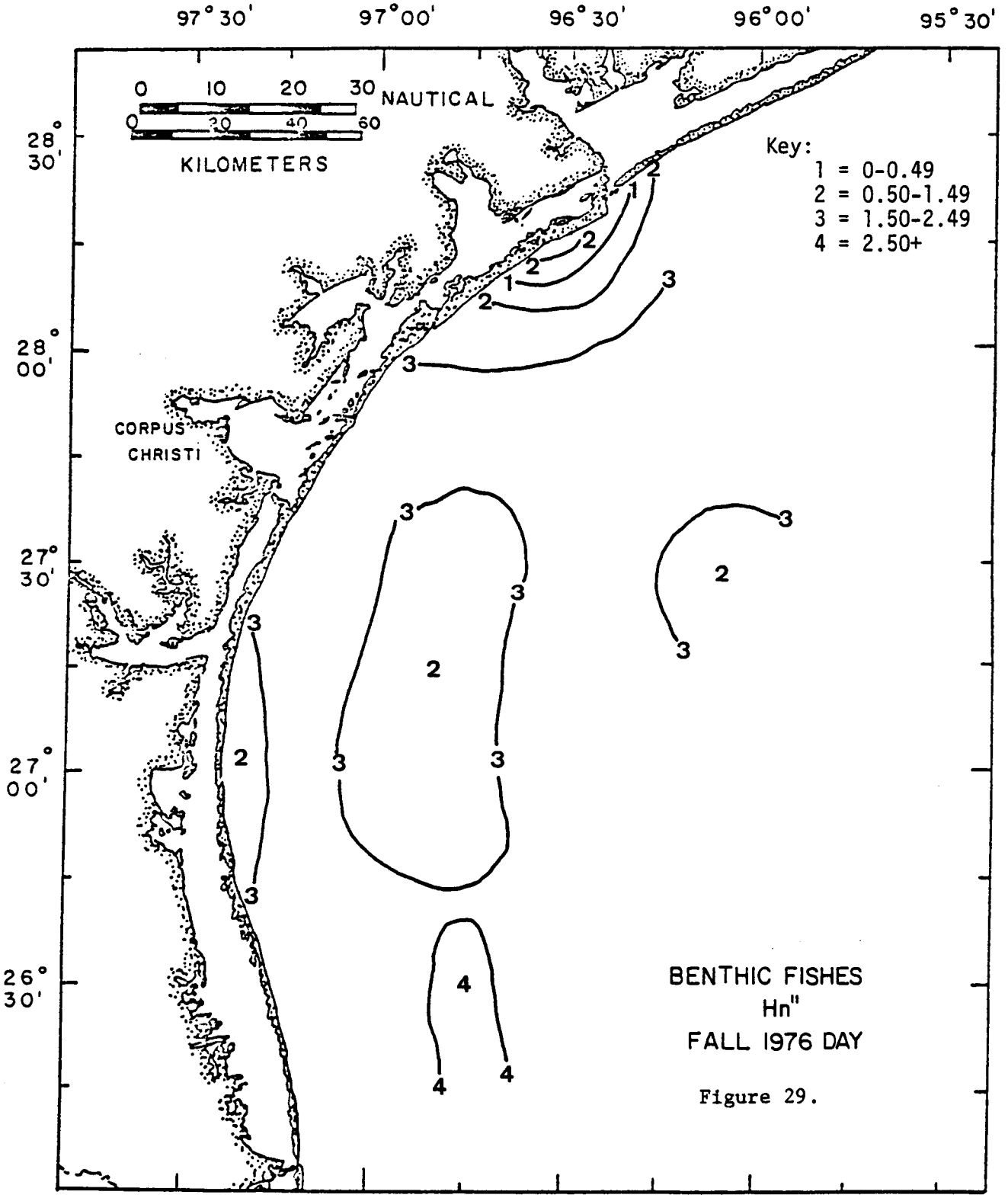
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-1.49
3 = 1.50-2.49
4 = 2.50+
BENTHIC FISHES
Hn"
SPRING 1976 NIGHT

Figure 28.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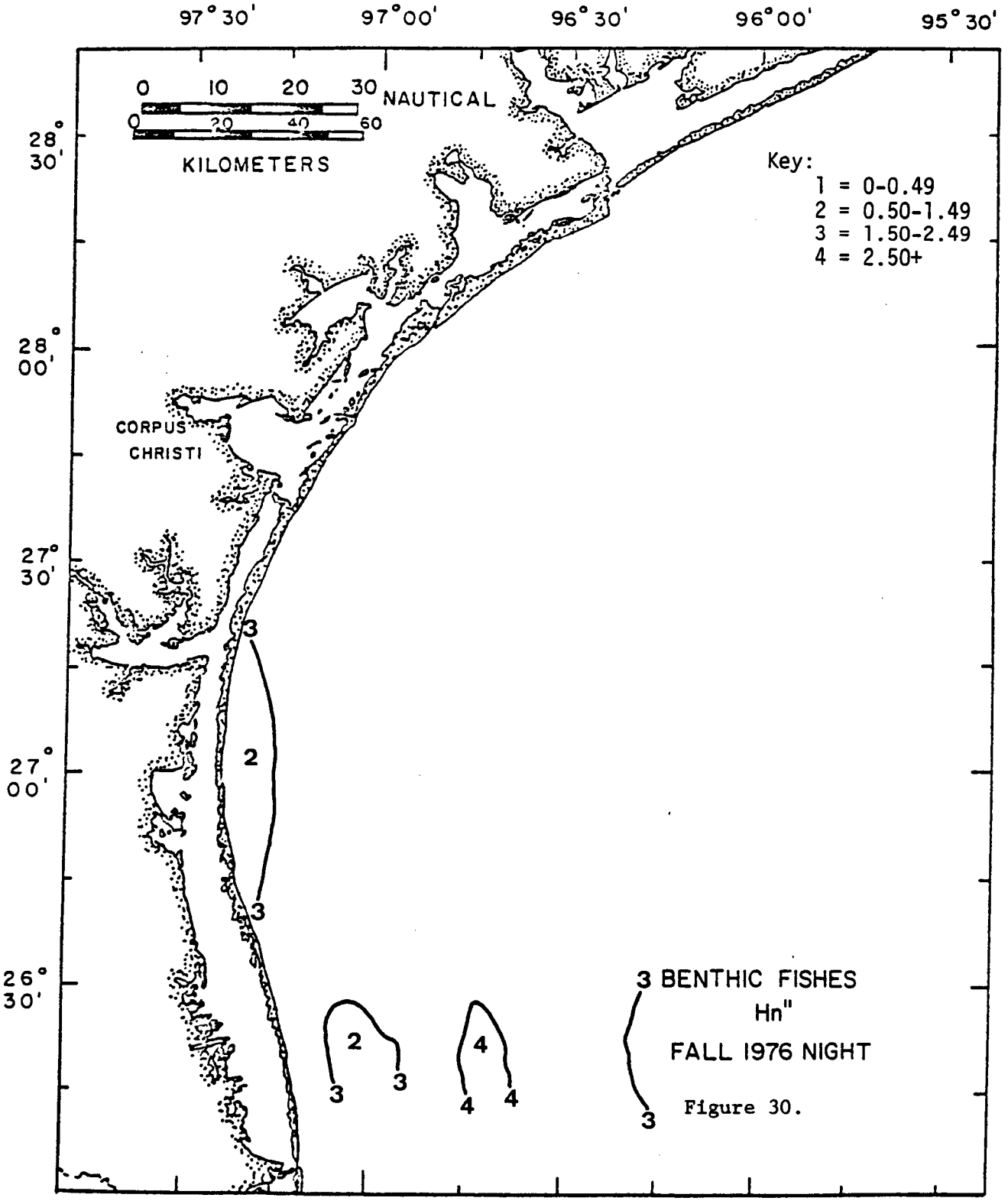
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-1.49
3 = 1.50-2.49
4 = 2.50+
BENTHIC FISHES
Hn"
FALL 1976 DAY

Figure 29.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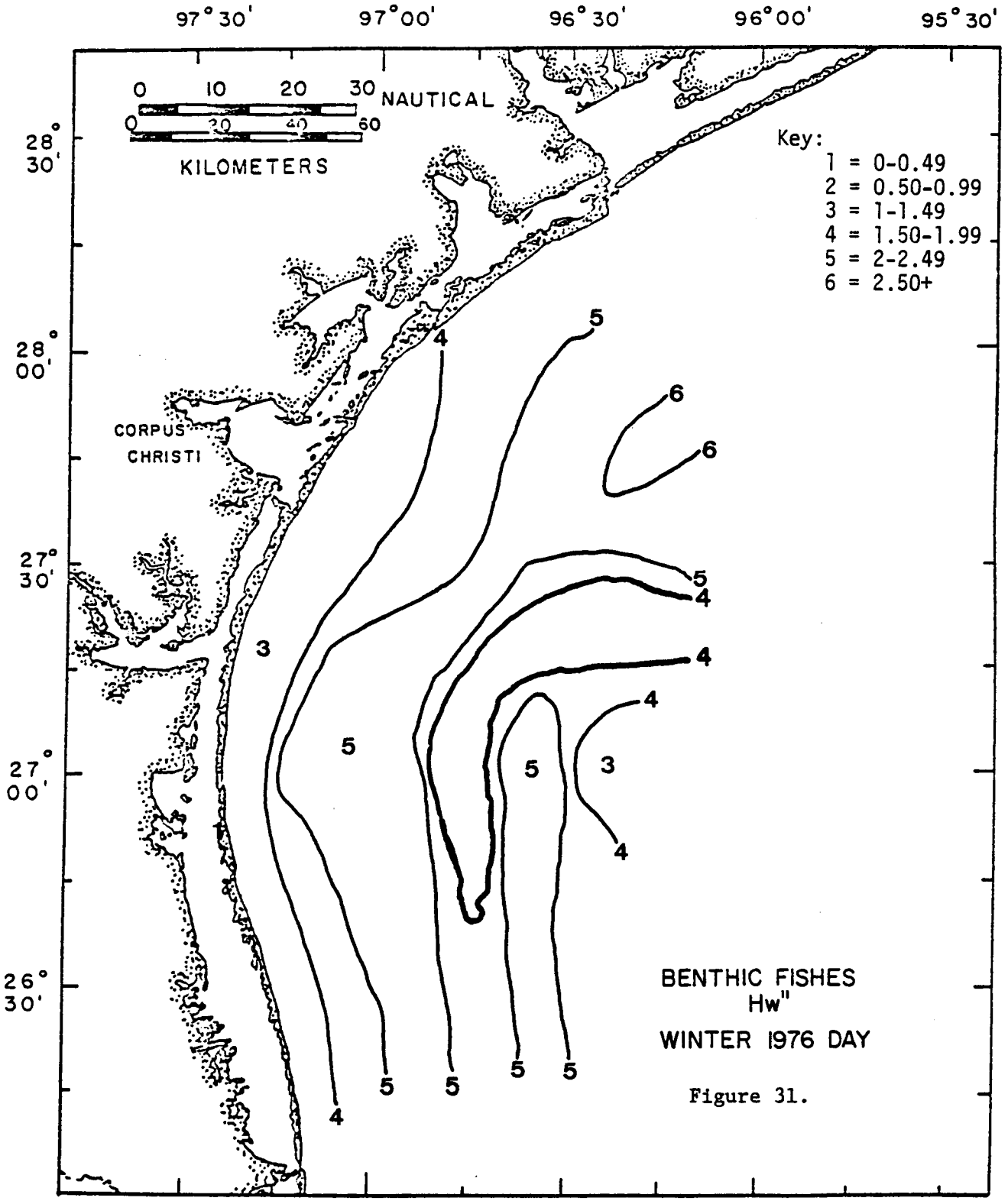
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
1 = 0-0.49
2 = 0.50-1.49
3 = 1.50-2.49
4 = 2.50+
CORPUS CHRISTI
3 BENTHIC FISHES
Hn"
FALL 1976 NIGHT

Figure 30.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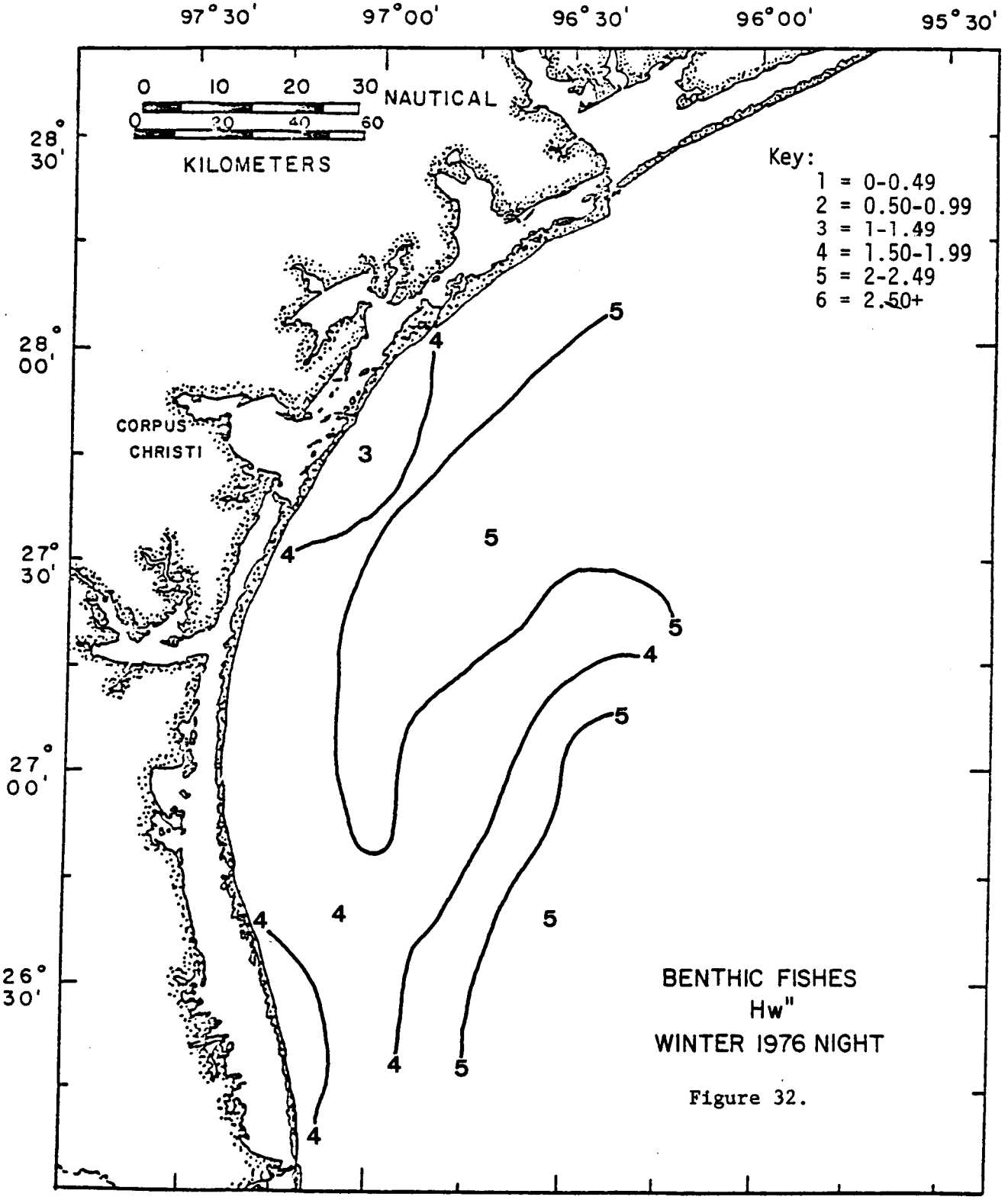
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-0.99
3 = 1-1.49
4 = 1.50-1.99
5 = 2-2.49
6 = 2.50+
BENTHIC FISHES
Hw"
WINTER 1976 DAY

Figure 31.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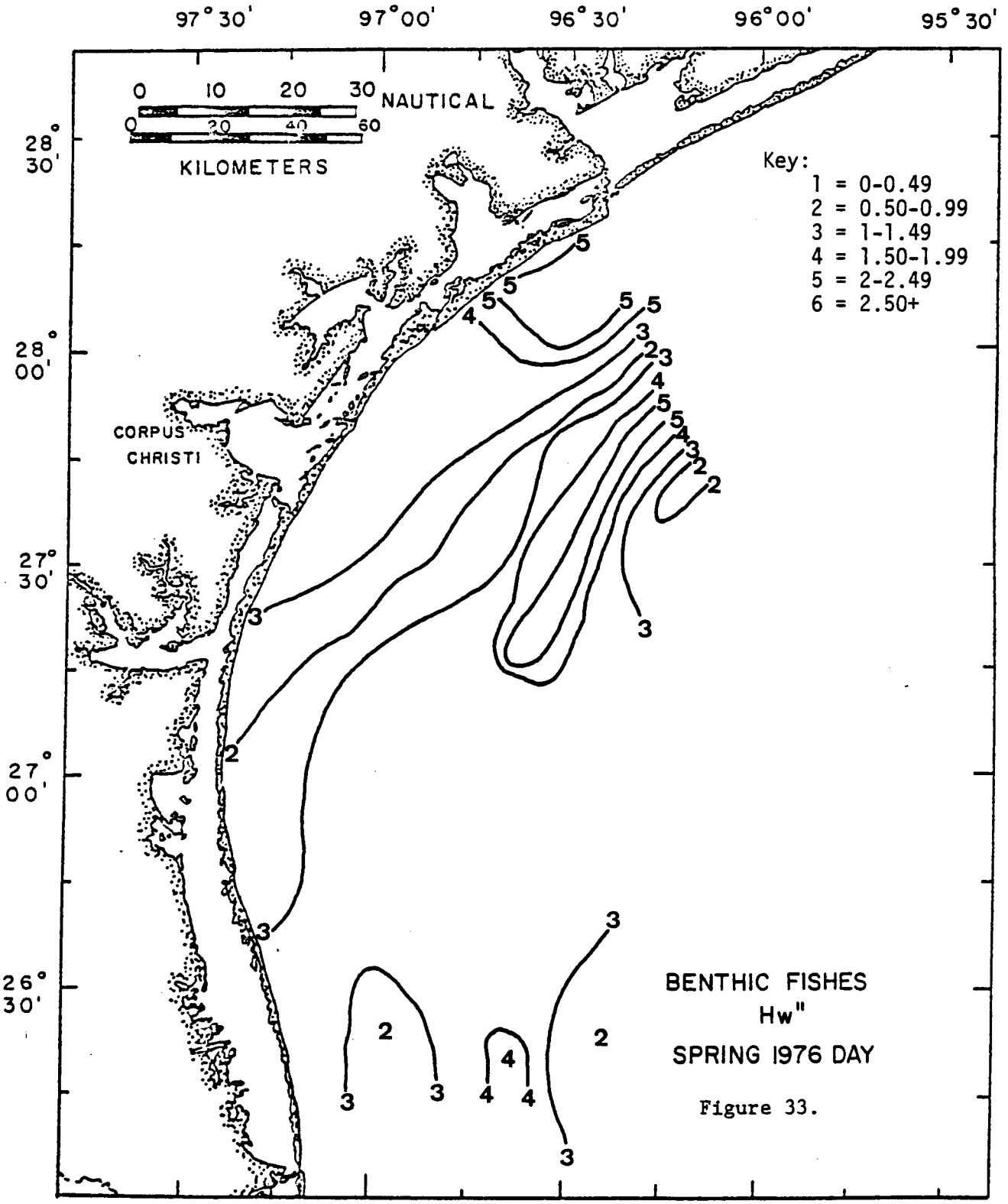
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-0.99
3 = 1-1.49
4 = 1.50-1.99
5 = 2-2.49
6 = 2.50+
BENTHIC FISHES
Hw"
WINTER 1976 NIGHT

Figure 32.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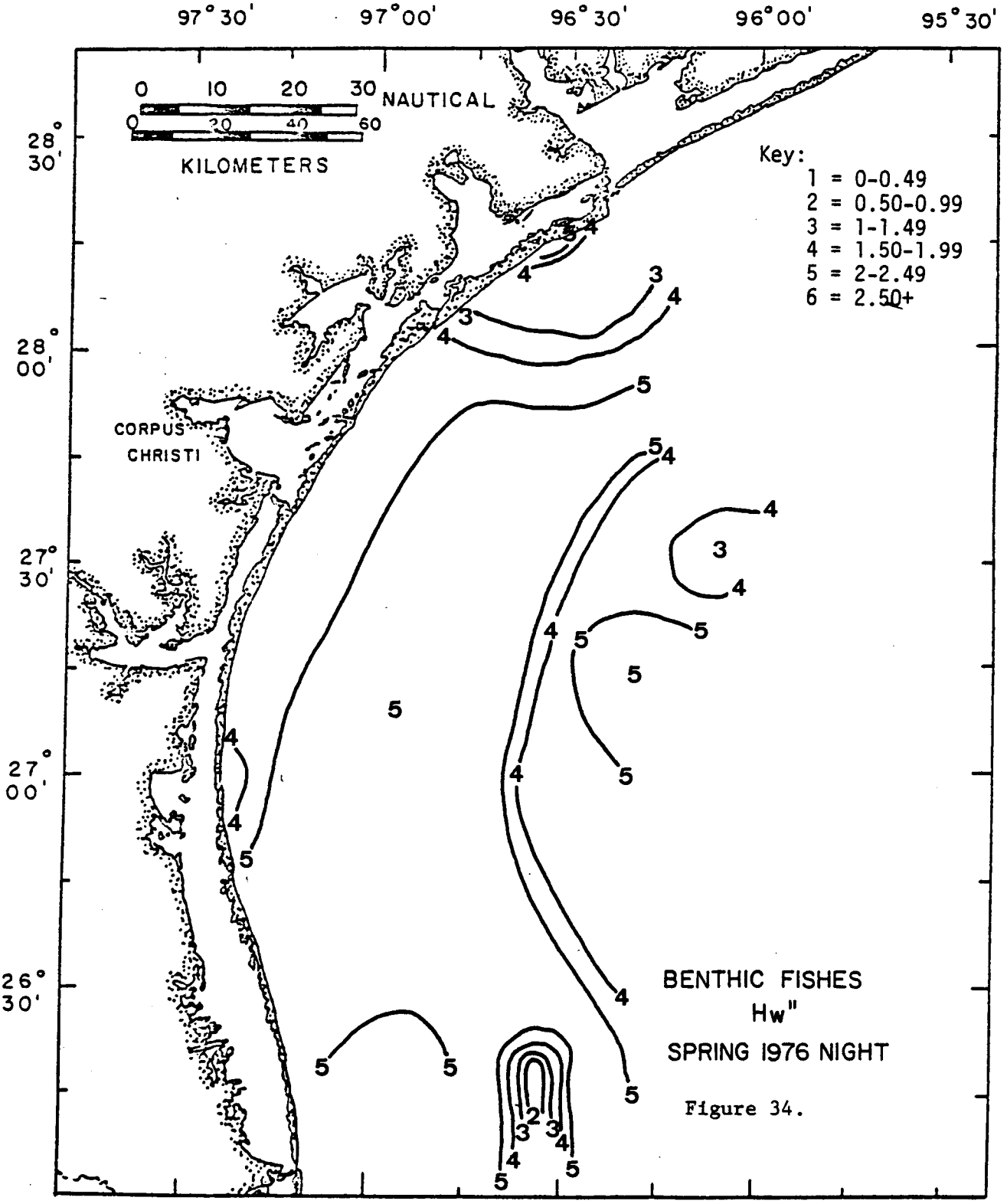
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-0.99
3 = 1-1.49
4 = 1.50-1.99
5 = 2-2.49
6 = 2.50+
BENTHIC FISHES
Hw"
SPRING 1976 DAY

Figure 33.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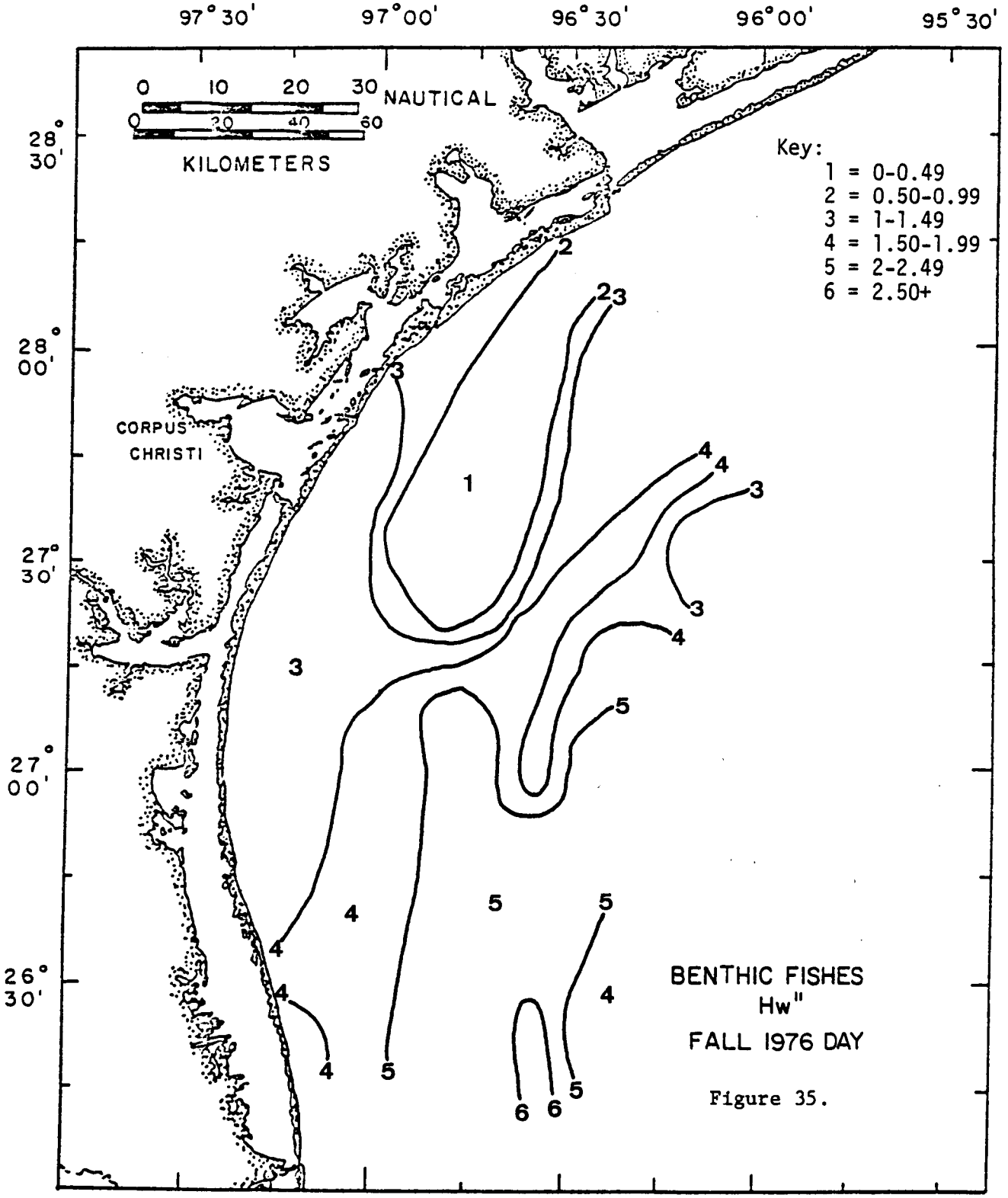
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-0.99
3 = 1-1.49
4 = 1.50-1.99
5 = 2-2.49
6 = 2.50+
BENTHIC FISHES
Hw"
SPRING 1976 NIGHT

Figure 34.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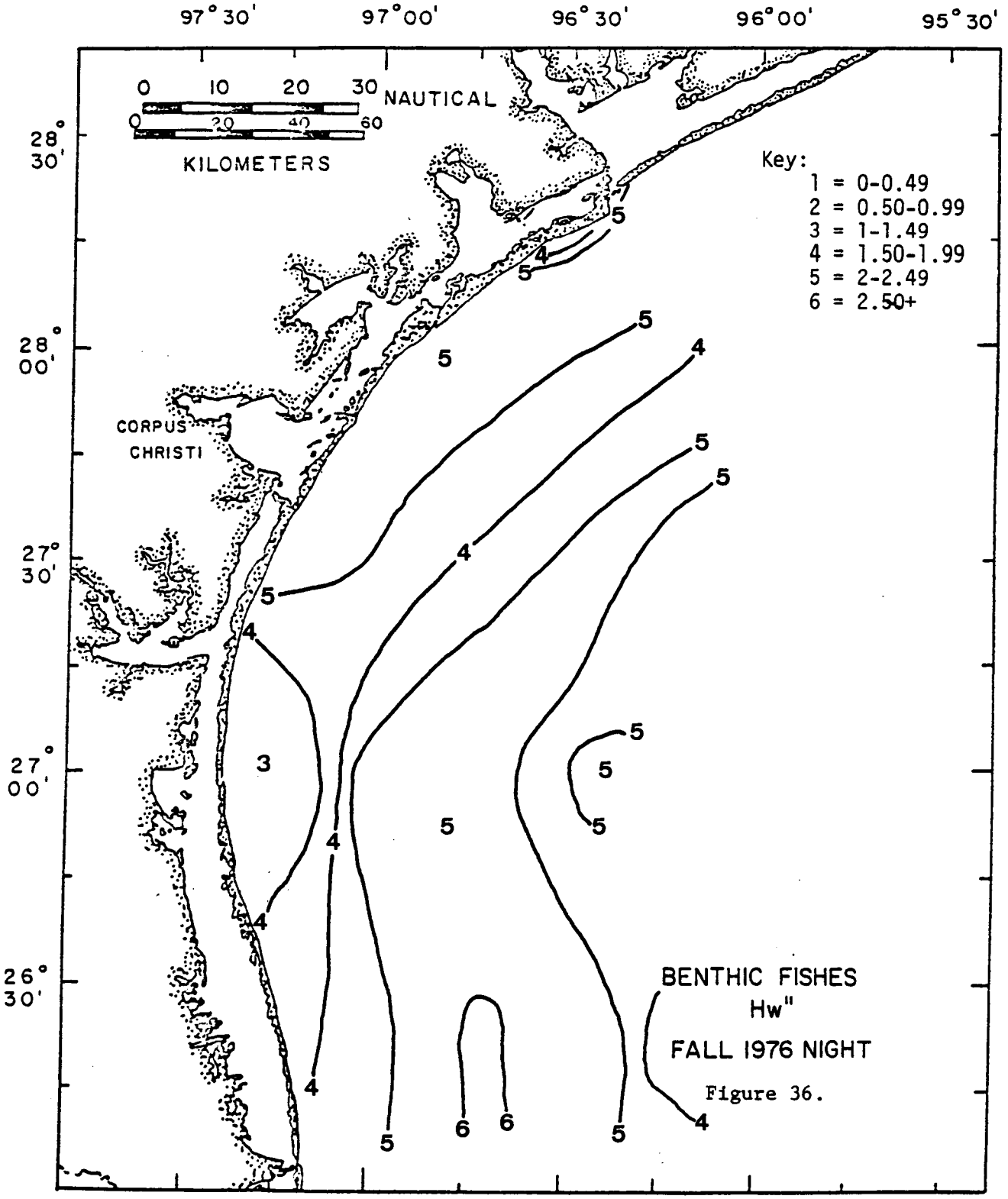
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-0.99
3 = 1-1.49
4 = 1.50-1.99
5 = 2-2.49
6 = 2.50+
BENTHIC FISHES
Hw"
FALL 1976 DAY

Figure 35.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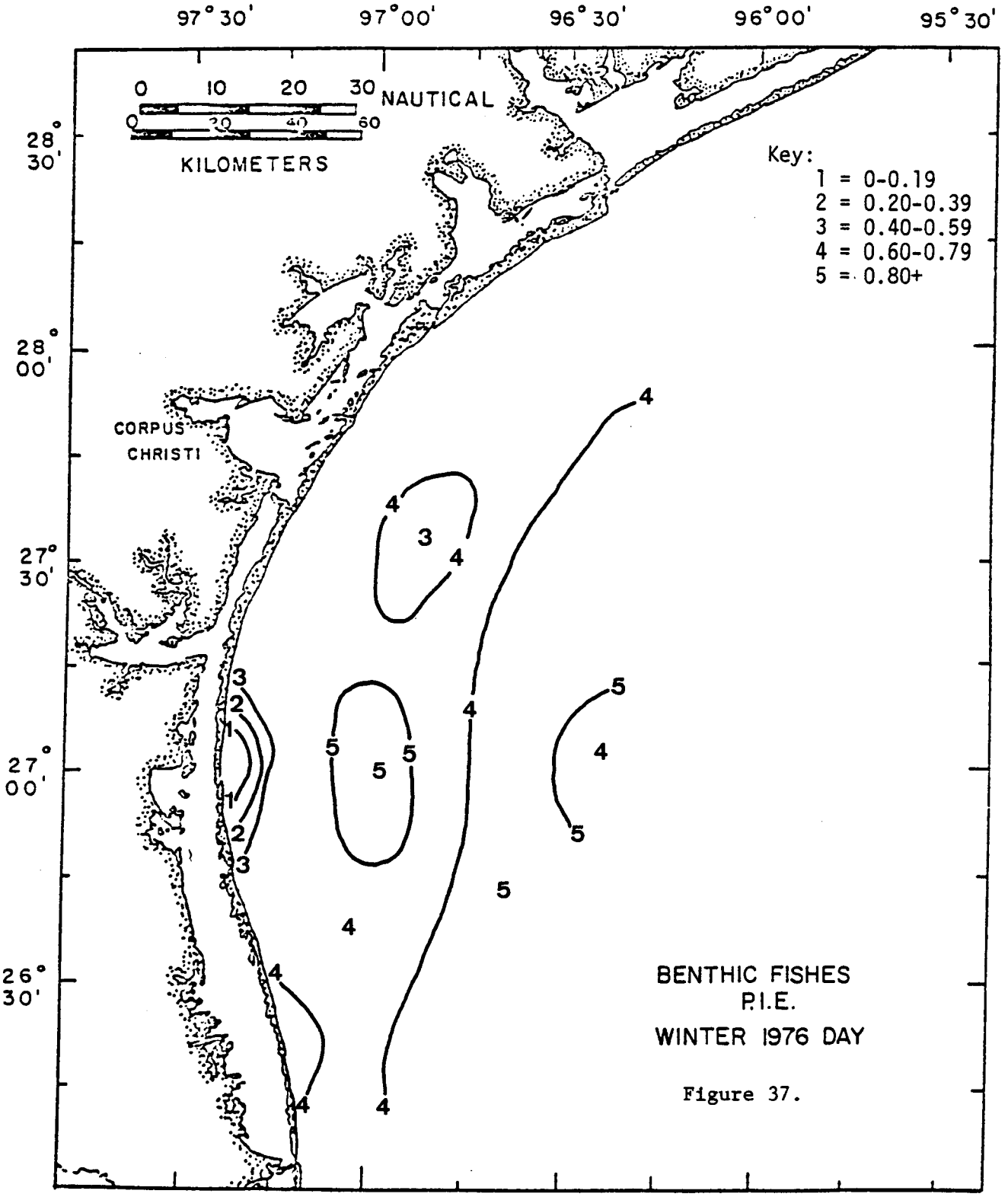
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.50-0.99
3 = 1-1.49
4 = 1.50-1.99
5 = 2-2.49
6 = 2.50+
BENTHIC FISHES
Hw"
FALL 1976 NIGHT

Figure 36.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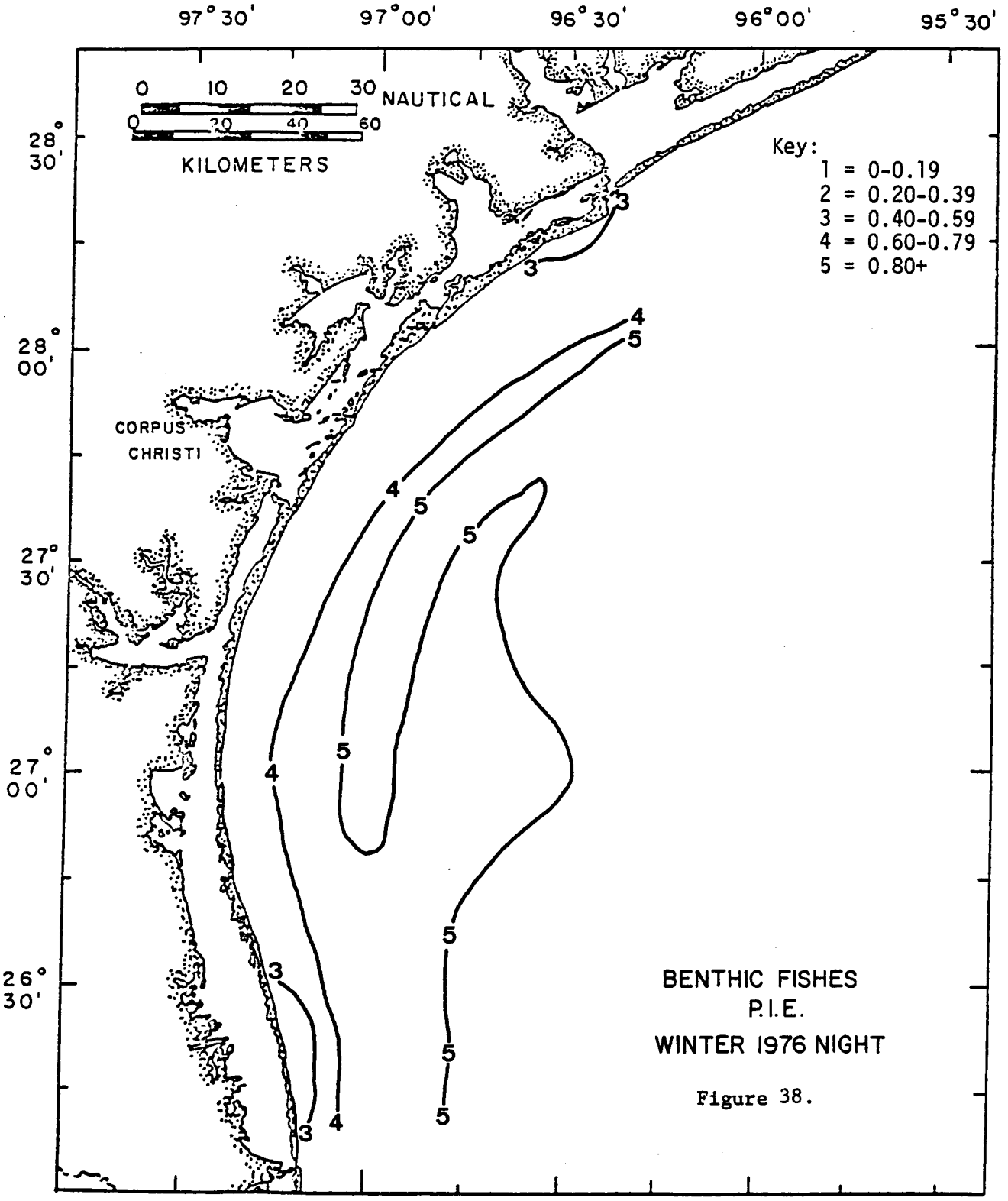
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.19
2 = 0.20-0.39
3 = 0.40-0.59
4 = 0.60-0.79
5 = 0.80+
BENTHIC FISHES
P.I.E.
WINTER 1976 DAY

Figure 37.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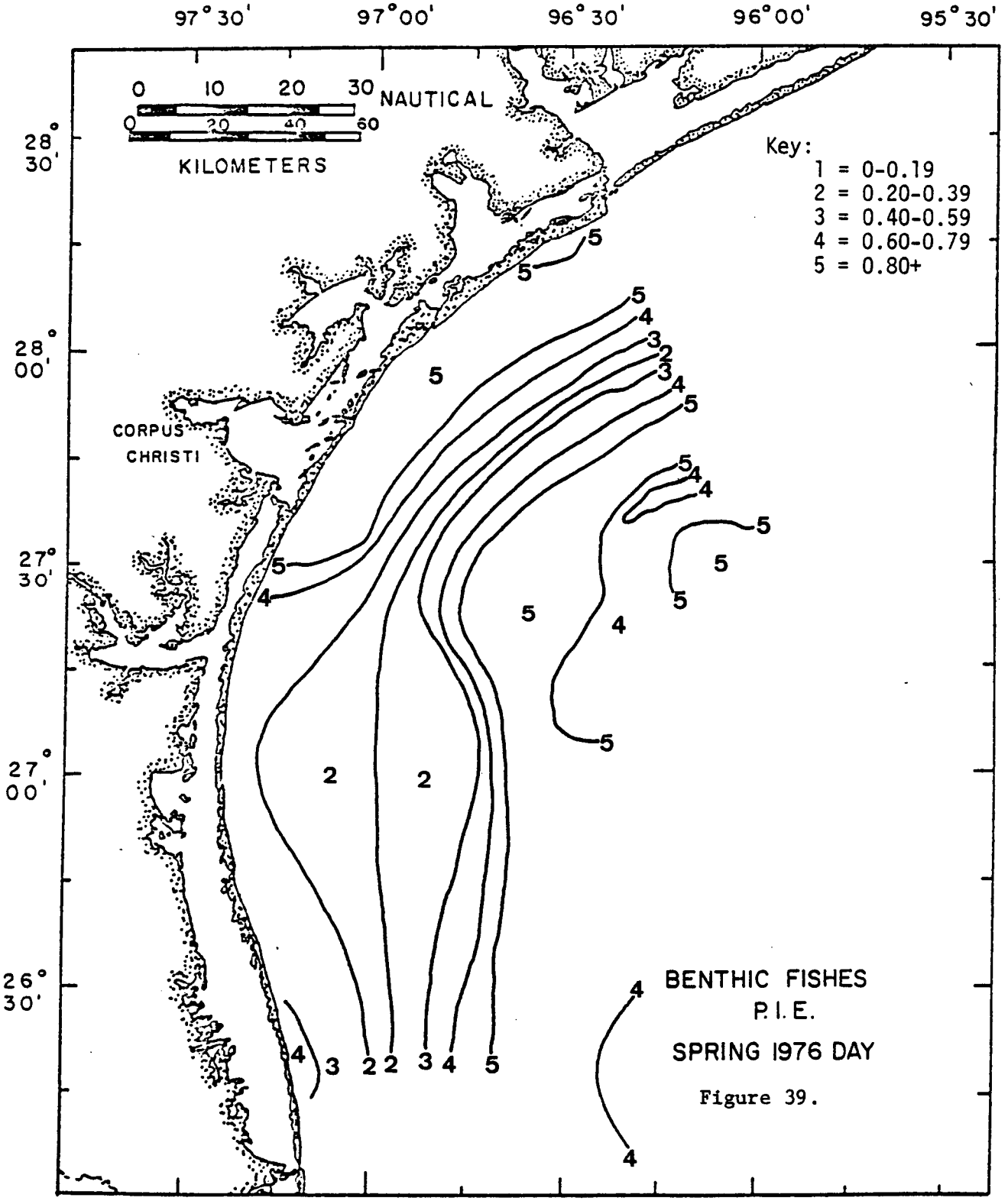
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.19
2 = 0.20-0.39
3 = 0.40-0.59
4 = 0.60-0.79
5 = 0.80+
BENTHIC FISHES
P.I.E.
WINTER 1976 NIGHT

Figure 38.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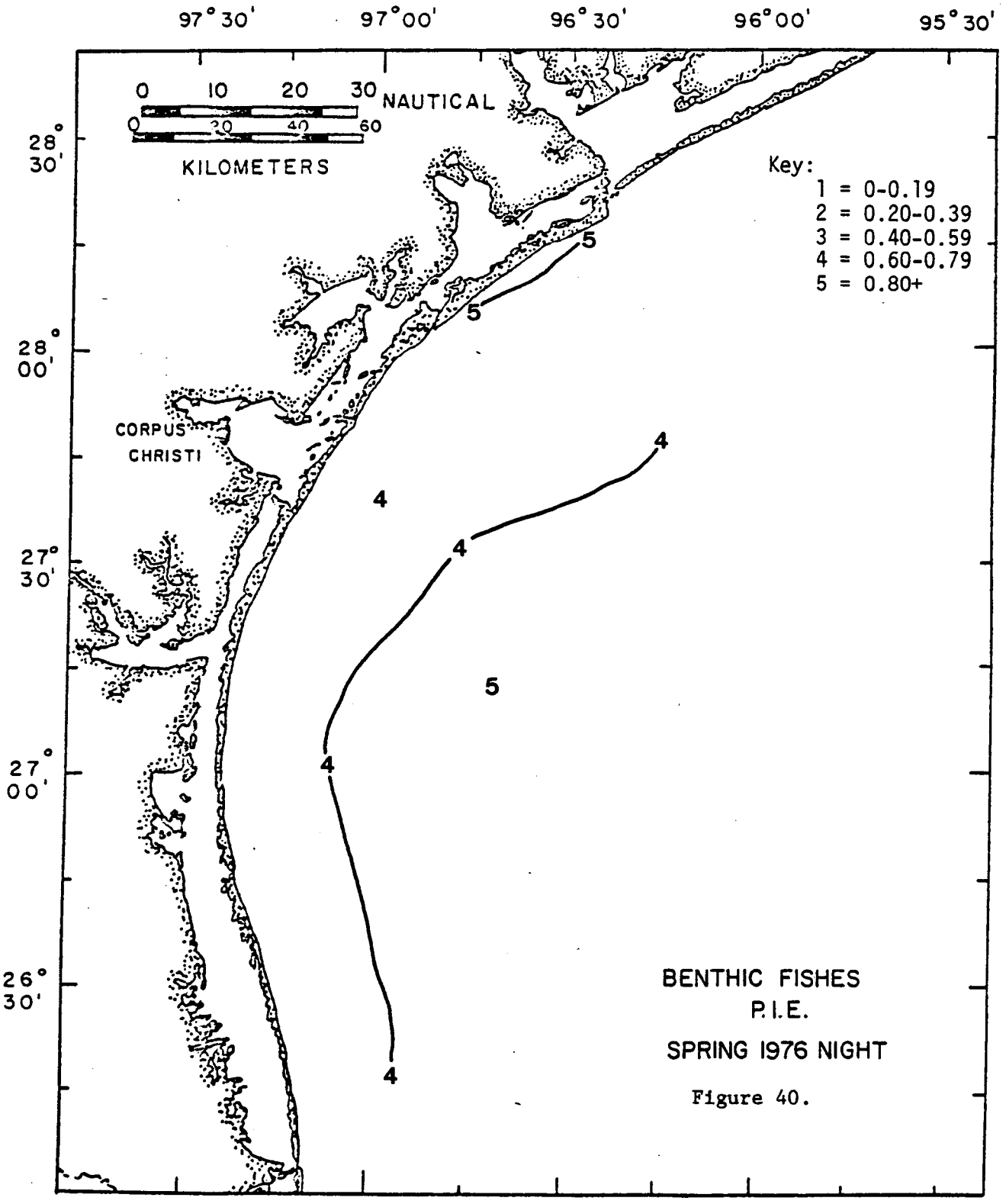
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
1 = 0-0.19
2 = 0.20-0.39
3 = 0.40-0.59
4 = 0.60-0.79
5 = 0.80+
CORPUS CHRISTI
BENTHIC FISHES
P.I.E.
SPRING 1976 DAY

Figure 39.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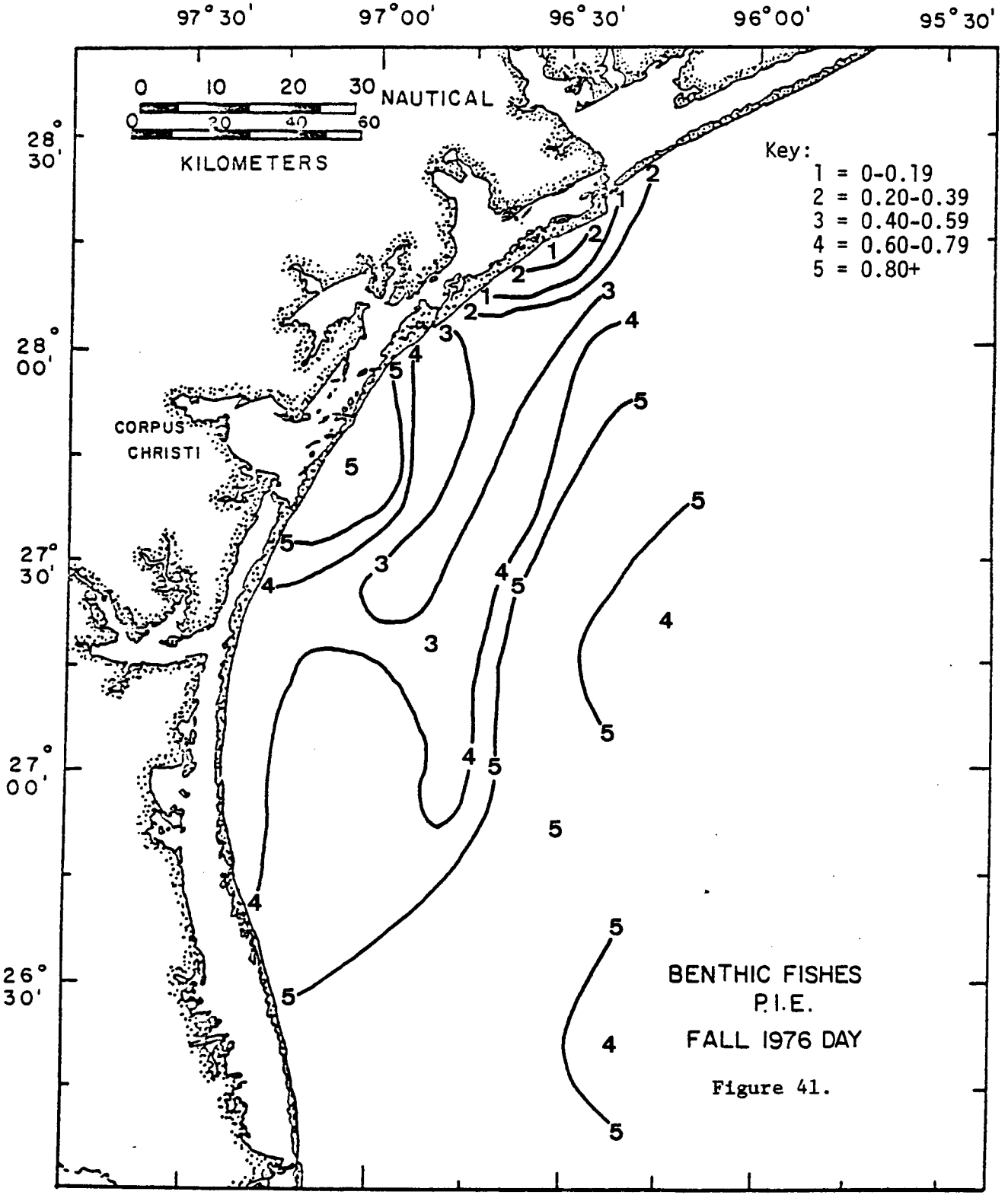
95° 30'
28° 30'
28° 00'
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
Key:
1 = 0-0.19
2 = 0.20-0.39
3 = 0.40-0.59
4 = 0.60-0.79
5 = 0.80+
CORPUS CHRISTI
BENTHIC FISHES
P.I.E.
SPRING 1976 NIGHT

Figure 40.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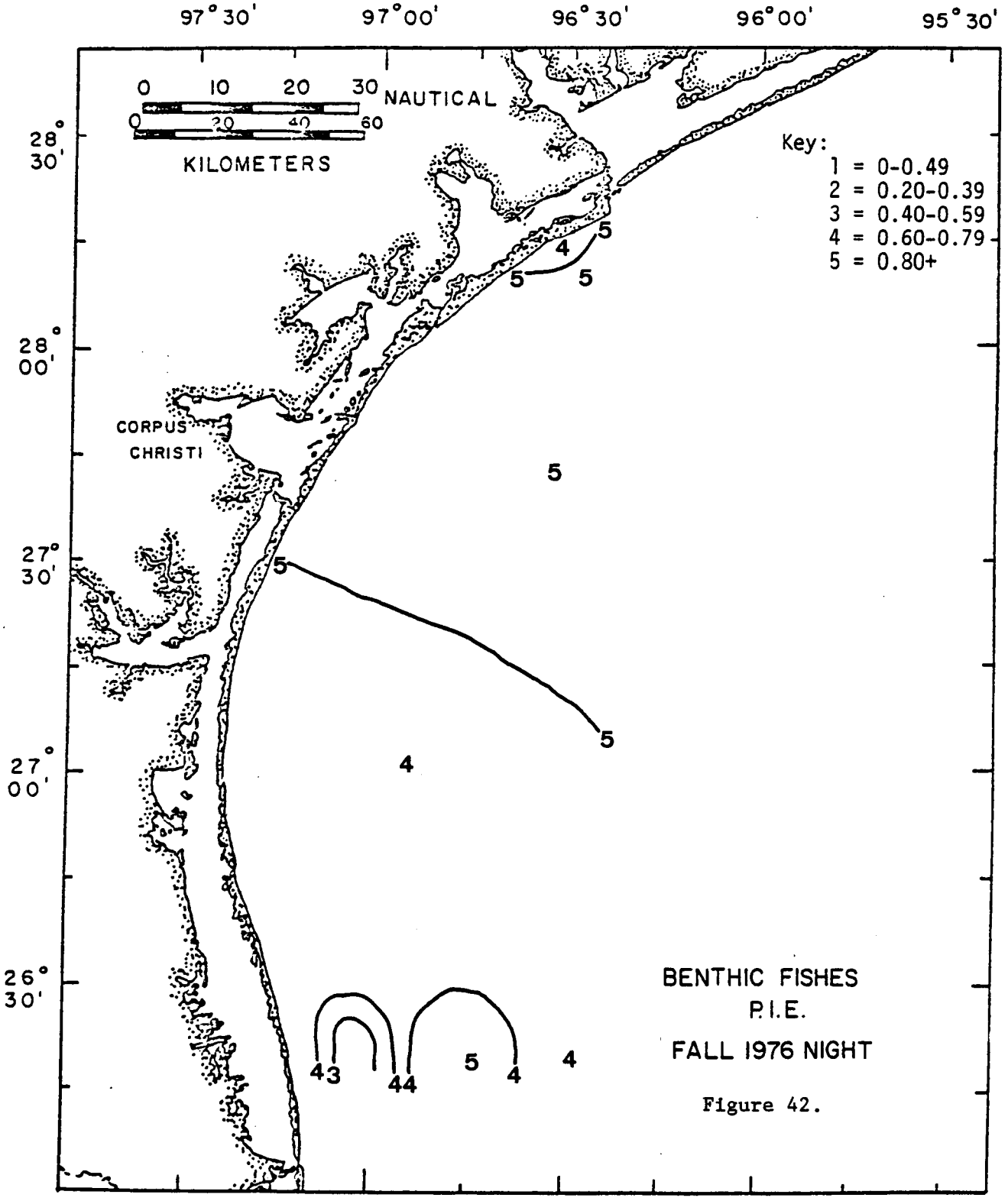
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.19
2 = 0.20-0.39
3 = 0.40-0.59
4 = 0.60-0.79
5 = 0.80+
BENTHIC FISHES
P.I.E.
FALL 1976 DAY

Figure 41.


97°30'
97°00'
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96°00'
95°30'
28° 30'
28° 00'
27° 30'
27° 00'
26° 30'
0 10 20 30 NAUTICAL
0 20 40 60
KILOMETERS
CORPUS CHRISTI
Key:
1 = 0-0.49
2 = 0.20-0.39
3 = 0.40-0.59
4 = 0.60-0.79
5 = 0.80+
BENTHIC FISHES
P.I.E.
FALL 1976 NIGHT

Figure 42.

in Table 7, Appendix H. For both numbers and weights of the individual species, the $(1 - \alpha)$ level, represented as (100% - α percent), is indicated with the appropriate ranges and the degrees of freedom for the comparisons. Zero range values indicate that there were no "ties" in the comparisons of the ranked sums. The designation "Day" or "Night" indicates statistical significance at or above the 80% confidence level ($P \leq 0.2$) and *ND* indicates statistical difference below the 80% level ($P > 0.2$) in Table 7, Appendix H. The comparisons in this table are for all 1976 data combined and for all seasonal and monthly (Transect II collections only) periods where collections of the species in question were made during at least one of the two trawls at each station.

Of the total number of species taken in 1976, 50 were predominantly caught at night and 16 predominantly during the day (at or below the $P \leq 0.20$ level). For data pooled over all seasonal or monthly periods in 1976, the species collected most frequently during the day with degrees of freedom, activity/associations, food habits, and feeding tactics are listed in Table 30. The behavioral information was derived from various literature sources.

In a similar manner, Table 31 is a listing of species collected most frequently during the night in 1976, while Table 32 includes those species occurring 10 or more times during 1976 for which no significant ($P \leq 0.20$) diel variation in trawl vulnerability was evident, based on analysis of data pooled over all seasonal and monthly periods. Note the much greater diversity of the nocturnal species in Table 31 as compared to either the diurnal species (Table 30) or the species indicating no significant diel differences (Table 32). While nocturnal species are represented by 8 orders, 27 families, and 50 genera (34 at $P \leq 0.05$), diurnal species include only 3 orders, 13 families and 16 genera (10 at $P \leq 0.05$), and no difference species were represented by only 5 orders, 9 families and 12 genera.

TABLE 30

SPECIES COLLECTED MOST FREQUENTLY IN DAY TRAWLS ($P \leq 0.20$) DURING 1976, BASED ON YEARLY-POOLED DATA. ASTERISK INDICATES $P < 0.05$

Taxonomic Categories	Degrees of Freedom	Activity and Associations	Food Habits	Feeding Tactic
Clupeiformes				
Clupeidae (Herrings)				
Harengula pensacolae	9	B3	C6	
Engraulidae (anchovies)				
**Anchoa mitchilli*	11	B3	C6	
Myctophiformes				
Synodontidae (lizardfishes)				
**Saurida brasiliensis*	127	B1	C1	D2
Perciformes				
Priacanthidae (big eyes)				
**Priacanthus arenatus*	25	B1-B2	C1	D1
Carangidae (jacks & pompanos)				
**Chloroscombrus chrysurus*	47	B3	C6	
Selar crumenophthalmus	3	B3	C6	
**Trachurus lathami*	135	B3	C6	
**Vomer setapinnis*	25	B3	C6	
Gerreidae (mojarras)				
Eucinostomus argenteus	3	-	C2	D4
Sciaenidae (drums)				
Pogonias chromis	3	B1-B2	C3	
Mullidae (goatfishes)				
**Upeneus parvus*	123	B1-B2	C2	
Sphyraenidae (barracudas)				
Sphyraena guachancho	3	B3	C1	
Trichiuridae (cutlass fishes)				
Trichiurus lepturus	27	B3	C1	
Scombridae (mackeral and tuna)				
**Scomber japonicus*	5	B3	C6	
Stromateidae (butterfish)				
**Peprilus burti*	79	B3	C6	
Tetraodontidae (puffers)				
**Lagocephalus laevigatus*	61	B1	C2	

TABLE 31

SPECIES COLLECTED MOST FREQUENTLY IN NIGHT TRAWLS
($P \leq 0.20$) DURING 1976, BASED ON YEARLY-POOLED DATA.
ASTERISK INDICATES COMPARISON AT $P < 0.05$.

Taxonomic Categories	Degrees of Freedom	Activity and Associations	Food Habits	Feeding Tactic
Anguilliformes:				
Muraenidae (morays)				
**Gymnothorax nigromarginatus*	27	A1 B1	C4	D1
Muraenesocidae (pike congers)				
**Hoplunnis macrurus*	47	A1 B1	C4	D1
Congridae (congers)				
**Congrina flava*	23	A1 B1	C4	D1
Ophichthidae (snake eels)				
**Ophichthus gomesi*	21	A1 B1	C4	D1
Myctophiformes:				
Synodontidae (lizard fish)				
Synodus poeyi	73	A2 B1	C4	D2
Batrachoidiformes:				
Batrachoididae (toad fishes)				
**Porichthys porosissimus*	105	A1 B1	C4	
Lophiformes:				
Antennariidae (frog fishes)				
**Antennarius radiosus*	51	A2 B1	C4	D2
Ogcocephalidae (bat fishes)				
**Halieutichthys aculeatus*	95	A3 B1		
Gadiformes				
Bregmacerotidae (codlets)				
**Bregmaceros atlanticus*	37			
Gadiidae (cod fishes)				
**Urophycis cirratus*	15		C4	D2
**Urophycis floridanus*	41		C4	D2
Ophidiidae (cusk eels and brotulas)				
**Brotula barbata*	49	A1 A5 B1	C1	D2
**Lepophidium graellsi*	101	A1 B1	C1	D2
**Ophidion welshi*	15	A1 B1	C1	D2
**Neobythites gilli*	11	A1 B1	C1	D2
Perciformes:				
Serranidae (seabasses)				
**Centropristis philadelphica*	161		C1	
**Hemanthias vivanus*	7		C1	
**Serranus atrobranchus*	165		C7	
Serraniculus pumilio	3	A4	C1	

TABLE 31 CONT.'D

Taxonomic Categories	Degrees of Freedom	Activity and Associations	Food Habits	Feeding Tactic
Apogonidae (cardinal fishes)				
**Apogon aurolineatus*	13	B1-B2	C7	
Synagrops spinosa	3	B1-B2	C7	
Branchiostegidae (tile fishes)				
Caulolatilus intermedius	53	A1-B1		
Lutjanidae (snappers)				
**Pristipomoides aquilonaris*	147		C1	
Lutjanus campechanus	69	B1-B2	C1	
Sparidae (porgies)				
**Stenotomus caprinus*	163		C4-C5	D4
Sciaenidae (drums)				
**Equetus acuminatus*	23	B1-B2	C1	D4
Cynoscion arenarius	49	B2	C1	D1
Larimus fasciatus	27		C1	
Menticirrhus americanus	27		C1	
Polynemidae (thread fins)				
Polydactylus octonemus	25	B1-B2	C1	D4
Uranoscopidae (stargazers)				
Kathetostoma albigutta	45		C1	D2
Gobiidae (gobies)				
**Bollmannia communis*	67	B1	C1	
Scorpaenidae (scorpion fishes)				
**Pontinus longispinis*	41	A1 B1	C1	D2
Scorpaena brasiliensis	9	A2 B1	C1	D2
Triglidae (sea robins)				
**Prionotus* larval	5			
Prionotus ophryas	7	B1	C1	D4
**Prionotus paralatus*	115	B1	C1	D4
**Prionotus rubio*	145	B1	C1	D4
**Prionotus salmonicolor*	15	B1	C1	D4
Pleuronectiformes:				
Bothidae (left eye flounders)				
**Citharichthys spilopterus*	31	A3 B1	C1	D1 D2
**Cyclopsetta chittendeni*	83	A3 B1	C1	D1 D2
**Engyophrys senta*	51	A3 B1	C1	D1 D2
**Etropus crossotus*	23	A3 B1	C1	D1 D2
**Syacium gunteri*	111	A3 B1	C1	D1 D2
**Trichopsetta ventralis*	103	A3 B1	C1	D1 D2
Soleidae (soles)				
Achirus lineatus	3	A3 B1	C1	D1 D2
Cynoglossidae (tongue fishes)				
**Symphurus parvus*	11	A3 B1	C1	D1 D2
**Symphurus plagiusa*	45	A3 B1	C1	D1 D2
Tetraodontiformes:				
Tetraodontidae (puffers)				
**Sphoeroides parvus*	97	B1-B2	C2,C4-C5	D4
Diodontidae (porcupine fishes)				
Chilomycterus schoepfi	3	B1	C2	D4

TABLE 32

SPECIES OCCURRING MORE THAN 10 TIMES DURING 1976
AND INDICATING LITTLE OR NO DIEL VARIATION
IN TRAWL VULNERABILITY (P > 0.20)
BASED ON YEARLY-POOLED DATA

Taxonomic Categories	Degrees of Freedom	Activity and Associations	Food Habits	Feeding Tactic
Clupeiformes:				
Engraulidae				
Anchoa hepsetus	39	B3	C6	
Myctophiformes:				
Synodontidae				
Synodus foetens	159	A2 B1	C1	D2
Lophiiformes:				
Ogcocephalidae				
Ogcocephalus species	63	A2 B1	C1	D2
Zalieutes mcgintyi	15	A2 B1	C1	D2
Perciformes:				
Serranidae				
Diplectrum bivittatum	63	A2, B1-B2	C4	D1 D2
Sparidae				
Lagodon rhomboides	39		C1	D1
Sciaenidae				
Cynoscion nothus	53	B2-B3	C1	D1
Leiostomus xanthurus	19		C1	
Micropogon undulatus	49	B2-B3	C4	D1 D4
Triglidae				
Prionotus stearnsi	117		C1	D4
Pleuronectiformes:				
Bothidae				
Ancylopsetta dilecta	41	A3 B1	C1	D1 D2
Ancylopsetta quadrocellata	23	A3 B1	C1	D1 D2
Soleidae				
Gymnachirus texae	67	A3 B1	C1	D1 D2

This indicates that it was more common for a species to show some diel variation in trawl vulnerability than to show no difference between day and night collections in 1976. These patterns are illustrated in Table 6, Appendix H, which reveals that, of the 62 species occurring nine or more times during 1976, only 13 were not collected in significantly greater numbers during either day or night. Of those 49 species occurring nine or more times and showing diel trends, 10 appeared more often during the day ($P \leq 0.20$ with eight having $P \leq 0.05$), while 39 species appeared more often at night ($P \leq 0.20$ with 27 having $P \leq 0.05$).

Tables 30 and 31 show that of those species different at less than the 5% level, one of the day species, *Scomber japonicus*, and two of the night species, *Prionotus* larvae and *Hemathias vivanus*, have associated degrees of freedom less than 10 but these species, while collected at very limited times of the year and at very limited numbers of localities were collected exclusively during the diel period to which they have been assigned.

Only Synodontidae and Tetrodontidae, and Sciaenidae contained genera in which one species was significantly diurnal and another nocturnal; of these, the significance of the nocturnal collections of both the offshore lizardfish, *Synodus poeyi* (Synodontidae) and the least puffer, *Sphoeroides parvus* (Tetraodontidae), was fairly low ($0.20 \leq P \leq 0.95$), as was that of the possibly diurnal black drum, *Pogonias chromis*.

For Tables 30-32, assignments of activity and association tendencies, food habits and food tactics were made based on literature reports, as indicated by numerical references to materials listed in the bibliography of this report. Assignments represent the following general tendencies as reported in the references:

1. Activity (A)

   A1. burrower

A2. sedentary
A3. shallow burrower
A4. somewhat sedentary
A5. secretive

2. Associations (B)

B1. solitary
B2. forms small schools
B3. forms large schools

3. Food habits (C)

C1. carnivore
C2. specialized carnivore (involving distinctive structural specialization, such as the chemosensory barbels of *Upeneus parvus*)
C3. molluscivore
C4. carnivore and scavenger
C5. omnivore
C6. planktivore (includes filter feeders as well as zooplanktivores)

4. Feeding tactic (D)

D1. stalker (generally visually oriented)
D2. ambusher
D3. cryptic angler
D4. searcher (may use other than visual means to locate prey, as in the Mullidae and Triglidae and Polynomidae)

The analysis of data by six periods (essentially two-month intervals) for those species that occur primarily at night ($P < 0.05$), is in Table 33, where the decreasing vulnerability of many species appears to occur toward the end of the year. For the diurnal species in Table 34, which is set up similarly to Table 33, the seasonal pattern is more irregular. Ten species that showed no significant differences between day and night vulnerability when the 1976 catch data were pooled did show significantly greater day or night differences within one or more of the seasonal and monthly periods as shown in Table 35.

TABLE 33

SEASONAL VARIABILITY IN TRAWL VULNERABILITY OF SPECIES COLLECTED PREDOMINANTLY AT NIGHT IN 1976 (BASED ON POOLED DATA). NUMBERS REPRESENT TABULATIONS FOR PERIODS WHERE P ≤ 0.05. DASH INDICATED SPECIES COLLECTED AT P ≥ 0.05; BLANK SPACES INDICATE NO COLLECTIONS

Species	Total Degrees of Freedom	Winter	March/ April	Spring	July/ August	Fall	November/ December
Prionotus rubio	145	39	-	29	17	25	13
Serranus atrobranchus	165	33	19	37	19	37	-
Lepophidium graellsi	101	29	17	27	5	15	-
Brotula barbata	49	5	9	23	3	9	
Bollmannia communis	67	17	17	13	-	5	-
Porichthys porosissimus	105	29	15	23	5	17	11
Hoplunnis macrurus	47	15	13	13		15	-
Ophichthus gomesi	21	7	-	9		-	
Congrina flava	23	5	-	7	-	-	
Bregmaceros atlanticus	37	17	3	13		-	
Antennarius radiosus	51	23	-	9	-	-	-
Etropus crossotus	47	15	3	13	-	-	-
Trichopsetta ventralis	103	27	-	19	-	19	-
Symphurus parvus	11	5		5			
Symphurus plagiusa	45	21	-	13		-	
Gymnothorax nigromarginatus	27	9	7	-			
Urophycis floridanus	41	13	-	9	-	-	
Cyclopsetta chittendeni	83	21	-	-	-	-	-
Apogon aurolineatus	13	3	9	-	-		-
Engyophrys senta	51	-	7	7	-	-	-
Stenotomus caprinus	163	-	17	37	23	39	-
Syacium gunteri	111	-	11	25	-	21	-
Sphoeroides parvus	97	-	-	19	9	25	9
Centropristis philadelphica	161	25N	-	37	-	39	-
Prionotus paralatus	115	-	-	29	13	21	-
Halieutichthys aculeatus	95	-	-	15	5	17	-
Citharichthys spilopterus	31	-	-	7	3	-	-
Ophidion welshi	15	-		9		-	
Pristipomoides aquilonaris	147	-	-	-	17	29	-
Pontinus longispinis	41	3	-	-	-	11	-
Equetus acuminatus	23	-	3	3	-	5	-
Hemanthias vivanus	7			-		5	
Prionotus Larvae	5	-		-			
Prionotus salmonicolor	15	-	-		-		
Neobythites gilli	11	-	-	-	-		
Cynoscion arenarius	49	-	-	-	-	-	7
Total at P ≤ 0.05		18	14	26	11	18	4
Totals - 35 species		35	32	35	27	30	23

TABLE 34

SEASONAL VARIABILITY IN TRAWL VULNERABILITY OF SPECIES COLLECTED PREDOMINANTLY DURING THE DAY IN 1976 (BASED ON POOLED DATA). NUMBERS REPRESENT DEGREES OF FREEDOM TABULATIONS FOR PERIODS WHERE $P \leq 0.05$. DASH INDICATES SPECIES COLLECTED AT $P > 0.05$; BLANK SPACES INDICATE NO COLLECTIONS

Species	Total Degrees of Freedom	Winter	March/ April	Spring	July/ August	Fall	November/ December
Upeneus parvus	123	25	17	-	11	-	11
Saurida brasiliensis	127	29	19	-	-	23	15
Trachurus lathami	135	17	-	41	-	37	-
Chloroscombrus chrysurus	17	5	-	9	-	19	3
Trichiurus lepturus	27	9	-	9			
Anchoa mitchilli	11	5	-	-			
Peprilus burti	79	-	-	27	15	15	-
Lagocephalus laevigatus	61	-	-	29	-	13	-
Vomer setapinnis	25	-	-	9	3	7	
Total at $P \leq 0.05$		6	2	6	2	6	3
Totals 9		9	9	9	7	8	6

TABLE 35

SEASONAL VARIABILITY IN TRAWL VULNERABILITY OF SPECIES COLLECTED DURING 1976 FOR WHICH NO DIEL VARIATION WAS APPARENT FROM ALL DATA POOLED ($P \geq 0.2$) BUT FOR WHICH DIEL VARIATIONS WERE APPARENT ($P \leq 0.05$) WITHIN ONE OR MORE SEASONS. NUMBERS REPRESENT DEGREES OF FREEDOM TABULATIONS FOR PERIODS WHERE $P \leq 0.05$. DASH INDICATES SPECIES COLLECTED AT $P \geq 0.20$; BLANK SPACES INDICATE NO COLLECTIONS

Species	Total Degrees of Freedom	Winter	March/ April	Spring	July/ August	Fall	November/ December
Symphurus diomedianus	7	5N				-	
Synodus poeyi	73	21D	-	15N	3D	-	-
Caulolatilus intermedius	53	-	7N	-	-	-	-
Lagadon rhomboides	39	-	5N	-	-	-	-
Pikea mexicana	11		5N			-	-
Anchoa hepsetus	39	-	-	13N	-	-	
Ancyclopsetta quadrocellata	23			5N			
Kathetostoma albigutta	45	-	-	11N	-	-	-
Prionotus stearnsi	117	-	19D	-	-	-	15D
Synodus foetens	159	-	-	-	-	-	19D
Total at $P \leq 0.05$		2	4	4	1	0	2
Total 10		8	8	8	7	9	7

Cluster Analyses of Sites and Species

Cluster Analysis

Test analyses were performed on spring data using the Bray-Curtis or the Canberra-Metric coefficient and group average or flexible sorting to determine the best method for the data. While group average sorting gave similar results to the flexible strategy in terms of station-groups, species associations were ill-defined and much more difficult to divide into meaningful groups. The Bray-Curtis coefficient gave more clearly definable results than the Canberra-Metric coefficient. The Bray-Curtis advantage is most likely due to the large number of zeros in the data matrices, a situation in which the Canberra-Metric measure has its most pronounced weakness. The dominance problem with the Bray-Curtis measure was minimized using a square root transformation of the raw data reducing the range of abundances from 0-2225 to 0-47. Flexible sorting with $\beta = -0.25$ combined with the Bray-Curtis coefficient gave the best results in terms of station-groups and species-groups and was chosen as the best method of analysis for the data.

Station-Groups (Normal Analysis)

The results of the normal analyses are presented in dendrograms in which the hierarchical relations of the groups are shown against a scale of relative dissimilarity. The scale of 0 to 200 actually represents dissimilarity values from 0 to 100 percent such that a reading of 80 would represent a dissimilarity value of 40 percent. Station dendrograms for the three seasonal collections are presented in Figures 43, 45 and 47 and maps showing the relative positions of stations within station-groups appear in Figures 44, 46 and 48. All three seasonal analyses showed strong depth relationships. In winter (Figures 43 and 44) the stations

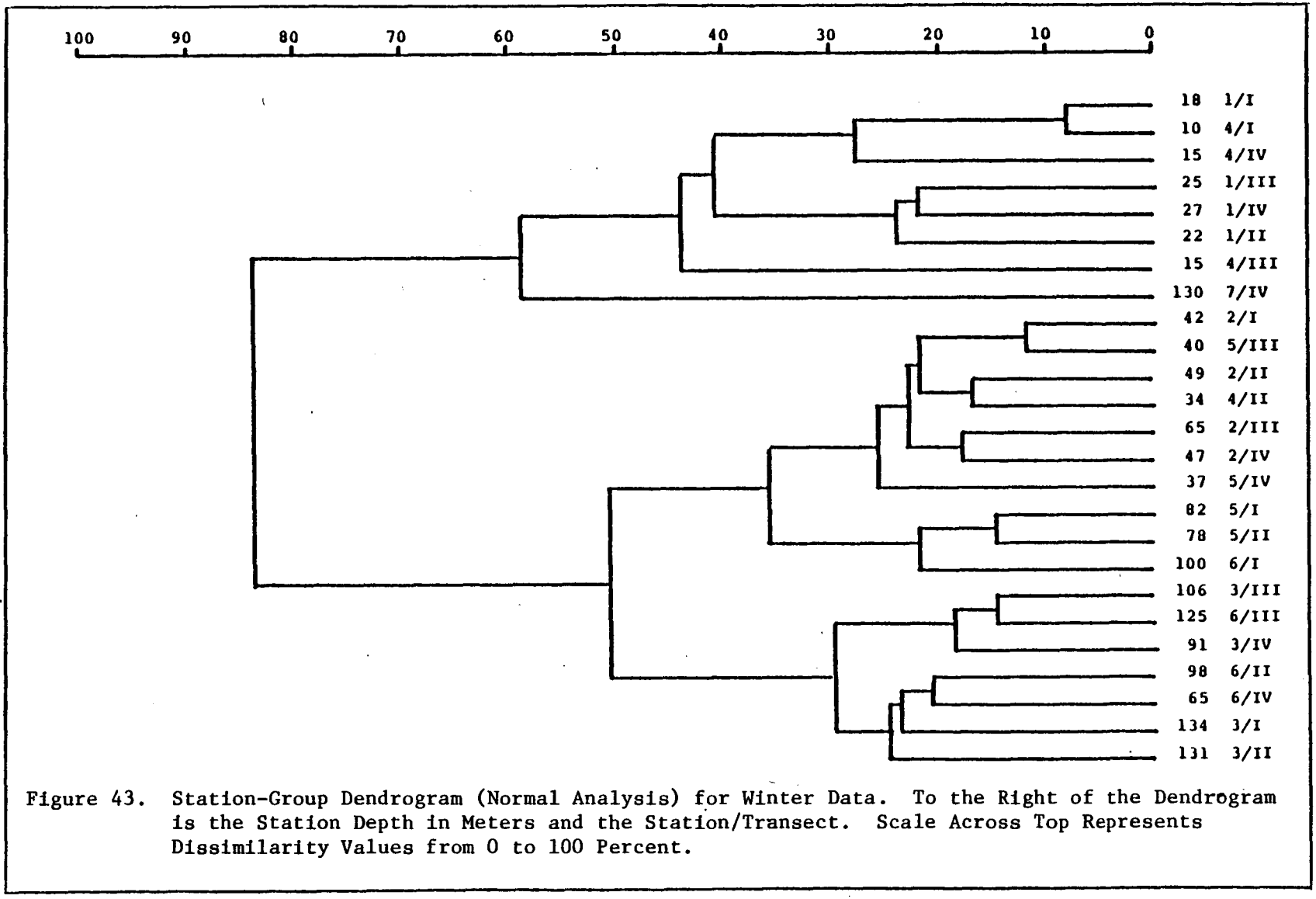

Figure 43. Station-Group Dendrogram (Normal Analysis) for Winter Data. To the Right of the Dendrogram is the Station Depth in Meters and the Station/Transect. Scale Across Top Represents Dissimilarity Values from 0 to 100 Percent.

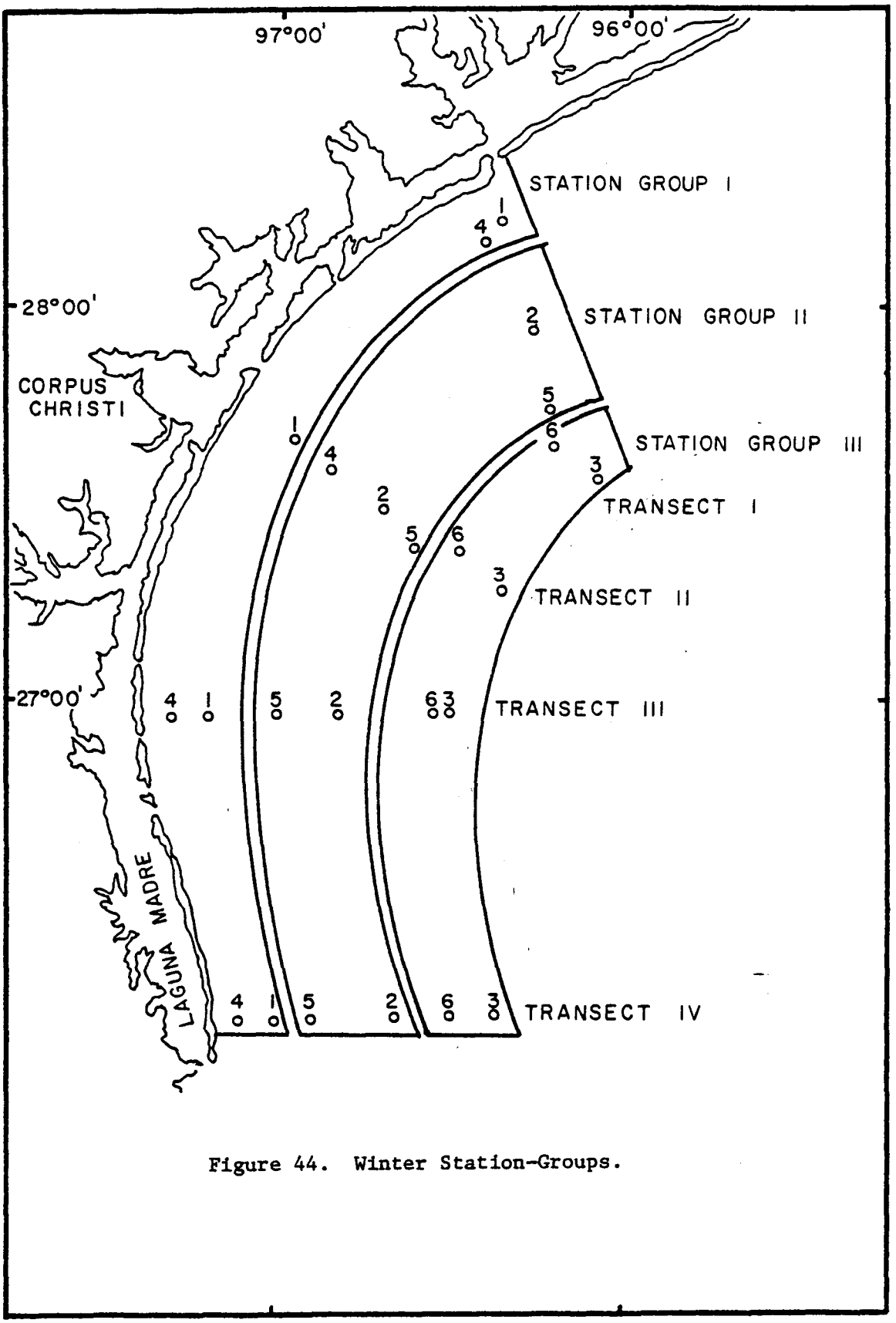

Figure 44. Winter Station-Groups.

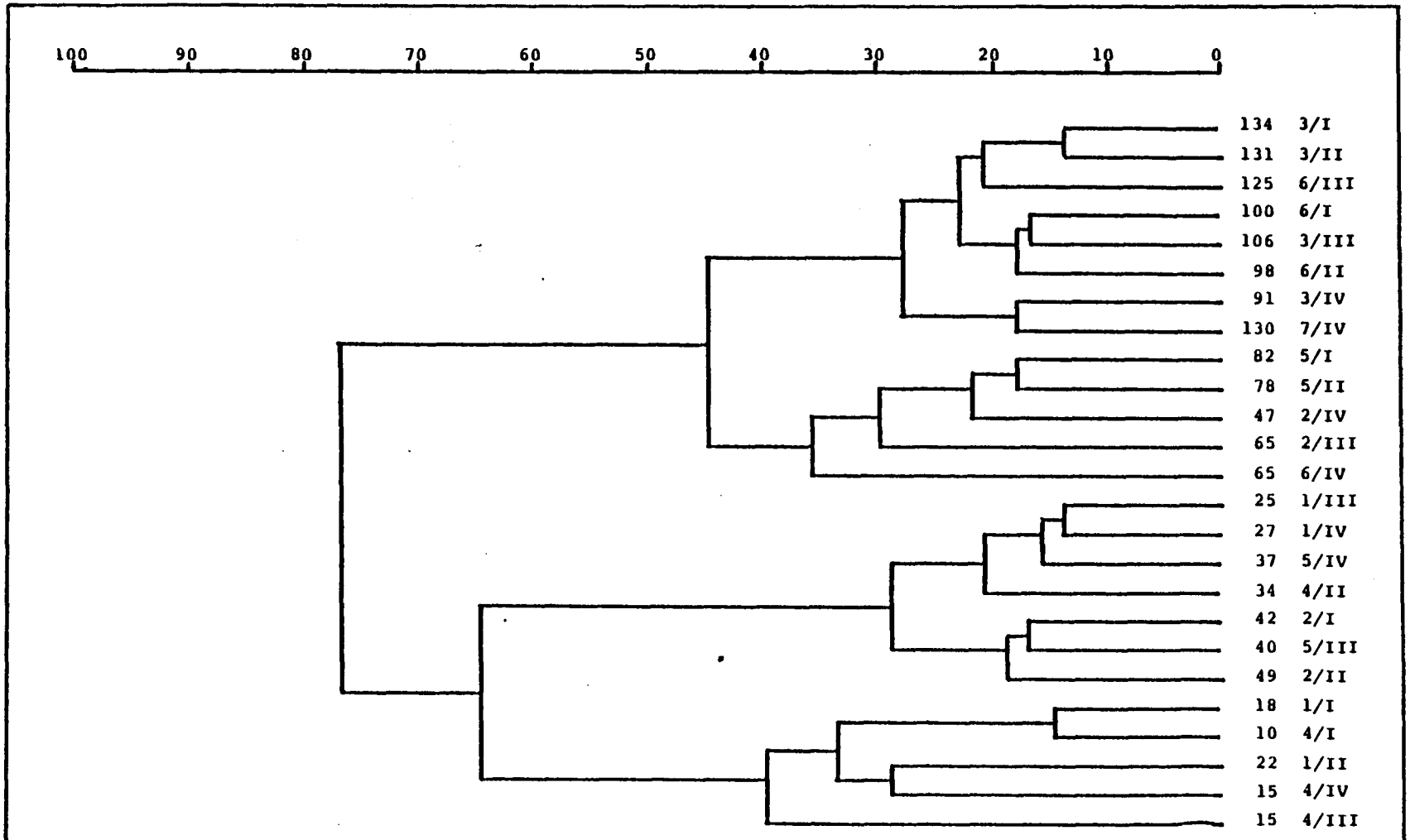

Figure 45. Station-Group Dendrogram (Normal Analysis) for Spring Data. To the Right of the Dendrogram is the Station Depth in Meters and Station/Transect. Scale Across Top Represents Dissimilarity Values from 0 to 100 Percent.

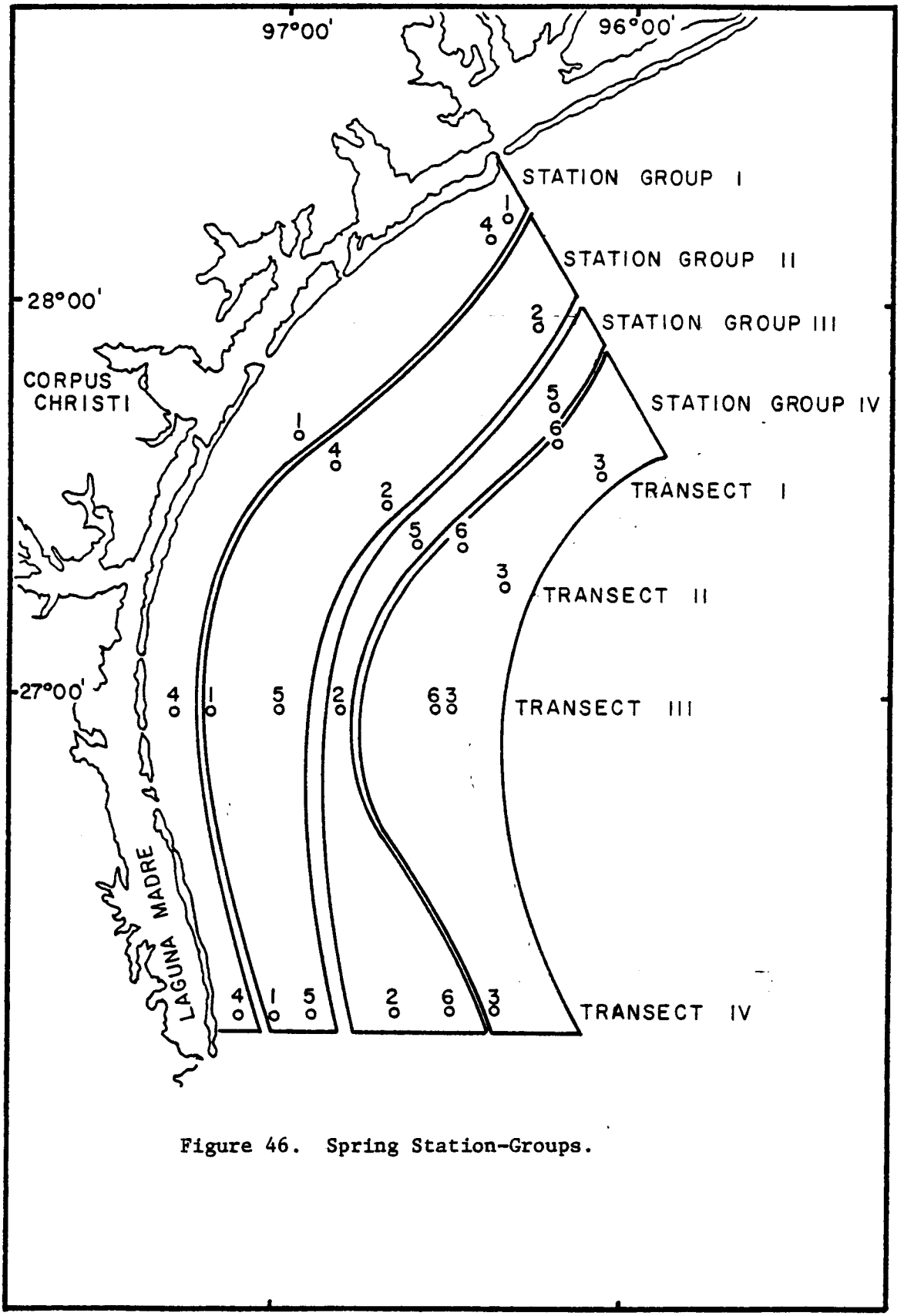

Figure 46. Spring Station-Groups.

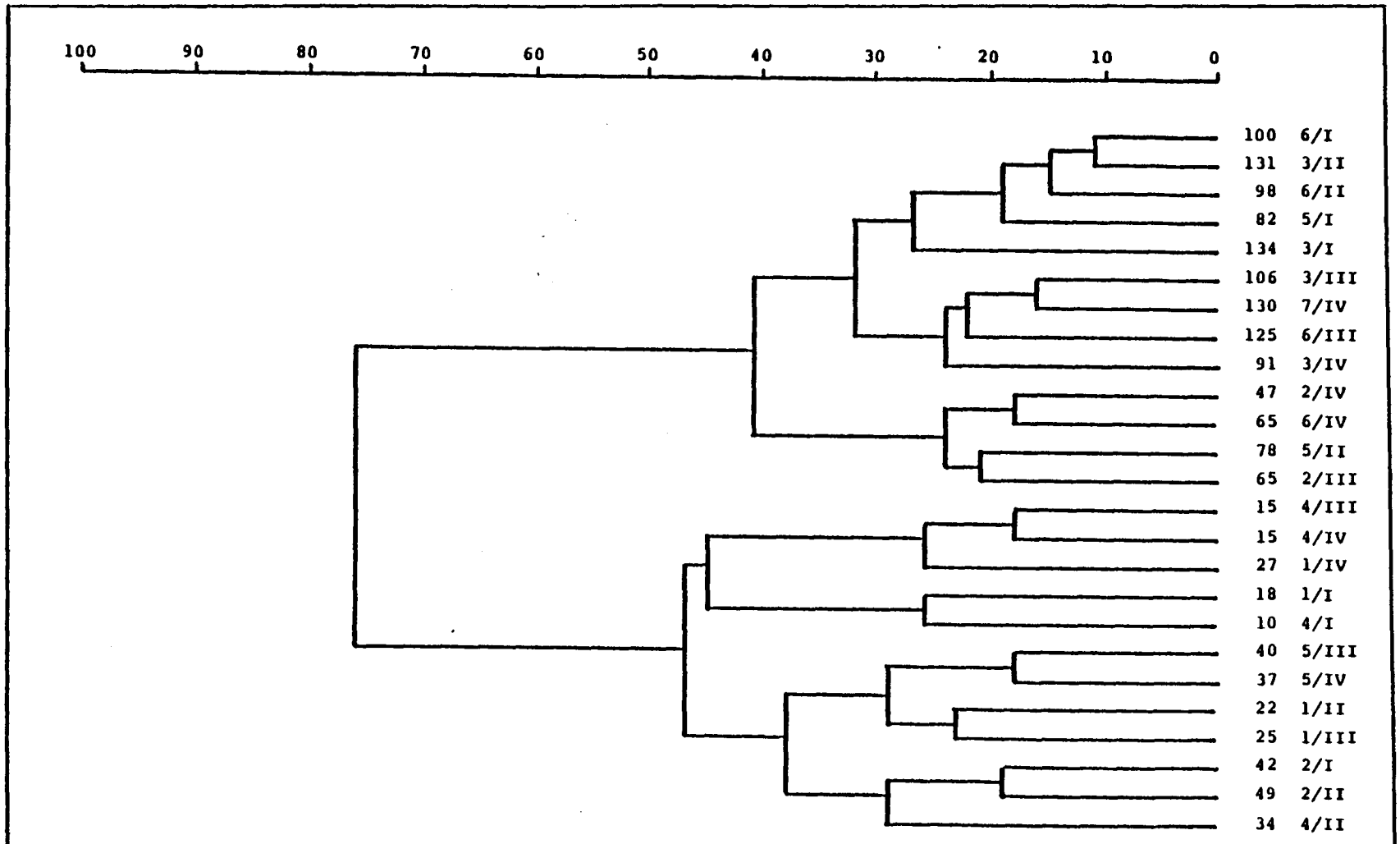

Figure 47. Station-Group Dendrogram (Normal Analysis) for Fall Data. To the Right of the Dendrogram is the Station Depth in Meters and the Station/Transect. Scale Across Top Represents Dissimilarity Values from 0 to 100 Percent.

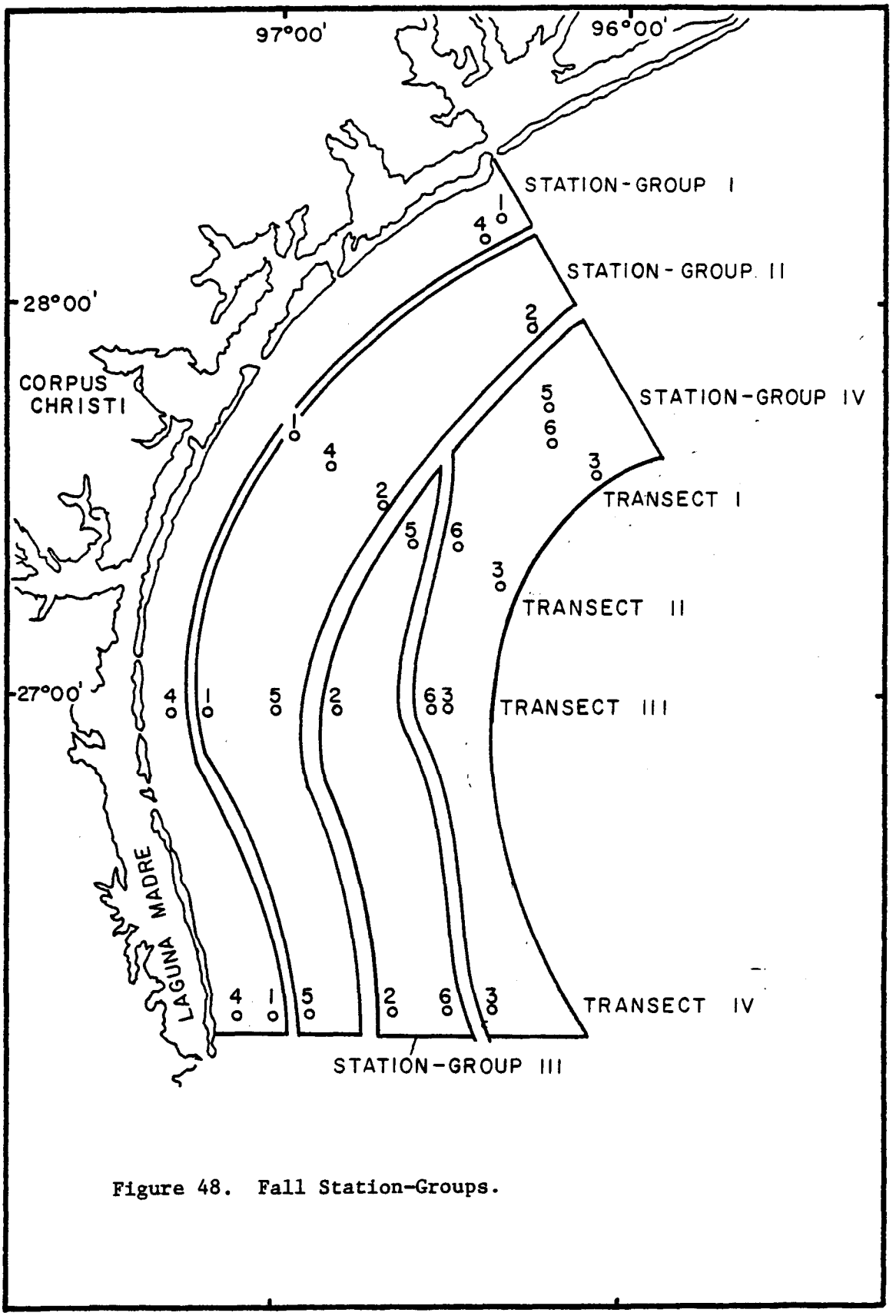

Figure 48. Fall Station-Groups.

fell into three main groups in depth ranges from 10-30 m (Station-Group I), 30-85 m (Station-Group II) and 65-135 m (Station-Group III). The inclusion of Station 6, Transect IV (6/IV), at 65 m in Group III caused the overlap in depth ranges between Groups II and III. All other stations in Group III ranged in depth from 90-135 m. Station-Groups II and III were more similar to each other than either was to Station-Group I making the most significant faunal division at about 30 m, separating the nearshore (turbulent zone) fauna from the mid to deep-water fauna.

Spring data showed four major groupings (Figures 45 and 46). Depth divisions were 10-25 m (Station-Group I), 25-50 m (Station-Group II), 50-85 m (Station-Group III) and 85-135 m (Station-Group IV). The most significant faunal break during the spring season occurred at about 50 m, separating Groups I and II from Groups III and IV. At the same time, Groups I and II showed more dissimilarity than Groups III and IV.

Four major depth groupings also occurred in the fall with almost identical depth divisions as in the spring (Figures 47 and 48). Depth ranges were 10-25 m (Station-Group I), 25-50 m (Station-Group II), 50-80 m (Station-Group III), and 80-135 m (Station-Group IV). Again, in the fall as in the spring, the most significant faunal break was at 50 m, separating the two inshore groups (I and II) from the two offshore groups (III and IV). Groups I and II also became more similar during the fall than they did in the spring so that the dissimilarity between Groups I and II were at about the same level as that between Groups III and IV, indicating that the turbulent zone fauna (Group I) which was significantly different from the Group II fauna in the winter and spring had begun to dissipate.

Two misclassifications occurred in the Station-Groups, both in the winter analysis. No collections were made at Station 7/IV because of

difficulties encountered in trawling on the shelf drop-off in 180 m of water. For the spring and fall sampling periods, the station was moved to the edge of the shelf-slope transition zone at 130 m depth. Since no data were collected at 7/IV in the winter, the station was grouped with the more depauperate inshore stations and should be disregarded. The other misclassification involved Station 6/I which was grouped with the mid-shelf stations (Station-Group II). After examination of the two-way table, the station was reclassified to Group III.

Studying the seasonal station dendrograms for any north-south division, none become evident until fall. During winter and spring months, the only stations which show polarization within a group were Stations 4/I and I/I in Station-Group I. During winter, large numbers of butterfish (*Peprilus burti*), atlantic croaker (*Micropogon undulatus*), silver seatrout (*Cynoscion nothus*), bay anchovies (*Anchoa mitchilli*) and banded drum (*Larimus fasciatus*) set these two stations apart from the other Group I stations. In spring, large numbers of croaker, anchovies and drum again appeared in large numbers at these two stations, while the butterfish moved offshore and spread out over the Group II stations. The silver seatrout still appeared in large numbers at Stations 4/I and 1/I, but becomes increasingly prevalent at the other Group I stations with the highest concentration of individuals appearing south at Station 4/IV.

The fall seasonal data showed a definite north-south split between Transects II and III. Station-Group I claimed no stations on Transect II and, once again, Stations 4/I and 1/I showed a relative dissimilarity to the other Group I stations. However, in the fall, this dissimilarity was due not to the presence of a few very abundant species as in the winter and spring, but rather to the relative absence of species and individuals.

Station-Group II showed dissimilarity between the southern (1/II, 1/III, 5/III and 5/IV) and the northern stations (2/I, 2/II and 4/II) based on the numbers of species and occurrences. Those stations in the southern section produced 37 species with 96 occurrences while the northern group of stations produced only 24 species with 43 occurrences, a significant difference even though there was one less station in the northern section of Group II. The similarity of Station 1/II with stations on Transects III and IV may be temperature related. Bottom temperatures for the three northern stations ranged from 23.6 to 27.5°C while the southern stations including 1/II showed a temperature range of 27.8 to 29.2°C (temperature data from Smith, 1976). Station-Group III claimed no stations on Transect I, possibly because there were no stations in the depth range (50-80 m) of Group III in the fall season. The northernmost station in this group was 5/II which showed fewer species (16) than the average number (28) at the southern stations (2/III, 2/IV and 6/IV). The deep-water group (IV) also supported the split between the northern depauperate stations and the richer southern stations. Stations 3/I, 5/I, 6/I, 3/II and 6/II had only 23 species with 63 occurrences, while the southern group with one less station (3/III, 6/III, 3/IV and 7/IV) produced 30 species with 62 occurrences. Overall, it appears that north-south differences were minimal until fall, or possibly late summer, when stations on the northern two transects (I and II) began to show a definite decline in the abundance of individuals and numbers of species.

Species Groups (Inverse Analysis)

The dendrograms from the inverse analyses showing species-groups for the three seasonal collection periods are presented in Figures 49-51. Two misclassifications occurred in the species-groups during the spring

data analysis. The blackfin searobin, *Prionotus rubio*, was grouped with dominant shallow-water species (Species-Group 2) while showing high constancy to the next deeper station-group as well. The misclassification most likely occurred as a result of the sensitivity of the Bray-Curtis measure to dominance since the searobin appeared in much larger numbers in Station-Group I than in Station-Group II. *Prionotus rubio* was reallocated to Species-Group 6 which contains species showing high constancy to Station-Groups I and II. The other misclassification involved the yellow conger, *Congrina flava*. Although the conger occurred only four times, it was grouped with species showing high constancy to Station-Groups II, III and IV (Species-Group 7), and was reclassified to Species-Group 5.

With the exception of these anomalies, the derived species-groups for the three seasonal analyses were generally acceptable in terms of their associations. The mid-to deep-water groups contained a number of species which occurred throughout the year, including such common species as the blackear bass (*Serranus atrobranchus*), wenchman (*Pristipomoides aquilonaris*), sash flounder (*Trichopsetta ventralis*), largescale lizardfish (*Saurida brasiliensis*), longspine porgy (*Stenotomus caprinus*), mexican searobin (*Prionotus paralatus*) and the shortwing searobin (*Prionotus stearnsi*). The shallow-water groups, however, changed considerably throughout the year with the most obvious change during late summer or fall. Abundant shallow species clustered together in the winter and spring, such as *Larimus fasciatus, Anchoa mitchilli, Cynoscion nothus,* the blackcheek tonguefish (*Symphurus plagiusa*) and the fringed flounder (*Etropus crossotus*), had either moved into deeper water or out of the study area by fall with the exception of the fringed flounder which

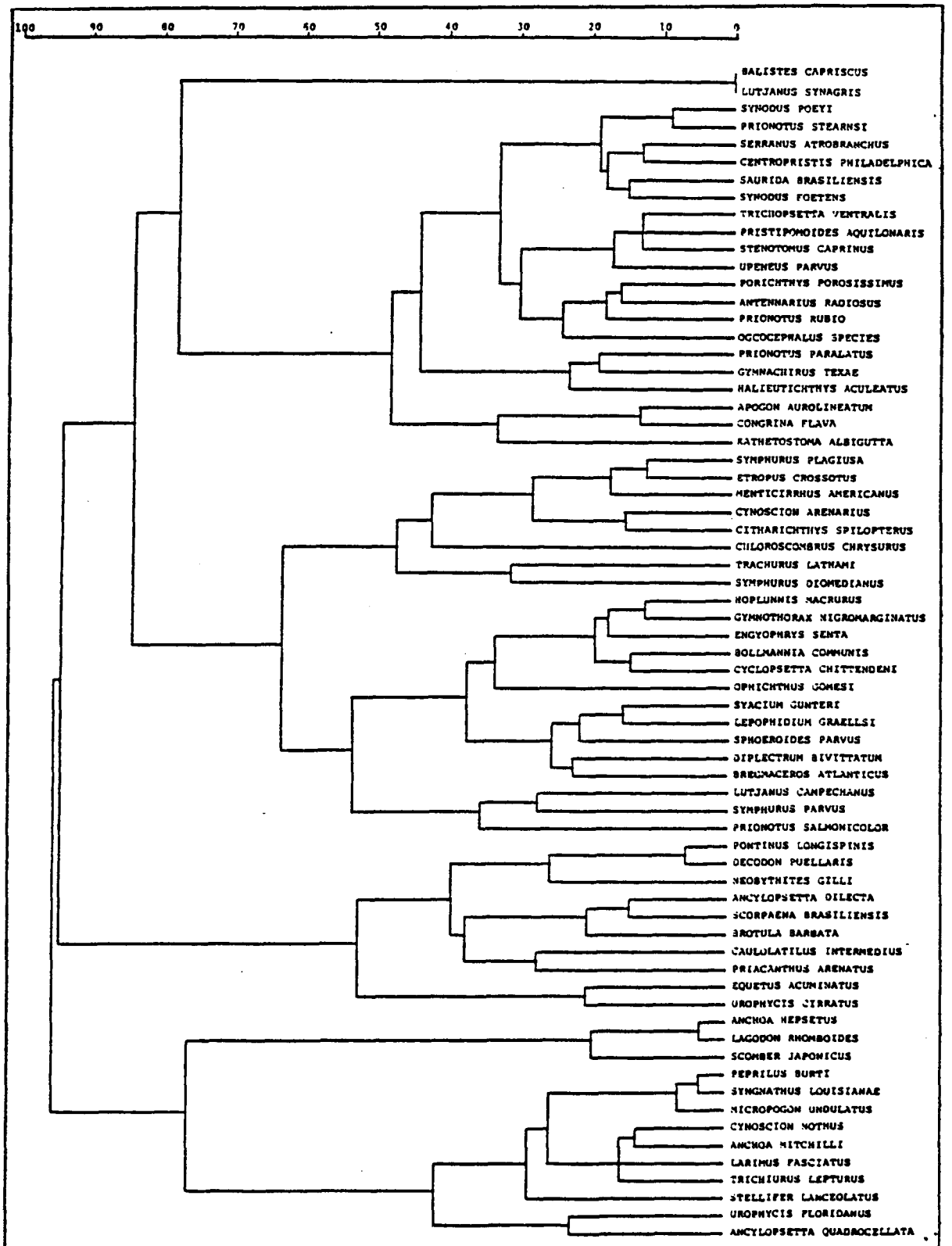

Figure 49. Species-Group Dendrogram (Inverse Analysis) for the Winter Data. Scale Across Top Represents Dissimilarity Values from 0 - 100 Percent.

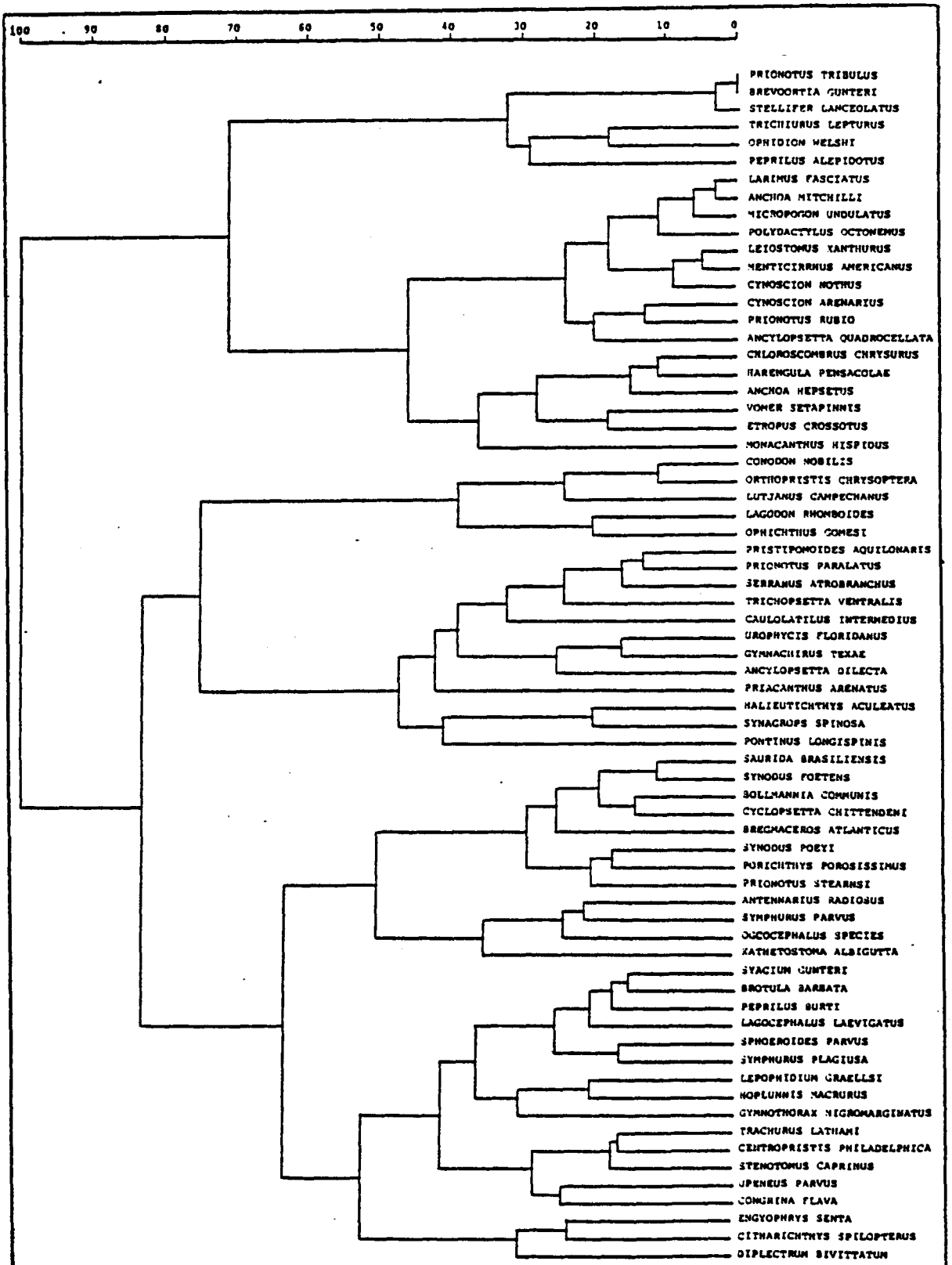

Figure 50. Species-Group Dendrogram (Inverse Analysis) for Spring Data. Scale Across Top Represents Dissimilarity Values from 0 - 100 Percent.

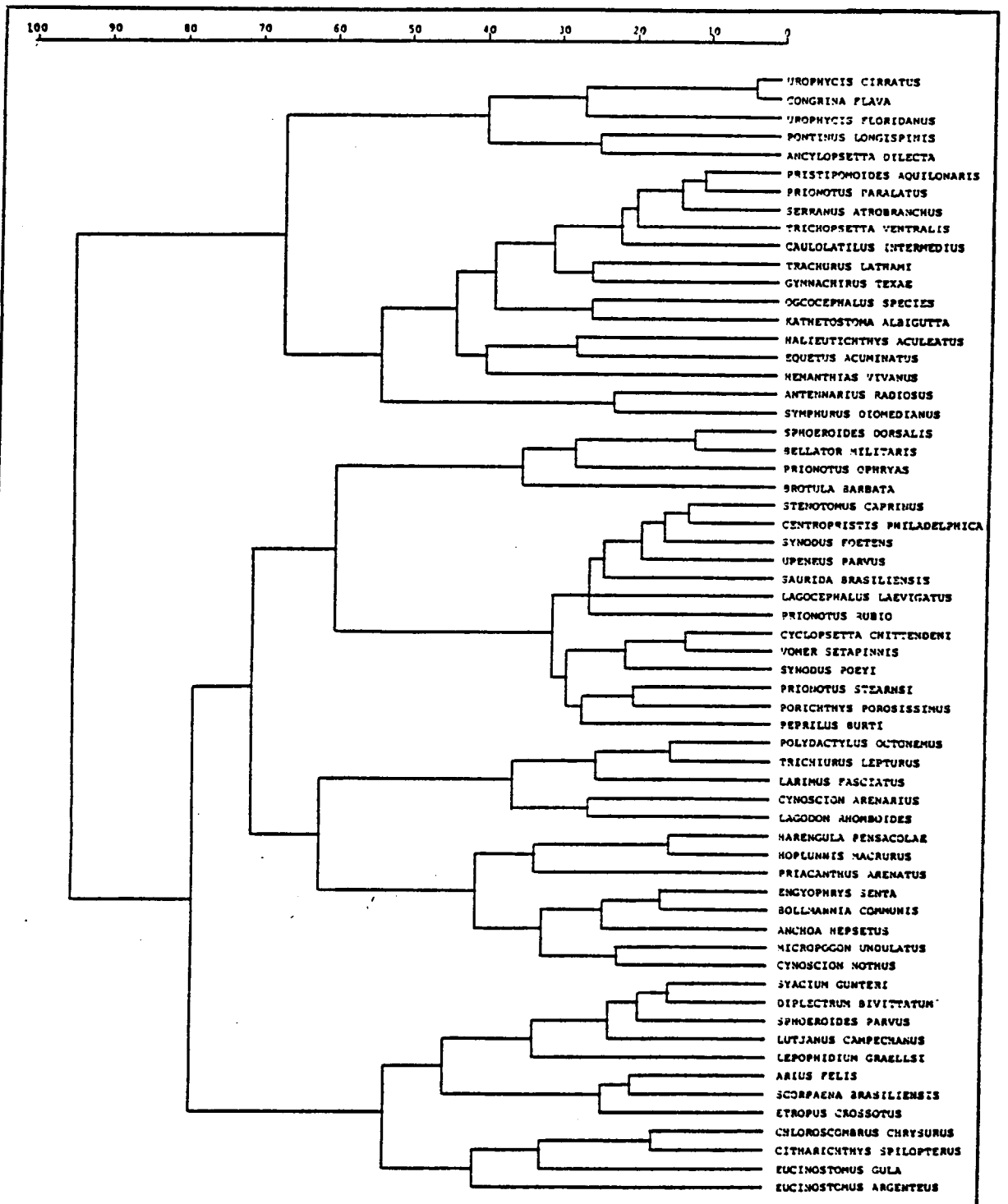

Figure 51. Species-Group Dendrogram (Inverse Analysis) for Fall Data. Scale Across Top Represents Dissimilarity Values from 0 to 100 Percent.

remained in somewhat smaller numbers in the shallow station-group. The red snapper (*Lutjanus campechanus*), which was captured occasionally in mid-to deep-water in the winter and spring, had moved inshore in sufficient numbers in the fall to show consistent occurrences at the stations in the shallow station-group.

Station-Species Coincidences (Two-Way Tables)

Tables 36-38 show information condensed from the seasonal two-way tables. Only those species showing VH or H constancy values and/or fidelity values are listed in the tables. Blocks show most representative station-group-species-group associations.

Table 36 for the winter data shows six of the seven species-groups and their relationships to the three station-groups. Species-Group 1, which was not included in the table, was composed of only two species with one occurrence each at Station 2/II. Since one occurrence does not constitute a faithful species for this study, both species and thus the group were eliminated. The major faunal division at 30 m was obvious with no blocks showing overlap between Station-Groups I and II. Species-Groups 3, 6 and 7 were associated with the shallow Station Group I. The striped anchovy (*Anchoa hepsetus*) in Group 6 could have been reallocated to Group 3, but those species in Group 3 showed a small number of occurrences in Station-Group II while the anchovy did not. It was thought best to leave the species in its own group. Although Species-Groups 3 and 7 represented the shallow station-group, they were separated because Group 7, including the butterfish, bay anchovy, silver seatrout and banded drum, showed no very high constancy values and was only indicative of Stations 4/I and 1/I. Group 3 species, including the fringed flounder, blackcheek tonguefish and southern kingfish (*Menticirrhus americanus*),

TABLE 36

INFORMATION CONDENSED FROM THE WINTER TWO-WAY TABLE SHOWING ONLY THOSE SPECIES HAVING VERY HIGH (VH = 95-100 PERCENT) OR HIGH (H = 66-94 PERCENT) CONSTANCY (C) AND/OR FIDELITY (F) VALUES. SPECIES-GROUPS APPEAR DOWN THE LEFT-HAND COLUMN AND STATION-GROUPS WITH APPROXIMATE DEPTH RANGE ACROSS THE TOP. BLOCKS SHOW MOST REPRESENTATIVE STATION-GROUP-SPECIES-GROUP ASSOCIATIONS.

	GROUP I 10-30 m		GROUP II 30-65 m		GROUP III 65-135 m	
	C	F	C	F	C	F
GROUP 2						
Synodus poeyi			H	H		
Prionotus stearnsi			H	H		
Serranus atrobranchus			VH		VH	
Centropristis philadelphica			H			
Saurida brasiliensis			VH			
Synodus foetens	H		VH		H	
Trichopsetta ventralis			H		VH	
Pristipomoides aquilonaris			H		VH	
Stenotomus caprinus			H			
Upeneus parvus			H			
Porichthys porosissimus			VH			
Antennarius radiosus			H	H		
Prionotus rubio	H		VH		H	
Prionotus paralatus					H	H
Apogon aurolineatum				VH		
Congrina flava				H		
GROUP 3						
Symphurus plagiusa	VH					
Etropus crossotus	VH	H				
Menticirrhus americanus	H	H				
Cynoscion arenarius		H				
Citharichthys spilopterus		H				
GROUP 4						
Hoplunnis macrurus			H	H		
Gymnothorax nigromarginatus				VH		
Engyophrys senta			H	H		
Bollmannia communis			H	H		
Cyclopsetta chittendeni			VH	H		
Ophichthus gomesi				H		
Syacium gunteri	H		H			
Lepophidium graellsi			H			
Sphoeroides parvus	H					
Diplectrum bivittatum				H		
Lutjanus campechanus				VH		
Symphurus parvus				VH		
GROUP 5						
Pontinus longispinis						VH
Decodon puellaris						VH
Neobythites gilli						VH
Ancylopsetta dilecta						H
Caulolatilus intermedius						H
Priacanthus arenatus						VH
Equetus acuminatus						VH
Urophycis cirratus						VH
GROUP 6						
Anchoa hepsetus	H	VH				
GROUP 7						
Peprilus burti	H	VH				
Sygnathus louisianae		VH				
Cynoscion nothus	H	H				
Anchoa mitchilli		VH				
Larimus fasciatus		H				
Trichiurus lepturus	H	VH				
Ancylopsetta quadrocellata		VH				

TABLE 37

INFORMATION CONDENSED FROM THE SPRING TWO-WAY TABLE SHOWING ONLY THOSE SPECIES HAVING VERY HIGH (VH = 95-100 PERCENT) OR HIGH (H = 66-94 PERCENT) CONSTANCY (C) AND/OR FIDELITY (F) VALUES. SPECIES-GROUPS APPEAR DOWN THE LEFT-HAND COLUMN AND STATION-GROUPS WITH APPROXIMATE DEPTH RANGE ACROSS THE TOP. BLOCKS SHOW MOST REPRESENTATIVE STATION-GROUP-SPECIES-GROUP ASSOCIATIONS.

		GROUP I 10-25 m		GROUP II 25-50 m		GROUP III 50-85 m		GROUP IV 85-135 m	
		C	F	C	F	C	F	C	F
GROUP 1									
	Prionotus tribulus		VH						
	Brevoortia gunteri		VH						
	Trichiurus lepturus	H							
	Peprilus alepidotus		VH						
GROUP 2									
	Larimus fasciatus		VH						
	Anchoa mitchilli		VH						
	Polydactylus octonemus	H	H						
	Leiostomus xanthurus		VH						
	Menticirrhus americanus		VH						
	Cynoscion nothus	VH	VH						
	Cynoscion arenarius	H							
	Ancylopsetta quadrocellata		H						
	Chloroscombrus chrysurus	H	H						
	Harengula pensacolae		VH						
	Anchoa hepsetus	H							
	Etropus crossotus	VH	H						
	Monacanthus hispidus		VH						
GROUP 3									
	Orthopristis chrysoptera		VH						
GROUP 4									
	Pristipomoides aquilonaris			H		H		VH	
	Prionotus paralatus			H				H	
	Serranus atrobranchus			VH		H		VH	
	Trichopsetta ventralis							VH	H
	Halieutichthys aculeatus							H	H
	Synagrops spinosa								VH
	Pontinus longispinis								VH
GROUP 5									
	Congrina flava				H				
	Saurida brasiliensis			VH					
	Synodus foetens			VH		H			
	Bollmannia communis			H	H				
	Cyclopsetta chittendeni			H	H				
	Bregmaceros atlanticus			H	H				
	Synodus poeyi					H			
	Porichthys porosissimus			H		H			
	Prionotus stearnsi					H			
	Symphurus parvus				H				
GROUP 6									
	Prionotus rubio	H		H					
	Syacium gunteri	VH		VH					
	Brotula barbata			VH					
	Peprilus burti	H		H					
	Lagocephalus laevigatus	VH		VH					
	Sphoeroides parvus	VH							
	Symphurus plagiusa	H							
	Lepophidium graellsi			H					
GROUP 7									
	Trachurus lathami			VH		VH		H	
	Centropristis philadelphica			VH		VH			
	Stenotomus caprinus			H		H		VH	
	Upeneus parvus			H		VH			
GROUP 8									
	Engyophrys senta				VH				
	Diplectrum bivittatum				VH				

TABLE 38

INFORMATION CONDENSED FROM THE FALL TWO-WAY TABLE SHOWING ONLY THOSE SPECIES HAVING VERY HIGH (VH = 95-100 PERCENT) OR HIGH (H = 66-94 PERCENT) CONSTANCY (C) AND/OR FIDELITY (F) VALUES. SPECIES-GROUPS APPEAR DOWN THE LEFT-HAND COLUMN AND STATION-GROUPS WITH APPROXIMATE DEPTH RANGE ACROSS THE TOP. BLOCKS SHOW MOST REPRESENTATIVE STATION-GROUP-SPECIES-GROUP ASSOCIATIONS.

		GROUP I 10-25 m		GROUP II 25-50 m		GROUP III 50-80 m		GROUP IV 80-135 m	
		C	F	C	F	C	F	C	F
GROUP 1									
	Urophycis cirratus								VH
	Congrina flava								VH
	Pontinus longispinis							H	VH
	Ancylopsetta dilecta								VH
GROUP 2									
	Pristipomoides aquilonaris					VH		VH	
	Prionotus paralatus					H		H	H
	Serranus atrobranchus			H		VH		VH	
	Trichopsetta ventralis					H		H	H
	Caulolatilus intermedius							H	H
	Trachurus lathami			H		H		H	
	Gymnachirus texae					H			
	Kathetostoma albigutta					H			
	Equetus acuminatus								H
	Hemanthias vivanus								VH
	Antennarius radiosus								H
GROUP 3									
	Sphoeroides dorsalis						H		
	Bellator militaris						VH		
GROUP 4									
	Stenotomus caprinus			VH		VH		H	
	Centropristis philadelphica	H		H		VH		H	
	Synodus foetens			H		VH			
	Upeneus parvus					VH			
	Saurida brasiliensis			H		H			
	Lagocephalus laevigatus					H			
	Prionotus rubio			H		H			
	Cyclopsetta chittendeni					VH			
	Synodus poeyi					VH	H		
	Prionotus stearnsi					VH			
	Porichthys porosissimus	H				H			
	Peprilus burti					H			
GROUP 5									
	Polydactylus octonemus				H				
	Larimus fasciatus				H				
	Bollmannia communis				VH				
	Micropogon undulatus			H	H				
	Cynoscion nothus				H				
GROUP 6									
	Syacium gunteri	H		H					
	Diplectrum bivittatum			H					
	Sphoeroides parvus	VH		H					
	Lutjanus campechanus	VH		H					
	Arius felis		H						
	Etropus crossotus		H						
	Chloroscombrus chrysurus			VH	H				
	Citharichthys spilopterus		H						
	Eucinostomus argenteus		H						

were found more consistently throughout the Station-Group I stations. Species-Group 4 showed a strong relationship to the 30-85 m depth group with six constant and nine faithful species out of a total of 14 in the group. Those species showing both constancy and fidelity to Station-Group II were the silver conger (*Hoplunnis macrurus*), spiny flounder (*Engyophrys senta*), naked goby (*Bollmannia communis*) and mexican flounder (*Cyclopsetta chittendeni*). Species-Group 5 consisted of 10 rare species, all with five or less occurrences. None were highly constant, but eight species were highly faithful to and thus characterize the deep Station-Group III. Species-Group 2 consisted of species which fairly consistantly represented the mid-to deep-water station-groups throughout the year. During the winter they were somewhat concentrated in Station-Group II with considerable overlap into Station-Group III. Fifteen of the 20 species in this group had high constancy grades in Station-Group II while only six had constant occurrences in the Group III stations. Five of the seven most abundant species in this group showed high constancy in both station-groups. The five species were *Serranus atrobranchus, Trichopsetta ventralis, Pristipomoides aquilonaris, Prionotus rubio* and the inshore lizardfish (*Synodus foetens*).

Table 37 shows the four site-groups and eight species-groups represented by the two-way table for spring. More station-group overlap by species-group began to appear and the major faunal break shifted from 30 m to approximately 50 m. Species-Groups 1, 2 and 3 all contained species highly constant or faithful to Station-Group I. Species-Group 1 consisted of six species, all with five or less occurrences. Three of the species were faithful to the station-group and were fairly rare species. The cutlassfish (*Trichiurus lepturus*) was the only species to show high constancy. This group of species was more representative of Station 4/I

than the rest of the station-group since all six species occurred at this station and no more than two of the species occurred at any other station in the station-group. Species-Group 2 consisted of species which were more consistent to the entire station-group but which were also most abundant at Stations 4/I and 1/I. Three species, the atlantic threadfin (*Polydactylus octonemus*), atlantic bumper (*Chloroscombrus chrysurus*) and *Cynoscion nothus* were highly constant and faithful to Station-Group I. Thirteen of the 16 species in Group 2 showed high constancy and/or fidelity values. All five of the species in Group 3 occurred six or less times and were not indicative of any station, except Station 4/IV where all five species were present. Only the pigfish (*Orthopristis chrysoptera*) showed a high fidelity value, but based on two occurrences only. Group 8 consisted of three species, two of which were very highly faithful to Station-Group II (25-50 m). Those two species, *Engyophrys senta* and the dwarf sand perch (*Diplectrum bivittatum*) were both highly faithful to the 30-85 m depth group in the winter. The remaining four species-groups (4, 5, 6 and 7) all showed station-group overlap. Group 6 showed eight species with high or very high constancy values for Station-Group I and/or II. None of the species were faithful to one station-group or the other, but four, *Prionotus rubio, Peprilus burti,* the shoal flounder (*Syacium gunteri*) and the smooth puffer (*Lagocephalus laevigatus*) were constant in both station-groups. Species-Group 5 contained six species constant and five species faithful to Station-Group II and four species constant and none faithful to Station-Group III. *Synodus foetens* and the midshipman (*Porichthys porosissimus*) were the only two species with constant occurrences over both station-groups. Species-groups 4 and 7 characterized Station-Groups II, III and IV (depth range 25-135 m) with Species-Group 4

leaning heavily towards the deeper water (85-135 m) and Group 7 more concentrated in the mid-depth station-groups (II and III). Group 4 had two species showing high constancy throughout the three station-groups, *Pristipomoides aquilonaris* and *Serranus atrobranchus*. *Trichopsetta ventralis* and the pancake batfish (*Halieutichthys aculeatus*) were both highly constant and faithful to Station-Group IV. Group 7 also showed two species with high constancy throughout the mid- to deep-water station-groups. Those species were the rough scad (*Trachurus lathami*) and longspine porgy (*Stenotomus caprinus*). The rock sea bass (*Centropristis philadelphica*) and dwarf goatfish (*Upeneus parvus*) occurred consistently in the two mid-depth station-groups (II and III).

Table 38 presents the four station-groups and six species-groups condensed from the fall seasonal two-way table. The most notable aspects of the fall data were the complete lack of any species-group which exemplified only the shallow-water station-group and the relatively smooth species transitions from station-group to station-group. Of the 26 species which characterized the shallow Station-Group I in the spring, only three *Syacium gunteri, Etropus crossotus* and the least puffer (*Sphoeroides parvus*)(Group 6), had high constancy or fidelity values for Station-Group I in the fall. Six other species *Lagocephalus laevigatus* and *Prionotus rubio* (Group 4) and *Chloroscombrus chrysurus, Cynoscion nothus, Polydactylus octonemus* and *Larimus fasciatus* (Group 5) which were associated with Station-Group I in the spring, had moved offshore with Group 4 showing constant occurrences in Station-Groups II (25-50 m) and III (50-80 m) and Group 5 showing five species faithful to Station-Group II with only one constant species (*Micropogon undulatus*). *Peprilus burti* which was found at a depth of 10-30 m in the winter and 25-50 m in the spring, occurred consistently at depths of 50-80 m in the fall.

The other 16 shallow-water species from the spring table appeared sporadically or not at all in the fall seasonal collections. Five species in Group 4 were consistently present at stations in Groups II and III. Those five species were *Stenotomus caprinus, Centropristis philadelphica, Synodus foetens, Saurida brasiliensis* and *Prionotus rubio.* Species-Group 3 characterized Station-Group III and consisted of four species with fewer than four occurrences each. Only two, the marbled puffer (*Sphoeroides dorsalis*) and the horned searobin (*Bellator militaris*) were faithful to the group. Species-Group 2, associated with Station-Groups III and IV (50-135 m), showed five highly constant species within the two station-groups: *Pristipomoides aquilonaris, Prionotus paralatus, Trichopsetta ventralis, Serranus atrobranchus* and *Trachurus lathami.* Group 1 species were fairly rare and only one, the longspine scorpionfish (*Pontinus longispinis*) showed high constancy and very high fidelity, while three other species, the gulf hake (*Urophycis cirratus*), the yellow conger (*Congrina flava*) and three-eye flounder (*Ancylopsetta dilecta*), had fidelity grades of very high.

DISCUSSION

General Distribution and Abundance

The overall 1976 collection records in Tables 1, 2 and 6, Appendix H. provide a good assessment of the samples in terms of species and their numerical and ponderal quantities. The species lists and relative abundances for 1975 and 1976 samples were quite similar. Of the 131 species collected in 1976, additions or absences from the 117 species collected in 1975 were ordinarily relegated either to relatively rare species or to "chance" occurrences of species which generally were invulnerable to trawl sampling. As in 1975, the 1976 frequency-rank plots were about the

same with about half the species occurring with less than 10 individuals each.

There appeared to be no unusual species occurrences and no unusual abundance patterns of individual species during the 1976 samplings, which were overall quite similar to the occurrences and patterns of the 1975 series.

The variability of the individual values for the replicates in Table 24 was rather large in many cases, with the numbers of individuals and weights in the triplicate samplings often varying by a factor of three or more. The nature of the calculations for the various indices, however, tended to make these values less variable among their replications.

A comparison of seasonal data for Stations 1, 2 and 3 in 1975 (Table 25) with comparable data in 1976 (Table 26) revealed some rather great year-to-year differences. In all categories, the means for the number of species and numbers of individuals were greater in 1975; the H_n" mean values were higher in all categories, but one, in 1975, while the opposite was true for the equitability E values. In only two cases were the PIE values higher in 1976. In all cases, the means of the various weight categories and the corresponding H_w" values were higher for the 1975 collections. The most likely initial explanation of the 1975-1976 differences in these means would be that the 1976 trawling was accomplished without the net liner that was used in 1975. An alternative explanation would be that the numbers, sizes and diversity indices actually declined after 1975. A third alternative explanation would involve statistical chance, which is less likely. Since the epibenthic invertebrates followed similar numerical, ponderal and species-specific declines in 1976

(Holland, this report), it is evident that any explanation of the differences between 1975 and 1976 at equivalent sampling stations would have to be related both to invertebrates and the fishes and both to sessile and vagile organisms.

Since the overall total number of fish species was slightly greater in 1976, the reduced diversity after 1975 is hard to account for, unless the fish in 1976 were generally larger in size in 1976 as a consequence of the larger mesh in the cod end of the trawl when the liner was absent. Currently, compilations of the length-frequency diagrams of the most abundant fishes over each of the seasons are being made for both 1975 and 1976. When these compilations are completed, a decision can be made whether or not the 1976 fishes averaged somewhat larger than those in 1975 and, thus, enable an evaluation of the effect of eliminating the trawl liner. On the other hand, the Shannon index H_n" should reflect diversity without bias due to sample size, according to its original usage (Peet, 1974, 1975; Goodman 1975). Heip and Engels (1974) noted that the Shannon index of diversity has a normal distribution, which would not be affected by sample size, and that the index has some degree of superiority over other indices. Thus, the data summarized in Tables 25 and 26 might logically differ in the event that there were real differences between 1975 and 1976 as a consequence of biotic and/or abiotic environmental changes over and above those due strictly to "uncontrolled" statistical variability. At the present stage of data analysis and synthesis between the two years, it is not likely that any simple explanation based on physical conditions, temperature, *e.g.*, could account for the differences, which implies that there may be multiple biotic-abiotic interactions involved.

On a comparable station basis, the collections in 1976 had fewer species and numbers of individuals, lesser biomasses, and lower ponderal and numerical diversities than in 1975. No completely tenable explanation has thus far appeared evident.

The analyses of variance for the various measurements and indices were not particularly different from 1975 (Table 27) to 1976 (Table 28) in which the single effects were not accompanied with especially large mean squares, except for "depth", especially in 1976. In 1975, the analyses showed little effect for "times" over the entire year; yet during winter and spring, night catches predominated but not in late summer-fall. In 1976, there were definitely more species taken at night and the diversity indices were also larger than in daytime. There is no good explanation why the "seasons" category had relatively larger mean squares in 1976 when none of the measurements and indices were statistically interesting with respect to "seasons" in 1975. (When individual species are considered in the later sections, it will be evident that major differences in single factor aspects of distribution and abundance are obvious.)

The most obvious characteristic of the two-factor effects in the analyses in Tables 27 and 28 are the several instances when "seasons" are included in the two-way interactions, especially in combinations of seasons with depths (stations), times of day, and (to some extent) transects. These two-way interactions are of particular interest because they agree quite well with field observations, plots of the original variates over the various seasons, an analysis of day-night differences, and cluster analyses by seasons. The combined analyses of 1975 and 1976 data as summarized in Table 29 are useful in that they reinforce the single

factor and double interaction effects for the 1975 and 1976 separate analyses, even though the variates are at different levels in the two years. Analysis of variance in this form without replications can also indicate the general level of the overall "error" variances in that the triple and quadruple interactions tend not to be statistically significant, and thus approach a reasonable estimate of the respective "error" variances that would be indicated by the replicated data from Transect II summarized in Table 24.

The analysis of variance for the various species counts, numbers and weights in individual collections and the various indices calculated from the samples are useful for a preliminary analysis of the overall effects of station depths, transects, time of day and season and various interactions of these effects.

The isopleth plots in Figures 1 to 42 are particularly revealing of seasonal and day-night attributes of distribution and abundance characteristics. Because of the obvious nature of the diagrams, no extended discussion is given here, although several qualitative conclusions are immediately evident.

It is obvious that patterns of abundance and distributional characteristics differed in practially all cases throughout the year for day-night pairs of isopleth diagrams. Also, the winter and spring gradients tended to be parallel and related to depth, while there was some indication of weak transect trends from north to south during the autumn. To expand on these conclusions, the preliminary master's thesis of Mrs. Elizabeth F. Vetter on day-night characteristics will be utilized in the following discussion, and the general clustering propensities discussed in the master's thesis of Mr. James F. Cole will be used more or less verbatim in the ensuing discussion.

Day-Night Comparisons

From the previous day-night isopleths and the analyses of variance, it is apparent that day-night differences existed between collection pairs, but data in a form such as that of Table 7, Appendix H, are required to separate individual species for day-night comparisons.

To explain why a given species was taken predominantly at a particular time on either a diel or seasonal basis, however, requires a considerable knowledge of both the behavioral patterns of the fish and the characteristics of the trawl. A few examples illustrate some of the major features of the dependence of catches on fish behavior. Some fishes are caught more readily at night because of visual inability to recognize and avoid the trawl (Woodhead, 1966). Emergence from or aggregation into diurnal shelter areas, dispersal or aggregation into defensively formed schools versus cases when individuals break away toward areas for feeding, among other factors, can be important in determining susceptibility to capture (Hastings *et al.*, 1976; Collette and Talbot, 1972). A large literature on the behavioral patterns around high diversity niches, such as coral reefs, artificial reefs, oil rigs, etc., is available for many species, but the literature on behavior for fishes on shelf plains with relatively soft bottoms is limited.

The fish taken predominantly in the day (Table 30) are quite commonly schooling fishes, whereas the more numerous, predominantly nocturnal species listed in Table 31 tend to be more solitary. At the $P = 0.05$ level or less, 10 species were taken predominantly during the day and 26 at night. Quite significantly, only the families Synodontidae, Tetraodontidae and Sciaenidae had genera in which one species was diurnal and one species was nocturnal.

Among all species that occurred ten or more times throughout 1976, only 13 species (listed in Table 32) definitely showed neither day or night trends in vulnerability to trawl sampling. Most of these species occurred much more than 10 times during the year.

When the 1976 collections are broken up into approximately two-month periods, the day (Table 33) and night collections (Table 34) indicate that there are pronounced seasonal changes that tend to explain the reason why the season-by-time interactions in the previous analyses of variance tend to be significant, while the time factor is ordinarily not statistically significant for data pooled over the entire year as indicated in Tables 27 and 28.

There is a tendency for both diurnal and nocturnal species to occur in the catches with increasing frequency from late fall and winter to the respective maxima in spring; this tendency is reversed in summer so that by autumn there are minima for both day and night predominating species.

When yearly pooled day-night comparisons were not statistically significant, the breakdown of comparisons of biomasses and numbers by seasons yielded seasonally significant differences ($P \leq 0.05$) for some additional species. There was a trend toward nocturnal prominence in the spring and a diminished tendency for either day or night prominence in the fall. To continue involved analyses of biomass changes seasonally between day and night collections would require additional analyses of the growth and changes in vulnerability of the individual species throughout the year. Complications would arise even in the simplest case where all the individuals of one species became vulnerable at one season and remained vulnerable throughout the year, in which case the continued mortality

through the year would gradually be reflected in progressively smaller catches.

When there was no general statistical significance between day and night catches for the 1976 pooled numerical occurrence data, the data, broken down into seasons on the basis of both numbers of individuals and biomass, yielded statistically significant ($P \leq 0.05$) day-night differences for a maximum number of species in the spring and for a minimum number of species in the fall.

There is insufficient knowledge of the life histories and behavioral characteristics of the various species to explain why they tend to reach their maximum vulnerabilities to trawling in terms of both day and night dominating species in the spring. Among the abiotic features, there is a need for further study of the effects of turbidity, light intensity, photoperiod and temperature as spring approaches. It is likely that these same features influence food supplies, especially the invertebrates, at this time. In addition to the biotic influences of feeding and food supply, the nature of feeding and growth rate along with spawning seasonality, needs ultimately to be investigated for each of the species to ascertain why they may tend to have an increase in either diurnal or nocturnal vulnerability to sampling in the spring and a decrease in the autumn.

Cluster Analyses of Sites and Species

General Remarks

In recent years, the utility of cluster analysis as applied to fishes has received some considerable emphasis. Horn and Allen (1976) give an excellent example of the application of cluster analysis in characterizing quantitatively the nature of California coastal environ-

ments in terms of species composition. Jumars (1976) evaluated species diversity in terms of scaling of the sampling techniques for different species taken at different places, and concluded that there may be subtle differences in vulnerability due to diverse biological characteristics of the species independently of their abundance. The problem of scaling quite obviously is in its initial stages of formulation; the solution to problems involved in scaling collections that have many species is not presently at hand, except in situations where all individuals can be censused. The following discussions are based on the combination of day and night collections with equal weight on each. Whether this unweighted combination is justified, collection for collection, locality by locality, and species by species, must await further theoretical and experimental research. However, the clustering techniques as applied by various disciplines are not altogether eristic, but are often in agreement with general knowledge and experience with reference to the distribution and abundance of the species.

Patterns of Distribution

General patterns showed rather strong shallow-water species isolation in the winter with the most abundant species occurring at Stations 4/I and 1/I just south of Pass Cavallo which is the Gulf access to Matagorda Bay. Four of the most common species at these stations (*Peprilus burti, Anchoa mitchilli, Micropogon undulatus* and *Cynoscion nothus*) are known to move into the bays or the shallow water near the passes in winter (Gunter, 1945), with *Micropogon undulatus* spawning in late fall or early winter and accounting for the huge numbers of small croaker found at these two stations in the spring. Nine of 11 of the constant species in the shallow-water group remained constant in the

spring and were joined by four other species which showed high constancy. One species-group (6) began to spread out in to the next deeper station-group with four species (*Prionotus rubio, Syacium gunteri, Peprilus burti* and *Lagocephalus laevigatus*) having high constancy values in both Station-Groups I and II. This small overlap caused the major faunal break to shift from 30 m in the winter to 50 m in the spring. By fall, however, only two of the highly constant species from winter and spring (*Sphoeroides parvus* and *Syacium gunteri*) remained constant in the shallow-water stations. The midshipman, rock sea bass and red snapper moved in from deeper water and were present consistently in the shallow-water group. The disappearance or reduction of abundance of the majority of the shallow species in the fall reduced the dissimilarity of the Group I stations and they became more similar to the Group II stations with the major faunal division remaining at 50 m. This faunal reduction in the fall was most likely due to the high, persistent temperatures of shallow water areas during this time. Winter sampling produced 12 species faithful to the shallow-water group, the spring 14 and the fall only four. Six species were repeated from winter to spring including: *Etropus crossotus, Menticirrhus americanus, Cynoscion nothus, Anchoa mitchilli, Larimus fasciatus* and the ocellated flounder (*Ancylopsetta quadrocellata*). *E. crossotus* was also highly faithful in the fall and was the only species to remain highly faithful to the shallow stations throughout the year.

The mid-depth station-groups representing a depth range of about 25-85 m showed a considerable amount of species shuffling throughout the year. The two mid-depth groups in both spring and fall (Station-Groups II and III) which range from 25-50 m and 50-85 m in the spring and 25-50 m and 50-80 m in the fall are discussed as single groups to allow comparisons to the winter 30-85 m depth group. Speaking only of species

which showed high constancy to the mid-depth stations and those stations along, *i.e.* without also being highly constant in another station-group, 9 species were represented in the winter, 12 in the spring and 11 in the fall. However, only six species were carried over from winter to spring, six from spring to fall, with only two represented throughout the year. Those two species were *Cyclopsetta chittendeni* and *Upeneus parvus*. Considering those species faithful to the mid-water groups, the winter collections showed 14 species, the spring collections seven and the fall collections nine. Six species retained their fidelity from winter to spring, but only one species (*Bollmannia communis*) remained faithful from spring to fall and was the only species with high fidelity in the mid-water groups throughout the year. It is interesting that in the spring there were no species highly faithful to Station-Group III (50-85 m) and only three species showed high fidelity to the equivalent station-group in the fall.

At the deep-water station-groups, only five species were found throughout the year which were highly constant to these groups alone. Those species were *Prionotus paralatus* (winter), *Trichopsetta ventralis* and *Halieutichthys aculeatus* (spring) and the blackline tilefish (*Caulolatilus intermedius*)(fall). Fidelity patterns had a higher number of coincidences between winter and fall rather than between winter and spring as in the rest of the station-groups already discussed. Six of the nine species which were faithful to the deep stations in winter were also faithful in fall. Only one faithful species from the winter was repeated in spring and that species (*Pontinus longispinis*) was faithful throughout the year. *Trichopsetta ventralis* was the only species, other than *P. longispinis*, to be highly faithful in spring and fall. Seven of

the nine faithful species which occurred in the winter were relatively rare so that the low repeatability in the spring was due either to absence of the species (four cases) or one or two occurrences outside the deep site-group (three cases).

All three of the depth zones showed considerable change throughout the year in terms of constant or faithful species when looked at individually, but considering the mid- to deep-water station-groups as a whole, there were a number of species which usually occurred with high constancy in more than one mid- or deep-water station-group throughout the year. These species were *Serranus atrobranchus, Synodus foetens, Trichopsetta ventralis, Pristipomoides aquilonaris, Stenotomus caprinus* and *Prionotus paralatus*. Although these were not the six most abundant species (abundance ranks for the year were 3, 24, 19, 15, 7 and 22, respectively) they were the most ubiquitous both in space and time.

Community Concepts

The only widespread dominant species found was *Serranus atrobranchus* which had the greatest abundance within its species groups at 10 of 17 associated stations in winter (Station-Groups II and III; 30-135 m), at 13 of 20 stations in the spring (Station-Groups II, III and IV; 25-135 m) and at 14 of 20 stations in the fall (Station-Groups II, III and IV; 25-135 m). Four minor dominant species appeared during winter and spring, but none appeared in both seasonal collections. In winter, *Syacium gunteri* dominated 7 of 9 stations within Station-Group II. Although *S. gunteri* remained constant at stations ranging from 10 to 85 m throughout the year, *Sphoeroides parvus* became increasingly prevalent in spring so that *S. gunteri* only dominated 5 of 12 associated stations while *S. parvus* dominated the other seven stations. In the spring, *Micropogon*

undulatus and *Polydactylus octonemus* from Species-Group 2 were dominant at Stations 4/I and 1/II, respectively, while both species were co-dominant at Station 1/I. By fall, both species had moved out to the next deeper station-group with the largest numbers of both appearing on Transects III and IV. *Trachurus lathami*, which dominated 6 and 7 stations in Station-Group II and showed consistent occurrences throughout Station-Group III and IV in spring, remained constant in the fall in all three station-groups but lost the dominant position to *Serranus atrobranchus*.

Many concepts of the community assume that a group of species will have similar ecological requirements and act as a unit in pattern of site distribution. No such groups were revealed in Petersen's original work. He sometimes listed a single species as characterizing a group of sites, sometimes it combined with one or more others, and elsewhere with yet others. The present study showed much the same, except that this analysis dealt in detail with many more species (62, 67 and 68 vs 12) which compounds the confusion. If the confusion is a real phenomenon then the community concept is weakened. These results support an interpretation of benthic assemblages as points in space and time with each species responding to its own individual set of environmental determinants (Boesch, 1973). Station-groups or "associations", therefore, seem to be segments of near continua of assemblages responding to complex environmental (including temporal) gradients (Whittaker, 1970). There is little justification for classifying the present species assemblages as tightly functioning units or as Petersen-type communities. However, a much more relaxed concept of what constitutes a community may be applicable. Mills (1969) defined a community as "a group of organisms occurring in a particular environment, presumably interacting with each other and the

environment, and separable by means of ecological survey from other groups".

Factors Affecting Distribution

The faunistic zones recognized on the South Texas Outer Continental Shelf appear to be related to depth. It would seem that the nature of the substrate is concerned, but attempts to relate distribution to sediment type have failed. Day *et al.* (1971) also failed to find any correlation between particle size and distribution of benthic invertebrates, while Field (1971) showed significant distribution-sediment type relationships and Day and Pearcy (1968) showed that zonation of benthic fishes was correlated to some extent with a subtle gradient in the sediment type and suggested that "faunal similarities are maintained in regions of sediment transition, and conversely, that factors other than sediment may govern the distribution of associations". Seasonality, with its associated temperature variations and programmed reproductive cycles, is a strong influence, especially on inshore populations which tend to move in and out of bays in response to these factors. Temperature variation for the most shallow stations was as much as 14°C while that for stations deeper than 50 m was less than 3°C (Smith, 1976). Since the temperature regime for offshore stations was rather stable throughout the year, seasonal variations in associations were most likely due to feeding habits or spawning migrations, suggesting that considerable life history work needs to be done before seasonal patterns can be fully understood.

Salinity is most likely not a factor in governing faunal distribution in deeper waters. Seasonal variation over all stations was only about 7 ppt, and variation within any one season was only slightly over

6 ppt (Smith, 1976). However, the much greater salinity variation in the bays and passes throughout the year was most likely a factor affecting those shallow-water species which move in and out of such areas.

Upon examining temperature ranges within station-groups and within seasons, there appeared to be some correlation to faunal zonation based on depth and station-group overlap by species-groups. Table 39 shows the station-group temperature ranges by season. In the winter, Station-Group I had a temperature range (14.3-17.2°C) significantly different from Station-Groups II or III which showed approximately the same temperature variation (17.8-20.8°C and 16.4-20.8°C, respectively). This was in accordance with the data presented in Table 36 showing no significant species overlap between Groups I and II or III. In spring, the Group I stations showed a considerable warming trend (22.8-26.6°C) with temperatures in all groups becoming more transitional. Group I was more dissimilar to Group II (5.5°C range) than Group III was to Group IV (3.5°C range) which agrees with Table 37 which shows strong shallow-water zonation with slight overlap into Group II and considerable species overlap between Groups II, III and IV. Overall temperature range was 9°C with the major faunal break occurring between Station-Groups II and III at about 21-22°C. By fall, the temperature range over all station-groups approached 13°C and the major faunal break occurred between 22° and 23°C. High inshore temperatures (28.3-29.3°C) seem to dissipate inshore fauna and the dissimilarity between Station-Groups I and II (5.7°C range) was at about the same level as that between Station-Groups III and IV (6°C range). The smooth transition of temperature over the larger temperature range was reflected by the smooth transition of species from station-group to station-group (Table 38).

TABLE 39

TEMPERATURE RANGES (°C) WITHIN STATION-GROUPS BY SEASON. STATION-GROUPS ARE REPRESENTED BY ROMAN NUMERALS. TEMPERATURE DATA ARE FROM SMITH (1976)

Winter		Spring		Fall	
I	14.3 - 17.2	I	22.8 - 26.6	I	28.3 - 29.3
II	17.8 - 20.8	II	21.1 - 23.3	II	23.6 - 29.2
III	16.4 - 20.8	III	20.8 - 22.1	III	19.8 - 22.4
		IV	17.6 - 20.5	IV	16.4 - 20.9

Depth Zonation

In comparing the depth zonation of the present study to numerical analyses of benthic invertebrates in False Bay, South Africa (Field, 1971) and on the shelf of North Carolina (Day, Field and Montgomery, 1971), similar results were found. In North Carolina, sampling was conducted at 10 stations from the intertidal to the continental slope at 200 m while in South Africa, sampling was in a bay using eight stations ranging in depth from 2 to 100 m. Using methods similar to those employed here, four faunistic zones were recognized in both studies, although two of the North Carolina zones (intertidal and slope) were beyond the range of this study, as well as the one in South Africa. The North Carolina turbulent zone (3-20 m) and the South African inner and outer turbulent zones (2-8 m and 16-23 m, respectively) correspond to the shallow-water turbulent zone in the present study (10-30 m), suggesting that a shallow-water faunal zone is most likely represented throughout the world's oceans in areas which are unprotected from turbulent wave action. The North Carolina outer shelf zone (40-120 m) and the outer shelf (38-58 m) and upper slope (80-100 m) zones of False Bay correspond to two groups in the winter (Station-Groups II and III; 30-85 m and 85-135 m) and three in spring and fall (Station-Groups II, III and IV; 25-50 m, 50-80(5) m and 80(5)-135 m) in the comparable analyses of South Texas OCS stations. Attempts to relate South Texas faunal zones with other benthic fish studies using similar methods resulted in problems dealing with much greater depth ranges. For example, Haedrich *et al.* (1975) sampled off New England at depths ranging from 140 to 1928 m, and Day and Pearcy (1968) sampled fishes off Oregon from 42 to 1829 m. Neither study sampled the turbulent zone fauna and the faunal zones

described were of a greater scope than the entire study area of the present study.

Sanders (1968) suggested that the diversity of a fauna increases as environmental stress decreases. It is interesting to see how this theory applies to faunistic groups along the South Texas Outer Continental Shelf. The mid-depth zone (25-85 m) appears to be the most diverse throughout the year, consistently showing higher numbers of highly constant species than either the shallow or the deep zones. According to Sanders (1968), harsh environments are populated by a few highly specialized species, though those that can tolerate such conditions may be present in large numbers since there is little competition. In areas where environmental conditions are less severe, there is more interspecific competition and the diversity rises. Changes in diversity in the present study support Sander's theory. A low diversity was found at the shallow stations where conditions were severe due to wave action, turbidity and extremes of temperature. The diversity increased as conditions improved and remained high until water depth reached about 85 m. This decrease in diversity on the outer edge of the shelf showed that conditions were poorer than on mid-shelf areas, but the reasons for this were not clear.

The seasonal faunistic zones recognized in this study appear to be depth and temperature dependent. There was no direct evidence that zonation was related to salinity or sediment type. It is not suggested that there are sharp changes between zones in nature, but the distribution of individual species suggest that they intergrade and the apparent distinctness of zones is caused in part by the distance between sampling stations.

CONCLUSIONS

The 1976 collections generally resembled those of 1975 with no unusual patterns of either abundance or distribution.

On a station to station basis, the 1976 collections had fewer species and numbers of individuals, lesser biomasses, and lower ponderal and numerical Shannon diversity indices than in 1975. No completely tenable explanation has appeared evident for the 1976 decreases in these measurements.

The analyses of variance for the various species counts, numbers and weights in individual collections, and the various indices calculated from the samples were useful for preliminary assessment of the general effects of station depths, transects, time of day, season and interactions among these effects. The most usual effects that had statistically significant levels involved interactions with seasons.

Numbers of species, biomasses, numbers of individuals, and the various indices of diversity for the individual stations plotted as isopleths differed in almost all cases between day and night throughout the year. The winter and spring gradients of these isopleths tended to be parallel and more or less related to depth, although there was some indication of north-south gradients during the autumn.

Fish taken predominantly during day sampling were commonly schooling species; predominantly nocturnal species tended to be solitary. Ten species were predominantly diurnal and 36 were nocturnal at a statistical level of $P \leq 0.05$. Synodontidae, Tetraodontidae and Sciaenidae were the only families in which one species was nocturnal and one species was diurnal. There was a pronounced tendency for both predominating diurnal and nocturnal species to be at a minimal abundance in autumn with a slow

rise to maximum abundance in spring. When there were no significant day-night differences among the pooled numerical occurrences, and when the data were broken down into seasons for the detection of differences in both numbers and biomasses, there were significant ($P \leq 0.05$) day-night differences for a maximum number of species in the spring and for a minimum number in the autumn. There appeared to be no simple explanation for the seasonal changes in diel sampling vulnerability.

Cluster analyses by use of the Bray-Curtis coefficient and flexible sorting technique with "normal analysis" with species as attributes of stations, indicated there were three station groupings in the winter and four groupings in the spring and autumn seasons. When stations were used as attributes of the species by "inverse analysis", there were seven species-groups formed from winter data, eight from spring data and six from autumn data. By use of two-way coincidence tables (see Tables 36, 37 and 38), the relationships between station-groups and species-groups became evident. The evaluation and interpretation of the cluster analyses also indicated that:

Zonation appeared to be depth related, with temperature and seasonal migration patterns influencing the species associations;

There was no direct evidence that zonation was related to salinity or sediment type;

The shallow-shelf turbulent zone has a low species diversity throughout the year, with especially high numbers of individuals in winter and spring;

The nearshore faunal associations dissipated during the late summer or autumn when shallow water temperatures were highest;

Mid- and deep-water associations were somewhat more stable throughout the year with the mid-shelf groups of species having the highest diversity;

North-south gradients were minimal except during autumn when weak species associations developed to show that the northern two transects were slightly different from the southern two transects; and,

All faunal zones had evidence of considerable species "shuffling" during the year, which suggested that Petersen-type species-dominated communities did not persist in the shelf areas that were studied.

LITERATURE CITED

Barnard, J. L., and F. C. Ziesenhenne. 1961. Ophiuroid communities of Southern California coastal bottoms. Pac. Nat. 21:131-152.

Boesch, D. F. 1973. Classification and community structure of macrobenthos in the Hamptons Roads area, Virginia. Mar. Biol. 21:226-244.

Bradley, J. V. 1968. Distribution free statistical tests. Prentice-Hall (ed.) New Jersey. pp. 105-117.

Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecol. Mongr. 27(4):325-349.

Buchanan, J. B. 1958. Bottom fauna communities across the continental shelf off Accra, Ghana. Proc. Zool. Soc. London 130:1-56.

Caswell, H. 1976. Community structure: a neutral model analysis. Ecol. Monogr. 46:327-354.

Chittenden, M. E., Jr., and J. D. McEachran. 1976. Composition, ecology and dynamics of demersal fish communities on the Northwestern Gulf of Mexico continental shelf, with a similar synopsis for the entire Gulf. TAMU-SG-76-208. pp. vii + 104.

_____, and D. Moore. 1976. Composition of the ichthyoplankton inhabiting the 100-m bathymetric contour of the Gulf of Mexico, Mississippi River to the Rio Grande. TAMU-SG-76-210, pp. v + 14.

Clifford, H. T., and W. Stepheson. 1975. An introduction to numerical classification. Academic Press, New York. 229pp.

Collette, B. B. and F. H. Talbot. 1972. Activity patterns of coral reef fishes with emphasis on nocturnal-diurnal change over. *In* Results of the Tektite Program: Ecology of coral reef fishes, pp. 98-124. Nat. Hist. Museum Los Ang. City, Sci. Bull. 14.

Day, D. S., and W. G. Pearcy. 1968. Species associations of benthic fishes on the continental shelf and slope off Oregon. J. Fish. Res. Bd. Canada 25(12):2665-2675.

Day, J. H. 1963. The complexity of the biotic environment. System. Assoc. Publ. No. 5, Speciation in the Sea. pp. 31-49.

_____, J. G. Field, and M. P. Montgomery. 1971. The use of numerical methods to determine the distribution of the benthic fauna across the continental shelf of North Carolina. J. Anim. Ecol. 40:93-126.

DeGroot, S. J. 1971. A review paper on the behavior of flatfishes. FAO Fish. Repts. No. 62(2):139-167.

Field, J. G. 1971. A numerical analysis of changes in the soft-bottom fauna along a transect across False Bay, South Africa. J. Exp. Mar. Biol. Ecol. 7(3):215-253.

_____. 1970. The use of numerical methods to determine benthic distribution patterns from dredgings in False Bay. Trans. R. Soc. Afr. 39:183-200.

_____. 1969. The use of the information statistics in the numerical classification of heterogeneous systems. J. Ecol. 57:564-569.

_____., and G. McFarlane. 1968. Numerical methods in marine ecology. I. A quantitative "similarity analysis" of rock shore samples in False Bay, South Africa. Zool. Afr. 3:119-138.

Goodman, D. 1975. The theory of diversity-stability relationships in ecology. Quart. Rev. Biol. 50:237-266.

Gunter, G. 1958. Population studies of the shallow water fishes of an outer beach in South Texas. Publ. Inst. Mar. Sci. Univ. Texas 5:186-193.

_____. 1945. Marine fishes of Texas. Publ. Inst. Mar. Sci. Univ. Texas 1:1-190.

_____. 1941. Relative number of shallow water fishes of the northern Gulf of Mexico, with some records of rare fishes from the Texas coast. Am. Midl. Nat. 26:194-200.

Haedrich, R. L., G. T. Rowe, and P. T. Polloni. 1975. Zonation and faunal composition of epibenthic populations on the continental slope south of New England. J. Mar. Res. 33(2):191-212.

Hartman, O. 1955. Quantitative survey of the benthos of the San Pedro Basin, Southern California. Allan Hancock Pac. Exped. 19:1-187.

_____., and J. L. Barnard. 1960. The benthic fauna of the deep basins off Southern California: continued studies in the seaward and deeper basins. Allan Hancock Pac. Exped. 22:217-235.

_____. 1958. The benthic fauna of the deep basins off Southern California. Allan Hancock Pac. Exped. 22:1-67.

Hastings, R. W., L. H. Ogren, and M. Mabry. 1976. Observations on the fish fauna associated with offshore platforms in the northwestern Gulf of Mexico. Fish. Bull. 74(2):387-402.

Heip, C., and P. Engels. 1974. Comparing species diversity and evenness indices. J. Mar. Biol. Ass. U. K. 54:559-563.

Hildebrand, H. H. 1954. A study of the fauna of the brown shrimp (*Penaeus aztecus* Ives) grounds in the western Gulf of Mexico. Publ. Inst. Mar. Sci. Univ. Texas 3(2):234-366.

Hobson, E. S. 1975. Feeding patterns among tropical reef fishes. Am. Sci. 63:382-392.

_____. 1974. Feeding relationships of teleostean fishes on coral reefs in Kona, Hawaii. Fish. Bull. U.S. 72:915-1031.

_____. 1973. Diel feeding migrations in tropical reef fishes. Helgoländer wiss. Meeresunters. 24:361-370.

_____. 1972. Activity of Hawaiaan reef fishes during the morning and evening transitions between daylight and darkness. Fish. Bull., U.S. 70:715-740.

_____. 1968. Predatory behavior of some inshore fishes in the Gulf of California. U.S. Fish. Wildl. Serv., Res. Repts. 73. 92pp.

_____. 1965. Diurnal-nocturnal activity of some inshore fishes in the Gulf of California. Copeia pp. 291-302.

_____., and J. R. Chess. 1976. Trophic interactions among fishes and zooplankters nearshore at Santa Catalina Island, California. Fish. Bull., U.S. 74:567-598.

Holt, S. A. 1976. Final report on temporal and spatial distribution of fishes in the upper Galveston Bay system with particular reference to the cooling water system of Cedar Bayou Generating Station. Thesis, Texas A&M Univ. 160pp.

Horn, M. H., and L. G. Allen. 1976. Numbers of species and faunal resemblance of marine fishes in California bays and estuaries. Bull. S. Calif. Acad. Sci. 75(2):159-170.

Hughes, R. N., and M. L. H. Thomas. 1971. The classification and ordination of shallow-water benthic samples from Prince Edward Island, Canada. J. Exp. Mar. Biol. Ecol. 7:1-39.

Hurlbert, S. H. 1971. A nonconcept of species diversity: a critique and alternative parameters. Ecol. 52:577-586.

Jumars, P. A. 1976. Deep-sea species diversity: Does it have a characteristic scale? J. Mar. Res. 34:217-246.

Kendall, M. G. 1962. Rank correlation methods. Charles Griffin and Co., London.

Lance, G. N., and W. T. Williams. 1967. A general thoery of classificatory sorting strategies. I. Hierarchical systems. Computer J. 9:373-380.

Lie, U., and J. C. Kelley. 1970. Benthic infauna communities off the coast of Washington and in Puget Sound: Identification and distribution of the communities. J. Fish. Res. Bd. Canada 27:621-651.

Lloyd, M., and R. J. Ghelardi. 1964. A table for calculating the "equitability" component of species diversity. J. Anim. Ecol. 33:217-225.

Longhurst, A. R. 1957. Density of marine benthic communities off West Africa. Nature 179:542-543.

_____. 1958. An ecological survey of the West African marine benthos. Fish. Publ. Colon. Off. London 11. 102pp.

May, R. M. 1975. Patterns of species abundance and diversity. pp 81-140. *In* Ecology and Evolution of Communities. M. L. Cody and J. M. Diamond (eds.) Belknap Press (Harvard Press), Cambridge and London.

Mayr, E., E. G. Linsley, and R. L. Usinger. 1953. Methods and principles of systematic zoology. McGray-Hill, New York.

Mills, E. L. 1969. The community concept in marine zoology, with comments on continua and instability in some marine communities: a review. J. Fish. Res. Bd. Canada 26:1415-1428.

Moore, D., H. H. Brusher, and L. Trent. 1970. Relative abundance, seasonal distribution and species composition of demersal fishes off Louisiana and Texas, 1962-1964. Contrib. Inst. Mar. Sci. 15:45-70.

Noy-Meir, I. 1973. Data transformation in ecological ordination. I. Some advantages of non-centering. J. Ecol. 61:329-341.

Parker, R. H. 1960. Ecology and distributional patterns of marine macro-invertebrates, Northern Gulf of Mexico, p 302-380. *In* Recent sediments, Northwest Gulf of Mexico, 1951-1958. Amer. Assoc. Petrol. Geol.

_____. 1956. Macro-invertebrate assemblages as indicators of sedimentary environments in the East Mississippi Delta Region. Bull. Mar. Assoc. Petrol. Geol. 40:295-376.

Peet, R. K. 1974. The measurement of species diversity. Ann. Rev. Ecol. Syst. 5:285-307.

_____. 1975. Relative diversity indices. Ecol. 56:496-498.

Petersen, C. G. J. 1918. The sea bottom and its production of fish food. Rep. Dan. Biol. Stn. 25:1-62.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. Jour. Theoret. Biol. 13:131-144.

Plante, R. 1967. Étude quantitative du benthos dans la region de Nosy-Bé note préliminaire. Cah. Office. Rech. Sci. Tech. Outre-Mer, Sér Océanogr. 5:95-108.

Pritchard, N. M., and A. J. B. Anderson. 1971. Observations on the use of cluster analysis in botany with an ecological example. J. Ecol. 59:727-747.

Robins, C. R. 1971. Distributional patterns of fishes from coastal and shelf waters of the tropical Western Atlantic. FAO Fish. Rept. 71 (2):249-255.

Sanders, H. 1968. Marine benthic diversity: a comparative study. Am. Nat. 102:243-282.

Seshappa, G. 1953. Observations on the physical and biological features of the inshore sea bottom along the Malabar Coast. Proc. Nat. Inst. Sci. India 19:257-279.

Shannon, C. E. 1948. A mathematical theory of communication. Bell. Syst. Tech. J. 27:372-423.

_____., and W. Weaver. 1963. The mathematical theory of communication. U. Ill. Press, Urbana. 117pp.

Smith, N. P. 1977. Hydrographic project. *In* Environmental assessment of the South Texas Outer Continental Shelf: Chemical and biological survey component. 1976 Draft Final Report to the Bureau of Land Management, Contract AA550-CT6-17.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. The principles and practice of numerical classification. Freeman, San Francisco. 537pp.

Springer, S., and H. R. Bullis. 1956. Collections by the OREGON in the Gulf of Mexico. Spec. Sci. Rept. U. S. Fish. and Wildl. Serv. 196: 1-134.

Stephenson, W. 1972. The use of computers in classifying marine bottom communities. UNESCO Oceanographic Symposium, Wellington, New Zealand.

_____., and W. T. Williams. 1971. A study of the benthos of soft-bottoms, Sek Harbor, New Guinea, using numerical analysis. Aust. J. Mar. Freshwat. Res. 22:11-34.

_____., and S. D. Cook. 1972a. Computer analysis of Petersen's original data on bottom communities. Ecol. Monogr. 42(4):387-415.

_____., and S. D. Cook. 1972b. The benthic fauna of soft-bottoms, Southern Moreton Bay. Ecol. Monogr. 42(2):155-178.

_____., and G. N. Lance. 1970. The macrobenthos of Moreton Bay. Ecol. Monogr. 40(4):459-494.

Stickney, R. R., G. L. Taylor, and R. W. Heard III. 1974. Food habits of Georgia estuarine fishes. I. Four species of flounders (Pleuronectiformes: Bothidae). Fish. Bull. 72:515-524.

Thorson, G. 1957. Bottom communities (sublittoral or shallow shelf). Geol. Soc. Am. Mem. 67(1):461-534.

_____. 1966. Some factors influencing the recruitment and establishment of marine benthic communities. Neth. J. Sea Res. 3:267-293.

Verdooren, L. R. 1963. Extended tables of critical values for Wilcoxon's test statistics. Biometrika. 50:177-186.

Whittaker, R. H. 1970. Communities and Ecosystems. MacMillan, London. 162pp.

Wiener, N. 1948. Cybernetics. John Wiley and Sons, N. Y. 194pp.

Wilhm, J. 1968. Use of biomass units in Shannon's forumla. Ecol. 49: 153-156.

Williams, W. T. 1971. Principles of clustering. Ann. Rev. Ecol. and System. 2:303-326.

Wohlschlag, D. E. 1976. Benthos Próject-Epifaunal fishes, pp. 250-330. *In* Environmental assessment of the South Texas outer continental shelf: chemical and biological survey component. Rept. to the Bureau of Land Management, Contract 08550-CT5-17. 598pp.

Woodhead, P. M. J. 1966. The behavior of fish in relation to light in the sea. Oceanogr. Mar. Biol. Ann. Rev. 4:337-403.

CHAPTER ELEVEN

HISTOPATHOLOGY OF INVERTEBRATE EPIFAUNA

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ABSTRACT

A total of 1152 organs from specimens representing 18 species were studied. There were 93 specimens of shrimp, representing five species and one stomatopod; 84 specimens of crabs, representing six species; 44 specimens of molluscs, representing four species; and 11 specimens of echinoderms, representing two species.

In the shrimp, the most common pathological condition of internal organs was due to nematodes. The most common symbionts in shrimp gills were ciliates. The most common gill pathology other than symbionts was deformed gills with excess cellularity and atrophy (etiology unknown). Shrimp had the lowest percentage of pathologies other than symbionts in internal organs.

In the crabs, nematodes were the most common symbiont of internal organs. The most common symbionts in the gill were unknown. The most common gill pathologies other than symbionts were small cell aggregates. Crabs had the lowest percentage of symbionts in tissues and the highest percentage of pathologies other than symbionts in internal organs.

Molluscs had the highest percentage and largest variety of symbionts in their internal organs. Trematode larvae were the most common symbiont. Molluscan gills were free from symbionts except for some bacteria infecting a few squid gills. Mollusc gills had the lowest percentage of pathologies other than symbionts.

No pathological conditions were found in the echinoderms.

INTRODUCTION

The purpose of this work element was to report the histopathologies observed in invertebrate epifauna collected at Stations 1, 2 and 3, Transect II. Five cruises were conducted (July 22-23, August 12-13, October 1-2, November 5-6 and December 3-4, 1976).

The collection of three species per station, five specimens per species and the examination of five organs per specimen was required. This was a total of 45 specimens and 225 organs per cruise, and 225 specimens and 1125 organs during the year. Whenever possible, extra species and specimens were collected to provide supplemental material if needed. Reported herein are 232 specimens (seven extra) and 1152 organs (27 extra).

The normal histology of most marine invertebrates has not been published. The majority of the reports in the literature are on species different from those of this study (Shaw and Battle, 1957). However, generalities can be made between the same tissues of a specific group of animals, such as bivalves.

Except for BLM environmental studies of regions other than the South Texas Outer Continental Shelf, no histopathological survey of marine invertebrates has been made to our knowledge. Examples of the only kinds of histopathological literature available are reports of a specific pathological condition in a single species (Barry *et al.*, 1971); a specific pathological condition in a particular group of animals (Solangi and Lightner, 1976); a general type of pathological condition in a particular group of animals (Sprague and Couch, 1971); and general reports which mention only a relatively few specific animals (Sparks, 1972). Most reports of pathological conditions were concerned with commercial species (Cheng, 1967). We studied only two commercial species.

In the literature concerning parasites of marine invertebrates, all of the parasites have been identified, at least to genus. Since the majority of pathological conditions found in the invertebrates in this study were due to parasites, identification of the parasites would be helpful. However, most parasites cannot be fully identified by means of sectioned material, but require the investigation of the whole parasite (helminths and arthropods) or live material (bacteria and most protozoans). Since these types of investigations are not possible in this work element, only general classification of the parasites were made.

MATERIALS AND METHODS

Invertebrates generally bury in the mud during daylight and emerge at night. Therefore, departure was at a time so as to arrive at Station 1 at dark, after which we proceeded to Stations 2 and 3. Specimens were collected in a 35-ft (10.7-m) otter trawl which was towed for 15-45 minutes, depending upon the availability of the animals. More than one trawl may have been taken per station.

Animals were removed from the otter trawl and sorted immediately. Usable specimens were placed in a tank with running seawater. One specimen at a time was brought into the dissecting area, measured, sexed (if possible) and various organs were dissected if the animal was large enough. If the animal was too small to dissect properly, it was opened to allow penetration of the fixative as follows: shrimp - the abdomen was severed from the cephalothorax, a small central dorsal portion of the carapace of the cephalothorax was removed after which the front of the head just behind the eyes was cut off; stomatopods - the abdomen was severed from the cephalothorax, sharp edges of abdominal segments and thoracic appendages were cut off and the front of the head just behind the eyes was removed; crabs - the legs

were removed, the dorsal carapace was opened from the posterior end, leaving the anterior end attached, and a cut was made into the muscle mass just anterior to the fourth leg; bivalves - specimens were carefully shucked and the adductor muscle was incised if necessary; if the specimens were too large to fix whole, they were cut into several portions; squid - the mantle was cut along the ventral midline and the animal spread open; heart urchin - the test was broken open on the aboral side; and, starfish - the arms were injected with fixative and severed. Organs not dissected on board ship were dissected in the laboratory with the aid of a dissecting microscope. Small organs were easier to dissect after fixation as less damage occurs to fixative-hardened tissues. Bivalves, regardless of size, were not dissected as they have a small coelomic cavity and most organs cannot be dissected without destroying the surrounding organs.

Different organs were studied in different types of animals as shown in Table 1. Since the organs of bivalves were not dissected, sections were made in the areas of the desired organs.

Dissected organs, parts of, or whole animals were placed in appropriate-sized tissue capsules, wrapped in cheese cloth or placed in perforated plastic bags with a label and immersed in Zenker's fixative (Lillie, 1965) for 12 to 24 hours. They were then rinsed in running tap water for 18 to 48 hours and placed in 70% ethyl alcohol until further treatment. At this time, dissection was completed. Further dehydration, clearing and infiltration with paraffin were completed in an automatic tissue processor (on loan from the Department of Biology, Texas A&M University) following a similar schedule as given in Humason (1972). Final paraffin embedding was done by hand.

Sections were cut at 6 μm, mounted on cleaned, labeled slides and

TABLE 1

ORGANS STUDIED

Animal	Gill	Digestive Gland	Intestine	Muscle	Renal Organ	Heart	Stomach	Other
crab	✓	✓	✓	✓	1st cruise only ✓	✓		
shrimp	✓	✓	✓	✓	1st cruise only ✓		✓	
stomapod	✓	✓	✓	✓			✓	
bivalve	✓	✓	✓	✓	✓			
squid	✓		✓	✓	✓			esophagus
heart urchin	(papula) ✓		✓	✓				epidermis & tube feet (podia)
starfish	(papula) ✓	✓	✓					epidermis & tube feet (podia)

dried at 35°C for about 24 hours. If the sections were small enough, six serial sections were mounted per slide, or six serial sections were mounted on the necessary number of slides. Each organ was stained with two different stains, two slides of each stain or 12 sections per stain, for a total of four slides or 24 sections per organ. Different stains were used for different tissues as listed in Table 2.

The Alcian Blue - PAS stain with hematoxylin demonstrates mucopolysaccharides, acid sulfated mucopolysaccharides, collagen, basement membranes, chitin and nucleic acids. Masson's Trichrome is a connective tissue stain and is designed to show nuclei, collagen, muscle, mucus, nerves, blood cells and other cytoplasmic elements. Chlorazol Black E is a good general stain, especially for chitin and muscle filaments. The Alcian Blue - PAS and Chlorazol Black E stains are performed by hand and the Masson's Trichome stain is carried out in an automatic tissue stainer. A special stain to aid in the identification of bacteria (Brown and Brenn, 1931) was also used. Slides were coverslipped with PERMOUNT mounting media. Slides were read with the aid of a Leitz Ortholux microscope.

Dr. John G. Mackin was a consultant and aided in the identification of parasites and other types of pathologies.

A total of 232 specimens representing 18 species were used (Table 3). BLM suggested collection of the brown shrimp (*Penaeus aztecus*), a bivalve and another species. It was possible to collect brown shrimp at least at one station each cruise. The most common bivalve collected was the paper shell scallop (*Amusium papyraceus*), but only when the otter trawl accidentally dug into the mud. No paper shell scallops were collected in August and only one in July, along with two specimens of the Gulf scallop (*Aequipecten glyptus*). The only other bivalve species collected was two

TABLE 2

STAINS USED FOR INVERTEBRATE TISSUES

Organ	[a]Alcian Blue - Periodic Acid Schiff	[b]Masson's Trichome	[c]Chlorozol Black E
Gill	✓		✓
Digestive Gland	✓	✓	
Intestine	✓	✓	
Muscle		✓	✓
Heart		✓	✓
Renal Organ	✓	✓	
Stomach	✓	✓	
Esophagus	✓	✓	
Epidermis	✓	✓	
Tube feet (podia)	✓	✓	

[a]Lillie, 1965

[b]Conn *et al.*, 1960

[c]Davenport, 1960

TABLE 3

NUMBER OF SPECIMENS STUDIED BY STATION AND MONTH

Species	July 1	July 2	July 3	Aug. 1	Aug. 2	Aug. 3	Oct. 1	Oct. 2	Oct. 3	Nov. 1	Nov. 2	Nov. 3	Dec. 1	Dec. 2	Dec. 3	Total
Shrimp:																
Penaeus aztecus	1	5		5	5		5	5		5	5			6		42
Penaeus setiferus													6			6
Trachypenaeus similis										5			5			10
Sicyonia dorsalis				6	5		5				2		6	6		30
Solenocera vioscai						4										4
Squilla chydaea										1						1
Crabs:																
Portunus spinicarpus			6						5			5			6	22
Portunus gibbesii										2						2
Callinectes similis		5		8	5			6			5					29
Anasimus latus			5			5						5			5	20
Raninoides louisianensis									5							5
Goneplacid crab	1	5														6
Molluscs:																
Amusium papyraceus			1					6	5			5			6	23
Aequipecten glyptus			2													2
Macoma pulleyi											2					2
Loligo pealei							6			5				6		17
Echinoderms:																
Brissopsis alta						6										6
Astropecten duplicatus											5					5
	2	15	14	19	15	15	16	17	15	18	19	15	17	18	17	232

specimens of *Macoma pulleyi* with the aid of a small biological dredge in November. Squid are not sedentary invertebrates, but since they are being used by Dr. Ramirez for chromosomal and gonadal studies, and by Drs. Presley and Boothe for heavy metals, a study of their histopathology was deemed useful.

The first cruise (July) was unsatisfactory with respect to the number of specimens collected at Stations 1 and 3. Only four specimens of the shrimp *Solenocera vioscai* were collected at Station 3 in August, but all other collections were adequate or more than adequate in terms of numbers of specimens/species. On the first cruise, collecting during the day was tried. Afterwards, collecting time was altered so as to arrive at Station 1 by sundown, then proceeding to Stations 2 and 3.

More organs than required (1152 vs 1125) were studied. Eight organs were lost in preparation of the tissue. Some were lost due to too large a hole in the tissue capsule (capsules with smaller holes were put in use), some to poor dissection on board ship (more dissection was done in the laboratory), and some due to shattering upon sectioning because of excessively hard material in the tissue block, such as intestinal contents containing sand (celloidin coating of intestine sections has been an added procedure).

RESULTS AND DISCUSSION

General

The majority of pathologies in the internal organs of all animals studied was due to symbionts, most of which were parasitic. Symbiosis is a general term used to describe all types of heterospecific associations, excluding predation (Cheng, 1967). Some of the symbionts found in this study are well known parasites, such as nematodes, but others may be

examples of commensalism, mutualism or phoresis. Information is not available for those not known as definite parasites so they cannot be definitely categorized. However, if a non-parasitic organism is present in large numbers in a region such as the intestine, it can definitely cause damage to the host by blocking the duct and possible damaging the epithelium (Sindermann, 1970). This same phenomenon can occur in the gills, where large numbers of nonparasites can cause loss of gill surface area exposed to the water for gaseous exchange as well as actual damage to the gill filaments such as breakage.

Damage to tissues by parasites or other symbionts is usually obvious, but the etiologies of other pathologies are unknown. There may be more than one cause for each type of pathology described in this study in the same or different species.

The results of this study are presented in Tables 4-19. The results are tabulated by season (Tables 4, 6, 8, 10, 12, 14, 16 and 18), summer for the July and August collections and fall for the October, November and December collections and by the three stations used along Transect II (Tables 5, 7, 9, 11, 13, 15, 17 and 19). In the tables, the term "between gill filaments" generally indicated a non-parasitic organism, or at least one which usually did not appear to cause damage to the gills. Symbionts recorded as "unknown" were generally ones in which only a small part of the organism appeared in only a few sections. Therefore, they could not be identified. When a symbiont or pathology was recorded as being in an organ, it may have been in the connective tissue immediately surrounding the organ rather than in the parenchyma of the organ itself. This was usually the case with symbionts in the heart. In tables for pathologies other than symbionts, "leucocytosis around parasites" indicated a reaction by the host to the parasite. If the leucocytes of the host were successful,

FOOTNOTES TO TABLES 4 THROUGH 9

Symbiont

A - coelenterate
B - bacteria
C - cestode larva
D - degenerate parasite
E - *Squilla* ciliates
F - flatworm
G - gregarines
K - PAS+ cells
L - large ciliate (stalked)
M - mysoxporidian cyst
N - nematode
P - copepod
Q - pinnotherid crab
R - unknown spore infection
S - small ciliate
T - trematode larva
U - fungal hyphae
V - dorivelleid polychaete in gill chamber
W - sporazoan
X - unknown
Y - protozoan or algae attached to gill
Z - Plasmodiophoralid fungi

Organ

a - mantle
b - esophagus
c - interstitial connective tissue
d - caecum
e - gill
f - between gill filaments
g - muscle
h - body wall connective tissue
i - stomach
j - renal organ

Season

*S - summer
**F - fall

TABLE 4

NUMBER OF SHRIMP WITH SYMBIONTS BY SEASON

Species	No. of Specimens		Gills		Digestive Glands		Intestine		Stomach		Gonad		Other	
	S*	F**	S	F	S	F	S	F	S	F	S	F	S	F
Penaeus aztecus	16	26	2T 1S 1L	4W 1Z 2L	7N 7C 3D	8N 4C 2D 1T	1N	1N 7G	8N 1T	4N	5N		1N[j] 1D[j] 1X[g]	
Penaeus setiferus		6				3C				2N				
Trachypenaeus similis		10		5S										
Sicyonia dorsalis	11	19	11S 4L	17S 6L 1X	2N	12N 2D	1N	1X	3N	8N 4D	2N 1D	2N 1D	1N[c]	
Solenocera vioscai	4		1S		1N				2N		1N			
Squilla chydaea		1		1E										

TABLE 5

NUMBER OF SHRIMP WITH SYMBIONTS BY STATION

Species	No. of Specimens			Gill			Digestive Gland			Intestine			Stomach			Gonad			Other		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Penaeus aztecus	16	26		4W 1Z	2T 3L 1S		6N 5C 3D 1T	9N 6C 2D		1N	1N 7G		6N 1T	6N		4N	1N		1X[g] 1N[j] 1D[j]		
Penaeus setiferus	6						3C						2N								
Trachypenaeus similis	10			5S																	
Sicyonia dorsalis	17	13		16S 4L 1X	12S 6L		8N	6N 2D		1N 1X			5N 1D	6N 3D		1N 1D	3N 1D			1N[c]	
Solenocera vioscai			4			1S		1N						2N			1N				
Squilla chydaea	1			1E																	

TABLE 6

NUMBER OF CRABS WITH SYMBIONTS BY SEASON

Species	No. of Specimens		Gills		Digestive Gland		Intestine		Heart		Gonad		Other	
	S*	F**	S	F	S	F	S	F	S	F	S	F	S	F
Callinectes similis	18	11	10X[f]	3X[f] 1A[f] 1Z	4M	3M 2N 1D	1M			3M	3M	2M 1N	1N[h] 1M[g] 1X[g] 1X[h]	
Portunus spinicarpus	6	16		3X[f]	1N 1C	1N				1N				
Goneplacid crab	6		2X[f]		3N 3D		3N 1D						1N[h]	
Anasimus latus	10	10	6X[f] 4Y 5V	4X[f] 1U 6V	3N 1W 2D		1N 1D	1N			1N 2D		1N[i]	
Raninoides louisianensis		5				2N		2G				1N		

TABLE 7

NUMBER OF CRABS WITH SYMBIONTS BY STATION

Species	No. of Specimens			Gills			Digestive Gland			Intestine			Heart			Gonad			Other		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Callinectes similis	8	21		6X[f]	1A[f] 7X[f] 1Z		4M	3M 2N 1D		1M				3M		3M	2M 1N		1M[g] 1X[h]	1N[h] 1X[j]	
Portunus spinicarpus			22			3X[f]			2N 1C						1N						
Goneplacid crab	1	5				2X[f]	1N	2N 3D		1N 1D	2N									1N[h]	
Anasimus latus			20			11V 4Y 1U[f] 10X[f]			3N 1W 2D			2N 1D						1N 2D			1N[i]
Raninoides louisianensis			5						2N			2G						1N			

TABLE 8

NUMBER OF MOLLUSCS WITH SYMBIONTS BY SEASON

Species	No. of Specimens		Digestive Gland		Intestine		Gonad		Stomach		Kidney		Other	
	S*	F**	S	F	S	F	S	F	S	F	S	F	S	F
Amusium papyraceus	1	22		5N 3C 3P 3R 1F 3X 1D		4P 2T 4D 1C		2C 1D 1P 1Z		2T 2X 2D 1N 1C 1P	1K	22K 1T 1W 1Z		2D[a]
Aequipecten glyptus	2		1N 1T 1D		2C 1P 1W		1N 1Z		1N 1T 1C				2T[a]	
Loligo pealei		17		1X		4T 1W 1X 1D				3T		1N 2T 2X[c]		1W[a] 1T[b] 1T[c] 1C[c] 2T[d] 2X[d] 5B[e]
Macoma pulleyi		2		1N 1D		1F								1Q

TABLE 9

NUMBER OF MOLLUSCS WITH SYMBIONTS BY STATION

Species	No. of Specimens			Digestive Gland			Intestine			Gonad			Stomach			Kidney			Other		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Amusium papyraceus		6	17		1N 1C 1P 2X	4N 2C 2P 1X 3R 1D 1F		1C	2T 4P 4D		1C	1C 1P 1D 1Z		1C 1X	2T 1N 1P 2D 1X		6K	17K 1T 1W 1Z			2D[a]
Aequipecten glyptus			2			1N 1T 1D			2C 1P 1W			1N 1Z			1N 1T 1C						2T[a]
Loligo pealei	11	6		1X			3T	1T 1W 1D 1X					1T	2T		1T 2X	1T 1N		1W[a] 1T[b] 1T[c]	2T[d] 1C[d] 2X[d] 5B[e]	
Macoma pulleyi		2			1N 1D			1F												1Q	

FOOTNOTES TO TABLES 10 THROUGH 19

Pathology

A - atretic egg
C - cyst
D - deformed hepatopancreatic tubules
E - degenerate eggs
H - hyperplasia
L - leucocytosis (etiology unknown)
L'-leucocytosis around parasites
S - swollen sarcolemma

Organ

a - mantle
b - esophagus
c - interstitial connective tissue
d - caecum
e - gill
i - stomach
j - renal organ

Season

*S - summer
**F - fall

TABLE 10

NUMBER OF SHRIMP WITH PATHOLOGIES OTHER THAN SYMBIONTS
IN INTERNAL ORGANS BY SEASON

Species	No. of Specimens		Digestive Gland		Stomach		Gonad		Renal Organ		Heart	
	S*	F**	S	F	S	F	S	F	S	F	S	F
Penaeus aztecus	16	26	6C	2C		1L' 1C	1L'		1L'	1L		
Penaeus setiferus		6		4C		3L 1C						
Sicyonia dorsalis	11	19	1C	3C 2L'		3C 4L'		1A				1L

TABLE 11

NUMBER OF SHRIMP WITH PATHOLOGIES OTHER THAN SYMBIONTS IN INTERNAL ORGANS BY STATION

Species	No. of Specimens			Digestive Gland			Stomach			Gonad			Renal Organ			Heart		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Penaeus aztecus	16	26		2C	6C		1C	1L'			1L'		1L	1L'				
Penaeus setiferus	6			4C			3L 1C											
Sicyonia dorsalis	17	13		2C	2C 2L'		1L'	3L' 3C		1A						1L		

TABLE 12

NUMBER OF CRABS WITH PATHOLOGIES OTHER THAN SYMBIONTS
IN INTERNAL ORGANS BY SEASON

Species	No. of Specimens		Digestive Gland		Intestine		Heart		Gonad		Renal Organ	
	S*	F**	S	F	S	F	S	F	S	F	S	F
Callinectes similis	18	11	3H 5H 1D	2H 3L 4C	1C 1L	3C 2L 2H	1L	1L 2C				1L
Portunus spinicarpus	6	16	1C	2C	1C			3S				
Goneplacid crab	6		2C 1L'		1L'							
Anasimus latus	10	10		1C		1C		5S 2C		1A		
Raninoides louisianensis		5						5S				

TABLE 13

NUMBER OF CRABS WITH PATHOLOGIES OTHER THAN SYMBIONTS IN INTERNAL ORGANS BY STATION

Species	No. of Specimens			Digestive Gland			Intestine			Heart			Gonad			Renal Organ		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Callinectes similis	8	21		3L 1D	5L 5H 4C			3L 2H 4C		1L	1L 2C						1L	
Portunus spinicarpus			22			3C			1C			3S						
Goneplacid crab	1	5			2C 1L'		1L'											
Anasimus latus			20			1C		1C				2C 5S			1A			
Raninoides louisianensis			5									5S						

TABLE 14

NUMBER OF MOLLUSCS WITH PATHOLOGIES OTHER THAN SYMBIONTS BY SEASON

Species	No. of Specimens		Gill		Digestive Gland		Intestine		Gonad		Other	
	S*	F**	S	F	S	F	S	F	S	F	S	F
Amusium papyraceus	1	22		3L		2C		8C 2L		3C 1L 1E		1C[c] 2C[i]
Aequipecten glyptus	2						2C					
Loligo pealei		17		2H 1L				1H 1C				
Macoma pulleyi		2				1C		2L				1C[j]

TABLE 15

NUMBER OF MOLLUSCS WITH PATHOLOGIES OTHER THAN SYMBIONTS BY STATION

Species	No. of Specimens			Gill			Digestive Gland			Intestine			Gonad			Other		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Amusium papyraceus		6	17			3L		1C	1C		2L 1C	7C		1C 1E 1L	2C		1C[c] 2C[i]	
Aequipecten glyptus			2									2C						
Loligo pealei	11	6		1H	1H 1L						1H 1C							
Macoma pulleyi		2						1C			2L						1C[j]	

TABLE 16

NUMBER OF SHRIMP WITH GILL PATHOLOGIES OTHER THAN SYMBIONTS BY SEASON

Species	No. of Specimens		Small Cell Aggregates		Deformed Filaments with excess Cellularity		Swollen Filaments		Cyst in Branchial Sinus	
	S*	F**	S	F	S	F	S	F	S	F
Penaeus aztecus	16	26		1	15	23			4	3
Penaeus setiferus		6				4				4
Trachypenaeus similis		10		2		9				1
Sicyonia dorsalis	11	19	1	3	11	18	7	17		
Solenocera vioscai	4				4					
Squilla chydaea		1				1				

TABLE 17

NUMBER OF SHRIMP WITH GILL PATHOLOGIES OTHER THAN SYMBIONTS BY STATION

Species	No. of Specimens			Small Cell Aggregates			Deformed Filaments with excess Cellularity			Swollen Filaments			Cyst in Branchial Sinus		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Penaeus aztecus	16	26			1		14	24					1	6	
Penaeus setiferus	6						4						4		
Trachypenaeus similis	10			2			9						1		
Sicyonia dorsalis	17	13		1	3		16	13		13	11				
Solenocera vioscai			4						4						
Squilla chydaea	1						1								

TABLE 18

NUMBER OF CRABS WITH GILL PATHOLOGIES OTHER THAN SYMBIONTS BY SEASON

Species	No. of Specimens		Small Cell Aggregates		Large Cell Aggregates		Deformed Filaments with excess Cellularity		Swollen Filaments		Cyst in Branchial Sinus		Deformed Filaments	
	S*	F**	S	F	S	F	S	F	S	F	S	F	S	F
Callinectes similis	18	11	16	7	7	1	12	7	6	5	8	5	3	2
Portunus spinicarpus	6	16	5	16	1	2		3	2	6	3	3		1
Portunis gibbesii		2		2						2		2		
Goneplacid crab	6		3						2		2			
Anasimus latus	10	10	6	9		3		7	1	2		4		1
Raninoides louisianensis		5		2								1		

TABLE 19

NUMBER OF CRABS WITH GILL PATHOLOGIES OTHER THAN SYMBIONTS BY STATION

Species	No. of Specimens			Small Cell Aggregates			Large Cell Aggregates			Deformed Filaments with excess Cellularity			Swollen Filaments			Cyst in Branchial Sinus			Deformed Filaments		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	3	3	1	2	3
Callinectes similis	8	21		8	15		3	5		4	15		3	8		4	9		1	4	
Portunus spinicarpus			22			21			3			3			8			6			1
Portunus gibbesii	2			2						2						2					
Goneplacid crab	1	5			3									2			2				
Anasimus latus			20			15			3			7			3			4			1
Raninoides louisianensis			5			2												1			

the parasite would die and be observed as a degenerating parasite in early stages or as a cyst in later stages. However, cysts may occur from unknown etiologies.

Symbionts may be single or multiple. The actual number of symbionts, for example, relatively long and sinuous nematodes, could not always be counted in sections. Since only sectioned material was studied most of the symbionts could not be positively identified. Animals collected but not listed in the tables did not have symbionts or pathologies. *Squilla chydaea*, a stomatopod, is listed with the shrimp.

Photomicrographs representing various common symbionts and pathologies are presented as Figures 1-20. Although not all species are represented, these figures aid in the understanding of various common diseases.

Symbionts

The percentages given in the following discussion are the number of animals with a given symbiont per total number of animals examined.

Nematodes (Figure 1) were the most common parasite of shrimp (76%) and crabs (29%). They were present (25%), but not the most common parasite in molluscs (Cheng, 1967). In shrimp, crabs and molluscs, the digestive gland had the highest infection rate. Nematodes occurred singly or in relatively large numbers and were often observed in and between the internal organs during dissection. Nematodes may become encysted in the host's tissues, a reaction by the host to the parasite, and may be attacked successfully by the host's leucocytes. These are the source of many of the tabulated "degenerating parasites" in shrimp and crabs.

Trematode larvae (Figure 2) were the most common parasite in molluscs (50%). There were very few trematode larvae in shrimp (4%) and none in crabs. These parasites may also become encysted and frequently degenerate.

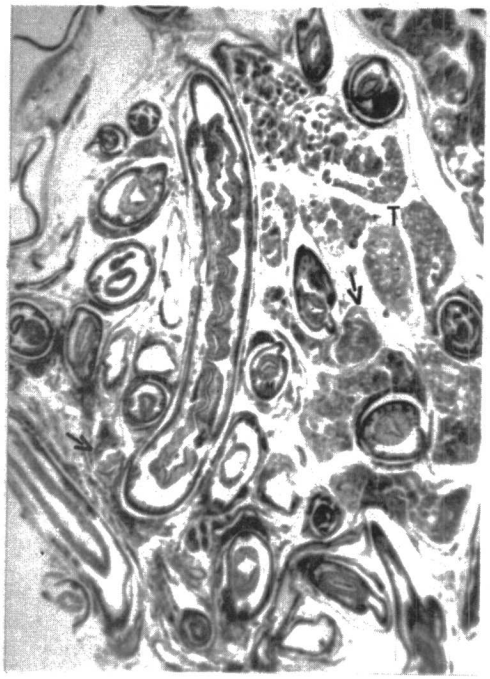

Fig 1. *S. dorsalis*. Section of live and degenerating (arrows) nematodes in the testis (T). 33X.

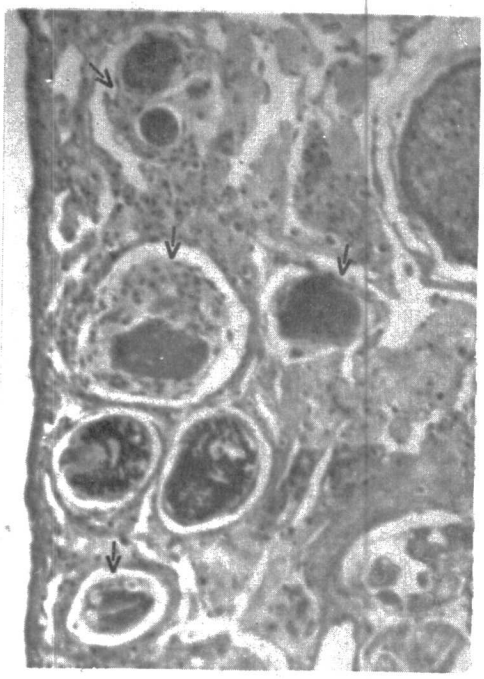

Fig. 2. *A papyraceus*. Live and degenerating (arrows) trematode larvae beneath the intestinal epithelium. 230X.

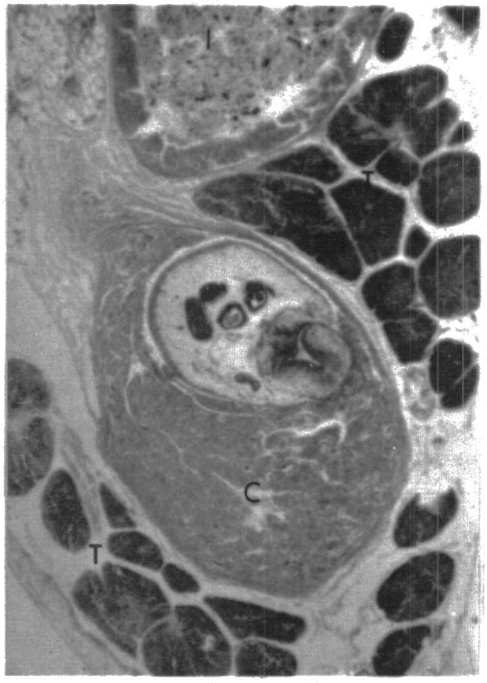

Fig. 3. *A. papyraceus*. Cestode larva by testis (T) and intestine (I) causing a large cystic reaction (C). 33X.

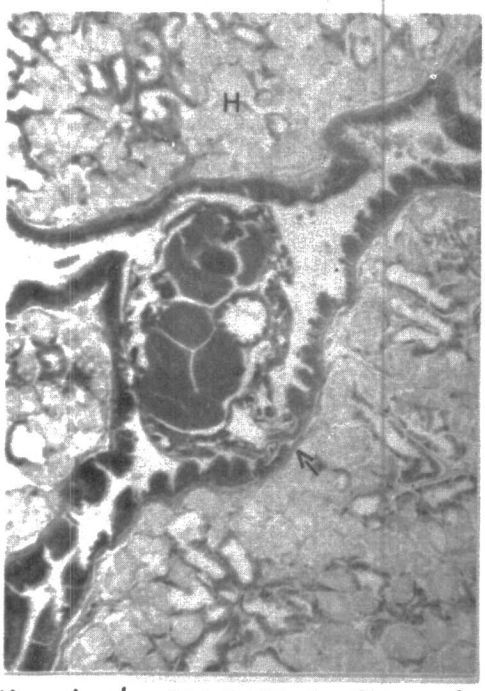

Fig. 4. *A. papyraceus*. Copepod in duct lumen in the hepatopancreas (H). Note legs attached to epithelium (arrow). 33X.

Cestode larvae (Figure 3) were only found in the hepatopancreas (or digestive gland) of shrimp (15%). Only one cestode larvae was found in the crabs, also in the hepatopancreas. They were found in the crabs, also in the hepatopancreas. They were found in several mollusc organs (25%). Even though these parasites were not present in large numbers, they were relatively large in size and appeared to cause damage to the surrounding tissue by exerting pressure. In molluscs, cestode larva frequently caused large cystic reactions (Figure 3).

Copepods (Figure 4) were only found in molluscs (23%) and usually in the intestine or digestive gland ducts. However, one was found encysted in the connective tissue between the seminiferous tubules of a scallop.

Gregarines (Figure 5) occurred in the intestine of various crustaceans but were not considered harmful to the host unless present in excessive numbers to cause blockage. They were only found in two of five specimens of *Raninoides louisianensis* and seven of 42 specimens of *Penaeus aztecus*.

Myxosporidian cysts (Figure 6) were only found in the lesser blue crab (*Callinectes similis*) where they were the most common symbiont in internal organs (59%).

All specimens of the paper shell scallop contained PAS+ (periodic-acid-Schiff positive) cells in their renal organs in varying numbers (Figure 7). The PAS+ cells could not be identified nor is it known whether they were parasitic.

Fungi in the order Plasmodiophorales (Figure 8) have been described as oyster parasites by J. Mackin (1962), our consultant. The lesions caused by these organisms resembled neoplasms due to their hyperplastic nature. They were found in both scallop species collected, in one brown

shrimp and in one lesser blue crab. They cannot be identified further without culture.

Small ciliates (Figure 9) were found only among the gill filaments of shrimp (38%) and were particularly abundant in *Sicyonia dorsalis* (93%). They did not appear to be parasitic or to cause any damage. A different ciliate which did appear to cause damage to the gills was found in the mantis shrimp.

Large ciliates (Figure 10) have a stalk which may attach to the thin chitin cuticle of the gills of shrimp (14%). Again, they were most abundant in *Sicyonia dorsalis* (33%).

An unknown parasite (an alga or protozoan) was found attached to the gills of four specimens of the spider crab *Anasimus latus* (Figure 1). It caused swelling of the gill filaments, leucocytosis and cuticle thickening. Several other unknown symbionts were found among crab gill filaments (34%), most of which did not appear to cause damage.

Pathologies Other Than Symbionts

Percentages given below are the number of animals with a given pathology per total number of animals examined.

All cysts found in the branchial sinuses of crabs (33%) and penaeid shrimp (21%) have unknown etiologies (Figure 12). They occurred as a simple cyst (Figure 12) or as one involving adjacent gill filaments.

Small cell aggregates composed of concentrically arranged cells were found (Figure 13). These obviously interfere with blood flow, and hence with gas exchange. They were particularly common in crab gills (79%), seldom seen in shrimp (8%), and were not observed in molluscs.

Large cell aggregates (Figure 14) which generally possessed a small central area of concentrically arranged cells with a large part of the

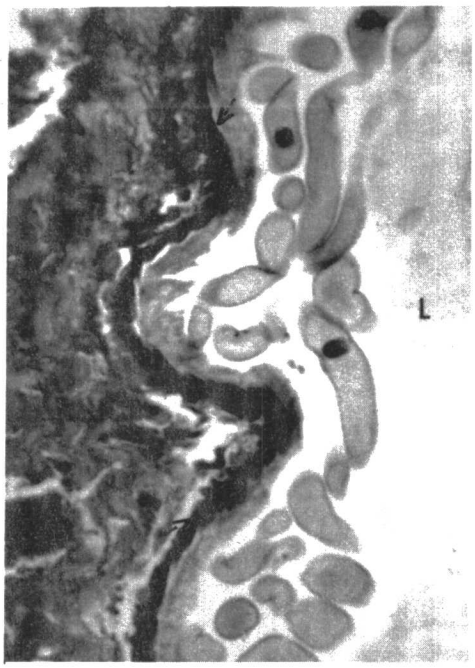

Fig. 5. R. *louisianensis*. Gregarines in the intestinal lumen (L). Elastic lamina of intestinal wall (arrows). 230X.

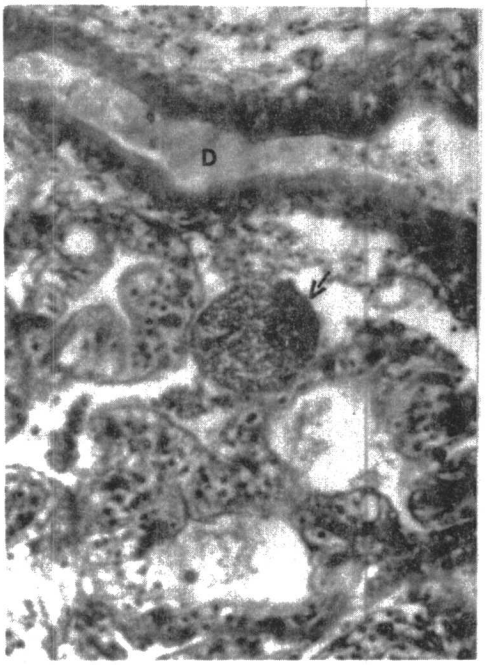

Fig. 6. C. *similis*. Myxosporidian cyst (arrow) by a digestive duct (D) in the hepatopancreas. 230X.

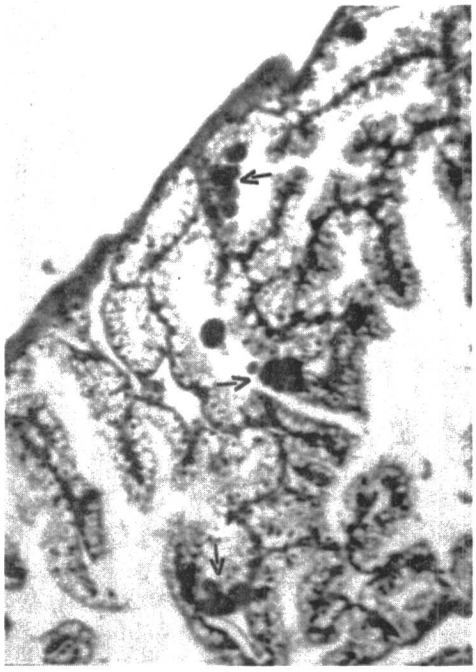

Fig. 7. A. *papyraceus*. An unknown PAS+ cell (arrows) infecting the renal organ. 190X

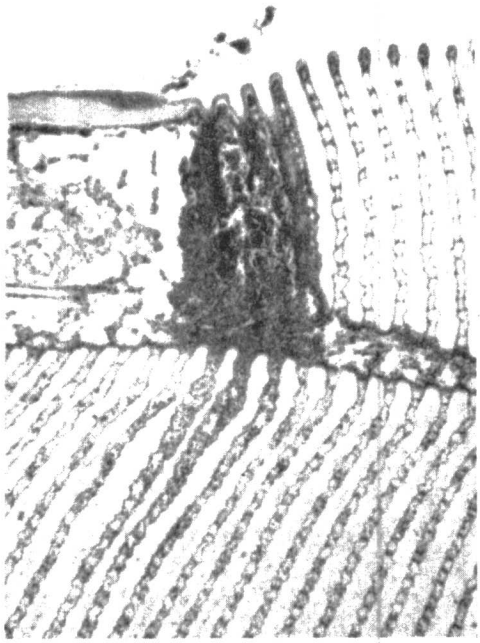

Fig. 8. C. *similis*. Hyperplasia in a gill caused by a Plasmodiophorales. 33X.

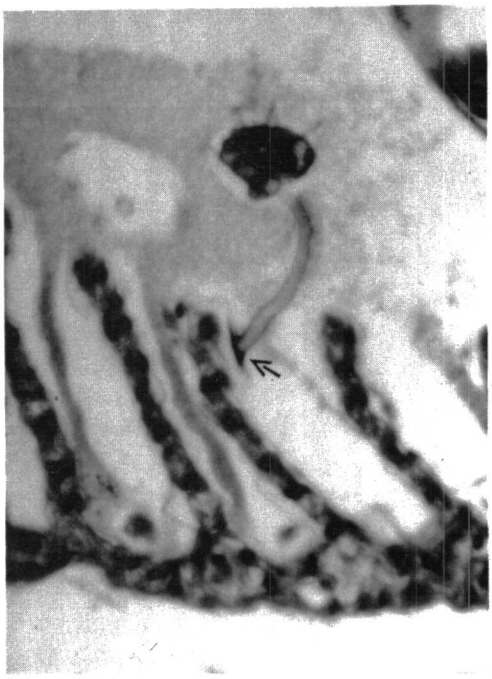

Fig. 9. *S. dorsalis*. Large stalked ciliate attached (arrow) to the thin chitin of a gill. 450X.

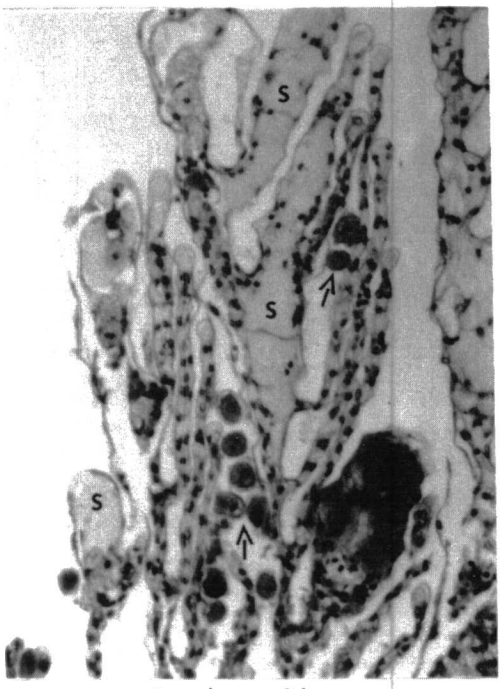

Fig. 10. *S. dorsalis*. Small ciliates (arrows) among gill filaments some of which are swollen (S) and one is abnormal. 230X.

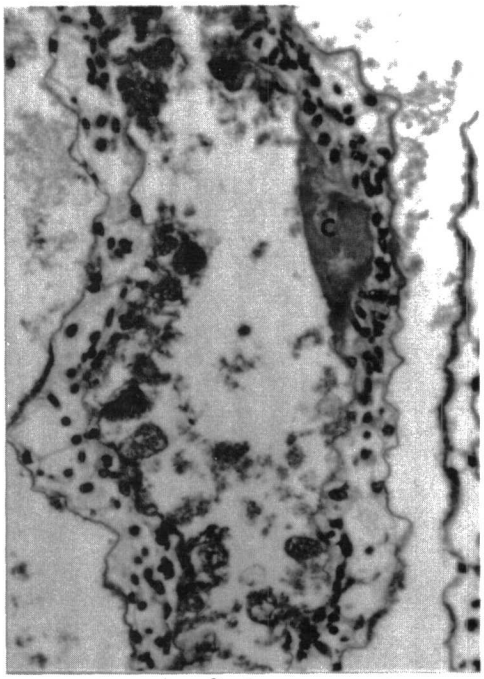

Fig. 11. *A. latus*. An algae or protozoan attached to the gill filaments causing swelling and cuticle thickening (C). 230X.

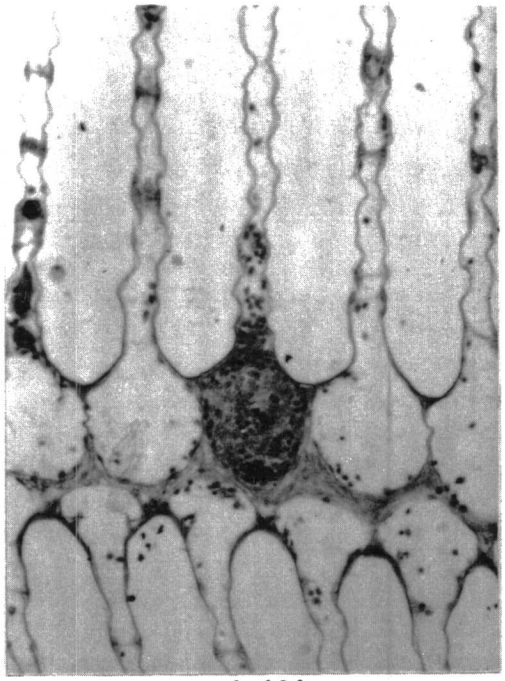

Fig. 12. *C. similis*. Cyst in the branchial sinus of a gill. 230X.

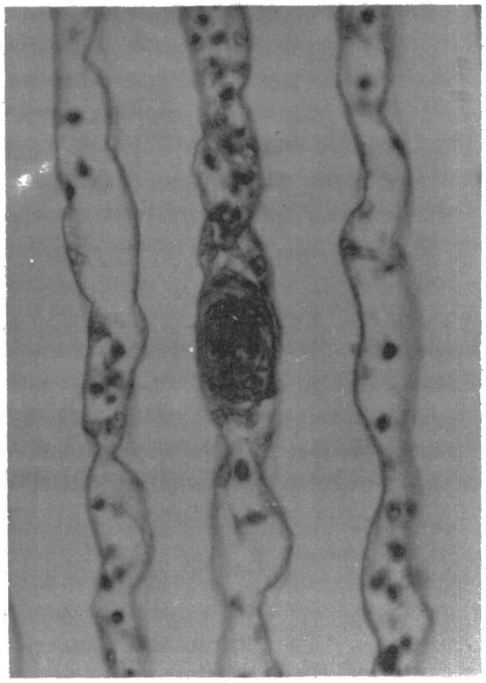

Fig. 13. *P. spinicarpus*. Small cell aggregate with concentrically arranged cells in a gill filament. 450X.

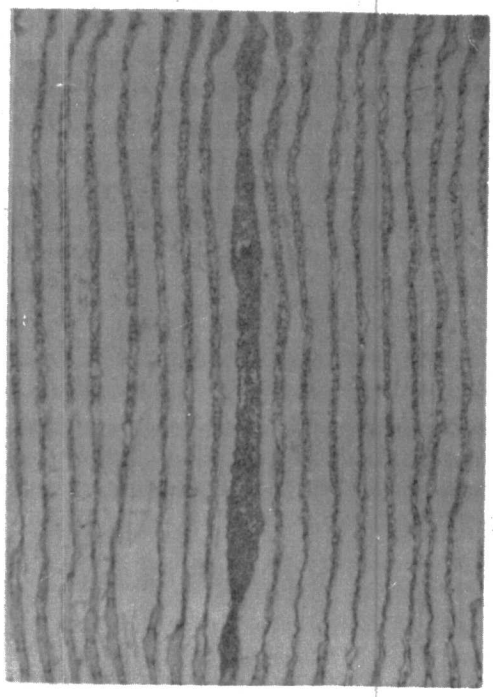

Fig. 14. *C. similis*. Large cell aggregate affecting the entire gill filament. 33X.

Fig. 15. *S. dorsalis*. Diseased gill filament with excess cellularity and distal atrophy. Note small ciliates. 230X.

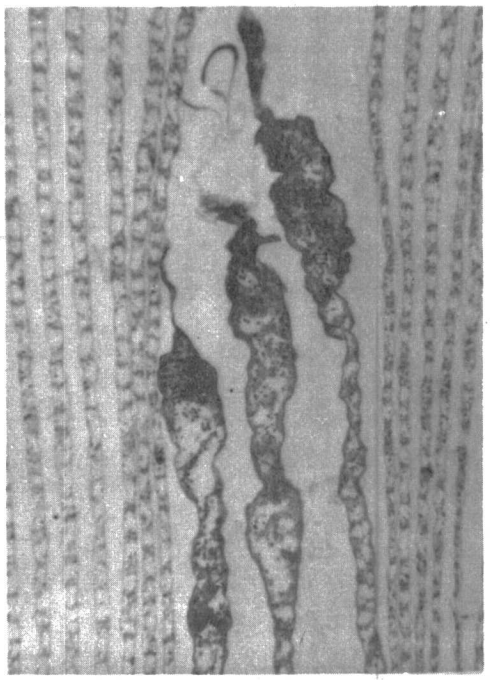

Fig. 16. *C. similis*. Three swollen gill filaments with excess cellularity and distal atrophy. 33X.

gill filament filled with leucocytes on each side of the area were present in crabs (17%). Whether these were later stages of some type of the small cell aggregates or an entirely new lesion is not known.

Deformed filaments with excess cellularity were observed (Figures 15 and 16). In shrimp (91%), progressive stages of this lesion were observed. A secondary gill filament (two or three may be involved) becomes swollen, filled with leucocytes and develops a necrotic area. This necrotic area increases in size and becomes atrophied, frequently forming a filamentous material with small dense areas in the periphery (Figure 15). A paraffin section consisting of parts of six to eight gills sometimes had more than 25 such lesions, particularly in the rock shrimp, *Sicyonia dorsalis*.

A similar type of lesion, one with swelling, leucocytosis and a necrotic area becoming atrophied distally, occurred in crabs (35%), particularly in *Callinectes similis* (66%). These lesions were observed near the end of a filament (Figure 16) or in the center of a filament with cell-filled swollen areas on each side of an atrophied region. Whether these lesions were advanced stages of large cell aggregates is not known, but no intermediate stages were observed.

Abnormal numbers of leucocytes (leucocytosis) in a specific area or throughout a tissue is an indication of injury or disease (Figure 17). When no etiology was observed in molluscs (20%), crabs (17%) and shrimp (6%), the condition was labeled leucocytosis (etiology unknown) in Tables 10-19. When leucocytes were aggregated around a parasite, it was an indication that the host was reacting to the invader attempting to destroy it. This was seen only in brown shrimp, rock shrimp and the goneplacid crab.

Cysts (Figure 18) may be caused by degenerating parasites or unknown injuries or diseases and were present in molluscs (48%), crabs (24%) and

shrimp (27%). They were frequently surrounded by masses of leucocytes.

Swelling of tissues (Figure 19) is an indication of injury or disease. Such swellings could have occurred when the animals were brought up in the trawl from deep waters or as a reaction to a fixative which was not isotonic to the tissue fluid. However, all tissues of the animal would show some effect, but this has not occurred with the animals studied. Blebbing of some of the sarcolemmas was observed in three crabs (Figure 19). Portions of some gill filaments were swollen, filled or not filled with hemolymph, in crabs (31%) and the rock shrimp (80%)(Figure 15).

Growth due to abnormal proliferation of cells (hyperplasia) can be caused by injury or disease, including neoplasia (Figure 20). Neoplastic growth is difficult to diagnose in invertebrates (Cheng, 1976). Cheng suggested that enzyme concentrations in the cells involved in a proliferative growth be determined to use as a future standard for diagnosing such growths. Whether the hyperplasia observed in the lesser blue crab and the squid in these studies is a true neoplasia or due to other diseases or injury is not known.

Animals

Shrimp

There were 93 specimens of shrimp examined, representing five species and one stomatopod. The brown shrimp (*Penaeus aztecus*) was caught most frequently (42 specimens) and the rock shrimp (*Sicyonia dorsalis*)(30 specimens) was second in abundance. Both species were only collected at Stations 1 and 2. *Trachypenaeus similis* was only collected in sufficient numbers (five specimens per station) in the late fall. *Penaeus setiferus* was only obtained at Station 1 and *Solenocera vioscai* only at Station 3. These facts agree with the report on epifauna by J. S. Holland. Infection of

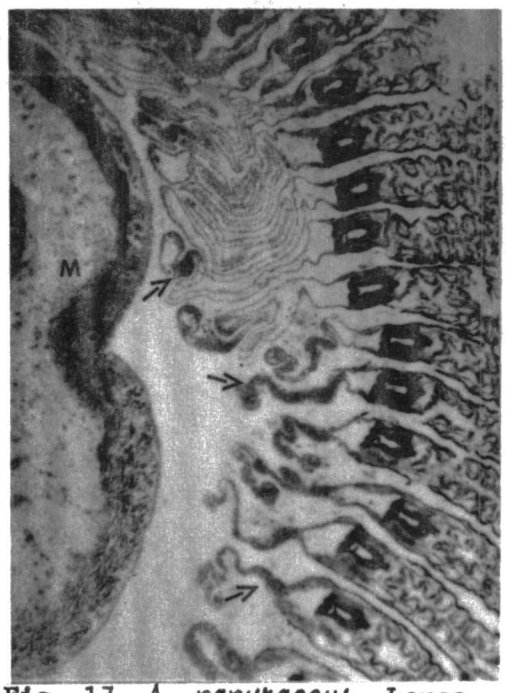

Fig. 17. A. *papyraceus*. Leucocytic infiltration (arrows) in the gill membranes that attach to the mantle (M). 33X.

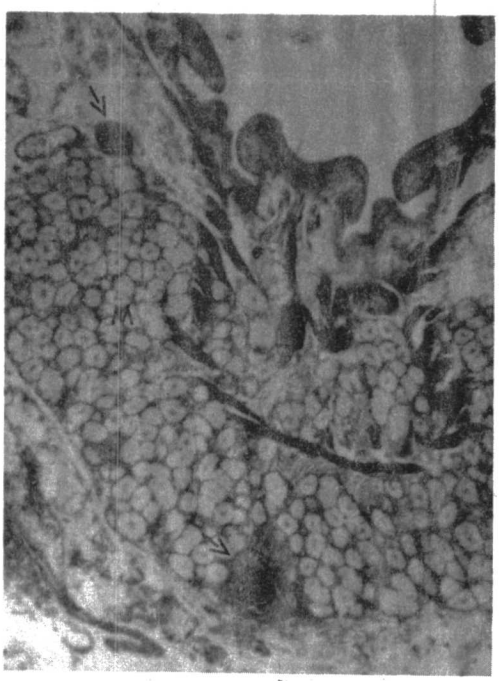

Fig. 18. C. *similis*. Cysts (arrows) in the intestinal mucous glands (M) with leucocytosis by the lower cyst. 33X.

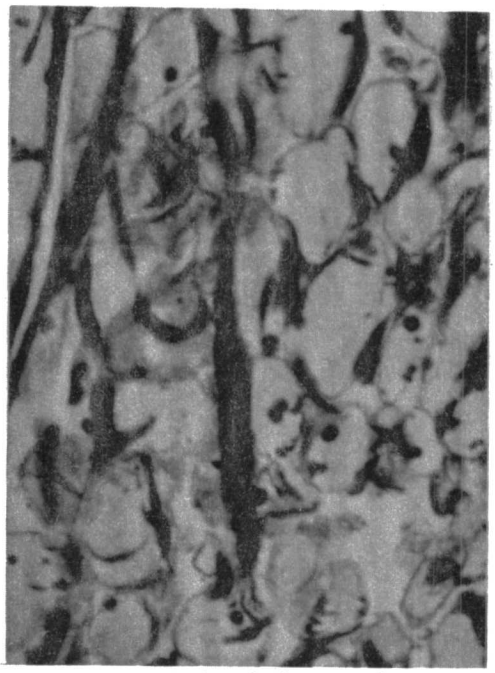

Fig. 19. R. *louisianensis*. The sarcolemma has ballooned away from the heart muscle filaments. 230X.

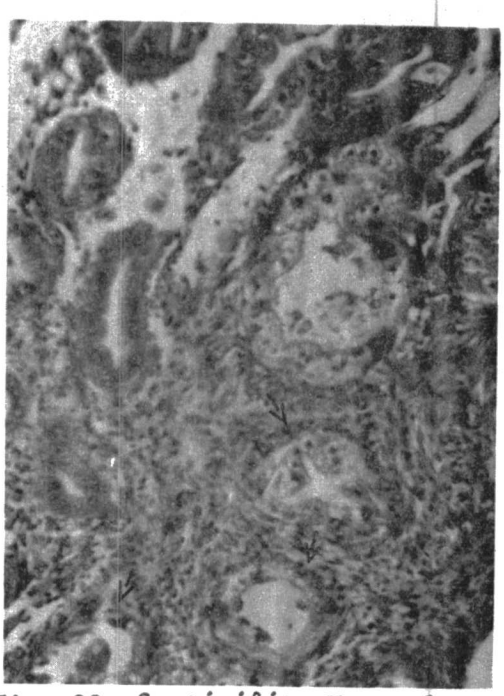

Fig. 20. C. *similis*. Hyperplasia of the connective tissue with abnormal hepatopancreatic tubules (arrows). 230X.

shrimp by nematodes was 53% higher in July and August than in the fall.

The most common symbionts in internal organs of shrimp were nematodes. In the gills, the most common symbionts were ciliates. The most common gill pathology other than symbionts was deformed gills with excess cellularity and atrophy. *Sicyonia dorsalis* had the highest percentage of symbionts and gill pathologies, and *Trachypenaeus similis* the lowest. Shrimp as a whole had the lowest percentage of pathologies other than symbionts in internal organs consisting of cysts, leucocytosis and one atretic egg.

Crabs

There were 84 specimens of crabs studied, representing six species. The lesser blue crab (*Callinectes similis*) was collected most frequently (29 specimens) and the spider crab (*Anasimus latus*), was next in abundance (20 specimens). *Callinectes similis* was only collected at Stations 1 and 2, and *Anasimus latus* only at Station 3. This agrees with the information by J. S. Holland on epifauna. Again, the highest percentage of nematode infections occurred in the summer.

Nematodes were the most common symbiont of internal organs. The most common symbionts in the gills were unknown symbionts. The most common gill pathologies other than symbionts were small cell aggregates. The gone-placid crab (we were unable to identify this crab further than the family level) had the highest percentage of symbionts with the spider crab second. The latter frequently (55%) had a dorivelleid polychaete living in one or both gill chambers. The spider crab had the highest percentage of unknown symbionts between gill filaments, but *Callinectes similis* had almost as high a percentage (50 and 45%, respectively). *Portunus spinicarpus* had the lowest percent of symbionts. Crabs had the lowest percentage of symbionts compared to other animals studied. *Portunus gibbesii* had the

highest percentage of gill pathologies other than symbionts, *Callinectes similis* the next highest percentage, and *Raninoides louisianensis* the lowest. Crab gill filaments are normally single, straight, rather long extensions from a central axis (see the normal filaments in Figures 8, 14 and 16), but some will occasionally branch or two filaments may fuse. These are listed in the tables as "Deformed Filaments" and may be genetic deformities as no evidence of injury, such as thickened cuticles, was observed. Such deformations were not observed in shrimp or molluscs. In internal organs, *Callinectes similis* had the highest percentage of pathologies other than symbionts, the goneplacid crab had none, and *Portunus spinicarpus* the lowest percentage.

Molluscs

There were 44 specimens of molluscs used consisting of four species. The paper shell scallop (*Amusium papyraceus*) was collected most frequently (23 specimens) and the squid (*Loligo pealei*)(17 specimens) was next in abundance. The paper shell scallop was collected at Station 2 in October, and at other times only at Station 3 which agrees with J. S. Holland's report on the epifauna.

Trematode larvae were the most common symbionts in internal organs. Cestode larvae, nematodes and copepods were also common symbionts in molluscan internal organs. The Gulf scallop (*Aequipecten glyptus*) had the highest percentage of symbionts in internal organs, but since only two specimens were obtained, this may be misleading. The paper shell scallop had the next highest percentage of such symbionts. Molluscs as a whole had the highest percentage of symbionts in their internal organs and the largest variety. The only symbionts in the gills were gram-negative bacteria infecting some of the squid gills. Molluscs had the lowest

number of other gill pathologies, consisting only of leucocytosis in the paper shell scallop and squid and two cases of hyperplasia in squid gills. Relatively few pathologies other than symbionts were found in internal organs. One paper shell scallop had a diseased ovary, with degenerating eggs, leucocytosis and proliferation of connective tissue into the region. The etiology was unknown. A pinnotherid crab was found in one of the small clams (*Macoma pulleyi*) caught in the biological dredge. Since only two specimens were caught, knowledge of the frequency of this symbiont residing in these clams is unknown.

Echinoderms

Two species of echinoderms were collected, six specimens of *Brissopsis alta* (heart urchin) at Station 3 in August and five specimens of *Astropecten duplicatus* (starfish) at Station 2 in November. The heart urchin was only collected at Station 3, according to J. S. Holland's report on epifauna. None of the echinoderms had any recognizable pathologies other than the heart urchin being filled with mud (the otter trawl had dug into the bottom). The starfish were very young specimens, which may account for their lack of pathologies.

CONCLUSIONS

Symbionts, particularly parasitic ones, apparently cause the most damage to marine invertebrates. Many instances of parasitism have been reported in the literature (Sinderman, 1970; Cheng, 1967; Hopkins, 1957) but these reports are mainly on commercial species, only two of which were collected in this study (*Penaeus aztecus* and *P. setiferus*). Many parasites of the animals used in this study cannot be identified without special culturing methods (Plasmodiophoralid fungi) and some may be entirely new to science (PAS+ cell in the paper shell scallop renal organ).

Scientists are just beginning to understand the cellular reactions to parasites in marine invertebrates (Cheng, 1976; Anderson and Good, 1976; Solangi and Lightner, 1976; Johnson, 1976). Understanding of other diseases and reactions of marine invertebrates to injury is also just starting (Sparks, 1972; Fontaine and Lightner, 1975; Rigdon and Baxter, 1970; Barry *et al.*, 1971; Cheng, 1976).

Since little in the literature is helpful in knowing the normal and pathologic conditions of most of the animals collected in this study, further histological baseline information is needed to understand the potential impact of man's altering the environment with offshore exploration and development. Even though most of the species used are not commercially valuable, the ecological balance of the oceans is delicate, and all forms of life in the sea are important to this balance.

LITERATURE CITED

Anderson, R. S., and R. A. Good. 1976. Opsonic development in phagocytosis by mollusc hemocytes. J. Invert. Pathol. 27:57-64.

Barry, M. M., P. P. Yevich, and N. H. Thayer. 1971. Atypical hyperplasia in the soft-shell clam *Mya arenaria*. J. Invert. Pathol. 17:17-27.

Brown, J. H., and L. Brenn. 1931. A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. Bull. Johns Hopkins Hospital. 48:69-73.

Cheng, T. C. 1967. Marine molluscs as hosts for symbioses: with a review of known parasites of commercially important species. Adv. Mar. Biol. 5:1-424.

_____. 1976. Identification of proliferative lesions in molluscs. Mar. Fish. Rev. 38:5-6.

Conn, H. J., M. A. Darrow, and V. M. Emmel. 1960. Staining procedures used by the Biological Stain Commission. Second Edition. The Williams and Wilkins Company, Baltimore. pp 70-72.

Davenport, H. A. 1960. Histological and histochemical techniques. W. B. Saunders Co., Philadelphia. pp 222-223.

Fontaine, C. T., and D. V. Lightner. 1975. Cellular response to injury in penaeid shrimp. Mar. Fish. Rev. 37:4-10.

Hopkins, S. H. 1957. Parasitism. Geol. Soc. Am. Mem. 67(1):413-428.

Humason, G. L. 1972. Animal tissue techniques. Third Edition, W. H. Freeman and Co., San Francisco. pp 45-47.

Johnson, P. T. 1976. Bacterial infection in the blue crab,*Callinectes sapidus*: course of infection and histopathology. J. Invert. Pathol. 28:25-36.

Lillie, R. D. 1965. Histopathologic technic and practical histochemistry. Third Edition. McGraw-Hill Book Co., New York. pp 49-52, 510-511.

Mackin, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publ. Inst. Mar. Sci., Univ. Tex. 7:132-229.

Rigdon, R. H., and K. N. Baxter. 1970. Spontaneous necroses in muscles of brown shrimp, *Penaeus aztecus* Ives. Am. Fish. Soc. Trans. 99:583-587.

Shaw, B. L., and H. J. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Can. J. Zool. 35:325-347.

Sinderman, C. J. 1970. Principal diseases of marine fish and shellfish. Academic Press, New York.

Solangi, M. A., and D. V. Lightner. 1976. Cellular inflammatory response of *Penaeus aztecus* and *P. setiferus* to the pathogenic fungus, *Fusarium* sp., isolated from the California brown shrimp, *P. californiensis*. J. Invert. Pathol. 27:77-86.

Sparks, A. K. 1972. Invertebrate pathology. Noncommunicable diseases. Academic Press, New York.

Sprague, V., and J. Couch. 1971. An annotated list of protozoan parasites, hyperparasites, and commensals of decapod crustacea. J. Protozool. 18:526-537.

CHAPTER TWELVE

HISTOPATHOLOGY OF DEMERSAL FISHES

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ABSTRACT

This report is concerned with the histopathologic analyses of 10 species of demersal fishes, South Texas OCS Monitoring Study, 1976. Observations pertain to five cruises: July, August, October, November and December, 1976. All samples were collected on Transect II, Stations 1, 2, and 3, and at the Southern Bank. The minimum sampling effort was five specimens of two fish species at each station and five specimens of one species at the Southern Bank. The organ sampling effort included the heart, stomach, kidney, muscle and liver.

Organ samples were prepared for histologic observation by routine histologic procedures. A total of 840 organ samples were collected. From these, 3150 hematoxylin and eosin and 1680 Verhoeff-Van Gieson stained slides were prepared.

Thirty-four percent (286) of the organ samples demonstrated lesions. The rock sea bass had the smallest percentage of lesions and the vermilion snapper the largest. The vermilion snapper also demonstrated the largest percentage of cardiac lesions. There was a tendency for kidney and muscle lesions to be more numerous in the sand seatrout. Kidney, muscle and liver lesions tended to be more numerous in the last three cruises than in the first two. Stomach and liver were more frequently involved pathologically than the muscle, kidney and heart, and this was apparently unrelated to species, station or cruise. The percentage of lesions was larger in fish obtained from the Southern Bank than at other stations, and the smallest percentage occurred in fish obtained from Station 2. These observations were due to the vermilion snapper being sampled at the Southern Bank and the rock sea bass at Station 2. All stations showed a tendency for the percentages of lesions to increase over the last three cruises (October, November and December).

Parasitism was the primary cause of lesions by both protozoan and helminth parasites. Parasitism caused varying degrees of necrosis, especially in the liver and stomach. The general integrity of the organ tissues was maintained adjacent to the lesions.

INTRODUCTION

This annual report is concerned with the histopathologic analyses of demersal fishes, South Texas OCS Monitoring Study, 1976. Qualitative and quantitative observations pertain to fish samples collected on five cruises: July 22-23; August 12-13; October 1-2; November 5-6; and December 3-4, 1976. All samples were collected on Transect II, Stations 1, 2 and 3, and at the Southern Bank. The minimum sampling effort for this study was five specimens of two fish species at each station and five specimens of one fish species at the Southern Bank.

The organ sampling effort for each specimen within each species included the heart, stomach, kidney, muscle and liver. Fish organ samples were collected by the principal and associate investigators on all cruises.

MATERIALS AND METHODS

Fish were collected in a 35-ft. (10.7-m) otter trawl at Stations 1, 2 and 3, and by hook and line at the Southern Bank. Trawl time was approximately 30 minutes each with two to four trawls per station. Fish from each trawl were sorted, identified by species, and placed in a live holding tank. Fish caught at the Southern Bank were also placed in a live holding tank.

Organ sample processing began immediately aboard ship in an effort to prevent postmortem changes. Samples of each organ, no greater than 5 mm in all directions, were placed in tissue capsules with identification information and placed in a fixative solution. Two fixatives were employed (buffered neutral formalin and Helly's solution, Appendix I, Table 1). Cardiac tissue, predominantly consisting of the ventricle, was fixed in Helly's solution primarily, and occasionally in formalin.

All organ samples were fixed for 24 hours.

Following fixation the organ samples were washed in running tap water for 24 hours and placed in 70 percent ethyl alcohol. Trimming of tissue to remove unnecessary connective tissue remnants and for blocking purposes occurred while they were in 70 percent alcohol. Kidney tissue, collected in situ, was placed in ethylenediaminetetraacetic acid (EDTA) for 8 hours to decalcify adjacent bone structures. All organ samples were then dehydrated with ethyl alcohol in increasing concentrations, cleared in xylene, infiltrated with paraffin[1] in a vacuum oven at 58°C for 3 hours, and embedded in paraffin[1].

Sectioning of the organs was done at 6 μm on an American Optical 820 rotary microtome. The sections, cut from both Helly's and formalin-fixed organs, were mounted on glass microscope slides. Section size permitting, six sections of formalin fixed tissues were mounted per slide. Two sections of Helly's fixed tissue were mounted per slide. A minimum of two slides were prepared for each organ within each fixative. The basic staining procedure employed was hematoxylin and eosin (Appendix I, Tables 2 and 3). Additional sections fixed in Helly's solution were also stained with Verhoeff and Van Gieson connective tissue stain (Appendix I, Tables 4 and 5). All slides were mounted with synthetic mounting media[2].

A total of 3150 hematoxylin and eosin-stained slides, and a total of 1680 Verhoeff-Van Gieson stained slides, have been prepared.

Problems associated with the collection of the various fish organs were minimal and generally related to the organs themselves. Fish muscle,

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[1]TISSUE PREP, Fisher Scientific Company, Houston, Texas 77001

[2]HISTOCLAD, Clay Adams, Parsipany, New Jersey, 07054

even in the fresh state, is friable and tends to pull apart. This was reflected in the muscle sections where the individual muscle fibers separated from each other. Liver tissue was also friable depending upon the amount of lipid present. As a result of decompression, the gastrointestinal tract often everted into the oral cavity. When this occurred it was necessary to gently straighten out the digestive tube before collecting stomach tissue. It also was necessary to collect the kidney samples in situ by including a segment of vertebrae which lies dorsal to the kidney. Fish kidney tissue cannot readily be collected otherwise, since it disintegrates when touched with instruments. It was necessary, therefore, to decalcify the kidney samples after fixation and prior to dehydration.

Coding of fish aboard ship was as follows. Each fish species was given a single Arabic letter, followed by a number to indicate the sequence of the specimens within each species. A Roman numeral preceding the Arabic letter indicates the station within Transect II. This is referred to as the Haensly code and will be used throughout this report. A computer code was also provided for each species, each specimen within each species, and each organ within each specimen, by the Data Management Staff at the University of Texas, Port Aransas Marine Laboratory, Port Aransas, Texas.

RESULTS AND DISCUSSION

Organ samples were collected from the following species of fish in the 1976 sampling effort:

Scientific Name	Common Name	Haensly Code
Pristipomoides aquilonaris	Wenchman	A

Scientific Name	Common Name	Haensly Code
Stenotomus caprinus	Longspine porgy	B
Cynoscion arenarius	Sand seatrout	C
Micropogon undulatus	Atlantic croaker	D
Peprilus burti	Butter fish	E
Centropristis philadelphica	Rock sea bass	F
Lutjanus campechanus	Red snapper	G
Trachurus lathami	Rough scad	H
Upeneus parvus	Dwarf goatfish	J
Rhomboplites aurorubens	Vermilion snapper	SN

Due to the unavailability of fish in the trawl, the following were not collected: one species of fish, Station 2, the July cruise; and two specimens of one species of fish, Station 1, the August cruise. All species and organ samples were successfully collected on the October, November and December cruises.

Quantitative Evaluation

Statistical analyses to evaluate the differences between observations were not conducted since significant differences may have little meaning in the data collected in 1976. Wide variation existed between species in sample size. Further, no fish species was consistently sampled at each station on all five cruises. The following evaluation of numbers and percentages, however, does illustrate some possible developing trends.

Table 1 is a summary of the number of lesions observed for the five organs within each species collected on the five cruises. Thirty-four percent of the 840 organ samples demonstrated lesions. The percentage

of lesions varied from a low of 17 percent (rock sea bass, F) to 47 percent (vermilion snapper, SN). Because sample size varied widely between species the percentage of lesions between species may have little meaning. When the species are grouped according to sample size, four groups are apparent: species H and J, with 25 organs each; species D, E and G with 50 to 75 organ samples; species C and F, with 90 and 100 organ samples; and species A, B and SN with organ samples of 125 or more. With two exceptions, the percentages of lesions between species within each of the above groups were similar. The rock sea bass (F) stood out as the species with the smallest percentage of lesions, while the vermilion snapper (SN) had the largest.

It is apparent from Table 1 that the stomach and liver were consistently larger in numbers and percentages of lesions than the other three organs, regardless of species. This may be a reflection of the system and functions with which those two organs are associated, making them more susceptible to infection. The number of lesions related to heart, kidney and muscle was generally small and more variable. Compared with the other nine species, however, the heart of the vermilion snapper (SN) stood out with the largest percentage of lesions. This cardiac difference presented by the vermilion snapper may be related to that species' environment (i.e. Southern Bank). Kidney and muscle lesions were more numerous in the sand seatrout (C). Why this should be characteristic of this species cannot be determined at this time.

Table 2 is a summary of the number of organs containing histopathologic conditions for each station for the five cruises. On a percentage basis, the smallest incidence of lesions occurred at Station 2, and the largest incidence at the Southern Bank. The frequency at Station 2 was

TABLE 1

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS WITHIN EACH SPECIES SAMPLED ON THE JULY, AUGUST, OCTOBER, NOVEMBER AND DECEMBER CRUISES, 1976, SOUTH TEXAS OCS MONITORING STUDY

	Species										
Organ/Species:	A	B	C	D	E	F	G	H	J	SN	TOTAL
Heart	4*(11)** / 35	3(12) / 25	0(0) / 18	2(13) / 15	1(10) / 10	1(5) / 20	0(0) / 10	0(0) / 5	0(0) / 5	16(64) / 25	27(16) / 168
Stomach	31(86) / 35	13(52) / 25	16(89) / 18	9(60) / 15	7(70) / 10	10(50) / 20	8(80) / 10	5(100) / 5	4(80) / 5	22(88) / 25	125(74) / 168
Kidney	0(0) / 35	4(16) / 25	6(33) / 18	2(13) / 15	0(0) / 10	0(0) / 20	1(10) / 10	0(0) / 5	2(40) / 5	1(4) / 25	16(10) / 168
Muscle	0(0) / 35	2(8) / 25	5(28) / 18	2(13) / 15	2(20) / 10	0(0) / 20	0(0) / 10	2(40) / 5	0(0) / 5	1(4) / 25	14(8) / 168
Liver	22(63) / 35	25(100) / 25	10(56) / 18	6(40) / 15	5(50) / 10	6(30) / 20	4(40) / 10	2(40) / 5	5(100) / 5	19(76) / 25	104(62) / 168
TOTAL	57(33) / 175	47(38) / 125	37(41) / 90	21(28) / 75	15(30) / 50	17(17) / 100	13(26) / 50	9(36) / 25	11(44) / 25	59(47) / 125	286(34) / 840

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

TABLE 2

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS WITHIN EACH STATION OF TRANSECT II ON THE JULY, AUGUST, OCTOBER, NOVEMBER AND DECEMBER CRUISES, 1976, SOUTH TEXAS OCS MONITORING STUDY

Organ	Station 1 (Species C,D,E,G,H)	Station 2 (Species A,E,F,G,J)	Station 3 (Species A,B)	Southern Bank (Species SN)	TOTAL
Heart	2*(4)** / 48	3(7) / 45	6(12) / 50	16(64) / 25	27(16) / 168
Stomach	39(81) / 48	27(60) / 45	37(74) / 50	22(88) / 25	125(74) / 168
Kidney	9(19) / 48	2(4) / 45	4(8) / 50	1(4) / 25	16(10) / 168
Muscle	9(19) / 48	2(4) / 45	2(4) / 50	1(4) / 25	14(8) / 168
Liver	20(42) / 48	22(49) / 45	43(86) / 50	19(76) / 25	104(62) / 168
TOTAL	79(33) / 240	56(25) / 225	92(37) / 250	59(47) / 125	286(34) / 840

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

low because species F (rock sea bass) was among the species sampled here. The factor responsible for the high incidence at the Southern Bank was the high incidence of cardiac lesions in the vermilion snapper (SN). The percentages of lesions at Stations 1 and 3 were distributed approximately centrally between that of Station 2 and the Southern Bank.

As was apparent in Table 1, Table 2 demonstrates that the stomach and liver were consistently higher than the other three organs in numbers and percentages of lesions, regardless of stations. Gastric lesions were consistently high at all stations. In contrast, the percentages of hepatic lesions at Stations 1 and 2 were considerably less than that at Station 3 and the Southern Bank. The higher frequency at Station 3 reflects the 100 percent incidence of liver lesions in species B (longspine porgy), which was sampled only at Station 3. While species J (dwarf goatfish), Station 2, also had 100 percent hepatic pathology, this species was sampled only once. The vermilion snapper (SN), sampled at the Southern Bank, ranked high, not only in cardiac lesion, but also in gastric and hepatic lesions.

The percentages of renal and muscle lesions at Stations 2, 3 and the Southern Bank were consistently low. This was in contrast to higher frequency of lesions in these two organs at Station 1. This latter incidence was apparently related to the consistently high frequency in species C (sand seatrout) and D (Atlantic croaker) which were sampled only at Station 1.

Table 3 is a summary of the number of organs containing histopathologic lesions for each of the five cruises in 1976. These data indicate that, overall, there was little difference between the various cruises. Since the vermilion snapper (SN) was sampled on all five cruises, the cardiac lesions tended to be more evenly distributed for each cruise.

TABLE 3

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS FOR THE JULY, AUGUST, OCTOBER, NOVEMBER AND DECEMBER CRUISES, 1976 SOUTH TEXAS OCS MONITORING STUDY

Organ	July (Species A,B,C, D, SN)	August (Species A,B,C, E,F, SN)	October (Species A,B,C, D,E,F, SN)	November (Species A,B,F, G,H, SN)	December (Species A,B,C, D,F,J, SN)	TOTAL
Heart	7*(23)** / 30	4(12) / 33	4(11) / 35	6(17) / 35	6(17) / 35	27(16) / 168
Stomach	25(83) / 30	22(67) / 33	21(60) / 35	26(74) / 35	31(89) / 35	125(74) / 168
Kidney	0(0) / 30	0(0) / 33	2(6) / 35	2(6) / 35	12(34) / 35	16(10) / 168
Muscle	0(0) / 30	1(3) / 33	7(20) / 35	2(6) / 35	4(11) / 35	14(8) / 168
Liver	15(50) / 30	15(45) / 33	24(69) / 35	23(66) / 35	27(77) / 35	104(62) / 168
TOTAL	47(31) / 150	42(25) / 165	58(33) / 175	59(34) / 175	80(46) / 175	286(34) / 840

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

Gastric lesion incidence was large for all cruises, and displayed no monthly pattern. Kidney, muscle and liver lesions increased in number and percentage in the last three months of the year. Monthly sampling may be a significant factor in renal, muscular and hepatic pathology.

Tables 4 through 8 show the histopathologic detail within each monthly sampling effort. The information presented in these five tables displays information already revealed in Tables 1, 2 and 3. The incidence of cardiac lesions was generally low except in the vermilion snapper (SN). Kidney and muscle lesion frequency, while generally low compared to other organs, increased in the last three cruises. The incidence of stomach lesions was large and variable from month to month. The incidence of liver lesions was large and variable over the five cruises for Stations 1 and 2, but tended to increase over the five cruises for Station 3 and the Southern Bank. Specific comparisons between Tables 4 through 8 becomes more difficult to make since no species was sampled each month at each station. When the percentages of lesions within each station were compared from month to month, however, a trend was evident. This trend showed that Station 2 had the fewest lesions, Stations 1 and 3 more than Station 2, and the Southern Bank the greatest percentage. Further, all stations tended to display an increased frequency in lesions in the last three cruises.

Qualitative Evaluation

The lesions observed in each of the five organs were predominantly related to parasitism caused by both protozoan and helminth parasites. Parasitism is a common observation in both freshwater and marine fish tissue. Among the nematodes infecting fish is *Anisakis*. A brief review of this parasite and its host fish species was recently reported

TABLE 4

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS PER NUMBER OF ORGANS SAMPLES ON THE JULY, 1976, CRUISE SOUTH TEXAS OCS MONITORING STUDY

	Station 1		Station 2	Station 3		Southern Bank	
Organ/Species:	C	D	A	A	B	SN	TOTAL
Heart	0*(0)** / 5	0(0) / 5	0(0) / 5	1(20) / 5	2(40) / 5	4(80) / 5	7(23) / 30
Stomach	5(100) / 5	3(60) / 5	5(100) / 5	4(80) / 5	3(60) / 5	5(100) / 5	25(83) / 30
Kidney	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 30
Muscle	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 30
Liver	3(60) / 5	1(20) / 5	3(60) / 5	1(20) / 5	5(100) / 5	2(40) / 5	15(50) / 30
Subtotal	8(32) / 25	4(16) / 25	8(32) / 25	6(24) / 25	10(40) / 25	11(44) / 25	
TOTAL	12(24) / 50		8(32) / 25	16(32) / 50		11(44) / 25	47(31) / 150

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

TABLE 5

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS PER NUMBER OF ORGANS SAMPLED ON THE AUGUST, 1976 CRUISE. SOUTH TEXAS OCS MONITORING STUDY

	Station 1		Station 2		Station 3		Southern Bank	
Organ/Species:	C	E	A	F	A	B	SN	TOTAL
Heart	0*(0)** / 3	0(0) / 5	1(20) / 5	0(0) / 5	1(20) / 5	1(20) / 5	1(20) / 5	4(12) / 33
Stomach	3(100) / 3	5(100) / 5	2(40) / 5	1(20) / 5	5(100) / 5	2(40) / 5	4(80) / 5	22(67) / 33
Kidney	0(0) / 3	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(8) / 5	0(0) / 33
Muscle	0(0) / 3	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	1(20) / 5	1(3) / 33
Liver	0(0) / 3	1(20) / 5	1(20) / 5	1(20) / 5	3(60) / 5	5(100) / 5	4(80) / 5	15(45) / 33
Subtotal	3(20) / 15	6(24) / 25	4(16) / 25	2(8) / 25	9(36) / 25	8(32) / 25	10(40) / 25	
TOTAL	9(22) / 40		6(12) / 50		17(34) / 50		10(40) / 25	42(25) / 165

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

TABLE 6

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS PER NUMBER OF ORGANS SAMPLED ON THE OCTOBER, 1976 CRUISE. SOUTH TEXAS OCS MONITORING STUDY

	Station 1		Station 2		Station 3		Southern Bank	
Organ/Species:	C	D	E	F	A	B	SN	TOTAL
Heart	0*(0)** / 5	1(20) / 5	1(20) / 5	0(0) / 5	0(0) / 5	0(0) / 5	2(40) / 5	4(11) / 35
Stomach	3(60) / 5	3(60) / 5	2(40) / 5	3(60) / 5	5(100) / 5	2(40) / 5	3(60) / 5	21(60) / 35
Kidney	1(20) / 5	1(20) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	2(6) / 35
Muscle	3(60) / 5	1(20) / 5	2(40) / 5	0(0) / 5	0(0) / 5	1(20) / 5	0(0) / 5	7(20) / 35
Liver	3(60) / 5	2(40) / 5	4(80) / 5	0(0) / 5	5(100) / 5	5(100) / 5	5(100) / 5	24(69) / 35
Subtotal	10(40) / 25	8(32) / 25	9(36) / 25	3(12) / 25	10(40) / 25	8(32) / 25	10(40) / 25	
TOTAL	18(36) / 50		12(24) / 50		18(36) / 50		10(40) / 25	58(33) / 175

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

TABLE 7

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS PER NUMBER OF ORGANS SAMPLED ON THE NOVEMBER, 1976 CRUISE. SOUTH TEXAS OCS MONITORING STUDY

	Station 1		Station 2		Station 3		Southern Bank	
Organ/Species:	G	H	F	G	A	B	SN	TOTAL
Heart	0*(0)** / 5	0(0) / 5	0(0) / 5	0(0) / 5	1(20) / 5	0(0) / 5	5(100) / 5	6(17) / 35
Stomach	4(80) / 5	5(100) / 5	2(40) / 5	4(80) / 5	5(100) / 5	1(20) / 5	5(100) / 5	26(74) / 35
Kidney	1(20) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	1(20) / 5	0(0) / 5	2(6) / 35
Muscle	0(0) / 5	2(40) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	0(0) / 5	2(6) / 35
Liver	1(20) / 5	2(40) / 5	4(80) / 5	3(60) / 5	4(80) / 5	5(100) / 5	4(80) / 5	23(66) / 35
Subtotal	6(24) / 25	9(36) / 25	6(24) / 25	7(28) / 25	10(40) / 25	7(28) / 25	14(56) / 25	
TOTAL	15(30) / 50		13(26) / 50		17(34) / 50		14(56) / 25	59(34) / 175

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

TABLE 8

NUMBER OF ORGANS CONTAINING HISTOPATHOLOGIC CONDITIONS PER NUMBER OF ORGANS SAMPLED ON THE DECEMBER, 1976 CRUISE. SOUTH TEXAS OCS MONITORING STUDY

	Station 1		Station 2		Station 3		Southern Bank	
Organ/Species:	C	D	F	J	A	B	SN	TOTAL
Heart	0*(0)** / 5	1(20) / 5	1(20) / 5	0(0) / 5	0(0) / 5	0(0) / 5	4(80) / 5	6(17) / 35
Stomach	5(100) / 5	3(60) / 5	4(80) / 5	4(80) / 5	5(100) / 5	5(100) / 5	5(100) / 5	31(89) / 35
Kidney	5(100) / 5	1(20) / 5	0(0) / 5	2(40) / 5	0(0) / 5	3(60) / 5	1(20) / 5	12(34) / 35
Muscle	2(40) / 5	1(20) / 5	0(0) / 5	0(0) / 5	0(0) / 5	1(20) / 5	0(0) / 5	4(11) / 35
Liver	4(80) / 5	3(60) / 5	1(20) / 5	5(100) / 5	5(100) / 5	5(100) / 5	4(80) / 5	27(77) / 35
Subtotal	16(64) / 25	9(36) / 25	6(24) / 25	11(44) / 25	10(40) / 25	14(56) / 25	14(56) / 25	
TOTAL	25(50) / 50		17(34) / 50		24(48) / 50		14(56) / 25	80(46) / 175

*Numerator: number of organs containing histopathologic conditions

Denominator: number of organs sampled

**Percentage of lesions

(Pippy and van Banning, 1975). Many different species of trematodes are pathogenic to many fish species, as indicated in several recent reviews (Holmes, 1971; Klein, Olsen and Bowden, 1969; Schell, 1975; 1976; and Scott, 1975). Cestode infections are also common to a large number of fish species (Sandeman and Burt, 1972). Among the protozoa that invade fish tissues, the most prominent are *Myxosporida* and *Ichthyophthirius* spp. (Daniels, Herman and Burke, 1976; Landolt, 1973; Love and Moser, 1976; Markiw and Wolf, 1974; Moser, Noble and Lee, 1976; Moser, Love and Jensen, 1976; Nigrelli, Polorny and Ruggieri, 1976). Most of the above reports were concerned with the identification of the whole parasite, and substantiate parasitism as a major pathologic condition in fish. Few reports, on the other hand, pertain to the identification of parasites, especially metazoa, in tissue sections.

Muscle

Histology

The muscle sections, sampled from blocks of tissue collected from the left dorsal trunk musculature, demonstrated striated muscle fibers cut obliquely and/or on cross section. The individual fibers were large and surrounded by small amounts of collagenous connective tissue fibers. Occasionally, nerve ganglia and nerve fibers were observed located between the muscle fibers. Blood vessels were also present in the interstitial connective tissue.

Histopathology

All of the muscular lesions observed were related to parasitism. The majority of these were protozoan cysts, with and without prominent capsules, located either between the muscle fibers (Figures 1 and 2) or within the muscle fibers (Figures 3 to 6). A few of the parasitic

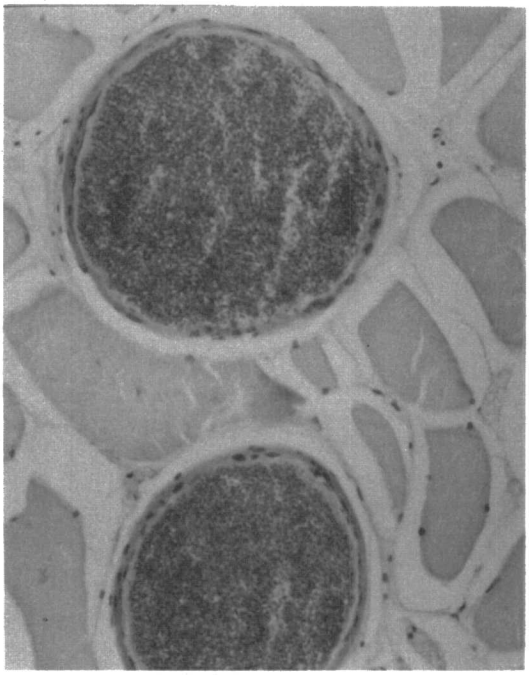

Fig. 1. S. caprinus, muscle. Extracellular protozoan cyst. H&E. 240X

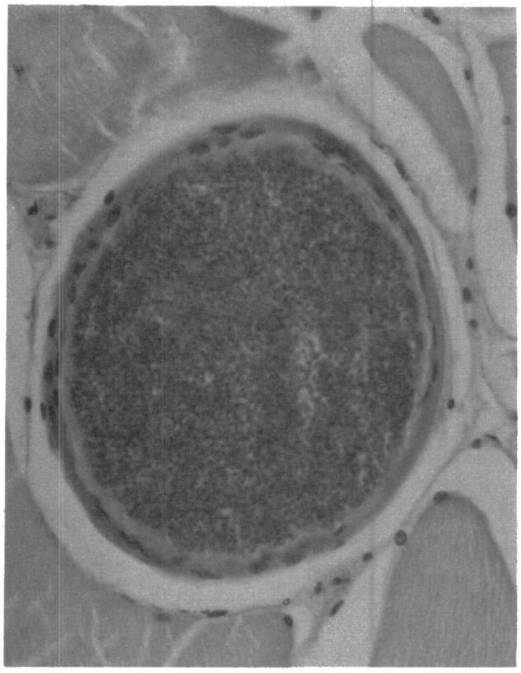

Fig. 2. S. caprinus, muscle. Same as Fig.1, but enlarged to show cyst detail. H&E. 384X

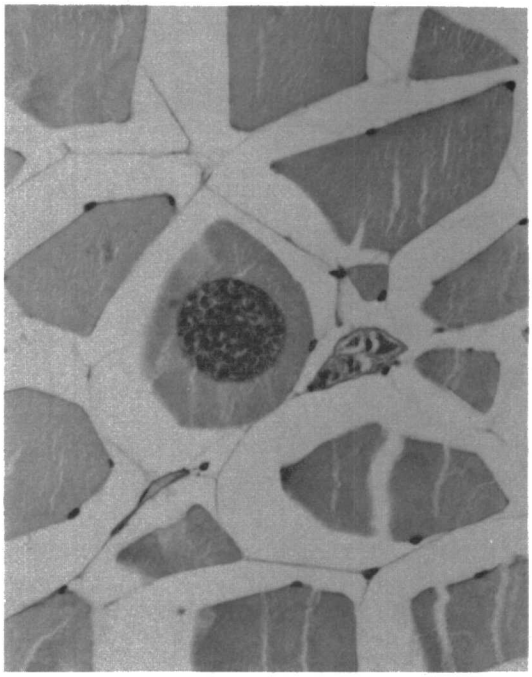

Fig. 3. T. lathami, muscle. Intracellular protozoan cyst. H&E. 384X

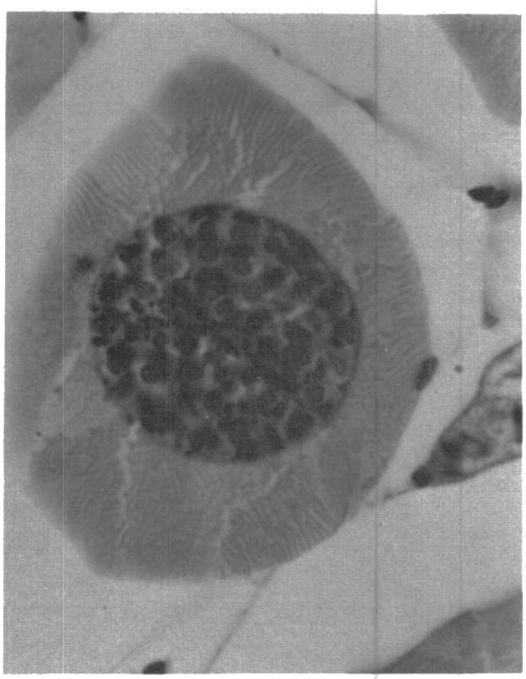

Fig. 4. T. lathami, muscle. Same as Fig.3, but enlarged to show cyst detail. H&E. 960X

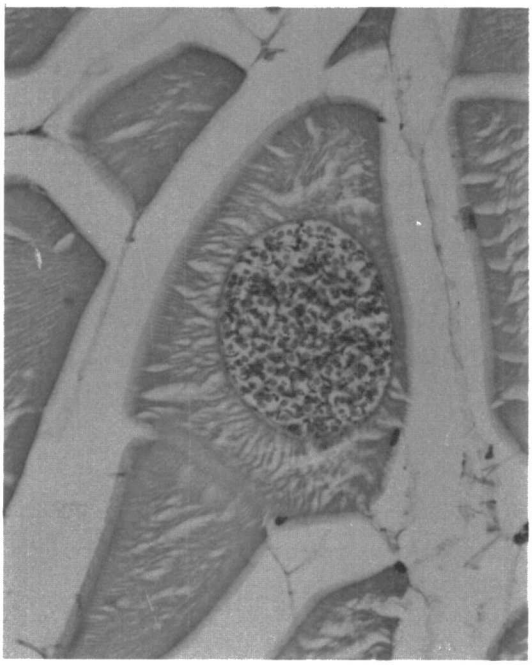

Fig. 5. T. lathami, muscle. Intracellular protozoan cyst in more advanced state of maturation than illustrated in Fig. 4. H&E. 384X

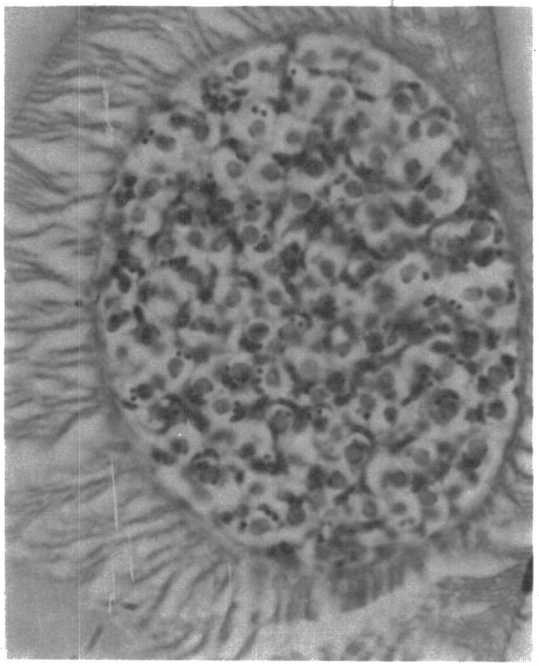

Fig. 6. T. lathami, muscle. Same as Fig.5, but enlarged to show detail. H&E. 960X

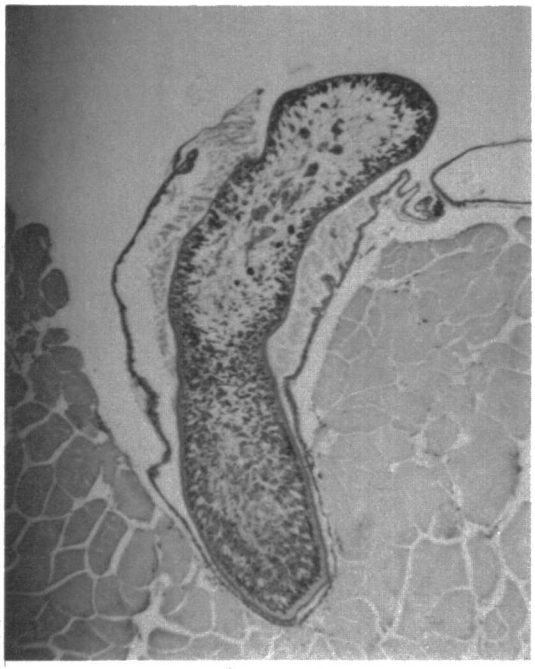

Fig. 7. P. burti, muscle. Helminth parasite. H&E. 60X

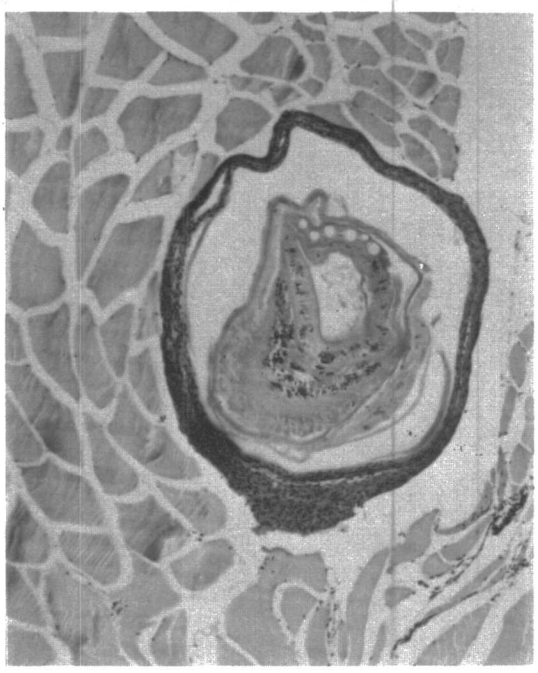

Fig. 8. S. caprinus, muscle. Helminth parasite. H&E. 96X

lesions were encysted helminths (Figures 7 and 8). None of the lesions were associated with inflammatory reactions or the accumulation of unusual cell types.

Kidney

Histology

The basic morphology of the renal tissue was the same for all species i.e., nephrons embedded in varying amounts of hemopoietic tissue. Glomeruli were located peripherally, dorsal and lateral, within the main renal mass. Renal tubules were numerous. Their structure appeared similar to mammalian renal tubules with a layer of simple cubiodal epithelial cells with round, central, basophilic nuclei surrounded by an eosinophilic cytoplasm. Wandering lymphocytes penetrating the tubular epithelium were seen only occasionally.

Scattered through the kidney sections were areas of pigment-containing cells (Figure 16). The pigmented areas varied in number from kidney to kidney and appeared to be associated with the hemopoietic tissue. Delicate strands of collagenous fibers supported the renal parenchyma. Connective tissue was not conspicuous in the kidney except adjacent to large blood vessels. Acidophil cells were occasionally observed in the renal interstitium. Even though the cytoplasm was eosinophilic, the structure of acidophil cells resembled that of mammalian mast cells and not granular leukocytes.

Histopathology

The lesions observed in the kidney samples were of a wide variety: protozoa in duct lumens (Figures 9 to 12), protozoa between duct epithelial cells (Figure 14) or thickly encapsulated in the parenchyma as xenomas (Figure 13), remnants of helminth parasites, and an apparent

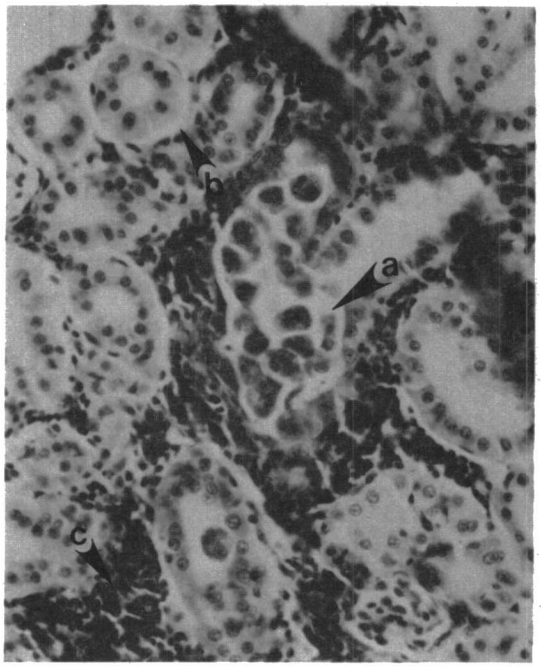

Fig. 9. C. arenarius, kidney. Protozoa within lumen of renal duct (arrow a). Normal renal tubule (arrow b) and hemopoietic tissue (arrow c). H&E 384X

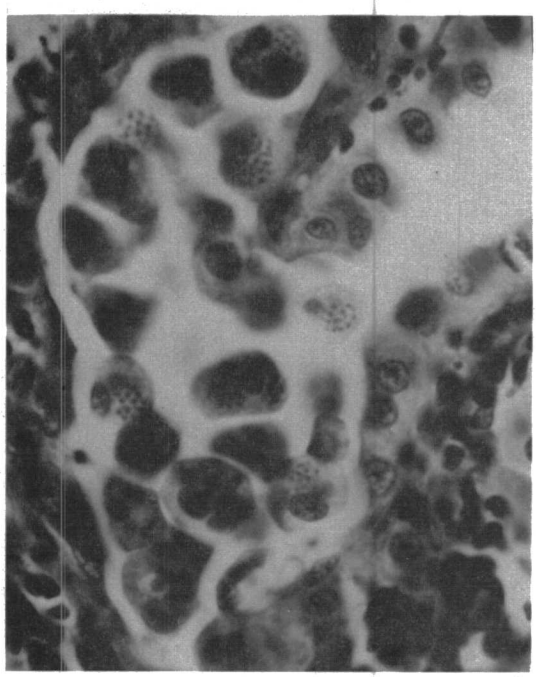

Fig. 10. C. arenarius, kidney. Same as Fig.9, but enlarged to show protozoa detail. H&E. 960X

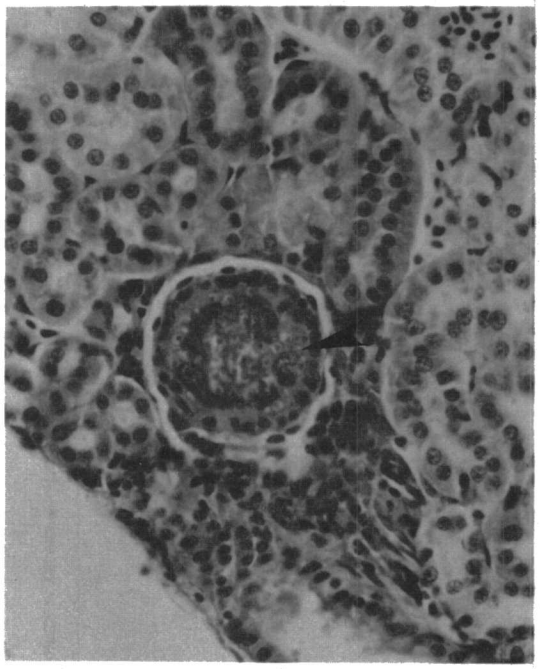

Fig. 11. L.campechanus, kidney. Protozoa within lumen of renal duct (arrow). H&E. 384X

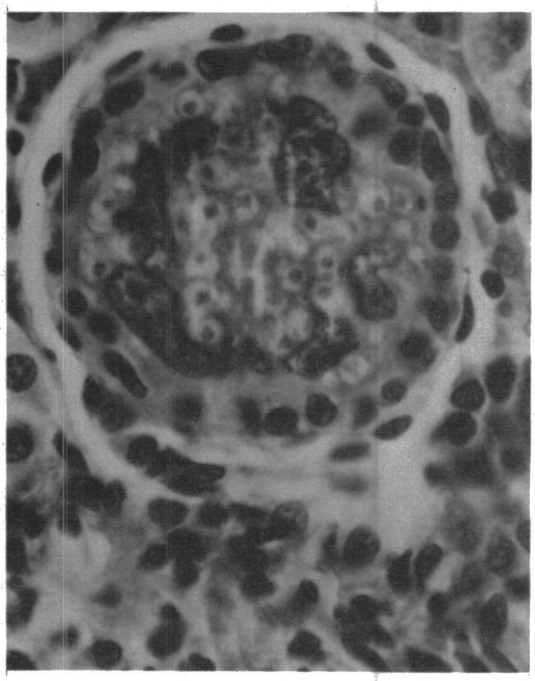

Fig. 12. L.campechanus, kidney. Same as Fig.11, but enlarged to show protozoa detail. H&E. 960X

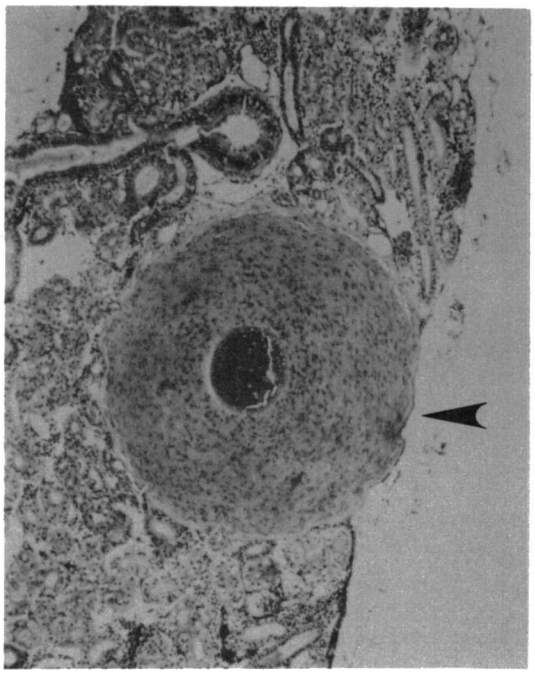

Fig. 13. C. arenarius, kidney. Thickly encapsulated protozoan cyst (arrow). H&E. 96X

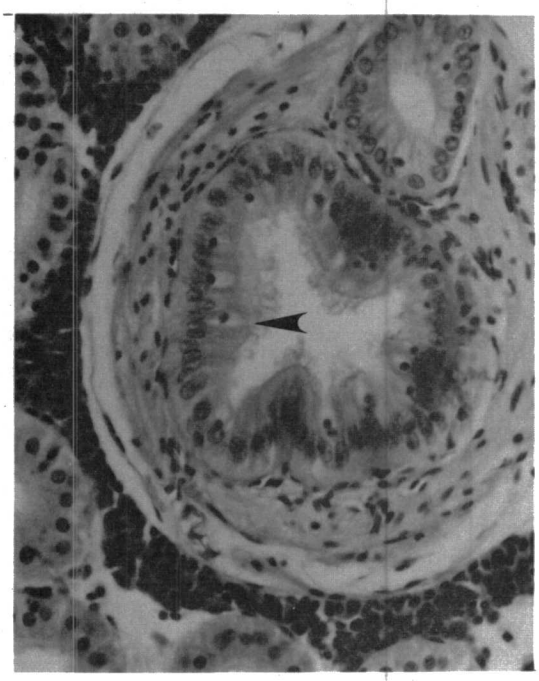

Fig. 14. M.undulatus, kidney. Protozoa in renal duct epithelium (arrow). H&E. 384X

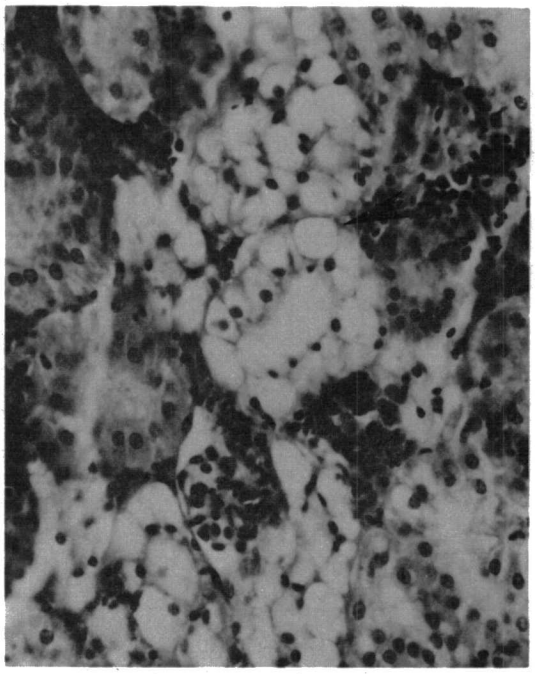

Fig. 15. S. caprinus, kidney. Renal tubular epithelial cells containing large amounts of lipid (arrow). H&E. 384X

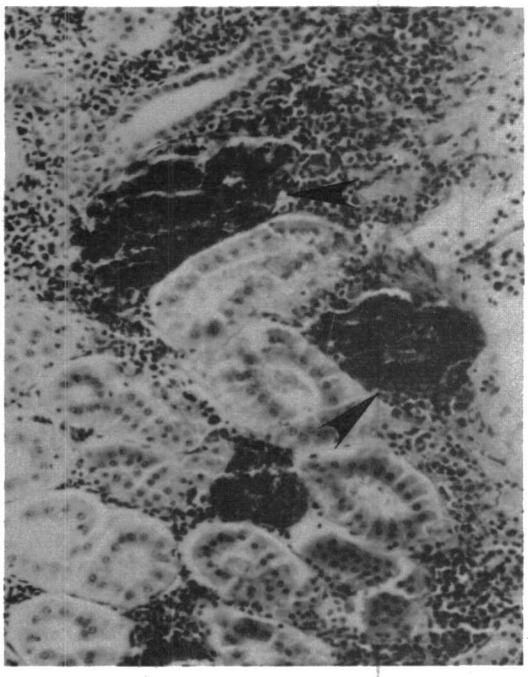

Fig. 16. U. parvus, kidney. Dense pigmented areas within renal tissue (arrows). H&E. 240X

deposition of large amounts of lipid in tubule epithelial cells (Figure 15). Protozoa, free or encysted, were the most numerous parasitic observation.

Heart

Histology

Sections of cardiac ventricular muscle revealed bundles of striated muscle fibers cut on one of three planes: cross-section, obliquely or longitudinally. Small amounts of collagenous connective tissue fibers supported the muscle cells and the abundant interstitial vascular supply. Occasional clumps of lightly pigmented cells were present, predominantly in the pericardium. Acidophil cells were occasionally observed in the pericardial region.

Histopathology

Encysted helminth parasites or their remnants were infrequently observed (Figures 17 and 18). The predominant cardiac lesions were approximately circular and measured about 24 μm in diameter (Figures 19, 20 and 21). The outer wall of these lesions was cellular and surrounded a central region of varying structure in apparent degenerative states. In the same hearts that contained the above lesions, there were protozoa-like cells aligned in rows along the cardiac muscle fibers (Figure 22), or arranged in aggregates between muscle fibers (Figure 23). An extensive myocarditis was associated with these lesions (Figure 19 and 24). It is suggested at this time that the protozoa-like structures were in fact protozoan, many of which were being walled off by connective tissue cells.

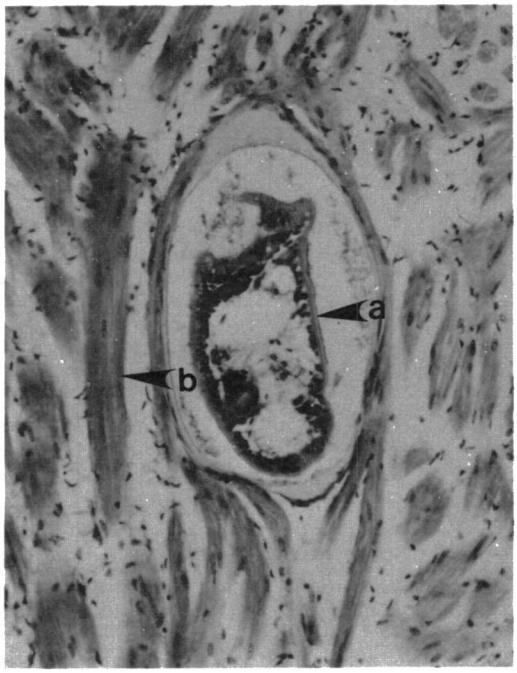

Fig. 17. P. burti, heart. Encysted helminth parasite (arrow a). Cardiac muscle fibers (arrow b). H&E. 240X

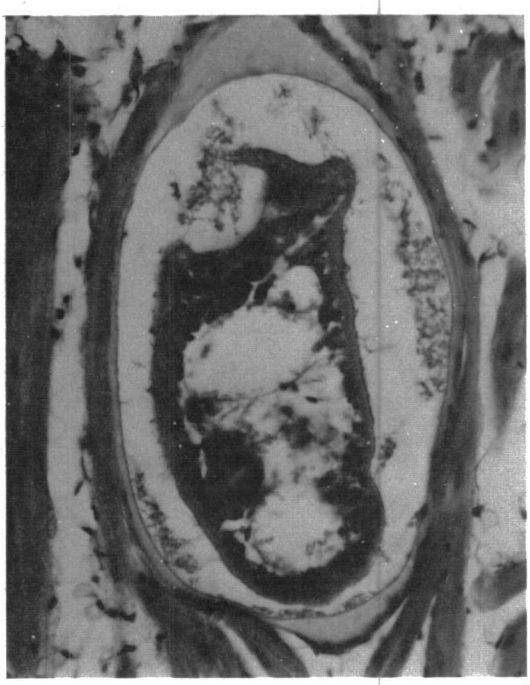

Fig. 18. P. burti, heart. Same as Fig. 17, but enlarged to show details of the parasite. H&E. 384X

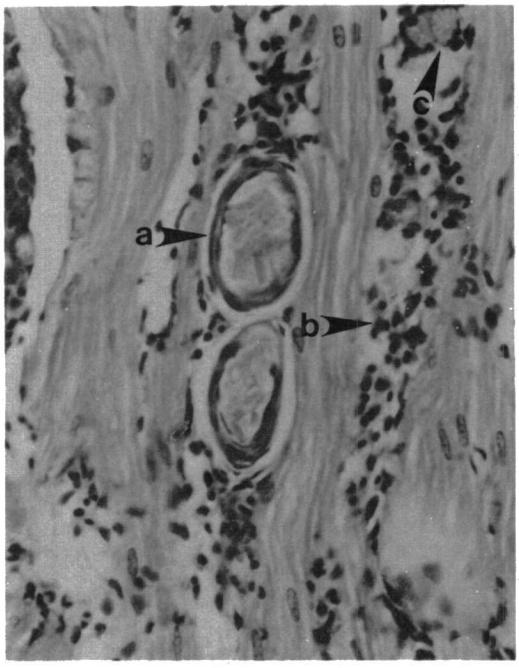

Fig. 19. R. aurorubens, heart. Cystic structure in interstitium (arrow a). Myocarditis (arrow b). Protozoa-like cells (arrow c). H&E. 384X

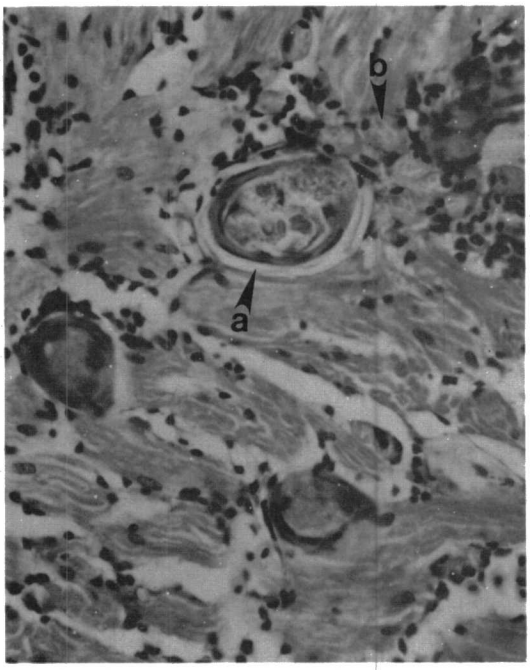

Fig. 20. R. aurorubens, heart. Interstitial cystic structure (arrow a) adjacent protozoa-like cells (arrow b). H&E. 384X

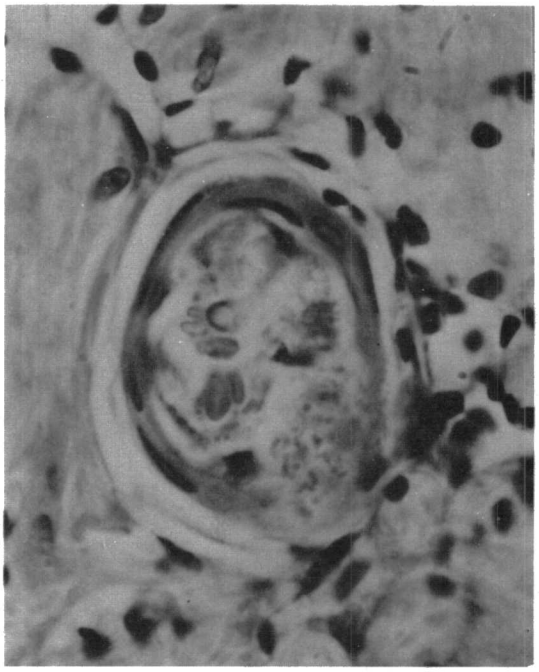

Fig. 21. R. aurorubens, heart. Same as Fig. 20, but enlarged to show detail of cystic structure. H&E. 960X

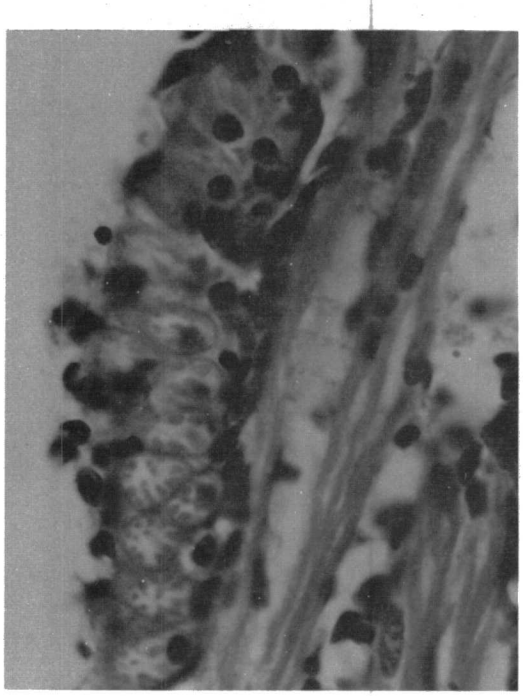

Fig. 22. R. aurorubens, heart. Protozoa-like cells arranged along cardiac muscle fibers. H&E. 960X

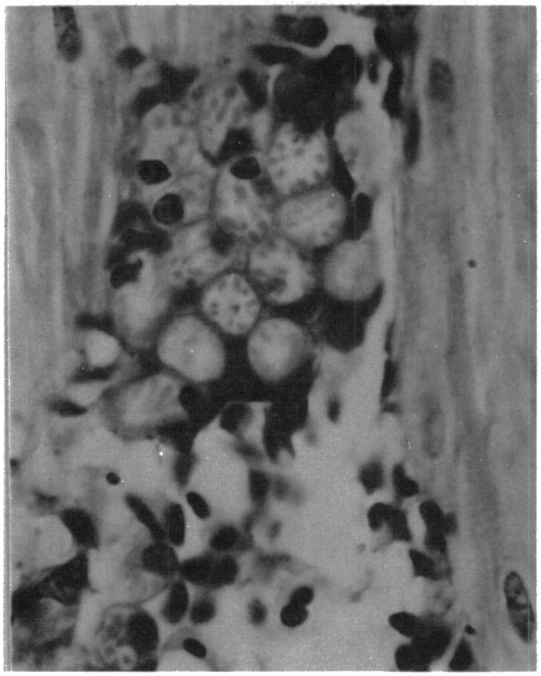

Fig. 23. R. aurorubens, heart. Aggregate of protozoa-like cells in cardiac interstitium. H&E. 960X

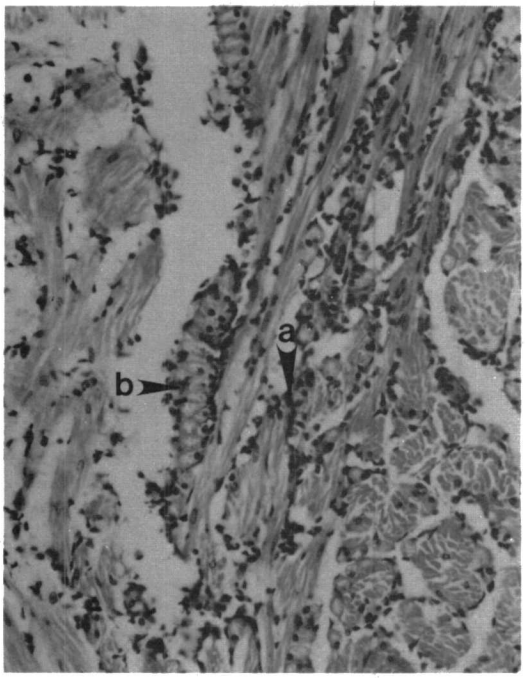

Fig. 24. R. aurorubens, heart. Myocarditis (arrow a) associated with protozoa-like infection (arrow b). H&E. 394X

Stomach

Histology

The basic structure of the stomach wall reviewed in this report was similar to that of carnivorous animals: a well-developed mucosa with columnar epithelium cells, gastric glands and muscularis mucosa, a well-developed submucosa, and a tunica muscularis (Figures 25 and 26). No parietal cells were observed in the glandular epithelium in any of the species examined. Occasionally, striated muscle fibers were present in the muscle tunic.

Histopathology

The majority of the histopathologic observations were lesions associated with parasitism. All gastric glands appeared normal except for three specimens. The glands in the latter three specimens were undergoing degeneration, apparently from pressure necrosis brought about by parasite-packed gastric lumens.

Helminth parasites were occasionally seen in the mucosa or attached to the mucosal surface (Figure 27). The most common mucosal-residing parasites were protozoa located among the gastric epithelial cells either as single cells or small groups (Figures 28 and 29).

Protozoa were also observed in the submucosa (Figure 30), but less frequently than helminths. Helminth parasites in the submucosa represented the most intensive gastric infection. These represented sections of encysted trematodes, cestodes and nematodes (Figures 31 to 36). In addition, many encapsulated areas of degenerating parasitic foci could be detected in the submucosa (Figures 37 to 40). Helminth parasites were observed in the tunica muscularis occasionally.

The submucosa of all stomachs exhibited varying amounts of inflam-

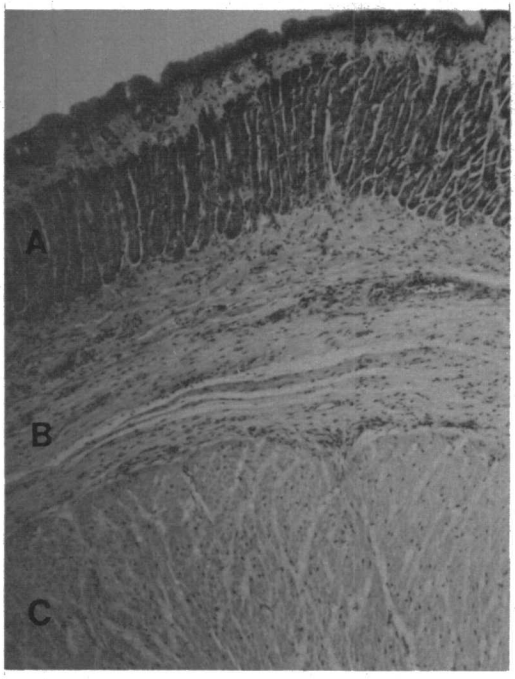

Fig. 25. C.arenarius, stomach. Cross section of gastric wall. Mucosa-A; submucosa-B; tunica muscularis-C. H&E. 96X

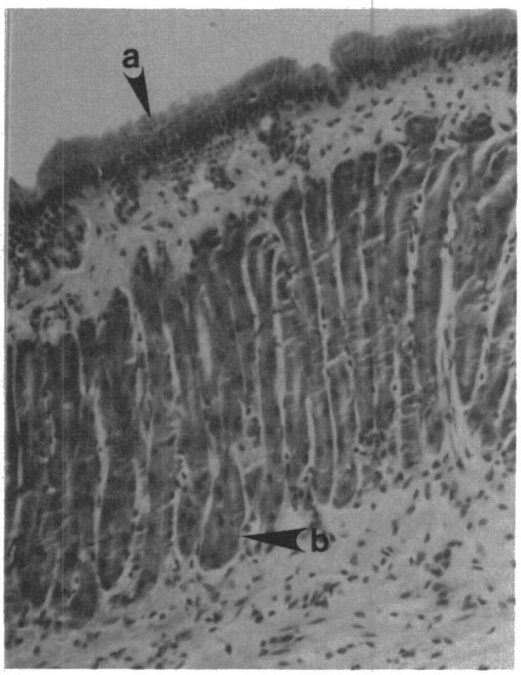

Fig. 26. C. arenarius, stomach. Mucosa. Gastric epithelium (arrow a) and gastric glands (arrow b). H&E. 240X

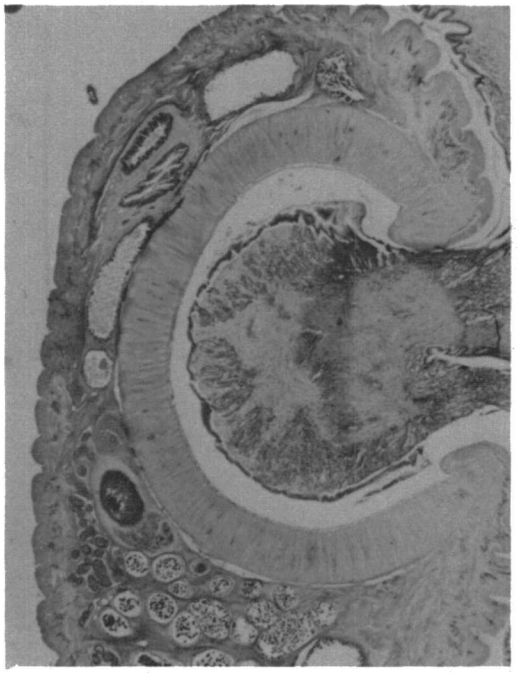

Fig. 27. R. aurorubens, stomach. Helminth parasite attached to gastric mucosa. H&E. 24X

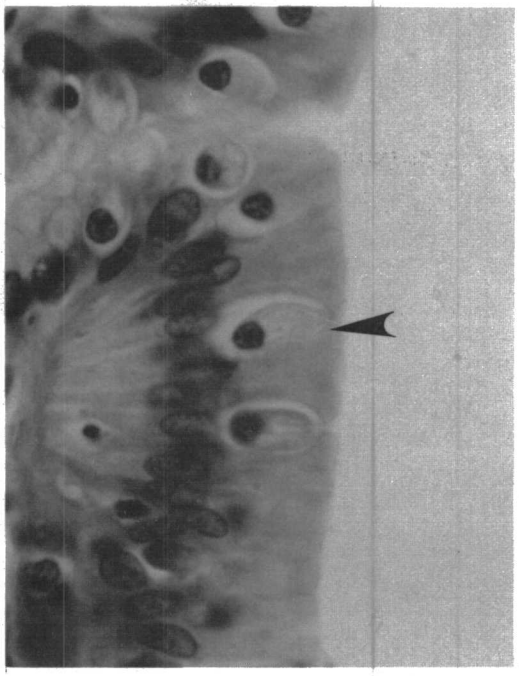

Fig. 28. P. aquilonaris, stomach. Protozoa in gastric epithelium (arrow). H&E. 960X

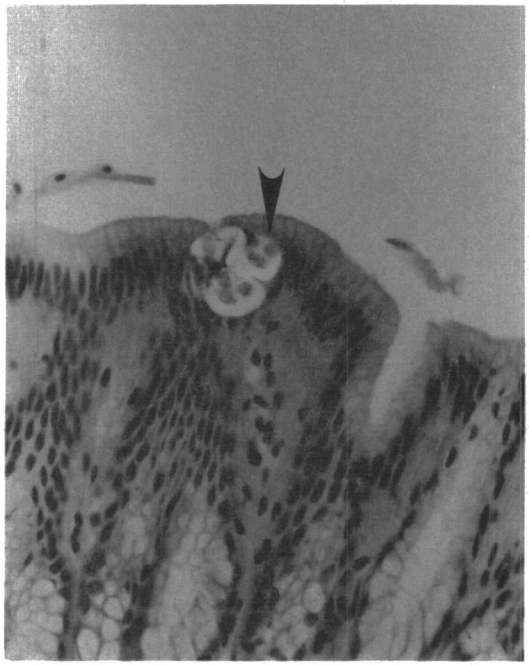

Fig. 29. C.philadelphica, stomach. Protozoa in gastric epithelium (arrow). H&E. 384X

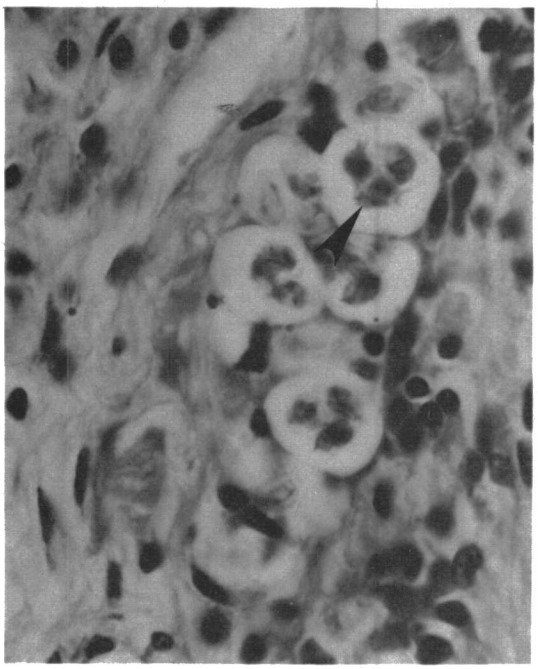

Fig. 30. M. undulatus, stomach. Protozoa in gastric submucosa (arrow). H&E. 960X

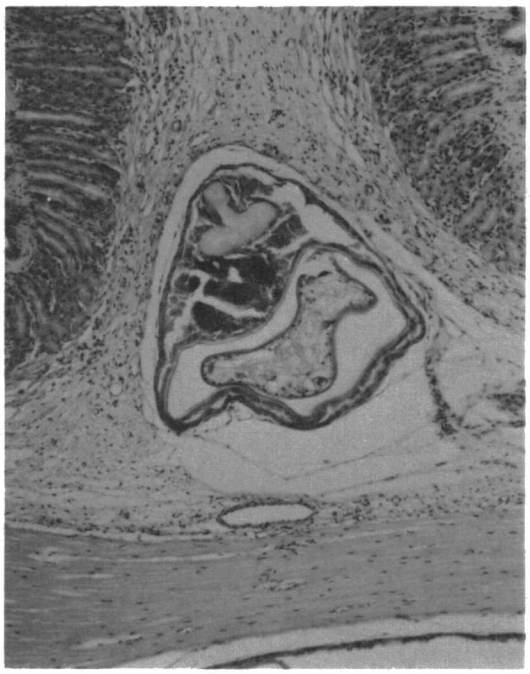

Fig. 31. C. arenarius, stomach. Helminth parasite in gastric submucosa. H&E. 96X

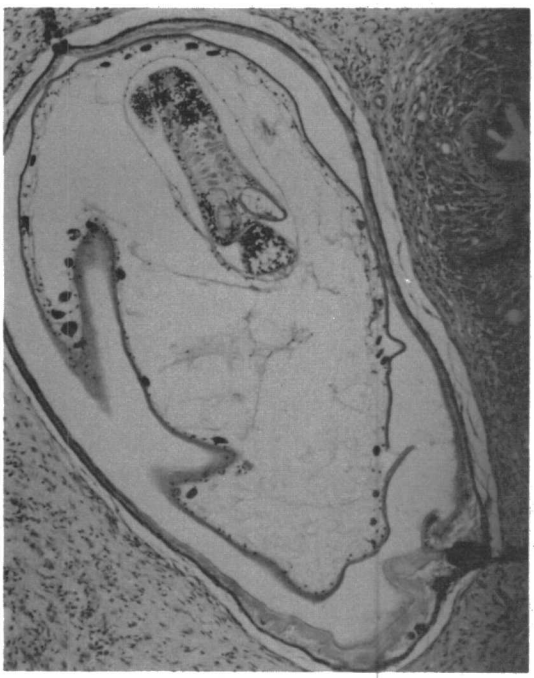

Fig. 32. C. arenarius, stomach. Helminth parasite in gastric submucosa. H&E. 96X

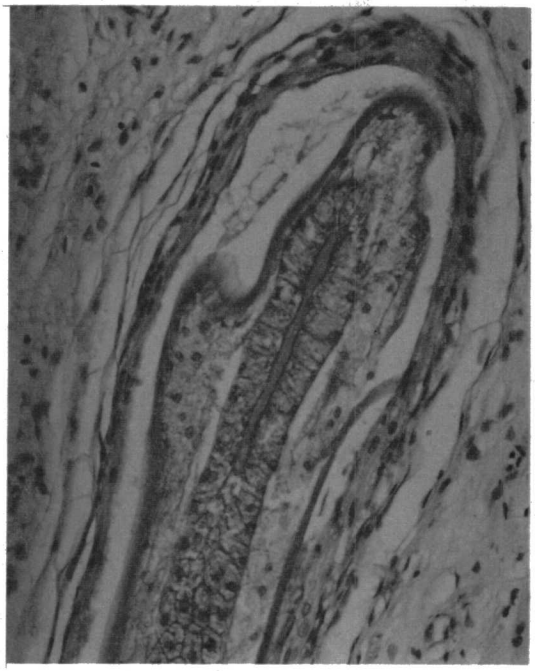

Fig. 33. P. aquilonaris, stomach. Helminth parasite in gastric submucosa. H&E. 240X

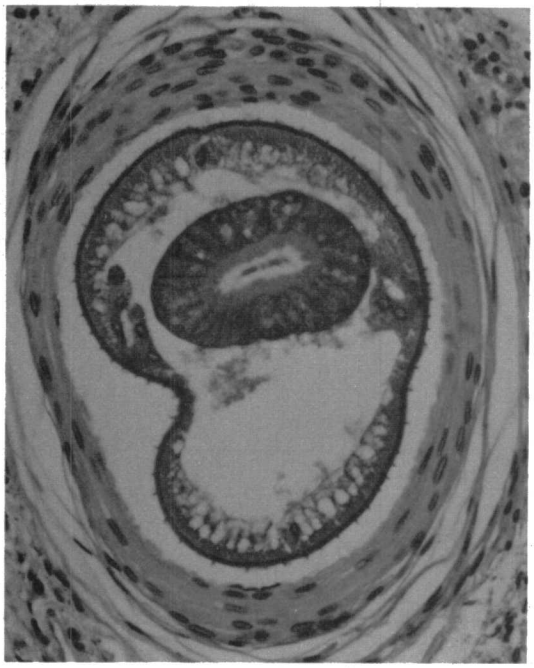

Fig. 34. P. aquilonaris, stomach. Helminth parasite in gastric submucosa. H&E. 384X

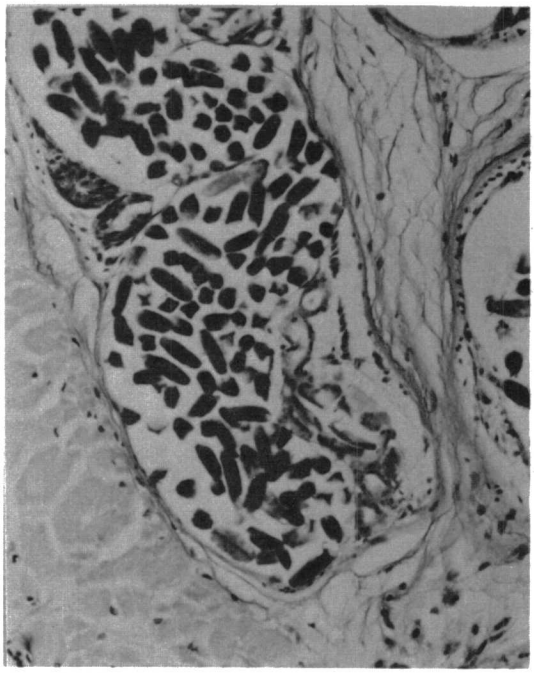

Fig. 35. P. aquilonaris, stomach. Helminth parasite in gastric submucosa. H&E. 240X

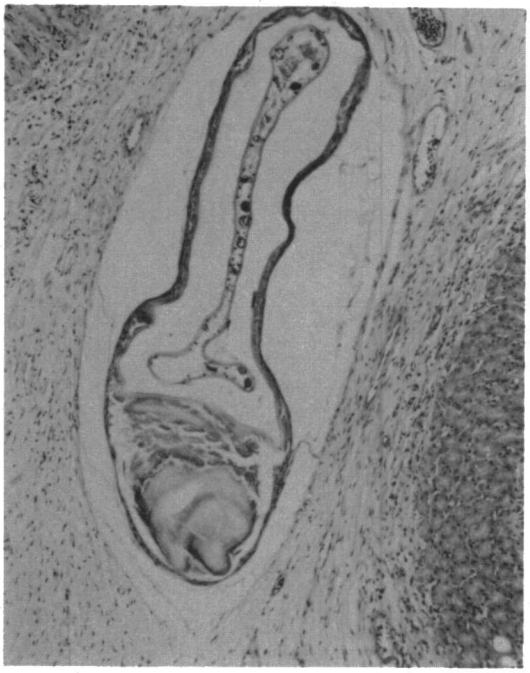

Fig. 36. C. arenarius, stomach. Helminth parasite in gastric submucosa. H&E. 96X

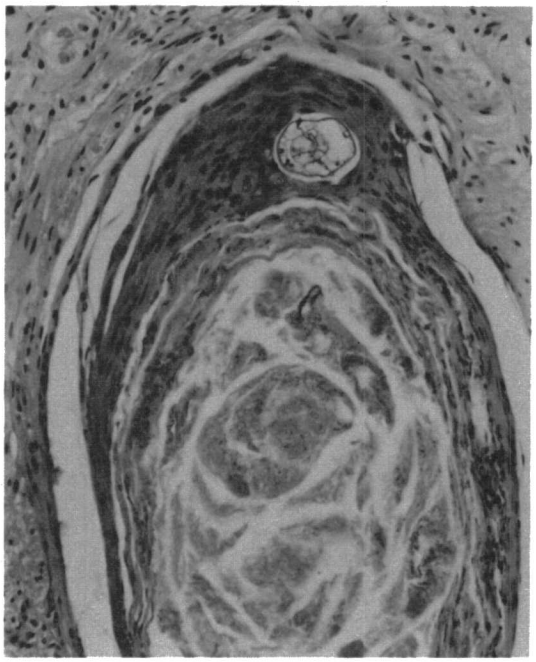

Fig. 37. R. aurorubens, stomach. Cystic structure in gastric submucosa. H&E. 240X

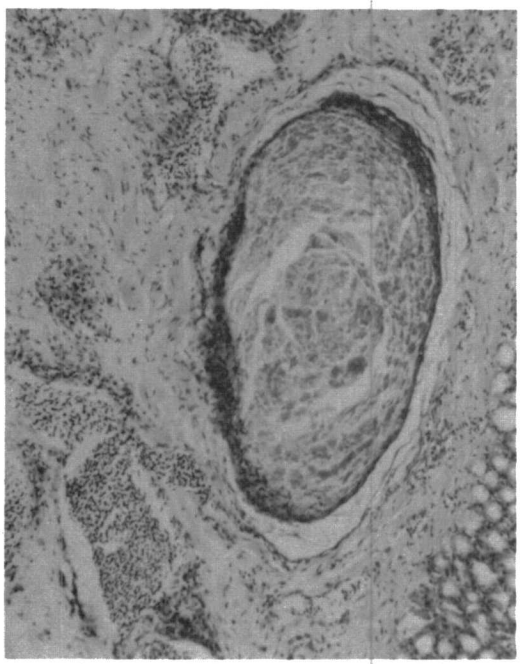

Fig. 38. P. aquilonaris, stomach. Cystic structure in gastric submucosa. H&E. 96X

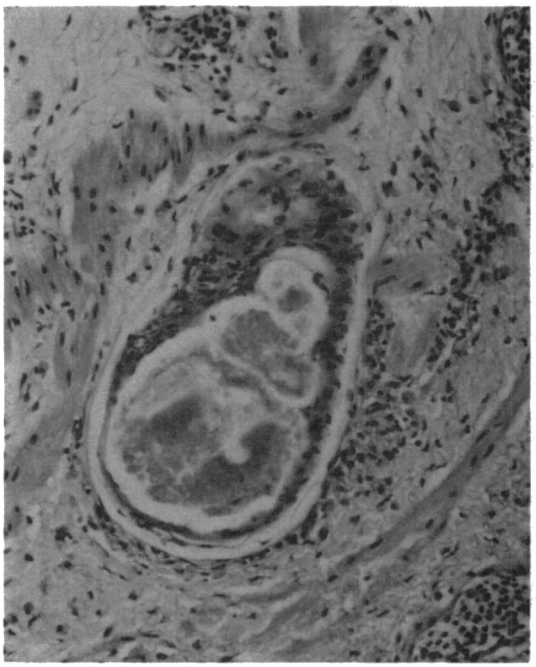

Fig. 39. L. campechanus, stomach. Cystic structure in gastric submucosa. H&E. 240X

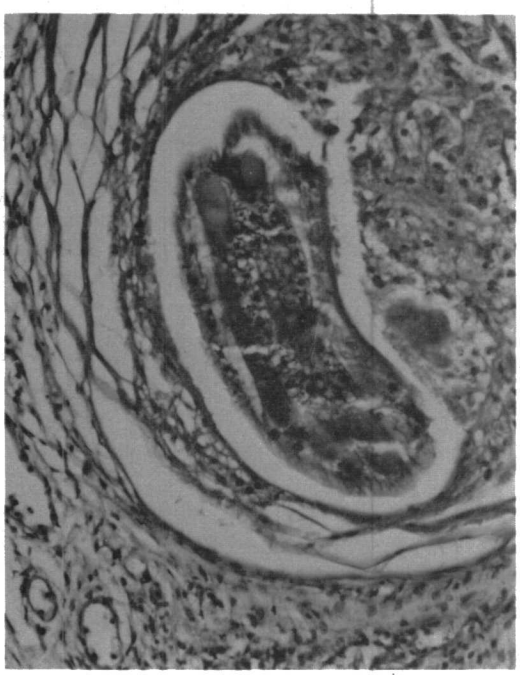

Fig. 40. P. aquilonaris, stomach. Cystic structure in gastric submucosa. H&E. 240X

matory reactions. Usually they were scattered foci of inflammatory cells, predominantly lymphocytes, plasma cells, or mixtures of these two cell types. Lymph nodules were not observed.

Acidophil cells were a commonly observed cell type in the gastric tissue. While they were predominantly located in the submucosa, they were also present in the other two wall layers. In this study the presence and numbers of acidophil cells in the stomachs appeared to be directly correlated with the degree of parasitism.

Liver

Histology

The general arrangement of the liver cytology was either tubular or muraliar. The tubular pattern consisted of six to eight hepatocytes surrounding a central sinusoid (Figure 41). The muralium arrangement consisted of interweaving sheets of hepatocytes two cells thick, marginated by sinusoids (Figure 42). The muralium arrangement has been reported in freshwater teleosts (Anderson and Mitchum, 1974; Bucke, 1971; Hale, 1965; Hinton, Snipes and Kendall, 1972; Mukherjee and Bhattacharya, 1975), while the tubular pattern was reported in more primitive marine Myxiniformes (Mugnaini and Harboe, 1967).

Hepatocytes were large cuboidal cells with prominent nuclei and nucleoli. The cytoplasm ranged from non-vacuolated (Figure 43) to extremely vacuolated (Figure 44). Vacuolation of the hepatocytes may have been due to glycogen and/or lipid accumulation.

Sinusoids were lined by discontinuous endothelium and Kupffer cells. The sinusoids were either dilated or closed. Sinusoid dilation may have been a shock reaction to trawl entrapment and rapid decompression.

Hepatopancreas (exocrine pancreas) units were scattered through the

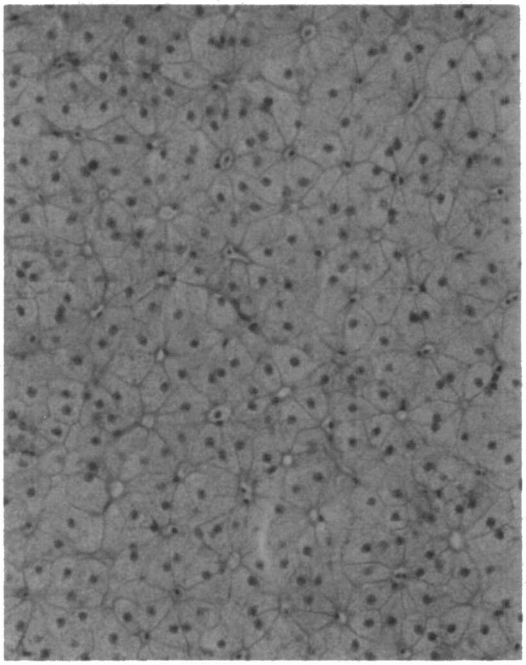

Fig. 41. C.philadelphica, liver. Hepatocytes arranged in a tubular pattern. H&E. 240X

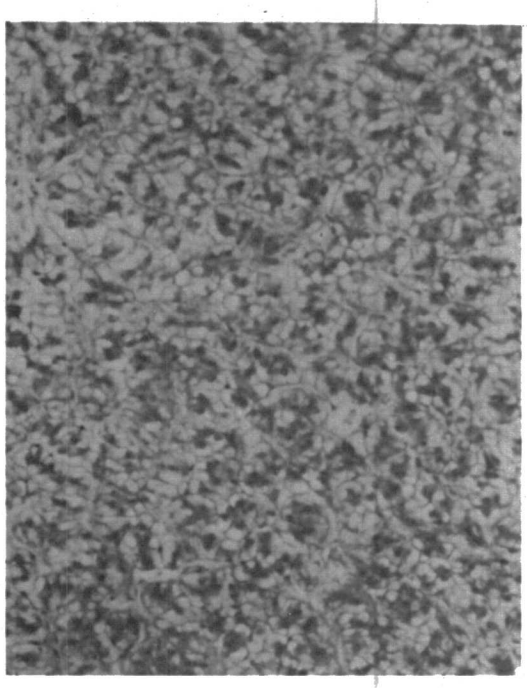

Fig. 42. U. parvus, liver. Hepatocytes arranged in a muralium pattern. H&E. 384X

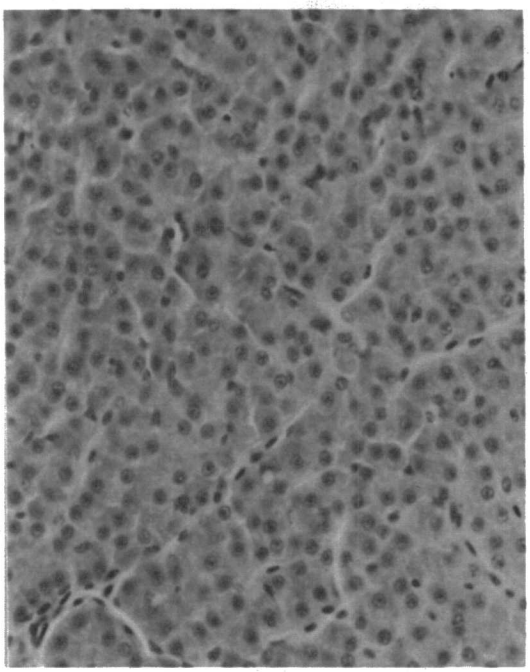

Fig. 43. P. aquilonaris, liver. Hepatocytes with very little cytoplasmic vacuolation. H&E. 384X

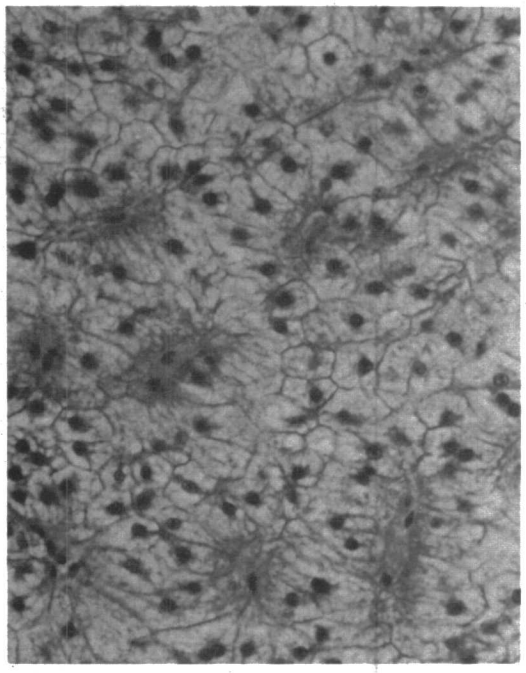

Fig. 44. C.philadelphica, liver. Hepatocytes with extreme cytoplasmic vacuolation. H&E. 384X

hepatic parenchyma in all species observed (Figure 45) except the rock sea bass (F), rough scad (H), and the dwarf goatfish (J). With hematoxylin and eosin stain, hepatopancreatic cells were basophilic with prominent eosinophilic zymogen granules at the apical end of the columnar cells.

Groups of pigmented cells were also found in the hepatic parenchyma. These cells appeared yellow to gold in color (Figure 45). Groups of pigmented cells have been reported in other species as ceroid cells and are considered a normal structure (Wood and Yasutake, 1956).

In the perihepatopancreatic space, cells with acidophilic granules and basophilic nuclei (acidophil cells) were sometimes observed (Figure 46).

Histopathology

Pathologic lesions were primarily parasitic in nature. Cross-sections of helminths were observed both in the liver parenchyma and hepatopancreas (Figure 47).

Four types of protozoa were observed in the hepatic tissue. One type was a basophilic, biconcave disc which appeared in groups of two to five in cysts (Figure 48). A second type, also encysted, had an eosinophilic plasma membrane and nuclei with basophilic nucleoli and colorless cytoplasm (Figure 49). The third type of protozoa, encysted in groups of three to five, had numerous eosinophilic granules (Figure 50). A fourth type, observed between bile duct epithelial cells, consisted of oval-shaped cells with an eosinophilic cytoplasm and basophilic nuclei (Figure 51).

Areas of necrotic hepatocytes with pyknotic nuclei were occasionally observed (Figure 52).

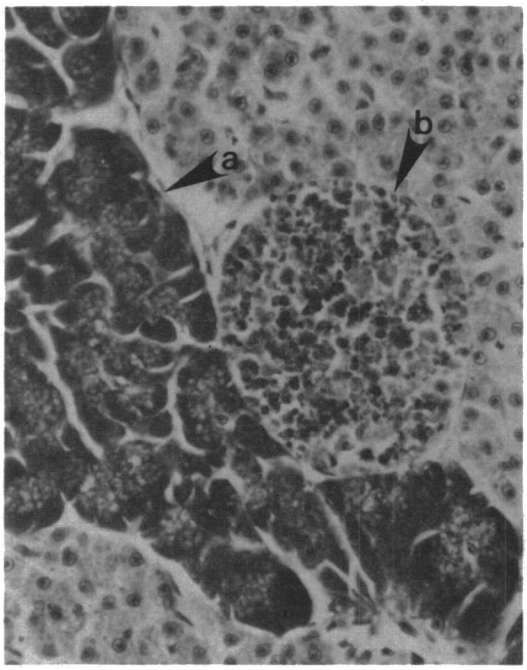

Fig. 45. P.aquilonaris, liver. Dark staining cells (arrow a) represent hepatopancreas. The group of cells at arrow b are pigmented cells. H&E. 384X

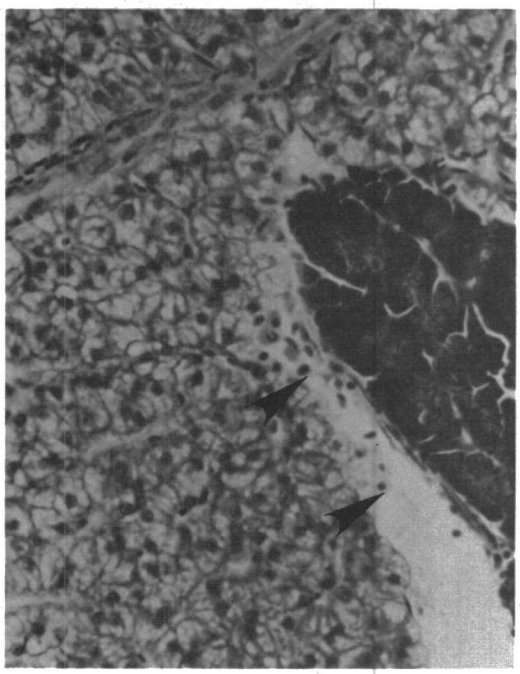

Fig. 46. C. arenarius, liver. The cells at the arrows are acidophils. H&E. 384X

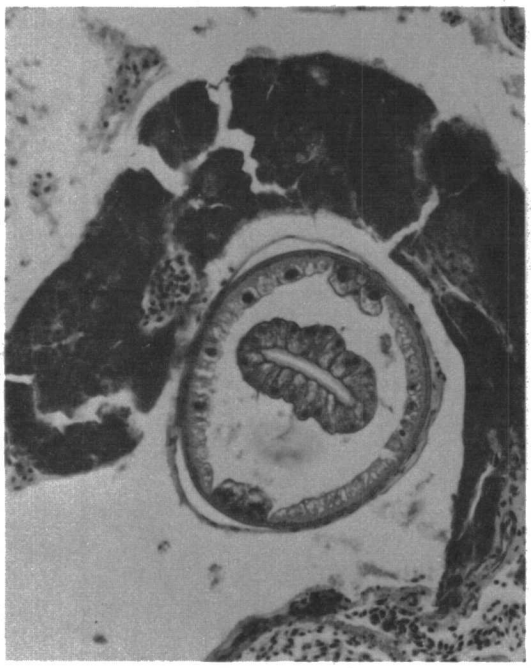

Fig. 47. U. parvus, liver. A helminth between hepatopancreas and hepatic parenchyma. H&E. 240X

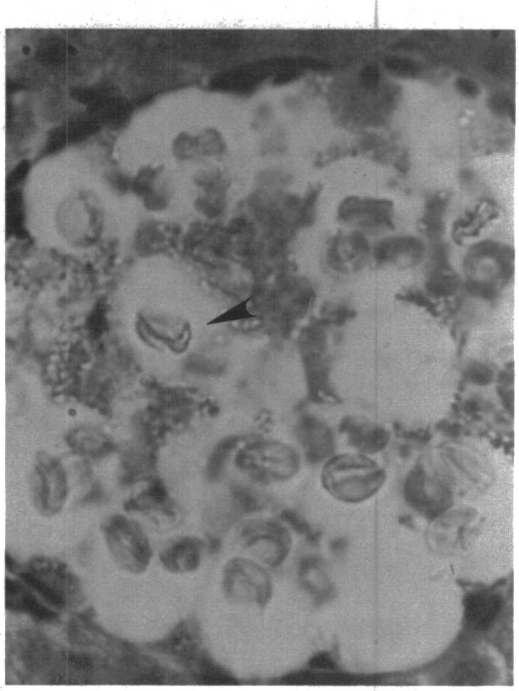

Fig. 48. S. caprinus, liver. Basophilic biconcave disc protozoon at arrow. H&E. 960X

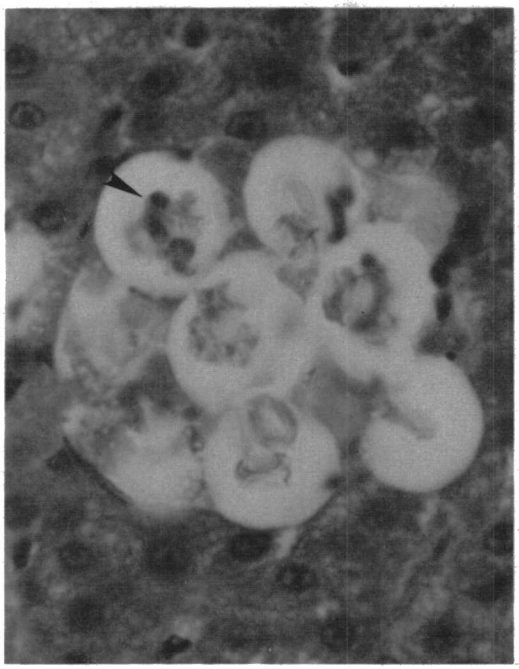

Fig. 49. S. caprinus, liver. Nucleated protozoon at arrow. H&E. 960X

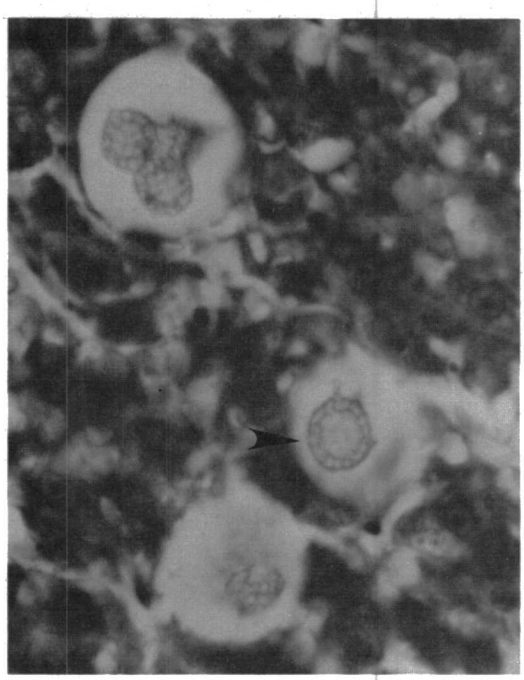

Fig. 50. U. parvus, liver. Protozoon with eosinophilic granules at arrow. H&E. 960X

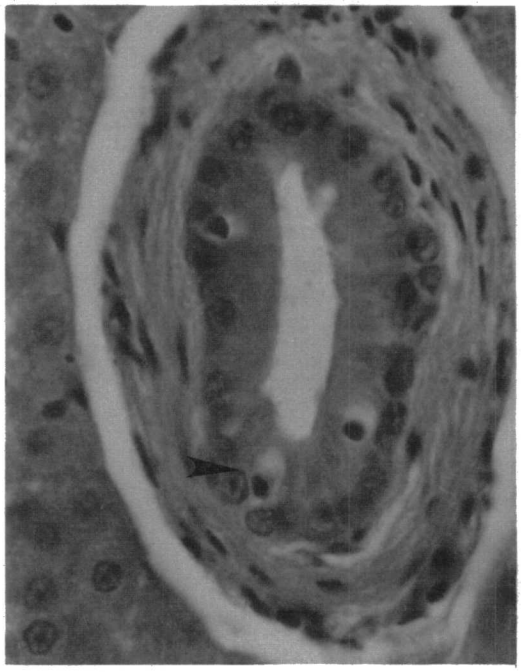

Fig. 51. S. caprinus, liver. Protozoon in epithelium of bile duct at arrow. H&E. 960X

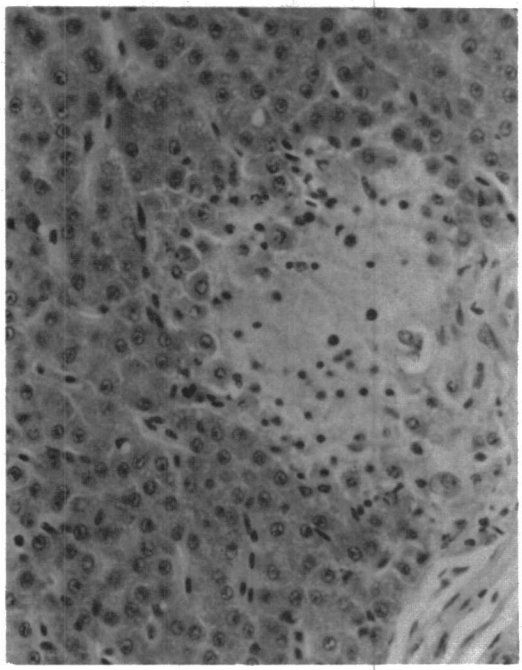

Fig. 52. P. aquilonaris, liver. Area of necrotic hepatocytes with pyknotic or absent nuclei. H&E. 384X

SUMMARY AND CONCLUSIONS

The qualitative and quantitative information obtained thus far in the histopathologic study of demersal fish demonstrated that parasitism was the major cause of lesions. Lesions that may be related to other etiologic agents were not observed. Parasitism caused varying degrees of necrosis, especially in the liver and stomach. Adjacent to their lesions, however, the general integrity of the organ tissues was maintained.

Although it may be too early to make significant comparisons regarding the histopathology of the fish used in this investigation, some trends may already be evident. Following is a summary of these observations.

1. About one-third of the organs collected demonstrated pathologic conditions, most of which were parasitic lesions.

2. The protozoan parasites appeared to be both microsporidia and myxosporidia.

3. Helminth parasites were larval trematodes, cestodes and nematodes.

4. Among the 10 species of fish examined, the rock sea bass had the smallest percentage of lesions and the vermilion snapper the largest.

5. The vermilion snapper also demonstrated the largest percentage of cardiac lesions.

6. There was a tendency for kidney and muscle lesions to be more numerous in the sand seatrout.

7. There was a tendency for kidney, muscle and liver lesions to be more numerous in specimens collected during the last three cruises, October, November and December, than during the first two cruises, July

and August.

8. The stomach and liver tissues were more frequently involved with parasitism than the muscle, kidney and heart. This was apparently unrelated to species, station or monthly cruise.

9. The overall percentage of lesions was larger in fish obtained from the Southern Bank than at other stations, while the smallest percentage of lesions occurred in fish obtained from Station 2. These observations were due to the vermilion snapper sampled at the Southern Bank and the rock sea bass sampled at Station 2.

10. All stations showed a tendency for the percentages of lesions in fish to increase over the last three monthly cruises, October, November and December.

LITERATURE CITED

Anderson, B. G., and D. L. Mitchum. 1974. Atlas of trout histology. Wyoming Game Fish Dept., Laramie, Wy. Pages 16-21.

Bucke, D. 1971. The anatomy and histology of the alimentary tract of the carnivorous fish the pike *Esox lucius* L. J. Fish Biol. :421-431.

Daniels, S. B., R. L. Herman, and C. N. Burke. 1976. Fine structure of an unidentified protozoon in the epithelium of rainbow trout exposed to water with *Myxosoma cerebralis*. J. Protozool. 23:402-410.

Hale, P. A. 1965. The morphology and histology of the digestive systems of two freshwater teleosts, *Poecilia reticulata* and *Gasterosteus aculeatus*. J. Zool. 136:132-149.

Hinton, D. E., R. L. Snipes, and M. W. Kendall. 1972. Morphology and enzyme histochemistry in the liver of the largemouth bass (*Micropterus salmoides)*. J. Fish Res. Board. Can. 29:531-534.

Holmes, J. C. 1971. Habitat segregation of sanguinicolid blood flukes (Digenea) of scorpaenid rockfishes (Perciformes) on the Pacific Coast of North America. J. Fish. Res. Board Can. 28:903-909.

Klein, W. D., O. W. Olsen, and D. C. Bowder. 1969. Effects of intestinal fluke, *Crepidostomum farionis*, on rainbow trout, *Salmo gairdneri*. Trans. Amer. Fish. Soc. 98:1-6.

Landolt, M. L. 1973. *Myxosoma cerebralis*: Isolation and concentration from fish skeletal elements-Trypsin digestion method. J. Fish. Res. Board Can. 30:1713-1716.

Love, M. S., and M. Moser. 1976. *Davisia reginae* Sp. N. (Protozoa: Myxosporida) from four California marine fishes. J. Parasitology 62:982-983.

Markiw, M. E., and K. Wolf. 1974. *Myxosoma cerebralis*: Isolation and concentration from fish skeletal elements-Sequential enzymatic digestions and purification by differential centrifugation. J. Fish. Res. Board Can. 31:15-20.

Moser, M., E. R. Noble, and R. S. Lee. 1976. The genus *Myxidium* (Protozoa: Myxosporida) in macrourid fishes. J. Parasitology 62:685-689.

_____, M. S. Love, and L. A. Jensen. 1976. Myxosporida (Protozoa) in California rockfish, *Sebastes* spp. J. Parasitology 62:690-692.

Mugnaini, E., and S. B. Harboe. 1967. The liver of *Myxine glutinosa*: A true tubular gland. Z. Zellforsch 78:341-369.

Mukherjee, S., and S. Bhattacharya. 1975. Histopathological lesions in the hepatopancreas of fishes exposed to industrial pollutants. Indian J. Exp. Biol. 13:571-573.

Nigrelli, R. F., D. S. Pôlorny, and G. D. Ruggieri. 1976. Notes on *Ichthyophythirius multifiliis*, a ciliate parasitic on fresh-water fishes, with some remarks on possible physiological races and species. Trans. Amer. Micros. Soc. 95:607-613.

Pippy, J. H. C., and P. van Banning. 1975. Identification of *Anisakis* (I) as *Anisakis simplex* (Rudolphi, 1809, det. Krabbe 1878) (Nematoda: Ascaridata). J. Fish. Res. Board Can. 32:29-32.

Sandeman, I. M., and M. D. B. Burt. 1972. Biology of *Bothrimonas* (=Diplocotyle)(Pseudophyllidea): Cestoda): Ecology, life cycle and evolution; a review and synthesis. J. Fish. Res. Board Can. 29:1381-1395.

Schell, S. C. 1975. The life history of *Plagioporus shawi* (McIntosh 1939) (Trematoda: Opecoelidae), an intestinal parasite of salmonid fishes. J. Parasitology 61:899-905.

_____. 1976. The life history of *Nezpercella lewis* Schell 1974 (Trematoda: Opecoelidae), a parasite of northern squawfish, and the smallmouth bass. J. Parasitology 62:894-898.

Scott, J. S. 1975. Incidence of trematode parasites of American plaice (*Hippoglossoides platessoides*) of the Scotian shelf and Gulf of St. Lawrence in relation to fish length and food. J. Fish. Res. Board Can. 32:479-483.

Wood, E. M., and W. T. Yasutake. 1956. Ceroid in fish. Am. J. Path. 32:591-603.

CHAPTER THIRTEEN

HISTOLOGICAL-HISTOPATHOLOGICAL SURVEY OF GONADAL TISSUE OF MACROEPIFAUNA AND DEMERSAL FISHES OF THE SOUTH TEXAS OUTER CONTINENTAL SHELF

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ABSTRACT

During this initial effort in the histological-histopathological survey of gonadal tissue of macroepifauna and demersal fishes of the South Texas Outer Continental Shelf, tissues were collected during the period July through December 1976. The Work Statement called for 5 samplings x 5 species x 5 specimens per species x 3 stations for a total of 375 specimens. Seventeen species and 456 specimens were collected. The additional species and specimens were necessary to maintain continuity and assure proper controls.

The purpose of this portion of the histopathological effort was twofold: first, to establish the normal seasonal (physiological) changes in the histology of the male and female gonads; and, second, to examine the gonads for pathological conditions. Seasonal changes in the histology of the gonads were only partially established, since the survey was conducted for only 6 months. Gonadal tissues were examined for pathological conditions and/or parasites. A number of pathological conditions was found in these tissues, but the incidence of pathology in the gonads was only 16.7 percent.

INTRODUCTION

The histological-histopathological baseline study of the macroepifauna and demersal fishes of the South Texas Outer Continental Shelf was initiated in the summer of 1976. During this initial partial effort, one seasonal and four monthly collections were made. Physiological conditions and pathological changes and/or occurrences were monitored and catalogued. This study consisted of the survey of: (1) invertebrate tissues from the gut, liver, kidney and muscles; (2) vertebrate tissues from the gut, liver, kidney, heart and muscles; (3) invertebrate and vertebrate gonads; and (4) karyotype analyses of vertebrate and invertebrate species. Items one and two are reported on elsewhere in this report by Drs. Haensly and Neff.

This report deals with the survey of histological-histopathological conditions of gonadal tissue. The karyotype analysis of vertebrate and invertebrate species met with technical difficulties. Karyotype analyses are included as an addendum to this report. The analysis of the gonad and gonadal tissues of vertebrates and invertebrates aids in the establishment of normal physiological changes. The survey also monitors any pathological conditions already occurring in these tissues.

It is important to first establish the normal reproductive cycle changes occurring in gonadal tissues. In contrast to other tissues not seasonally dependent, the gonads are constantly changing according to the breeding season. Since gonadal tissues are constantly changing throughout the reproductive cycle (Andrew and Hickman, 1974; Bloom and Fawcett, 1975; Hoar, 1969; Sadlier, 1973), it is important to know what changes normally occur before attempting to assign a pathological condition to such changes. Each species has its own inherent reproductive

cycle that puts it at a different phase at any given period (Burns, 1976; Larson, 1972; Liley, 1969; Fuller et al., 1976; Schwassmann, 1971). This makes it difficult to generalize concerning tissue condition. An additional problem is the fact that individuals within the same species may be at different phases in the reproductive cycle. The gonadal tissues being of two types, male and female, further compound the problem of establishing normal conditions. The collection of additional individuals per species is required to insure equal (or close to equal) distribution of male and female specimens. A partial reproductive cycle is reported herein for those species collected during the majority of the collecting efforts. The other species provide back-up data since it is difficult to predict which species will be available during any given collecting effort.

The pathology of the ovary and testes as reported herein is primarily limited to certain major conditions. Parasites are normally easy to identify and their effects on the tissue and/or organ readily seen. Without an established histological reproductive pattern, it is difficult to know if conditions such as interstitial cell and fibroblast infiltration are part of the normal cycle or pathological.

The karyotype analysis was initiated without any reportable success. Due to technical problems, the analysis of chromosomes is planned as a separate effort to establish proper methodology under controlled conditions not possible in this initial effort.

METHODS AND MATERIALS

Sampling

Samples for histopathological analysis were taken during five trawling cruises, one seasonal and four monthly. Collections were taken at Stations

1, 2 and 3, Transect II, with a 35-ft (10.7 m) standard otter trawl set to sample epifauna and near-surface infauna; and at Southern Bank demersal fish were collected with a hook and line. Whenever possible, 10 individuals of each species were collected to insure equal distribution of male and female specimens (Tables 1-13).

The BLM contract called for minimal sampling of 3 stations x 5 species x 5 individuals per species x 5 months for a total of 375 specimens. Total samples collected were 456. Since all species were not evenly distributed spatially or temporally, additional species were collected to insure sampling continuity from one cruise to another. This provided an overlap in the species available during different cruises. Since a goal of this study is to provide a catalogue of seasonal changes as well as any pathological conditions, continuity must be maintained. Tables 1-13 show that the species collected were not present in every trawl. By increasing the number of species sampled, continuity was maintained. The vermilion snapper (*Rhomboplites aurorubens*), collected by hook and line at the Southern Bank Station, was a good representative of a demersal fish species as it was always caught during such sampling efforts (Table 7),

In this study, not only was gonadal histology investigated but seasonal changes in male and female tissues as well. The need for up to 10 specimens per species is important in obtaining equal numbers of males and females, since secondary sexual characteristics are not always evident. Also, it is important to collect, whenever possible, the same species every cruise.

Collecting

Specimens selected for sampling were processed on board ship to avoid any post-mortem histological changes (Galigher and Kozloff, 1971;

TABLE 1

WENCHMAN (*Pristipomoides aquilonaris*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	3 m	2 m	d
	-	2 f	3 f	e,l,y,v
August	-	1 m	6 m	d,s
	-	4 f	4 f	e
October	-	-	4 m	d
	-	-	6 f	l
November	-	-	4 m	d
	-	-	6 f	e,l
December	-	-	3 m	d
	-	-	2 f	e,l

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 2

LONG SPINE PORGY (*Stenotomus caprinus*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	-	3 m	gc
	-	-	2 f	e
August	-	-	3 m	gc
	-	-	4 f	e
October	-	-	4 m	gc,d
	-	-	1 f	e
November	-	-	2 m	d,s
	-	-	8 f	e,l,y,v
December	-	1 m	3 m	d,s
	-	1 f	2 f	l,y

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 3

SAND SEA TROUT (*Cynoscion arenarius*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	3 m	-	-	s
	3 f	-	-	l,y
August	0 m	-	-	-
	3 f	-	-	e,v
October	7 m	-	-	s
	3 f	-	-	e
November	0 m	-	-	-
	1 f	-	-	e
December	0 m	-	-	-
	5 f	-	-	e

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 4

ATLANTIC CROAKER (*Micropogon undulatus*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	1 m	-	-	s
	4 f	-	-	1
August	-	-	-	-
	-	-	-	-
October	6 m	-	-	s
	4 f	-	-	e,1
November	2 m	-	-	s
	0 f	-	-	-
December	1 m	-	-	s
	3 f	-	-	e

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
1 = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 5

BUTTERFISH (*Peprilus burti*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	-	-	-
	-	-	-	-
August	0 m	-	-	-
	3 f	-	-	e
October	-	4 m	-	s
	-	6 f	-	e
November	-	-	-	-
	-	-	-	-
December	-	-	-	-
	-	-	-	-

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 6

ROCK SEA BASS (*Centropristis philadelphica*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	-	-	-
	-	-	-	-
August	-	0 m	-	-
	-	6 f	-	e
October	-	0 m	-	-
	-	5 f	-	1
November	-	0 m	-	-
	-	5 f	-	e,l,y
December	-	0 m	-	-
	-	5 f	-	e,l,y

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 7

VERMILION SNAPPER (*Rhomboplites aurorubens*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH AT SOUTHERN BANK TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	S.B.	Gametogenic Stage
July	4 m	d,s
	3 f	e
August	4 m	d,s
	2 f	e,l,y
October	6 m	gc,s,v
	4 f	e
November	3 m	gc,s,v
	2 f	e
December	4 m	gc
	2 f	e

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 8

RED SNAPPER (*Lutjanus campechanus*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH AT SOUTHERN BANK TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	S.B.	Gametogenic Stage
July	-	-
	-	-
August	4 m	gc
	2 f	e,v
October	-	-
	-	-
November	2 m	d,s
	0 f	-
December	-	-
	-	-

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 9

BROWN SHRIMP (*Penaeus aztecus*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	1 m	-	gc,d,s,v
	-	2 f	-	1
August	0 m	-	-	-
	5 f	-	-	1
October	4 m	2 m	-	d
	6 f	6 f	-	1,y
November	6 m	3 m	-	-
	5 f	5 f	-	1,y
December	1 m	1 m	-	d
	1 f	5 f	-	1,y

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development.

f = Female
e = Early Development, Small Oocytes
1 = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 10

BLUE CRAB (*Callinectes similis*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	2 m	-	d,s,v
	-	0 f	-	-
August	8 m	3 m	-	gc,d,s,v
	2 f	4 f	-	1
October	-	3 m	-	d
	-	5 f	-	1,y,v
November	-	1 m	-	d
	1	4 f	-	1,y
December	-	-	-	-
	-	-	-	-

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
1 = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 11

PORTUNID CRAB (*Portunus spinicarpus*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	-	-	-
	-	-	-	-
August	-	-	-	-
	-	-	-	-
October	-	-	6 m	d
	-	-	o f	-
November	-	-	2 m	d
	-	-	5 f	l,y,v
December	-	-	8 m	d
	-	-	2 f	y

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 12

SQUID (*Loligo pealei*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	2 m	-	gc,d,s
	-	3 f	-	l,y
August	3 m	0 m	-	gc,d,s
	3 f	1 f	-	l,y
October	2 m	3 m	1 m	gc,d,s
	5 f	3 f	0 f	l,y
November	5 m	3 m	2 m	gc,d,s
	3 f	2 f	0 f	l,y
December	-	3 m	1 m	gc,d,s
	-	4 f	0 f	l,y

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

TABLE 13

PAPER SCALLOP (*Amusium papyraceus*) - DISTRIBUTION OF SPECIMENS COLLECTED EACH MONTH PER STATION ALONG TRANSECT II DURING JULY THROUGH DECEMBER 1976 AND STAGES OF GAMETOGENIC DEVELOPMENT

	1/II	2/II	3/II	Gametogenic Stage
July	-	-	-	-
	-	-	-	-
August	-	-	-	-
	-	-	-	-
October	-	-	4 m	gc
	-	-	4 f	e,v
November	-	-	6 m	d
	-	-	6 f	e,l
December	-	-	10 m	d,s
	-	-	10 f	l,y

m = Male
gc = Predominantly Germ Cells
d = Developing - Predominantly Spermatogonia, Primary and Secondary Spermatocytes
s = Maturing Stage - Predominantly Spermatids
v = Variable - Several stages present; or different animals varying in stage of gamete development

f = Female
e = Early Development, Small Oocytes
l = Large Oocytes, No Yolk Platelets
y = Vitellogenesis - Large Oocytes with yolk platelets; large follicle cells; chorion and zona radiata present
v = Variable - Several sizes of oocytes; or different animals varying in stage of gamete development

Gurr, 1962; Guyer, 1953). This became critical in samples from Station 3, Transect II, because these were from deeper waters than at Stations 1 and 2, and tended to undergo rapid changes due to pressure changes. Whenever possible, the animals were kept alive in a holding tank until processed.

Gonads were removed from the animals as soon as possible. Fish gonad size ranged from 2 to 10 mm in diameter and from 10 to 50 mm in length. Initially the gonads were sectioned into short segments (5 mm^2) and fixed. Subsequently (second cruise), gonads were fixed *in toto*. This change in technique was necessary due to the texture of the fish gonads. Fish gonads tend to roll back upon themselves when sectioned due to the highly elastic tunica (Hoar, 1969; Nelsen, 1953). This resulted in severely distorted tissues. Fixing gonads *in toto* does not alter the procedure significantly since most gonads are less than 10 mm in diameter and the fixatives can penetrate into the tissues rapidly. More importantly, *in toto* fixation prevents tissue distortion. Secondly, it enables the study of germinal development for possible sequential maturation of germinal cells. By preserving the entire gonad and maintaining orientation, material can be obtained from the distal, medial and proximal areas and any variation in germ cell maturation may be analyzed.

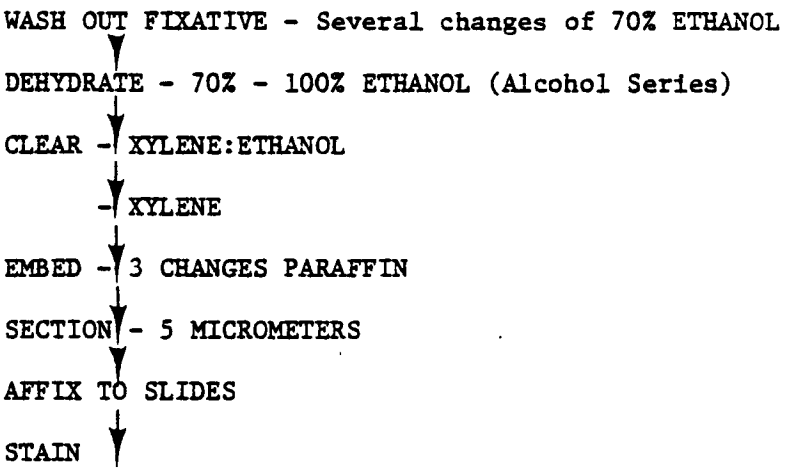
Invertebrate gonadal tissues did not present the same problem as fish gonadal tissue. Invertebrate animals were dissected on board, the gonads removed, sectioned and fixed. In several cases (shrimp and crabs), it was necessary to fix the animal *in toto*, so the animal was decapitated, the abdomen removed, the exoskeleton peeled off and the thorax only placed in the fixative.

Fixatives used were Bouin's (picric-acetic-formalin), Dietrich's (acetic-alcohol-formalin) and buffered formalin (Conn *et al.*, 1965; Galigher and Kozloff, 1971; Gurr, 1962; Guyer, 1953). These three fixatives

were chosen and used because of the versatility allowed in staining reactions and their fixing capabilities.

Laboratory Processing

Tissues were processed according to standard procedures for a given fixative. Tissues were trimmed, or further trimmed, prior to laboratory processing. Fixatives were washed from the tissues, and the tissues were dehydrated and embedded according to the following flow chart:



Tissues were sectioned at 5 μm. Some tissues were sectioned at 10 μm due to their texture or nature. Slides of each specimen were stained in Heidenhain's iron-hematoxylin/eosin (Galigher and Kozloff, 1971) and additional slides of each specimen stained with the Feulgen nuclear reaction (Guyer, 1953). Mallory's triple stain (Galigher and Kozloff, 1971; Gurr, 1962), a connective tissue and all-purpose stain was employed to detect high infiltration of fibroblasts and connective tissue. For study of the normal reproductive cycle in each species, a total of six slides were prepared. Sections were prepared from three areas of each ovary and testes to ascertain if differential and/or subsequential development was occurring in any of the specimens.

RESULTS

Seventeen (17) species (456 samples) were collected between July and December and 13 species (383 samples) are reported herein. The four species omitted were collected either once during the five sampling efforts, or several times but in limited numbers. Since one of the objectives was to establish normal reproduetive cycles (histological changes), these four species were processed and archived for later use should they recur in ample numbers in the collecting effort.

Tables 1 through 8 list the fish collected during this study period and their gametogenic stage. The wenchman (*Pristipomoides aquilonaris*), longspine porgy (*Stenotomus caprinus*) and vermilion snapper (*Rhomboplites aurorubens*) were the most abundant and consistent species. Reproductive cycles for these species are partly established. For many species, the reproductive cycle is not synchronous (Blaxter, 1969; Burns, 1976; Dorfman, 1976; Fuller *et al.*, 1976; Hoar, 1969; Sadleir, 1973) and in a given month, the reproductive phase will vary over a wide range of development. Two to three years must be sampled to establish this pattern. No hermaphroditic fish were observed (Dorfman, 1976; Yamamoto, 1969).

Tables 9 through 13 present the invertebrate species collected in 1976. One shrimp (*Penaeus aztecus*, brown shrimp), two crabs (*Callinectes similis*, blue crab; and *Portunus spinicarpus*, portunid crab) and two molluscs (*Loligo pealei*, squid; and *Amusium papyraceus*, paper scallop) were collected. Two additional shrimp species were collected but are not included in this report. Of the invertebrate species collected, the squid was the only species collected every time at the various stations (Table 12). As with the fishes, two or three years of sampling will be necessary to establish the reproductive cycles of the invertebrate species.

A variety of pathological conditions was observed (Figures 1-20). Of the 383 samples analyzed, 64 individuals (16.7%) demonstrated some pathological condition. In several cases, more than one condition was observed. The most commonly observed condition was an amorphous material (acellular mass)(Figures 11-14) in most of the longspine porgy specimens, both male and female. These were large spherical masses occupying various areas of the gonad. No distribution patterns were observable. The masses varied considerably in size, stained poorly with hematoxylin-eosin, were Feulgen reaction negative (no DNA), and stained faintly with Mallory's triple stain. Under high magnification, the masses appeared as irregular spheres, were crystalline-like and possessed no cellular characteristics. In some cases, "mitotic-like" Feulgen positive (DNA positive) figures were present (Figure 14). In the testes these masses were usually surrounded by connective tissue of fibroblast cells, while in the ovaries they were usually within the ovigerous fold, surrounded by follicular tissue (Figure 12, Table 14).

The second most common condition was the presence of nematode parasites (Figures 5, 15, 17). In several cases the nematode had penetrated and invaded the gonad, while in other cases, the parasite was associated with the gonad on the periphery or in the connective tissue or mesentery supporting the gonad. The brown shrimp specimens (*P. aztecus*) usually had parasites embedded in the gonadal tissues, but they were frequently observed peripherally, mainly in the hemocoel. In such cases, the parasite was also invading other tissues or moving freely within the hemocoel.

Gonads that failed to develop, whether male or female, often appeared to be associated with nematode parasitism (Figures 3, 4, 5). In such cases, the parasite invasion was often not in the gonad, but in the supporting tissue. The parasites appeared to block normal blood flow.

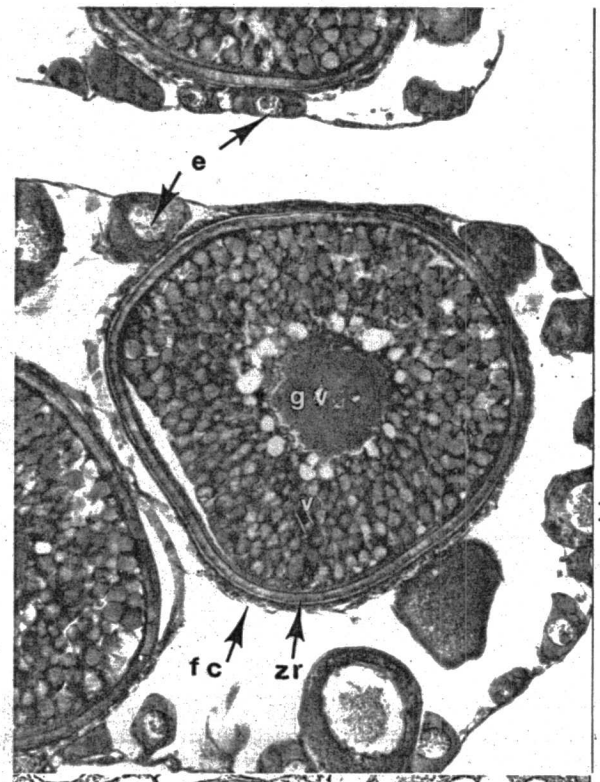


Figure 1 - Vermillion Snapper Normal oocytes in ovigerous fold. (e) early developing oocyte; (fc) follicle cells; (zr) zona radiata & chorion; (y) yolk platelets; (gv) germinal vesicle. 220X Mallory's Triple Stain

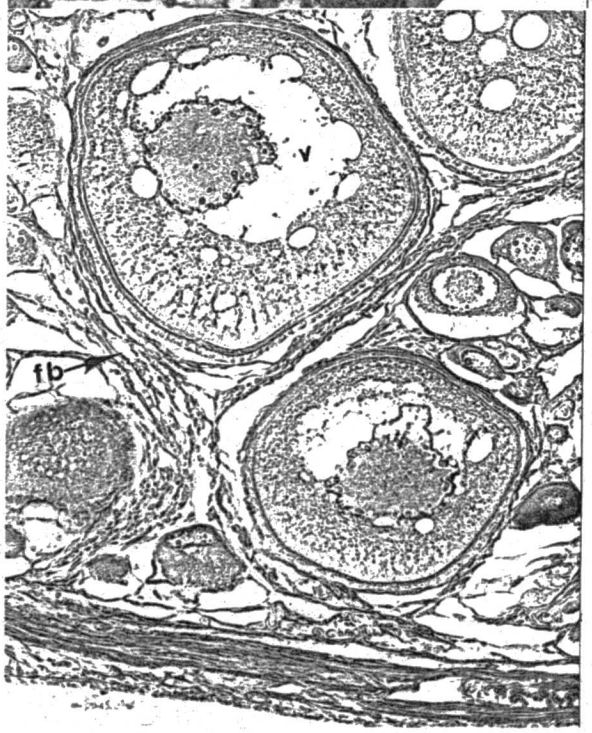


Figure 2 - Wenchman-Atretic oocytes with large vesicles (v) and large amount of fibroblast (fb). 220X Mallory's Triple Stain

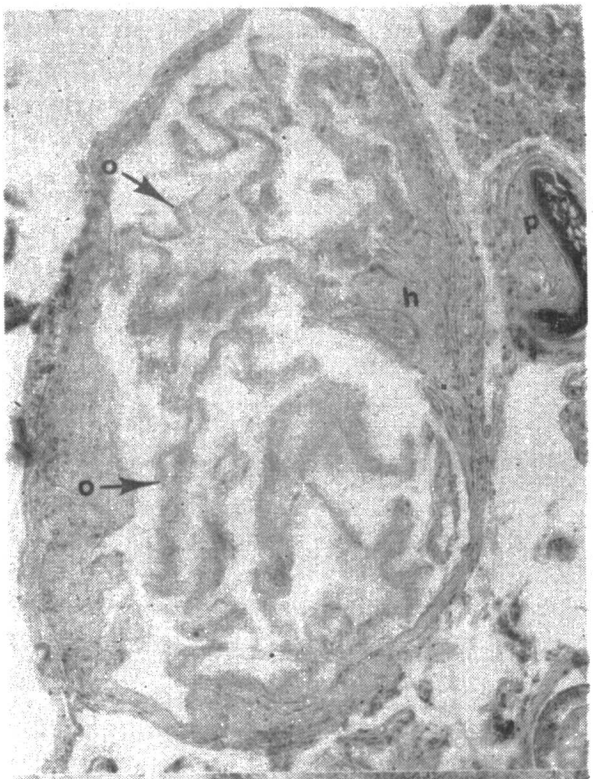


Figure 3 - Wenchman-Degenerated ovary due to parasite infestations in blood vessels and mesenteries; (o) ovigerous folds; hyperplasia (h); parasites (p). 220X H&E

Figure 4 - Wenchman-Hyperplastic cells (h) in degenerated ovary. 540X H&E

Figure 5 - Wenchman-Hyperplastic growth (h) and cross section of nematode parasite (p) in connective tissue supporting gonad. 540X H&E.

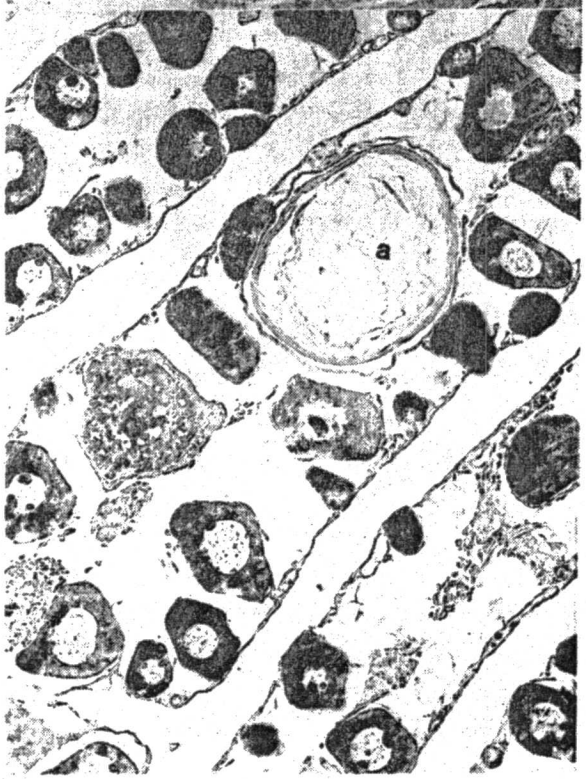


Figure 6 - Wenchman-Ovigerous fold with several developing stages of oogenesis plus an atretic oocyte (a) that has been reabsorbed leaving behind a cyst. 220X H&E.

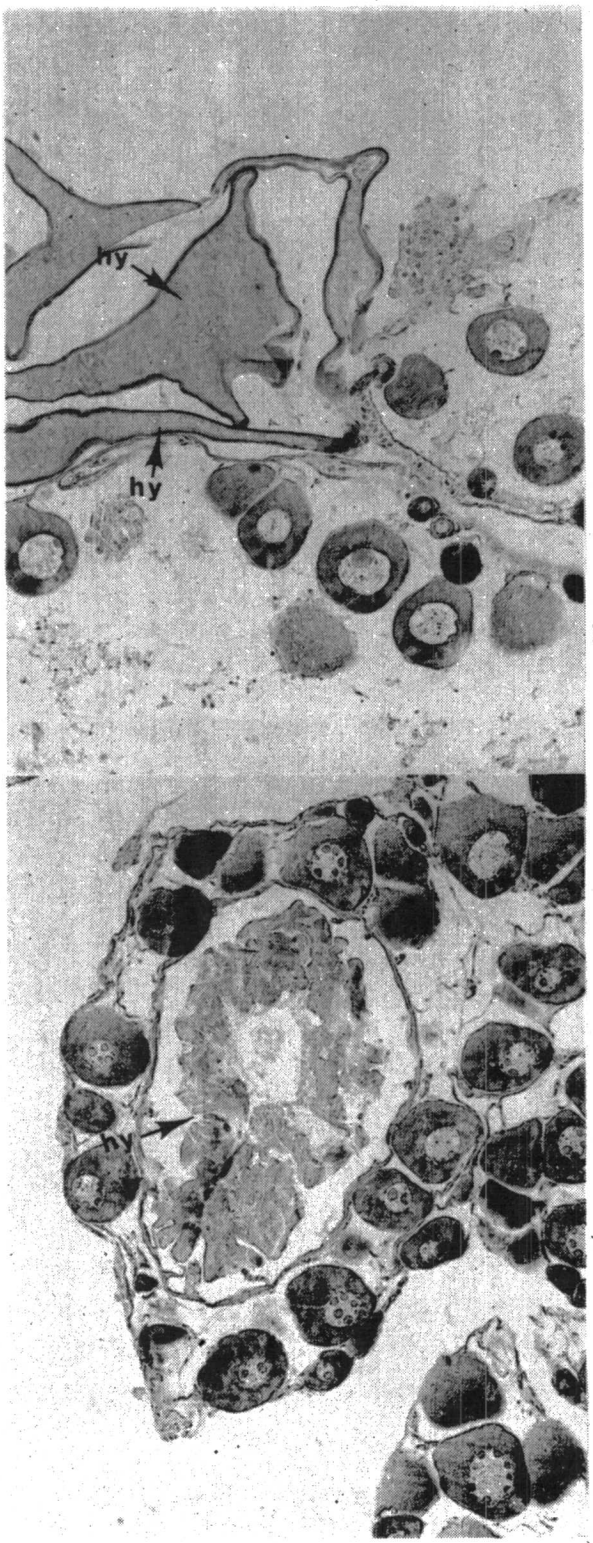


Figure 7 - Wenchman-Hyalinization (hy) of connective tissue between ovigerous fold and ovarian wall. 220X H&E

Figure 8 - Butterfish-Large oocyte that has been reabsorbed and undergone hyalinization (arrow). 220X H&E.

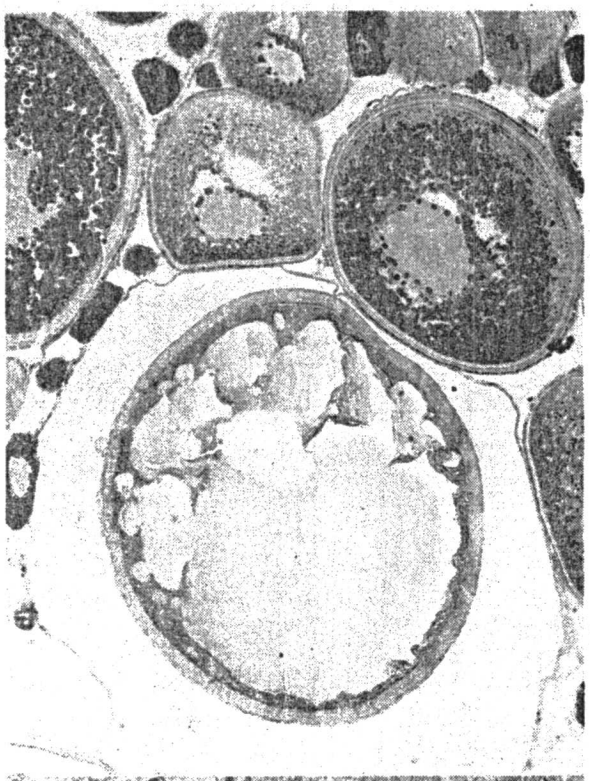


Figure 9 - Rock Bass-Large oocyte that has been hyalinized surrounded by large yolk filled oocytes. 135X H&E

Figure 10 - Atlantic Croaker - High magnification (540X) of ovary parasitized by nematodes, showing neoplastic growth and high amount of vascularization (arrows). H&E

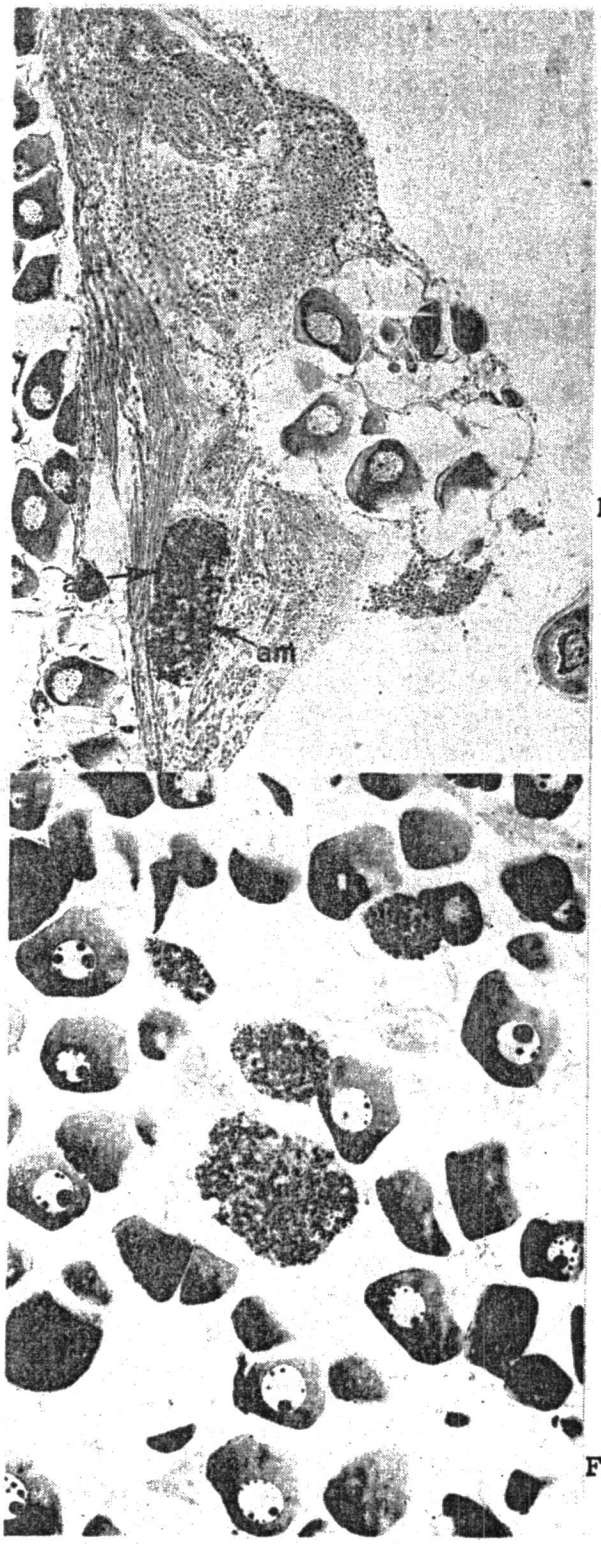


Figure 11 - Long-Spined Porgy ovary with outpocketing growth. Ovarian wall with large amount of blood cells infiltration & vesiculation. Thickened wall with acellular mass (?), amorphous material (arrows). 135X H&E

Figure 12 - Long Spined Porgy- Amorphous material in ovigerous fold. 220X H&E

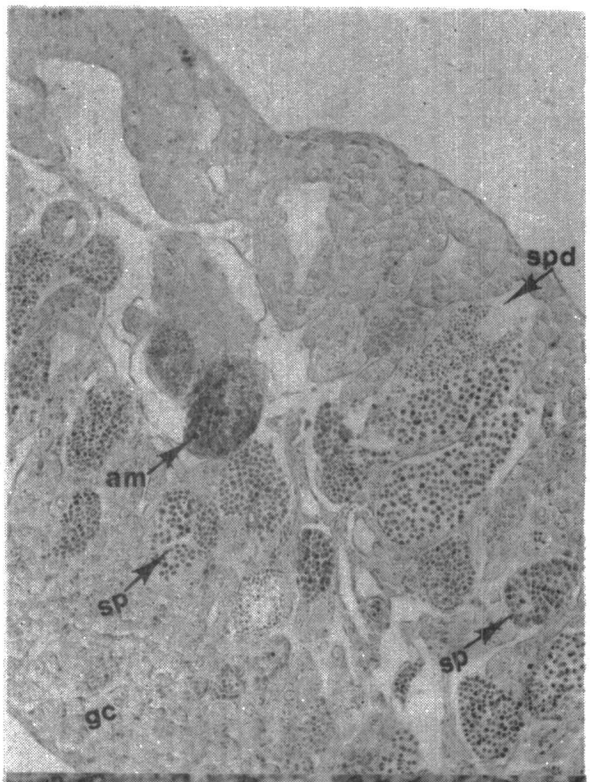


Figure 13 - Long Spined Porgy
Several stages of spermatogenesis are shown, (gc) germ cells; (sp) spermatocytes; (spd) spermatids, and amorphous material (am). 220X H&E

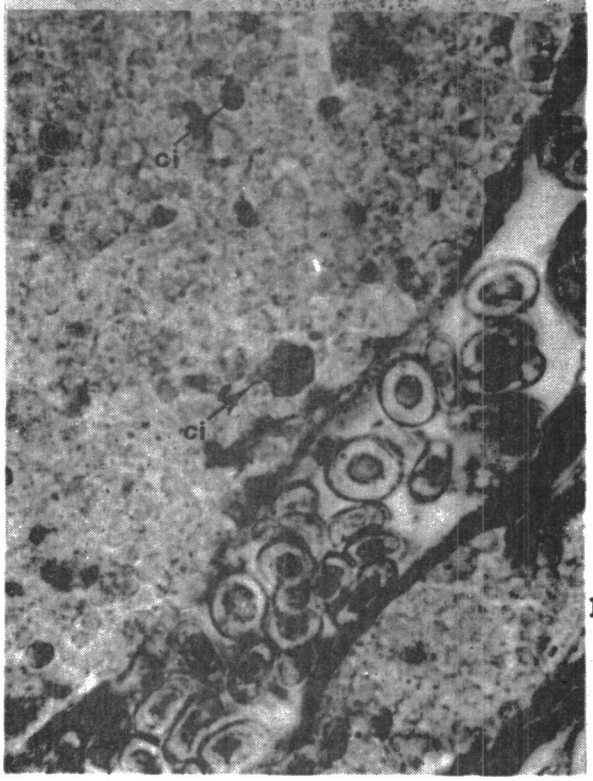


Figure 14 - Long Spined Porgy
High magnification (1320X) amorphous material with blood vessel through it and possible crystaline inclusions (arrows)

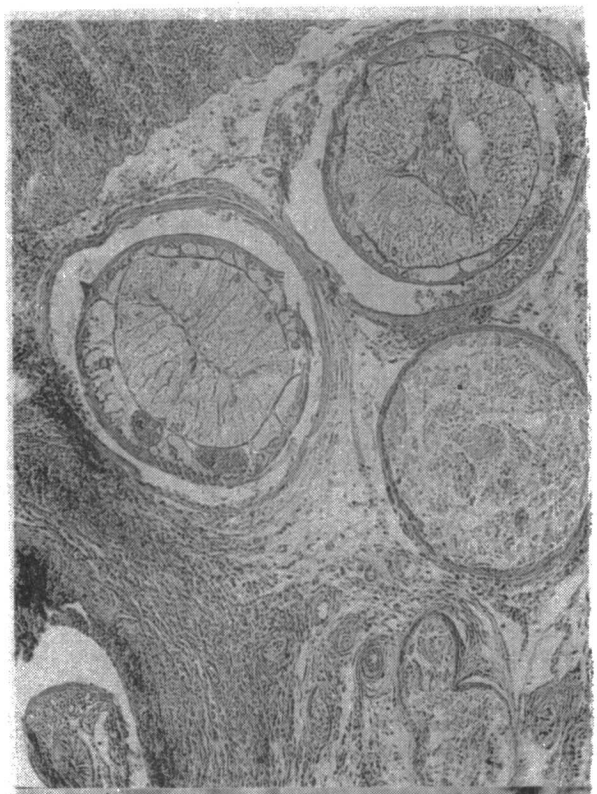


Figure 15 - Butterfish-Fibroblast surrounded nematodes in connective tissue adjacent to testes. 135X H&E

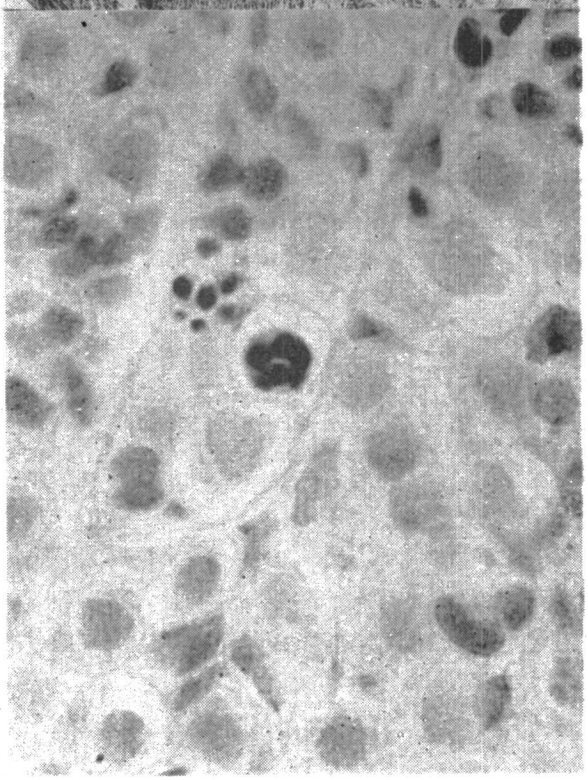


Figure 16 - Vermillion Snapper Dark staining (Feulgen) cells enclosed in cyst in germ cell follicles of testes. 1320X

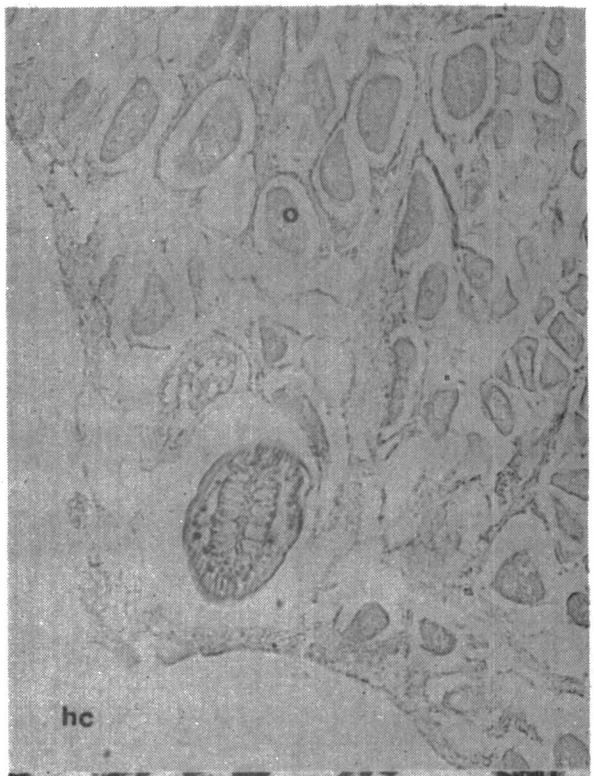


Figure 17 - Brown Shrimp-Nematode embedded in ovary. (hc) hemocoel; (o) oocyte. 220X, Feulgen

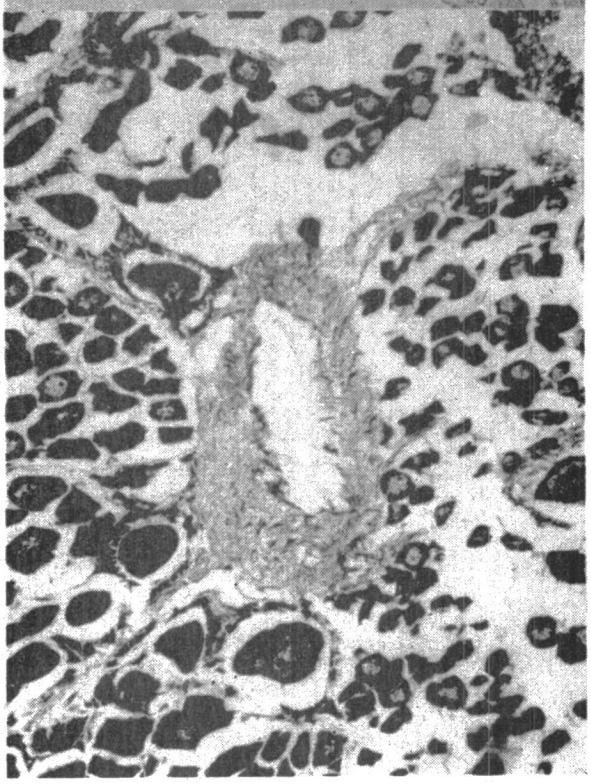


Figure 18 - Brown Shrimp-Fibrous ovarian cyst. 220X H&E

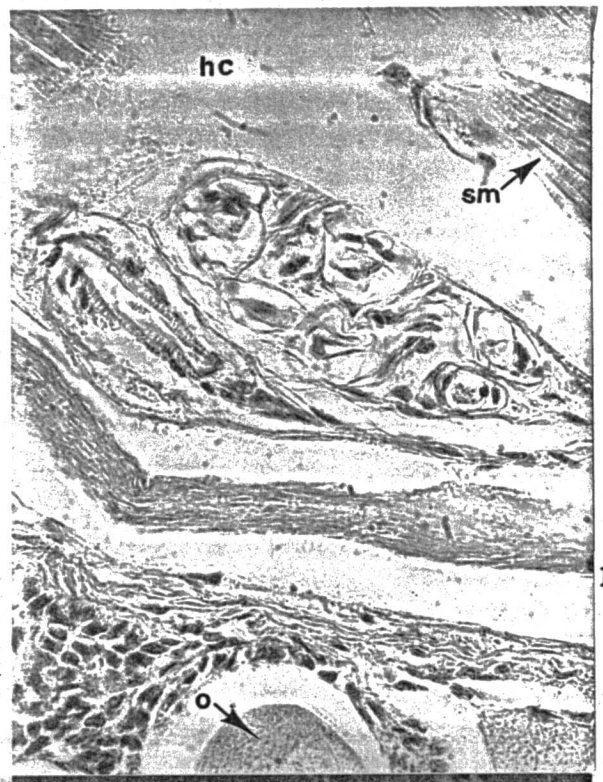


Figure 19 - Brown Shrimp
Large cyst containing several protozoan parasites in ovary wall. (o) oocyte; (hc) hemocoel; (sm) striated muscle. 540X Feulgen

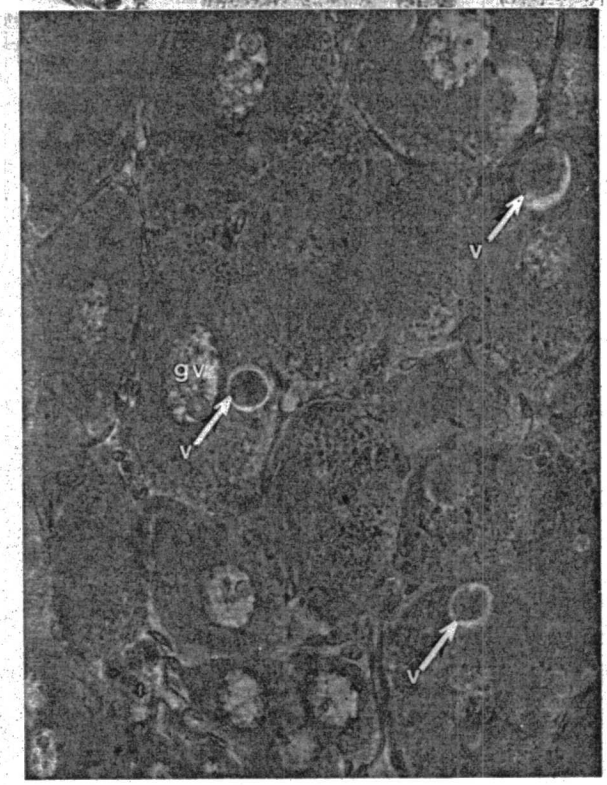


Figure 20 - Blue Crab-Maturing oocyte with large vesicle, possibly oil or yolk material (arrows); (gv) germinal vesicle. 540X Feulgen

TABLE 14

HISTOPATHOLOGICAL CONDITIONS AND PARASITES OBSERVED IN THE GONADS OF ANIMALS COLLECTED ALONG TRANSECT II DURING THE 1976 BASELINE STUDY OF THE SOUTH TEXAS OUTER CONTINENTAL SHELF

		JULY	AUGUST	OCTOBER	NOVEMBER	DECEMBER	TOTAL ANIMALS
Wenchman							
Pristipomoides	male	0	0	0	0	0	0
aquilonaris	female	0	a,g,i,j/2	j/1	f,g,j/1	0	4
Longspine Porgy	male	e,i/2	a,k/1	e,i/3	0	e,i/1	7
Stenotomus caprinus	female	e,i/2	e,h,i,j,k,l/4	e,i,j/1	a,e,h,i,j,l/6	e,g,i,l/3	16
Sand Seatrout	male	0	0	0	0	0	0
Cynoscion arenarius	female	0	0	0	0	0	0
Atlantic Croaker	male	0	-	0	0	0	0
Micropogon undulatus	female	a,f,h,i,k/1	-	0	-	0	1
Butterfish	male	-	0	a/1	-	-	1
Peprilus burti	female	-	0	a,d,e,f/1	-	-	1
Rock Bass							
Centropristis	male	-	-	-	-	-	0
philadelphica	female	-	0	0	0	d,f,j/1	1
Vermilion Snapper	male	b,d,g/2	0	b,d/1	0	b,d/2	5
Rhomboplites aurorubens	female	0	f,j,l/1	0	0	0	1
Red Snapper	male	-	0	-	0	-	0
Lutjanus campechanus	female	-	g,i,j/2	-	0	-	2
Brown Shrimp	male	0	-	n/1	a/3	a/1	5
Penaeus aztecus	female	a/1	a,c/2	a,b,d/2	a/2	a/3	10

TABLE 14. CONT.'D

		JULY	AUGUST	OCTOBER	NOVEMBER	DECEMBER	TOTAL ANIMALS
Blue Crab	male	0	0	0	0	-	0
Callinectes similis	female	0	a,g/2	0	1/2	-	4
Portunid Crab	male	-	-	-	0	0	0
Portunus spinicarpus	female	-	-	0	0	0	0
Squid	male	e/1	0	0	0	0	1
Loligo pealei	female	0	g/1	0	0	0	1
Paper Scallop	male	-	-	g/4	0	0	4
Amusium papyraceus	female	-	-	g/4	0	0	4

Condition/# of Organisms

a - Nematodes
b - Protozoa
c - Lesions
d - Cysts
e - Amorphous Material
f - Hyalinization
g - Fibroblast Infiltration
h - Blood Cell Infiltration and/or vascularization
i - Hyperplasia
j - Atretic Oocyte
k - Degenerated Gonad
l - Vesiculation of Oocytes
m - Amorphus Material
n - Other/Unknown
0 - No Observable Abnormality

Various cysts were observed (Figures 6, 16, 18). Neoplastic growths were also seen associated either with the gonadal wall (Figures 3, 4, 10, 11) or supportive connective tissue (Figure 5). Except in cases such as the degenerated gonad (Figure 3), neoplastic growth did not appear to have any effect on gamete formation.

Several other abnormalities were also observed. Blood cell infiltration and vascularization were seen in the longspine porgy and Atlantic croaker (Figures 10, 11). This condition was usually associated with neoplastic growth and/or fibroblast infiltration with no apparent effect on gamete development.

The ovaries often showed large numbers of oocytes that appeared to be atretic, degenerating, hyalinized and/or reabsorbed (Figures 2, 6, 8, 9). As compared to normal developing oocytes (Figure 1), atretic oocytes had a greater amount of vesiculation. In some species, such cells were surrounded by what appeared to be follicular debris and/or fibroblast cells (Figure 2). Not having a fully established ovarian cycle, it was difficult to establish if such was the norm.

Table 14 summarizes the histopathological and parasitic condition of the invertebrate and vertebrate gonads. The longspine porgy showed the greatest number and diversity of pathological/parasitic conditions. Sixteen of 18 female and 11 of 16 male longspine porgy had some histopathological condition. The gonads of vermilion snapper, sand seatrout and Atlantic croaker were relatively free of any pathological/parasitic condition.

The portunid crab and squid showed the lowest numbers of parasites and/or histopathology. The shrimp showed a high degree of nematode infiltration plus a few additional conditions (Table 14).

DISCUSSION

The reproductive cycles of fish and invertebrates can be established fairly well by histologically and behaviorally tracing the pattern of gamete formation. Field studies of reproductive cycles of fish are based on the gonadosomatic ratio (gonad weight/body weight)(Baerends, 1971; Burns, 1976; Fuller *et al.*, 1976; Larson, 1972; Liley, 1969). These studies showed the gonads at different stages of development, but made no attempt to trace the process histologically (Leake, 1975; Sadlier, 1973).

Ideally, this would be easy if all animals sampled were in synchrony. The invertebrates appear to be more synchronous than fish which show a greater variability from station to station, and occasionally within specimens at a given station. Due to this variability by station, month and species, a greater number of specimens per species per station must be sampled to provide a representative statistical base. Continued sampling will also be necessary for another year or two to provide the baseline data necessary to establish the reproductive cycle for each species.

With the present data, a tentative reproductive cycle can be developed for the wenchman (Table 1), longspine porgy (Table 2), female sand seatrout (Table 3), vermilion snapper (Table 7), brown shrimp (Table 9) and squid (Table 12). But even for organisms collected every sampling effort, too much variation was noted to make any such cycle totally reliable.

The observed variation, such as for the female wenchman, showed that animals collected were in early, intermediate and mature stages of development. Furthermore, it is not known if animals observed in a given

stage of development are developing rapidly or slowly. Correlation with temperature and light period was attempted. The developmental data available were insufficient to make any assumptions about the reproductive cycle and behavior (Baerends, 1971; Bieniarz and Epler, 1976; Burns, 1976; Fuller *et al.*, 1976; Larson, 1972; Lily, 1969; Schwassmann, 1971).

In surveying for pathological conditions, a similar problem was encountered. Since gonads are constantly changing (dependent on the duration of the reproductive cycle), it is difficult to know if the invasion of interstitial and fibroblast cells is a normal condition or abnormal until the normal pattern and histology are established. A similar case occurs in gonads having large amounts of connective tissue within the gonad. Until the normal pattern and histology are established for each species being surveyed, it will be difficult to specify whether it is the normal or abnormal condition.

Certain pathological conditions such as hyperplastic growth, cysts, vascularization, parasites and other conditions can easily be surveyed. Of 385 animals surveyed, only 64 (or 16.7 percent) demonstrated some type of pathology. Many of the animals demonstrated more than one type of condition. Overall, the testes and ovaries did not appear to be susceptible to parasites and/or other abnormalities. This may be due to the rapid turnover of tissue, especially in the cystovarian type of ovary characteristic of teleosts where the contents are emptied out at maturity leaving behind germinal epithelium (susceptible to hyperplastic condition) and ovigerous folds (Hoar, 1969; Leake, 1975; Nelsen, 1953; Sadlier, 1973). Male gonads have cystic spermatogenesis. Complete cysts develop synchronously and empty out almost completely, leaving germ cells behind to start the process again.

Invertebrates tend to be affected more by parasites than fish. This is because the gonads are in the hemocoel which contains or allows easy movement of parasites among the various organs (Sparks, 1972).

CONCLUSIONS

The 1976 histological-histopathological collecting effort was the initial sampling period for this study. One seasonal and four monthly collections were made. Seventeen (17) species (456 samples) were collected and 13 species (383 samples) are reported herein. Eight species of fish and five species of invertebrates were analyzed.

The reproductive cycles of the invertebrates and fishes were partially established. The fishes showed a great variability from station to station and occasionally within specimens at a given station. Invertebrates showed a greater degree of synchrony. Due to this variability it is necessary that a larger number of specimens per species per station be sampled to provide a representative statistical base. Sampling will be necessary for at least 2 more years to provide the baseline data necessary to establish the reproductive cycle for each species.

Pathological conditions observed in the fish and invertebrate gonads were hyperplastic growth, cysts, vascularization, hyalinization, degenerated condition, fibroblast infiltration and other abnormal conditions. Sixty-four of the 385 animals analyzed had some type of pathological and/or parasitic condition.

LITERATURE CITED

Andrew, W,. and C. P. Hickman. 1974. Histology of the vertebrates, a comparative text. C. V. Mosby Co., Saint Louis.

Baerends, G. P. 1971. The ethological analysis of fish behavior. Pages 279-370 *in* W. S. Hoar and D. J. Randall, eds. Fish physiology. Vol. 6. Academic Press, New York.

Bieniarz, K., and P. Epler. 1976. Preliminary results of the *in vivo* studies on ovarian resorption in carp (*Cyprinus carpio*, L.). J. Fish Biol. 8:449-451.

Blaxter, J. H. S. 1969. Development: eggs and larvae. Pages 177-252 *in* W. S. Hoar and D. J. Randall, eds. Fish physiology. Vol 3. Academic Press, New York.

Bloom, W., and D. W. Fawcett. 1975. A textbook of histology. W. B. Saunders Co., Philadelphia.

Burns, J. R. 1976. The reproductive cycle and its environmental control in the pumpkinseed, *Lepomis gibbosus* (Pisces; Centrarchidae). Copeia 3:449-455.

Conn, H. J., M. A. Darrow, and V. M. Emmel. 1965. Staining procedures used by the Biological Stain Commission, 2nd edition. Williams and Wilkins Co., Baltimore.

Dorfman, D. 1976. Occurrence of a hermaphroditic white perch (*Morone americana*). Prog. Fish Cult. 38:45.

Fuller, J. D., D. B. C. Scott, and R. Fraser. 1976. The reproductive cycle of *Coregonus lavaretus* (L) in Loch Lomond, Scotland, in relation to seasonal changes in plasma cortisol concentrations. J. Fish Biol. 9:105-117.

Galigher, A. E., and E. N. Kozloff. 1971. Essentials of practical microtechnique. Lea and Febiger, Philadelphia.

Gurr, E. 1962. Animal micrology. Univ. of Chicago Press, Chicago.

Higgs, D. A., E. M. Donaldson, H. M. Dye, and J. R. McBride. 1976. Influence of bovine growth hormone and L-thyroxine on growth, muscle composition, and histological structure of the gonads, thyroid, pancreas and pituitary of coho salmon (*Oncorhynchus kisutch*). J. Fish Res. Bd. Can. 33:1585-1603.

Hoar, W. S. 1969. Reproduction. Pages 1-72 *in* W. S. Hoar and D. J. Randall, eds. Fish physiology. Vol. 3. Academic Press, New York.

Larson, G. L. 1972. Gonad maturation of brook trout (*Salvelinus fontinalis*) in a high mountain lake under a modified photoperiod. J. Fish Res. Bd. Can. 29:1209-1211.

Leake, L. D. 1975. Comparative histology. Academic Press, New York.

Liley, N. R. 1969. Hormones and reproductive behavior in fishes. Pages 73-116 *in* W. S. Hoar and D. J. Randall, eds. Fish Physiology. Vol. 3. Academic Press, New York.

Nelsen, O. E. 1953. Comparative embryology of the vertebrates. McGraw-Hill Book. Co., New York.

Sadleir, R. M. F. S. 1973. The reproduction of vertebrates. Academic Press, New York.

Sparks, A. K. 1972. Invertebrate pathology, noncummunicable diseases. Academic Press, New York.

Schwassmann, H. O. 1971. Biological rhythms. Pages 371-428 *in* W. S. Hoar and D. J. Randall, eds. Fish physiology. Vol. 6. Academic Press, New York.

Yamamoto, T. 1969. Sex differentiation. Pages 117-175 *in* W. S. Hoar and D. J. Randall, eds. Fish physiology. Vol. 3. Academic Press, New York.

ADDENDUM

KARYOTYPE OF DEMERSAL FISHES AND INVERTEBRATE EPIFAUNA

INTRODUCTION

As an extension and corollary study of the histopathology baseline effort, a survey of chromosomal karyotypes of demersal fishes and invertebrates of the STOCS area was initiated. The survey largely consisted of a study of chromosomes of the animals being studied under the histopathology program, treating the chromosomes as a subsample of these species.

The purposes of the chromosomal survey are as follows:

1. To establish the normal karyotype of the species found within the STOCS study area;

2. To monitor these species for chromosomal aberations that result under normal conditions;

3. To monitor these species for chromosomal aberations that may result from environmental pressures or due to low level chronic pollution.

In this report, a preliminary chromosomal analysis of marine fishes and invertebrates is given. Methodology is still the biggest problem in adapting and/or modifying existing techniques to marine invertebrates and fishes.

METHODS AND MATERIALS

Sampling

Samples for chromosomal analysis were taken from offshore areas near the University of Texas Marine Science Institute at Port Aransas and from trawl samples collected during the histopathology cruises along Transect II. The fish and invertebrate organisms sampled and analyzed are listed in Table 1. A total of 38 organisms representing five species of fish

TABLE 1

SPECIES SAMPLED AND CHROMOSOME COUNTS

SPECIES	CHROMOSOME NUMBER
FISH:	
Sand seatrout - *Cynoscion arenarius*	48
Vermilion snapper - *Rhomboplites aurorubens*	—
Barred grunt - *Conodon nobilis*	—
Silver perch - *Bairdiella chrysura*	48
Gulf butterfish - *Peprilus burti*	—
INVERTEBRATES:	
Blue crab - *Callinectes sapidus*	24
Squid - *Loliguncula brevis*	24
Brown shrimp - *Penaeus aztecus*	22
Paper scallop - *Amusium papyraceus*	12

and four species of invertebrates were analyzed.

Tissue Sampling

Karyotypes were attempted from different tissue sources. From the fish samples, scale epithelium, liver, kidney, spleen and gill epithelium tissues were utilized. Tissues from invertebrate organisms varied and were not as consistent, although the gill epithelium and glandular tissue provided the best sources.

Chromosomal Analysis

The basic protocol used in obtaining suitable material for chromosomal analysis was either a direct squash of the tissue or hypotonic treatment prior to fixation and squashing. Whenever possible, the animals were injected with 0.25% colchicine and pretreated for varying times and doses. The animal was then sacrificed. The tissues were excised and placed in a hypotonic solution of either 0.075 M KCl, 0.1 M $CaCl_2$ or distilled water followed by fixation in 50% acetic acid. The tissue was then squashed in aceto-orcein stain and examined under the microscope. If a direct squash was done, the tissues were maintained in its own body fluids or physiological saline and small pieces of tissue dabbed on the slide and air dried prior to staining. Chromosomal spreads were stained with Giemsa and countable chromosome spreads photographed through a Zeiss research microscope.

RESULTS AND DISCUSSION

Five marine fish and four marine invertebrates (Table 1) were used in the initial chromosomal analysis effort. The majority of chromosomal analyses have been done on fresh water fish (Denton, 1973; Wharton *et al.*, 1977) and only a limited number of marine species have had their chromosome number established (Lee and Loh, 1975; Nicholson and Byrne, 1973;

Regan *et al.*, 1968; Wharton *et al.*, 1977). Similarly, with marine invertebrates, a limited number of species have been karyologically analyzed (Ahmed and Sparks, 1967, 1970; Menzel and Menzel, 1965, 1968).

Several problems had to be solved before chromosomes could be counted. Some of the animals sampled did not produce analyzable material. This was due to several factors including: (1) wrong colchicine dosage; (2) wrong treatment time; (3) improper hypotonic treatment; (4) improper spreading techniques; and (5) use of tissue with high mitotic activity. These factors will vary from species to species; from tissue to tissue and with size and/or age of animal. The factors for species listed on Table 1 have been established. Chromosome counts have been made and analyzable karyotypes are being developed for these species.

The basic protocol for chromosomal analysis used is a modification of the bone marrow and tissue techniques developed by Ford *et al.* (1956). Modifications of this technique have been applied to other tissues. Gill epithelium, liver, kidney, spleen and scale epithelium have successfully been used for fresh water fish (Denton and Howell, 1969; McPhail *et al.*, 1966). Gill epithelium provided the best results in the marine fish used in this study.

Five species of marine teleost fish have been analyzed. The silver perch (*Bairdiella chrysura*) and sand seatrout (*Cynoscion arenarius*) have been analyzed completely. Both of these species have been counted and karyotyped. Each of these species have 48 chromosomes and the majority of chromosomes are telocentric. Tentative chromosome counts have been made on the other three species, but not enough replicates have been done to establish the exact number. The chromosome number of these species fall within a range of 44 to 54.

Invertebrate chromosome counts reported in the literature have been

made on either oocytes or balstula cells. For this study, adult tissue was used. Different tissues were tried, such as glandular tissue and gill epithelium. These two tissues have proven effective in obtaining countable chromosomal spreads. The squid, *L. brevis*, has 22 to 24 chromosomes. The major problem in these organisms is the small size of the chromosomes. The squid and scallop chromosomes range from 0.25 μm to 4 μm in size, making the identification of the chromosomes difficult.

Replicates of these species are being done to confirm initial counts and to obtain analyzable chromosomes.

LITERATURE CITED

Ahmed, M. and A. K. Sparks. 1967. A preliminary study of chromosomes of two species of oysters (Ostrea lurida and Crassostrea gigas). J. Fish. Board Can. 24:2155-2159.

Ahmed, M. and A. K. Sparks. 1970. Chromosome number, structure and autosomal polymorphism in the marine mussels Mytilus edulis and Mytilus californianus. Biol. Bull. 138:1-13.

Denton, T. E. 1973. Fish Chromosome Methodology. Charles Thomas, Springfield, Ill.

Denton, T. E. and W. M. Howell. 1969. A technique for obtaining chromosomes from the scale epithelium of teleost fishes. Copeia 2:392.

Ford, C. E. and J. L. Hamerton. 1956. The chromosomes of man. Nature 178:1020-1023.

Lee, M. H. and P. C. Loh. 1975. Some properties of an established fish cell line from the marine fish, Caranx mate (Omaka). Proc. Soc. Exp. Biol. Med. 150:40-48.

Lieppman, M. and C. Hubbs. 1969. A karyological analysis of two cyprinid fishes. Texas Reports Biol. Med. 27:427.

Longwell, A. C., S. S. Stiles and D. G. Smith. 1967. Chromosome complement of the American oyster Crassostrea virginica as seen in meiotic and cleaving eggs. Can. J. Genet. Cytol. 9;845-856.

McPhail, J. D. and R. L. Jones. 1966. A simple technique for obtaining chromosomes from teleost fishes. J. Fish. Res. Bd. Can. 23:5-8.

Menzel, R. W. 1968. Chromosome number in nine families of pelecypod mollusks. Nautilus 82:45-58.

Menzel, R. W. and M. Y. Menzel. 1965. Chromosomes of two species of quahog clams and their hybrids. Biol. Bull. 129:181-188.

Nicholson, B. L. and C. Byrne. 1973. An established cell line from the Atlantic salmon (Salmo salar). J. Fish Res. Bd. Can. 30:913-916.

Regan, J. D., M. M. Sigel, W. H. Lee, K. A. Llamas, and A. R. Beasley. 1968. Chromosomal alterations in marine fish cells in vitro. Can. J. Genet. Cytol. 10:448-453.

Wharton, J. H., R. D. Ellender, B. L. Middlebrooks and P. K. Stocks. 1977. Fish cell culture; characteristics of a cell line from the silver perch, Bairdiella chrysura. In Vitro 13:389-397.

CHAPTER FOURTEEN

SEDIMENT TEXTURE

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ABSTRACT

Sediment textures (grain size distribution) were measured on 777 samples, including seven replicates from 25 stations sampled during three seasons, winter, spring, and fall, and seven replicates from six stations sampled during six additional monthly periods. Textural analysis was chiefly for comparison with the results of meiofauna, shelled microbenthos and macroinfauna studies.

Variability of textures within sampling stations was greatest in the Rio Grande delta region and at Station 5/I - possibly related to the ancestral Colorado-Brazos delta to the north of the study area. Textural variability was least in the outer-shelf clays. Significant seasonal variability was scattered, but most consistent in the spring loss of fine clay from the outer-shelf regions.

Coulter Counter and pipette methods were shown to give highly correlatable results so that both should show any significant relationships between textures and biological or chemical parameters. However, Coulter Counter techniques resulted in considerably coarser and better-sorted apparent textures due to the different computational procedures required with the data.

INTRODUCTION

Purpose

The purpose of this study was to provide sediment grain size distribution (texture) data for comparison with results of biological and chemical analyses of bottom samples, especially analyses for meiofauna, shelled microbenthos and macroinfauna. The replication of samples on station and through time also permitted analysis of the variance of sediment texture within sampling stations and analysis of seasonal trends in grain size characteristics.

A further result was the comparison of results of different analysis techniques, specifically, comparison of the results of the pipette method with results of the same raw data processed by the computational procedure necessitated by the Coulter Counter method.

Literature Survey and Previous Work

The geology and sedimentology of the South Texas shelf have had two extensive surveys, API Project 51 reported in the symposium volume, Recent Sediments, Northwest Gulf of Mexico (Shepard *et al.*, 1960) and the BLM-supported USGS studies begun in 1975 and reported in various BLM contract reports (Berryhill, 1976; 1977). The availability of these reports makes further review unnecessary. Grain size measurement techniques are discussed in several standard texts (*e.g.* Carver, 1971) and manuals (*e.g.* Folk, 1974). A more recent comparison of pipette and Coulter Counter techniques was given by Shideler (1976).

METHODS AND MATERIALS

Sediment texture samples were taken from Smith-MacIntyre grab samples. The sampling effort consisted of 7 replicates x 25 stations x 3 seasons

and 7 replicates x 6 stations x 6 months for a total of 777 samples. Each sediment texture sample consisted of not less than 100 g sediment.

Textural analyses were done by the rapid sediment analyzer method (Schlee, 1966) for the sand-sized fraction and by the pipette method (Folk, 1974) for the mud fraction. The following procedure was used:

The sample was homogenized by kneading in the plastic sample bag. Twenty (20) cc were extracted, dispersed in hydrogen peroxide, diluted to about 0.5 l and allowed to stand for 2 to 3 days. The clearer supernate was decanted through a 1.2 μm MILLIPORE filter and the filtered sediment was returned to the beaker. The sediment was resuspended and poured through a 0.062 mm screen. The screen (preweighed) and trapped sand were dried, weighed, and set aside for later settling tube analysis.

The mud fraction was transferred to a graduated cylinder and the sample was diluted to 1 l, stirred, and allowed to stand overnight. If no flocculation occurred (none ever did), the temperature was measured, settling rates were calculated by Stoke's Law, and withdrawal times and depths were calculated to obtain nine intervals from 4 to 10 phi. Twenty (20) ml samples were pipetted at the appropriate times, transferred to preweighed beakers, oven dried, weighed, and size fraction weights calculated.

A representative portion of the sand fraction was introduced into a settling tube and fall times were recorded continuously. Graphs were read for the proportion of sand at each 0.25 phi interval.

These data were used to compute moment and graphic grain size parameters by standard methods (McBride, 1971).

Of particular concern for data comparison purposes was the handling of data for the fine clay fraction. The last pipette measurement defines

the quantity of material finer than 10 phi. Extrapolation of the cumulative curve beyond 10 phi very commonly indicates that all of the sediment is coarser than 14 phi. Therefore, a common computational procedure is to extrapolate cumulative curves to 100 percent at 14 phi (Folk, 1974). One set of grain size distribution parameters was done on this basis.

The Coulter Counter technique defines the relative abundances of material in each of several size classes over the range of measurement. However, it does not determine how much of the mud fraction is within that range of measurement and how much is finer than the lower limit of the smallest class measured (10.6 phi). Therefore, the computational procedure assumes all of the mud is coarser than the finest size measured. This, in effect, redistributes the fine clays over all the silt and clay classes, and therefore, computes coarser mean sizes. To determine the extent of this and other possible effects, the raw data were used to compute grain size parameters by the technique necessitated by the Coulter Counter analysis technique.

RESULTS

Tabulation

The mean grain size, standard deviation, skewness, kurtosis, percent sand, silt and clay, sand/mud ratio, silt/clay ratio, and percent of sample finer than 10.6 phi for each of the 777 grab samples computed by moment measures and using the 14 phi extrapolation convention are given in Table 1, Appendix J. These data are considered the most accurate representations of the sediment textures and were used for analysis of station and seasonal variances. The same percentages and moment measures calculated as though the raw data were obtained by the Coulter Counter method are presented in Table 3, Appendix J. Tables 2 and 4, Appendix J,

are of the same measures calculated by the graphic methods of Folk (1974) rather than by moments. Table 5, Appendix J, summarizes the data by station and time of sampling. Each value in Table 5 is the average (from Table 1) of seven grab samples and is accompanied by the standard deviation of the seven values. These standard deviations are direct measures of the variability of textural characteristics within the limits of navigational coincidence of sampling points at each station.

Station Variability

One-quarter of the 75 seasonal sampling stations (25 stations x 3 seasons) had variations of mean grain size of over 1 phi and two-thirds of the samples had variations of over 0.5 phi. The greatest variability in sample means occurred at Stations 5/I and 5/IV. Station 5/IV is within the Rio Grande delta where sediment distributions are the most complex. Stations 2, 3, 5 and 6, Transect IV, showed moderately high variability between grab means. A similar variability occurred at Stations 1/III, 1/II, 4/II, 1/I and 4/I where sand, silt and clay modes were mixed. Moderately low variability existed at the sandiest, inshore stations (4/III, 4/IV and 1/IV) and the intermediately silty clay parts of Transects I, II and III (Stations 2/I, 6/I, 2/II, 5/II, 6/II, 2/III and 5/III). The clayiest, outermost stations (3/I, 6/I, 3/II, 3/III, 6/III and 7/IV) had the least variability of mean sizes.

While the variability of sediment sorting (standard deviation) within stations was also low for the clayiest stations, it was equally low for intermediate stations of the northern three transects (Stations 1/III, 1/II, 2/II, 4/II, 2/I). Sorting variability was somewhat higher through the Rio Grande delta and a few intermediate shelf stations on Transect III (Stations 1/IV, 2/IV, 3/IV, 4/IV, 4/III and 4/I). Station 5/I was, again,

unusually variable.

The higher moments of skewness and kurtosis varied regularly across the shelf with the coarsely skewed, outer-shelf muds being least variable (Stations 3/I, 6/I, 3/II, 6/II, 3/III, 2/III, 6/III and 7/IV), the mid-shelf, slightly fine-skewed silts being moderately variable (Stations 2/I, 5/I, 2/II, 5/II, 1/III, 5/III, 1/IV, 2/IV, 3/IV, 5/IV and 6/IV) and the strongly fine-skewed, inner sandy sediments most variable (Stations 1/I, 4/I, 1/II, 4/II, 4/III and 4/IV).

Seasonal Variability

About half of the 25 stations showed mean grain size changes of as much as 0.25 phi between seasonal resurveys. The significance of the variations depended, of course, on the variance within stations. Numerous T tests showed that if the pooled estimate of the standard deviation of a station sampled at two different times was equal to or less than the change in mean grain size, the change was significant at greater than the 90 percent confidence level. The pooled estimate of the standard deviation was always somewhat less than the greater of the two standard deviations of station means. At some stations, mean changes well over 0.25 phi were not significant, while at others, changes of as little as 0.1 phi were significant.

Between the winter and spring surveys, the most significant changes were the coarsening of eight clayey, outer-shelf stations (3/I, 5/I, 6/I, 3/II, 6/II, 3/III, 6/III and 7/IV). The mean also became much coarser at Station 1/II. The inner, sandy stations generally became finer in texture, but only the change at Station 4/I was significant. Fining of low significance apparently occurred through a mid-shelf zone, including Stations 5/II, 2/III, 2/IV and 3/IV.

Between the spring and fall surveys of the same outer-shelf group of stations, fining of texture also occurred although only three of the eight changes were significant. The mid-shelf zone also reversed its spring trend with coarsening apparent at Stations 2/I, 5/I, 2/II, 5/II and 2/III (coarsening at the underlined stations was significant). On the inner shelf, fining occurred in the northern part of the study area (Stations 1/I, 4/I, 1/II, 4/II and 4/III) while coarsening occurred in the southern part (Stations 1/III, 1/IV and 4/IV).

The Rio Grande delta appeared to be the most dynamic area, but the most drastic changes might be the result of station labeling errors. Assuming that station labels were correct, there was, between winter and spring, extreme coarsening at Stations 2/IV and 5/IV and extreme fining at the next two seaward Stations, 3/IV and 6/IV. Between spring and fall, the changes reversed with strong fining at Stations 2/IV and 5/IV and strong coarsening at Stations 3/IV and 6/IV. Both of these events involved mean grain size changes of more than 3 phi for some stations and this was considered quite drastic. All seasonal textural changes would be less than 1.0 phi on Transect IV if the results for Stations 2 and 3 and for Stations 5 and 6 (especially the first pair) were exchanged on the spring survey. This would produce alternating coarsening and fining on the outer four stations (the only ones with significant changes), with reversal of trends for the two seasonal intervals, and a net predominance of coarsening.

The variance between grab sample means within stations followed a weak seasonal trend with the outer, clayiest stations becoming more variable from winter to spring and less variable from spring to fall, with most of the rest of the stations showing the opposite trend. However,

F tests showed that almost all of the variance differences between seasons were statistically insignificant.

Seasonal changes in sorting were limited. The largest changes were at Stations 2/IV and 3/IV which increased the suspicion that samples from these stations were misidentified. Otherwise, there was only a very weak suggestion of a decrease in sorting in the outermost clayey stations between winter and spring and an increase between spring and fall. On the other hand, the innermost sandy stations became somewhat better-sorted, especially on Transects III and IV between winter and spring, and worse-sorted between spring and fall. Almost all of the seasonal sorting changes were less than 0.2 phi and most were not statistically significant.

Technique Differences

Using at least one set of seasonal samples (7 grabs x 25 stations = 175 values) correlation coefficients for sediment parameters calculated from settling tube and pipette data according to standard moment procedures and by Coulter Counter-necessitated procedures were: means (M) 0.99, standard deviations (SD) 0.94, skewnesses (Sk) 0.97, and kurtosises (K) 0.98. Regression equations for the correlated parameters were:

$$M_p = 1.43\ M_c - 1.2 \qquad M_c = 0.70M_p + 0.84$$

$$SD_p = 1.19SD_c + 0.77 \qquad SD_c = 0.84SD_p - 0.65$$

$$Sk_p = 1.00Sk_c - 0.08 \qquad Sk_c = 1.00Sk_p + 0.08$$

$$K_p = 1.12\ K_c - 0.31 \qquad K_c = 0.89K_p + 0.28$$

where subscripts c and p are for Coulter Counter and pipette, respectively. Samples of the scatter of individual measurements around these regression lines are shown in Figures 1-4.

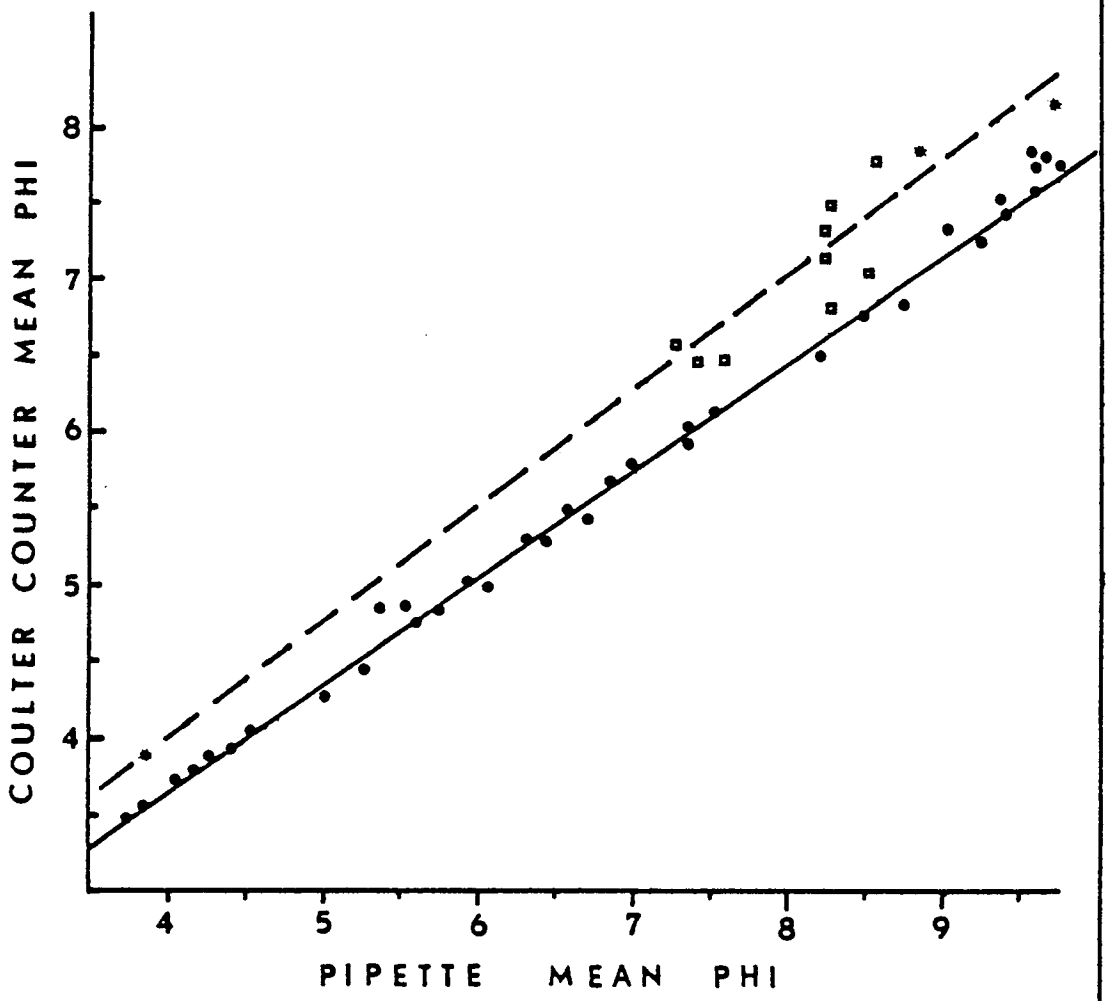

Figure 1. Mean grain size by Coulter Counter and pipette computational methods using pipette raw data (solid dots and solid line). Squares, stars, and dashed line are for samples actually analysed by the two different methods. Solid line equation is $C = 0.70P + 0.84$; dashed line equation is $C = 0.76P + 0.94$.

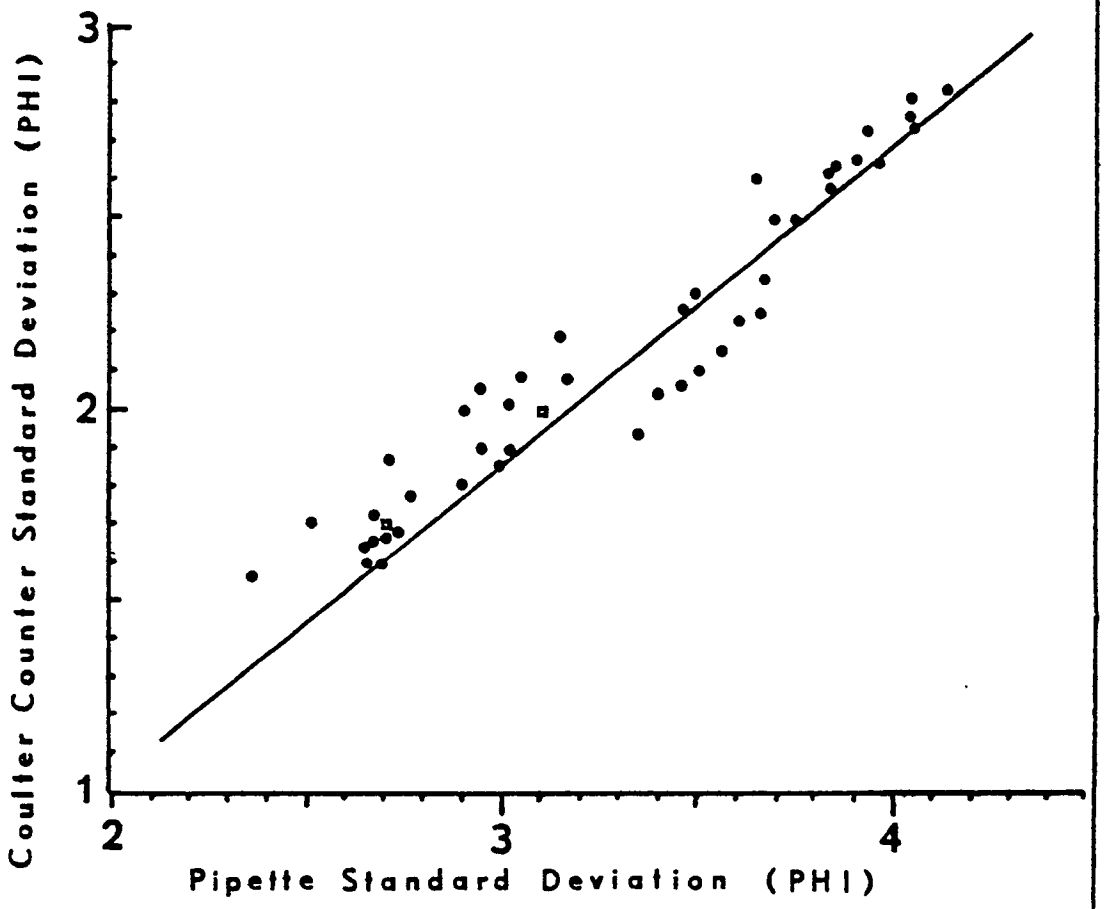

Figure 2. Moment standard deviations by Coulter Counter and pipette computational methods using pipette raw data (solid dots). Squares are for interlaboratory calibration samples actually analysed by the two methods. Solid line equation is $C = 0.84P - 0.65$.

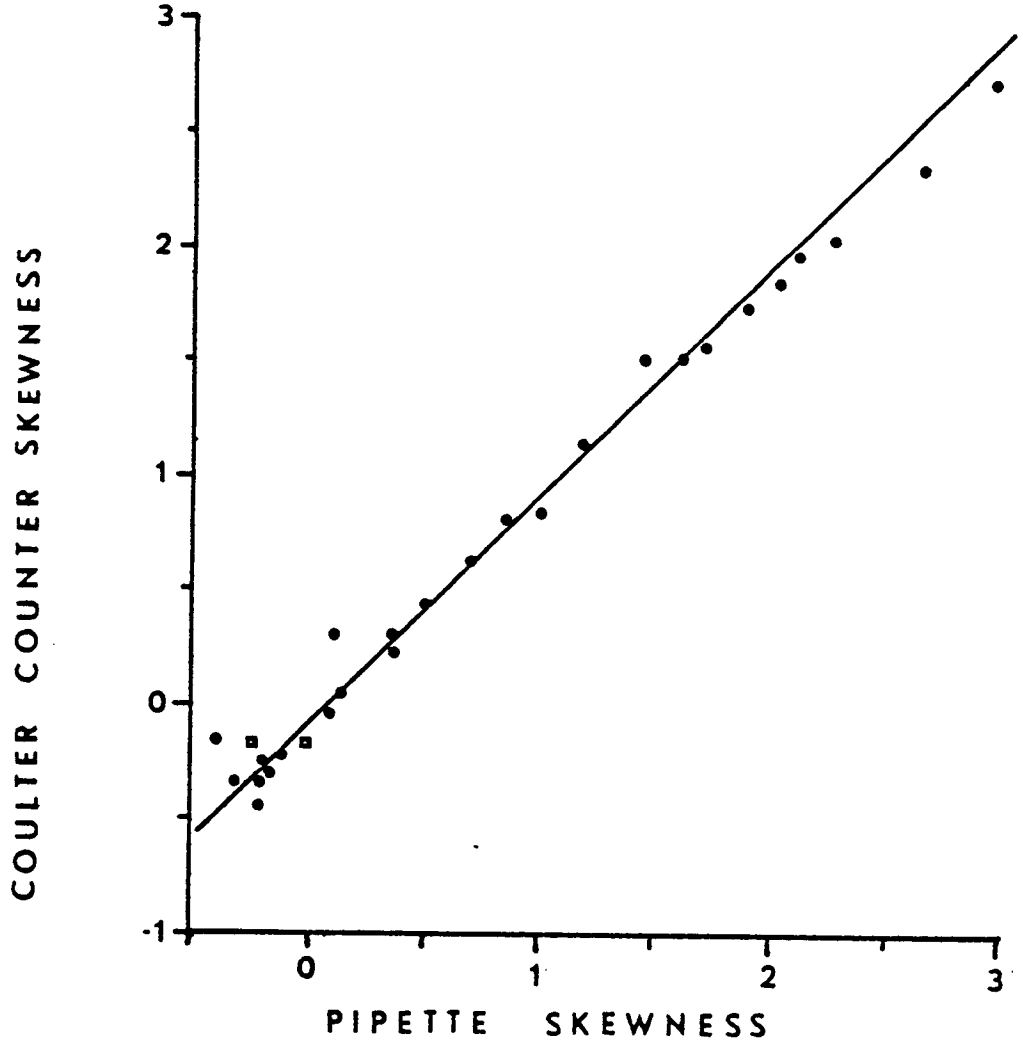

Figure 3. Moment skewness calculated by Coulter Counter and pipette techniques using pipette raw data. Squares are for samples actually analysed by the two methods. Equation for solid line is C = 1.00P - 0.08.

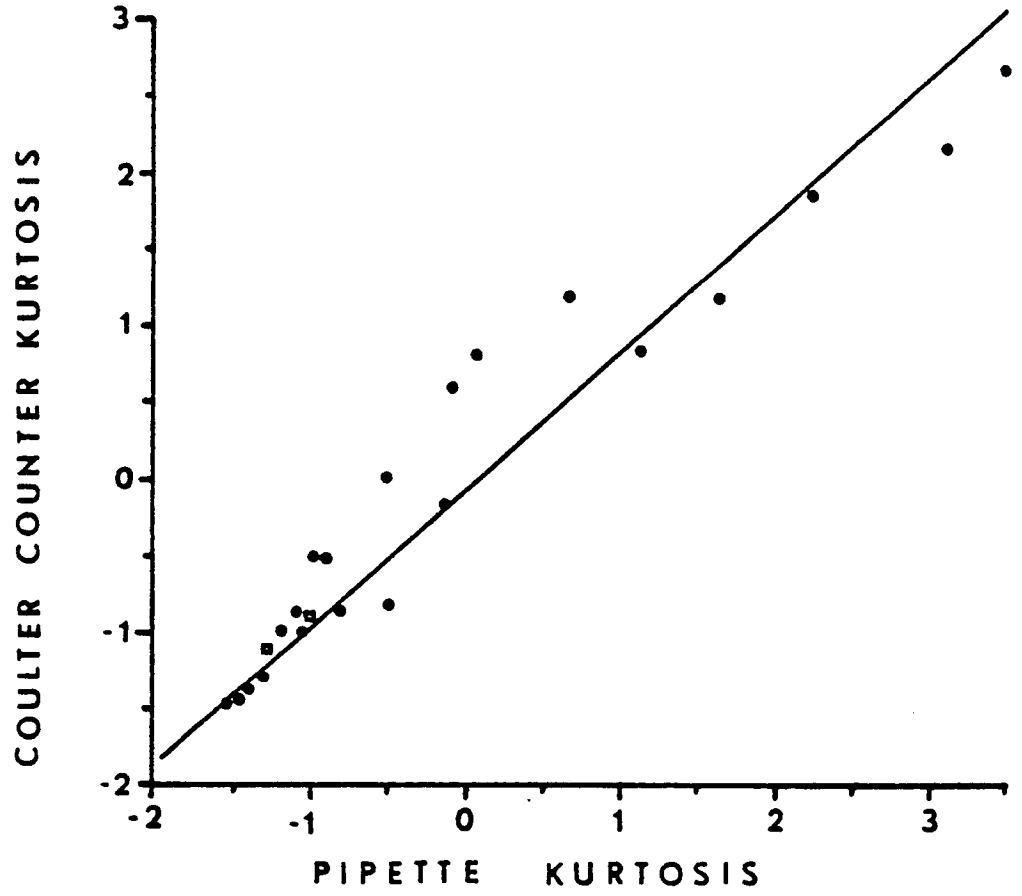

Figure 4. Moment kurtosis calculated by Coulter Counter and pipette techniques using pipette raw data. Squares are for interlaboratory calibration samples actually analysed by the two different methods. Equation for solid line is $C = 0.89P + 0.28$.

The fine clay (ignored by the Coulter Counter technique) which accounts for the differences between the various values generally constituted between 30 and 70 percent of the total clay. In many cases, the real amount of fine clay as more than the total amount of clay determined by the Coulter Counter technique.

DISCUSSION

Station Variability

Within station variability may be a major factor contributing to the considerable variability of benthic populations. Mean grain size variability is obviously less in the uniform, outer-shelf clays and increases as the bottom is exposed to a greater part of the wave orbital motion spectrum by shoaling, until it is within a sufficiently continuously agitated zone to produce more predominantly sandy means. Sorting variability follows a similar trend, but is highest where muds and sands are mixed in subequal amounts just beyond the subaqueous toe of the barrier island sands.

In addition to being related to physical energy intensity and variability, sediment texture variability is also related to variety of source material. Thus, where older sediments are being reworked into more recent material on the Rio Grande delta, there is relatively high variability. The highly variable station on Transect I (5/I) may be similarly related to the ancestral Colorado-Brazos delta sediments at the northern margin of the study area.

The degree of in-station variability did not correlate closely with significant seasonal textural changes. In fact, significant seasonal changes tended to occur most in regions which had the most uniform sorting. This suggested that seasonal changes were due to active processes

rather than to variability of repositioning stations on successive surveys. An attempt to correlate sediment variability with station navigation variability should be made to assess this suggestion.

The variability summarized was not based on all samples from every station. At about one-third of the seasonally-sampled stations, one or two of the samples were clearly not from the same populations as other samples from the same station. Eighteen of these were considered minor or moderate differences where mean grain sizes of the odd samples were less than 1.0 phi different from the mean for the rest of the samples. These could be real variations in sediment texture, especially at Stations 5/II, 6/II, 1/III, 2/III and 4/III where this phenomenon occurred in at least two, and in most cases, all three seasonal samplings. Large monthly variability within Station 1/II also suggested that the winter and spring variations there were real, although large. Other stations in this category were: winter 6/IV; spring 4/I, 2/IV; fall 3/I, 6/I; and 3/II. At five other stations (winter 4/IV, spring 5/IV, 7/IV, fall 4/III and 1/IV), large differences in grain size among the seven grabs per station remain unexplained. It should be noted, however, that all of these stations are within or adjacent to the Rio Grande delta where sediment distribution is more irregular than in other parts of the study area. Thus, they may represent real variations in sediment texture.

Seasonal Variability

The most consistent pattern of significant seasonal variation was the spring coarsening of texture at outer-shelf Stations 5/I, 6/I, 3/I

6/II, 3/II, 6/III, 3/III and 7/IV. A large portion of the coarse material in this region was skeletal debris from plankton and this coarsening might be the result of spring population blooms of these organisms. However, the textural changes consisted not of addition of a coarse component, but of loss of the finest (greater than 10.6 phi) clays. Thus, the coarsening seemed to result from an increase in bottom water movement which winnowed away some of the finest sediment.

On the other hand, the fining of all inner-shelf, sandy stations from winter to spring resulted primarily from loss from the sand mode. Thus, a zone of coarsening of inner- to mid-shelf stations (1/I, 1/II, 4/II, 5/III and 1/IV) may have been due to seaward transport of coarse material (probably very fine sand and coarse silt) during the spring seasonal peak in wind intensities.

From spring to fall, the outer-shelf coarsening trend seemed to move shoreward to the vicinity of Stations 2/I, 2/II and 2/III, and the outermost shelf sediments became finer again. The generally calmer weather during summer may have allowed deposition of clays to accomplish this. Indeed, the coarse clay mode showed a general increase at these stations by fall.

Other seasonal changes were more localized and had low statistical significance.

Technique

The different computation procedure required by Coulter Counter data make all previous BLM shelf sediment representations appear coarser and better-sorted than the sediments really are. For example, about one-half of the 25 stations in this study had mean grain sizes in the clay range, whereas none of the stations were represented as clays in the USGS

Final Report for 1975. Likewise, according to the same report, only five stations would have had standard deviations over 2.5 phi, whereas all 25 stations really do. Any representations of clay and silt distributions would be similarly different. However, either type of data would show the same relative differences between stations. The few stations used in this study relative to the USGS sample grid does not warrant remapping the sediment textural parameters. The relationships established between those parameters should enable conversion of the USGS data if desired. The high degree of correlation between the data computed by the two different methods probably means that any statistical comparison of other biological or chemical parameters would show very similar degrees of correlation with either type of sediment data. The specific values which would correlate would be different, but the relative relationships should remain similar when large samples of the populations under study are used.

Results from an interlaboratory calibration exercise and other samples actually analyzed by the two different methods by Shideler (1976) are included in Figures 1-4. Although the amount of data is small, it shows that for muddy samples, the computational differences account for all the significant differences in values of kurtosis, skewness, and standard deviation. For mean grain size, the actual Coulter Counter results give finer grain sizes than would be predicted using pipette results and the empirical relationship between calculated parameters. The regression equations show that the parameters for the two methods would be equal when the mean grain size is between 3 and 4 phi, *i.e.*, when the sediments entered the sand range. This suggests that the relationships are good, because there would be little or no fine clay in such samples, and thus, the techniques would produce the same results.

The equations would be invalid, of course, for coarser sediments.

CONCLUSIONS

The variability of sediment textural parameters on the scale of station maintenance and reoccupation navigability (probably tens of meters) followed approximately the uniformity or sorting of the texture itself as measured by the parameter of standard deviation. The significant number of stations with at least one sample whose texture was quite different from other closely-grouped samples from the same station suggested that a degree of patchiness of textures existed in much of the study area. Whether the scale of this patchiness was mottles of sand or clay in an otherwise much more uniform sediment, or larger scale features such as sand waves or biologically effected microenvironments could not be determined from the data at hand. Replicate sampling of grab samples would at least partially answer this question.

Although seasonal changes were expectedly subtle, the windier spring period seemed to correlate with winnowing of some of the finest clays from the outer most shelf, removal of sands from the innermost and coarsening on the mid-shelf. Weak evidence suggested that this trend reverses between spring and fall. Continued seasonal sampling should clarify these trends, although the large degree of known annual variance in weather patterns might require several years of record to achieve this clarification.

Coulter Counter and pipette analysis methods give very comparable results if the major difference in computational methods is taken into account. Pipette data can be used to compute sediment textural parameters either way, but Coulter Counter data must be processed in such a way that an unknown amount of fine clay is redistributed among the silt

and coarse clay classes. This causes mean grain sizes to appear considerably coarser than reality and sorting to appear better than is real. Skewness is apparently not affected. Kurtosis values are decreased and made somewhat more positive. Correlations between the two types of parameters are high, so that possible relationships of other variables with sediment textures should be accurately tested if large samples are used.

LITERATURE CITED

Berryhill, H. L., Jr., (Ed.) 1976. Environmental studies, south Texas outer continental shelf, 1975, geological investigations. A report to the Bureau of Land Management, Contract AA550-MU5-20. U.S. Geological Survey, Corpus Christi, Texas.

Berryhill, H. L., Jr., (Ed.) 1977. Environmental studies, south Texas outer continental shelf, 1975: an atlas and integrated summary. A report to the Bureau of Land Management, New Orleans OCS Office, New Orleans, Louisiana. University of Texas, Texas A&M University, Rice University, National Oceanic and Atmospheric Administration, and U.S. Geological Survey, Corpus Christi, Texas. 303pp + 114 Figs. and 17 Tables.

Carver, R. E., ed. 1971. Procedures in sedimentary petrology. New York, Wiley-Interscience. 653pp.

Folk, R. L. 1974. Petrology of sedimentary rocks. Austin, Hemphill. 182pp.

McBride, E. F. 1971. Mathematical treatment of size distribution data. Pages 109-127. *in* Carver, R. E., ed. Procedures in sedimentary petrology. New York, Wiley-Interscience. 653pp.

Schlee, J. 1966. A modified Woods Hole rapid sediment analyzer. Jour. Sed. Petrology. 36:403-413.

Shepard, F. P., F. B. Phleger, and T. H. van Andel. 1960. Recent sediments, northwest Gulf of Mexico. Amer. Assoc. Petroleum Geologists, Tulsa. 394pp.

Shideler, G. L. 1976. A comparison of electronic particle counting and pipette techniques in routine mud analysis. Jour. Sed. Petrology, 46:1017-1025.

CHAPTER FIFTEEN

SELECTED WATER COLUMN MEASUREMENTS: LOW-MOLECULAR-WEIGHT HYDROCARBONS, NUTRIENTS AND DISSOLVED OXYGEN

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ABSTRACT

This report contains a comprehensive tabulation and analysis of all low-molecular-weight hydrocarbon, dissolved oxygen, and nutrient data taken in the South Texas OCS area in 1976. The above-mentioned parameters were measured at three stations on each of four transects tangent to the South Texas coastline three times during the year (winter, spring, fall). In addition to these samplings, one transect near Port Aransas was sampled six times monthly. Southern Bank and Hospital Rock were also sampled nine times during the year. At least two depths for LMWH analyses and three for dissolved oxygen and nutrients were sampled at each station. However, at most stations, several additional samples were taken at intermediate depths to adequately define water column characteristics.

LMWH were analyzed by a modification of the Swinnerton and Linnenbom (1967) method. Some supplemental samples were analyzed by McAuliffe's (1971) method. Dissolved oxygen and nutrients were determined following the methods outlined by Strickland and Parsons (1972).

Methane showed considerable variation in the STOCS area. Near-bottom seepage was detected in bottom waters at some stations (*e.g.*, Station 3/IV). At mid-depth there was a seasonal maximum that developed following stratification of the water column in summer and fall. Methane seemed to show a good correlation with transmissometry (suspended material) traces. Ethene and propene showed the same general trend as productivity; low surface values were observed in the winter with higher values in the spring, summer and fall. Ethene generally showed a maximum at some shallow depth (20 to 40 meters) in the water column. The olefins generally dominated over their saturated analogs in the STOCS area. The lower Texas shelf is relatively "clean", as most of the light hydrocarbons of this shelf are presently derived from natural sources.

Throughout the year, oxygen levels in the upper 60 meters of the STOCS area were controlled mainly by physical processes (seasonal changes in temperature and salinity) rather than productivity. Surface and near-shore values were highest in winter and lowest in summer. The intrusion of 200-300 m Western Gulf Water was always observed below 70 meters.

Nutrient concentrations were typically low, being representative of open Gulf surface water. Nitrate was limiting to productivity and disappeared during the summer and early fall. Phosphate and silicate were affected by the spring increase in productivity but were generally regenerated by fall. Increased continental runoff during the spring months was reflected by high silicate values at near-shore stations.

INTRODUCTION

Purpose

The purpose of the low-molecular-weight hydrocarbon analysis program is to establish baseline levels for the C_1-C_4 hydrocarbon components in the South Texas OCS region. These components are very sensitive indicators of spilled petroleum hydrocarbons and natural seeps. Once a light-hydrocarbon baseline has been established, a light-hydrocarbon monitoring program will indicate spilled oil and be a potential tracer of the more highly toxic components of petroleum.

Methane in unpolluted areas of the shelf is also a good indicator of suspended (organic) matter levels in the water column. Subsurface suspended matter profiles correlate well with methane profiles. An understanding of suspended matter variations is important since trace metals and petroleum hydrocarbons released during drilling can be adsorbed onto the surfaces of particulate material where they may be assimilated by filter and detrital feeders. Methane and other light hydrocarbon profiles also indicate areas of gas seepage from the sediment-sea interface. These seeps may indicate the presence of subsurface oil or gas reservoirs. The seeps may also affect sediment stability since gas saturation in sediments has been shown to destabilize sediments which can lead to failure of bottom-mounted structures.

The olefin levels (*e.g.*, ethene and propene) correlate well with biological productivity parameters. Thus, the concentrations of these components in the water column are related to biological

activity in the area.

In addition to the hydrocarbon analyses mentioned, nutrient and dissolved oxygen concentrations are determined to support primary productivity measurements. Nutrient and dissolved oxygen concentrations are affected by the hydrology of a region as well as biological uptake and release. In turn, the concentration of nutrients and dissolved oxygen in the water column greatly affect the species diversity and standing crop of phtyo- and zooplankton.

Literature Survey

Dissolved gaseous hydrocarbons, methane through the butanes, in marine waters are derived from natural processes and/or man-related activities. Although microbially-produced methane has been known to exist in high concentrations (>>0.1 ml CH_4/l) in anaerobic waters such as isolated fjords and basins (Atkinson and Richards, 1976; Lamontagne *et al.*, 1973), the processes that control the concentrations of light hydrocarbons in the aerobic portion of the ocean are only recently beginning to be understood. Prior to determinations by Swinnerton and Linnenbom (1967) of C_1-C_4 hydrocarbons in two open ocean profiles, there had been no published data for these components in the open ocean. The reported analyses of gaseous hydrocarbons in the open ocean have been mainly restricted to our work over the past seven years in surveys of surface water from over 5000 miles of cruise tracks and analyses of several thousand discrete samples (Brooks and Sackett, 1973, 1977;

Brooks *et al.*, 1973, 1977; Sackett and Brooks, 1974, 1975; Brooks, 1975, 1976) and that of Naval Research Laboratory (Swinnerton and Linnenbom, 1967; Swinnerton *et al.*, 1969; Lamontagne *et al.*, 1971, 1973, 1974; Swinnerton and Lamontagne, 1974). MacDonald (1976) and Scranton and Brewer (1977) have recently reported light hydrocarbon concentrations in the Beaufort Sea and Eastern Tropical North Atlantic, respectively.

In the Gulf of Mexico, our laboratory has identified ports and estuaries with their associated commercial and petrochemical activities, offshore petroleum operations, and shipping activities, as the major man-derived sources of low-molecular-weight hydrocarbons. Of these, the underwater venting of waste gases and brine discharges, both associated with offshore production platforms, are the major sources of non-methane light hydrocarbons to upper Gulf coastal waters. These sources are apparently responsible for the two orders of magnitude increase in Louisiana Shelf waters over open ocean levels of the light hydrocarbons with average concentrations of 3100, 31 and 22 nl/l of methane, ethane, and propane, respectively (Brooks *et al.*, 1977).

Natural sources of light hydrocarbons to coastal waters include seepage of gas out of sediments and *in situ* production in the water column. The gas seeping from sediments can originate from either (1) bacterial catalysis, involving the reduction of CO_2 or fermentation of organic compounds in anoxic environments yielding principally methane, and (2) abiotic cracking, either thermal or catalytic, yielding a large spectrum of aliphatic and

aromatic hydrocarbons. Processes controlling the *in situ* production in the water column of methane and olefins are not well defined in the literature.

Previous Work

This report represents the second year of baseline low-molecular-weight hydrocarbon (LMWH), dissolved oxygen (DO), and nutrient data in the South Texas OCS region. During the first contract year, LMWH were analyzed at three depths in the water column along the four transects seasonally. The first year effort revealed areas of natural seepage in the STOCS region and distinct methane maxima at mid-depths during the spring and fall samplings. The second contract year LMWH were analyzed at only two depths (surface and near-bottom) along the four transects seasonally and along Transect II monthly. However, we have supplied many mid-depth analyses of methane so that the important subsurface maxima could be illustrated.

Although nutrient and dissolved oxygen concentrations are analyzed in all BLM OCS programs, LMWH have only been measured in a few. LMWH were analyzed by our group at Texas A&M in the MAFLA OCS area during 1974 and subsequently in the South Texas OCS area during 1975 and 1976. They are currently being analyzed in the Northeast Gulf of Alaska and the Southeastern Bering Shelf by Pacific Marine Environmental Laboratories for the Bureau of Land Management.

METHODS

Low-Molecular-Weight Hydrocarbons

LMWH were analyzed in samples from 12 stations (three stations on each of four transects tangent to the South Texas coast line) and

two bank stations in the STOCS area during 1976. The 12 stations were sampled three times during the year (*e.g.*, winter, spring and fall). Although an attempt was made to sample the area synoptically, the physical sampling of the twelve stations was over a period ranging from six days (*e.g.*, fall seasonal) to over one month (*e.g.*, winter seasonal). Thus, rigorous interpretations of areal distributions should take sampling periods into account. Transect II was also sampled on six monthly cruises in addition to the three seasonal cruises. The monthly samplings of Transect II required no more than three days. The bank stations (*e.g.*, Hospital Rock and Southern Bank) were also sampled monthly, but due to an incomplete Work Statement at the time of the winter sampling, no bank station samples were obtained.

Samples were taken by standard hydrographic casts using a series of Niskin or Nansen bottles. After retrieval, the seawater samples were transferred by gravity flow into 1-l ground-glass stoppered bottles. The bottles were stoppered in a manner to avoid entrapment of gas bubbles. Samples were poisoned with sodium azide to prevent bacterial alteration.

The contract called for LMWH to be analyzed at two depths (surface and near-bottom) at each station. These samples were analyzed by a modification of the Swinnerton and Linnenbom (1967) method. The 1-l samples of seawater were purged by a hydrocarbon-free helium stream and the LMW hydrocarbons were adsorbed on a trap cooled to liquid nitrogen temperature. The trap was then isolated, heated, and coupled by a slider valve to the gas chromatographic stream for analysis. The hydrocarbons were separated on a 1.8-m, 1.5-mm-I.D. POROPAK Q column and detected with a Flame Ionization Detector (FID). Sensitivity of the method was 0.1 nl hydrocarbons/l seawater, and precision was generally better than ± 5%.

In addition to the above samples, intermediate depths at most stations were sampled. These supplemental samples were taken in 125-ml narrow-mouth bottles with screw-top caps. The bottles were poisoned and stored upside-down until analysis. They were analyzed by McAullife's (1971) method of multiple phase equilibrium. McAullife's method involves equilibrating 25 ml of purified helium with 25 ml of sample water in the 50-ml syringe with a LUER-LOK stopcock. Since 96+% of the light aliphatic hydrocarbons partition into the gas phase, analysis was performed by injecting part of the equilibration helium into the chromatographic stream by means of a sample injection valve. The sensitivity of the method is ±5 nl of CH_4 per liter of seawater. These intermediate samples provide more detailed vertical distributions of methane and ethene, but since the sensitivity of the method is approximately 5 nl/l, few of the other C_2 and C_3 hydrocarbon levels were measured at intermediate depths.

Dissolved Oxygen

Samples were analyzed using the Winkler method, as outlined by Strickland and Parsons (1972). Precision is somewhat dependent on the technician doing the analysis, but is generally better than ±0.01 ml/l.

Nutrients

Phosphate, nitrate, and silicate samples were taken in separate 6 oz Whirl-Pak plastic bags and frozen. Samples were analyzed using a single-channel TECHNICON AUTOANALYZER, following the methods of Strickland and Parsons (1972) as modified by Atlas *et al.* (1971).

Temperature

Temperatures were measured with deep-sea reversing thermometers

attached to Nansen bottles. The thermometers were calibrated yearly to ±0.005 °C. Two reversing thermometers were attached to each Nansen bottle and each thermometer was read separately by two observers. The thermometer readings from each depth were averaged and reported to an accuracy and precision of ±0.02 °C.

Salinity

Seawater for salinity measurements was drawn from the sampling bottles after LMW hydrocarbon and oxygen samples were taken. The water was stored in 500-ml citrate bottles for measurement on a PLESSEY 6210 inductive salinometer. Accuracy and precision are typically ±0.001 ppt.

Precision of Replicates

In accordance with the BLM contract for 1976, 20 samples were taken in triplicate for each selected parameter during the November and December sampling. The measured concentrations for each of these triplicate samples are tabulated in Appendix K, Tables 8 and 9, for LMWH and Appendix K, Tables 18 and 19, for dissolved oxygen, nitrate, phosphate and silicate. A simple statistical analysis was performed on the values reported for the LMWH samples, and is shown on Table 1. For each set of triplicate samples the station number and depth are tabulated along with mean values, standard deviations, and relative errors in percent. Average percent relative errors for each parameter are also listed. Table 1 shows that average relative errors in the sampling and analytical technique for the light hydrocarbons were ±6.4, 8.3, 5.6, 6.8 and 7.6% for methane, ethene, ethane, propene and propane, respectively.

RESULTS AND DISCUSSION

Table 2 shows the number of observations, mean, minimum and maximum

TABLE 1

REPLICATE ANALYSES OF TWENTY SAMPLES FOR LMWH. THE MEAN (X) AND STANDARD DEVIATION (S.D.) ARE BASED ON THREE REPLICATE ANALYSES AT EACH STATION. RELATIVE ERROR (R.E.) IS THE QUOTIENT OF THE STANDARD DEVIATION AND THE MEAN GIVEN IN PERCENT. ALL CONCENTRATIONS ARE GIVEN IN nl/l (NPT).

SEASON	STATION	DEPTH	METHANE (nl/l)			ETHENE (nl/l)			ETHANE (nl/l)			PROPENE (nl/l)			PROPANE (nl/l)		
			X	S.D.	R.E.	X	S.D.	R.E.	X	S.D.	R.E.	X	S.D.	R.D.	X	S.D.	R.E.
Nov. 76	11/1	0 m	91.33	2.309	2.53	4.697	0.196	4.18	0.633	0.015	2.41	1.497	0.040	2.70	0.550	0	0
		18 m	77.33	12.423	16.06	3.137	0.241	7.68	0.543	0.035	6.46	1.180	0.291	24.66	0.540	0.062	11.56
	11/2	0 m	83.67	6.028	7.20	2.677	0.093	3.47	0.433	0.021	4.70	0.817	0.045	5.52	0.500	0.046	9.17
		40 m	104.33	10.970	10.51	3.757	1.106	29.43	0.500	0.017	3.46	0.983	0.015	1.55	0.483	0.021	4.31
	11/3	0 m	83.33	10.693	12.83	4.127	0.523	12.68	0.363	0.038	10.42	0.920	0.070	7.61	0.430	0.079	18.46
		120 m	161.33	11.719	7.26	0.590	0.121	20.55	0.423	0.015	3.61	0.193	0.025	13.02	0.503	0.057	11.30
	SB/4	0 m	83.67	4.619	5.52	5.167	1.218	23.58	0.440	0.010	2.27	0.943	0.029	3.06	0.440	0.010	2.27
		70 m	449.67	11.590	2.58	2.227	0.156	6.99	0.473	0.029	6.10	0.313	0.029	9.21	0.490	0.030	6.12
	HB/2	0 m	77.67	11.846	15.25	4.12	0.127	3.09	0.387	0.049	12.76	0.933	0.025	2.70	0.470	0.046	9.75
		70 m	415.33	19.348	4.66	3.597	0.075	2.09	0.557	0.021	3.74	0.497	0.025	5.07	0.500	0.017	3.46
Dec. 76	11/1	0 m	107.33	7.23	6.74	2.32	0.20	8.80	0.58	0.04	6.22	0.44	0	0	0.51	0.02	4.56
		18 m	106.00	6.56	6.19	2.09	0.21	10.22	0.62	0.05	7.41	0.45	0.02	3.85	0.49	0.01	2.37
	11/2	0 m	97.67	4.73	4.84	2.65	0.10	3.73	0.63	0.01	1.84	0.66	0.02	2.33	0.59	0	0
		40 m	88.33	0.58	0.65	2.28	0.22	9.49	0.55	0.03	4.60	0.60	0.01	0.97	0.51	0.02	4.11
	11/3	0 m	106.67	6.11	5.73	2.41	0.12	4.77	0.45	0.02	5.09	0.46	0.02	3.77	0.38	0.03	8.11
		125 m	102.00	8.00	7.84	0.63	0.09	14.33	0.16	0.03	8.85	0.15	0.05	32.22	0.54	0.10	18.29
	SB/1	0 m	106.00	3.00	2.83	2.27	0.03	1.27	0.80	0.05	6.14	0.53	0.02	4.38	0.59	0.05	8.80
		70 m	122.67	2.52	2.05	2.37	0.06	2.64	0.45	0.02	5.09	0.53	0.01	2.17	0.55	0.06	11.27
	HB/1	0 m	132.67	5.86	4.42	2.43	0.09	3.73	0.52	0.03	5.77	0.57	0.06	9.77	0.48	0.07	15.18
		70 m	133.33	2.31	1.73	2.39	0.08	3.39	0.49	0.03	6.12	0.54	0.01	1.08	0.43	0.02	3.58
	AVERAGE				6.37			8.81			5.65			6.78			7.63

TABLE 2

SELECTED PARAMETERS IN THE STOCS (1976)

Variable	Number of Observations	Mean	Minimum	Maximum
Methane (nl/l)	400	99	41	500
Ethene (nl/l)	293	4.6	0.09	25.0
Ethane (nl/l)	155	0.4	0.10	1.30
Propene (nl/l)	154	0.9	0.14	2.49
Propane (nl/l)	154	0.4	0.19	0.83
R (methane)	378	2.4	0.92	12.4
Depth (m)	487	37.3	0	130
Temperature (°C)	448	22.5	13.87	29.98
Salinity (ppt)	452	35.42	27.914	36.754
Oxygen (ml/l)	472	4.71	2.32	6.36
Phosphate (μM)	369	0.26	0	2.43
Nitrate (μM)	368	1.5	0	22.1
Silicate (μM)	369	2.5	0.10	15.8

values for each of the LMWH measured in the STOCS region in 1976. These values are tabulated in Appendix K, Tables 1 through 10.

Areal Distribution of Methane

Figure 1 shows near surface methane concentrations during the seasonal cruises in the STOCS area in 1976. The processes controlling the surface concentrations of methane on the STOCS appear to be similar to those occurring in the open ocean waters of the Gulf of Mexico. In the open Gulf of Mexico, concentrations are controlled mainly by biological processes occurring in the water column and exchange across the air-sea interface. Biological and physical processes (*e.g.*, salinity and temperature variations) are more pronounced in the coastal zone resulting in greater variability than observed in the open Gulf. The lower Texas shelf is generally free of the large man-derived additions that control surface methane and other LMWH concentrations on the upper Texas-Louisiana shelf. These man-related sources include offshore drilling and production, transportation losses, river runoff, and inputs associated with bays and harbors. The STOCS region is relatively clean with respect to man-induced LMWH since large-scale drilling and production has not commenced on this part of the Texas shelf.

Surface concentrations of methane on the STOCS are influenced by air-sea exchange. If there were no man-derived or natural inputs of methane, surface concentrations would be at equilibrium with the atmosphere. The concentration of a dissolved non-reactive gas in seawater is determined according to Henry's Law by its solubility coefficient at the temperature and salinity during water mass formation and its partial pressure in the atmosphere. For the LMWH, only the partial pressure of methane (1.4±0.1 ppmv for the atmosphere over the entire earth) is

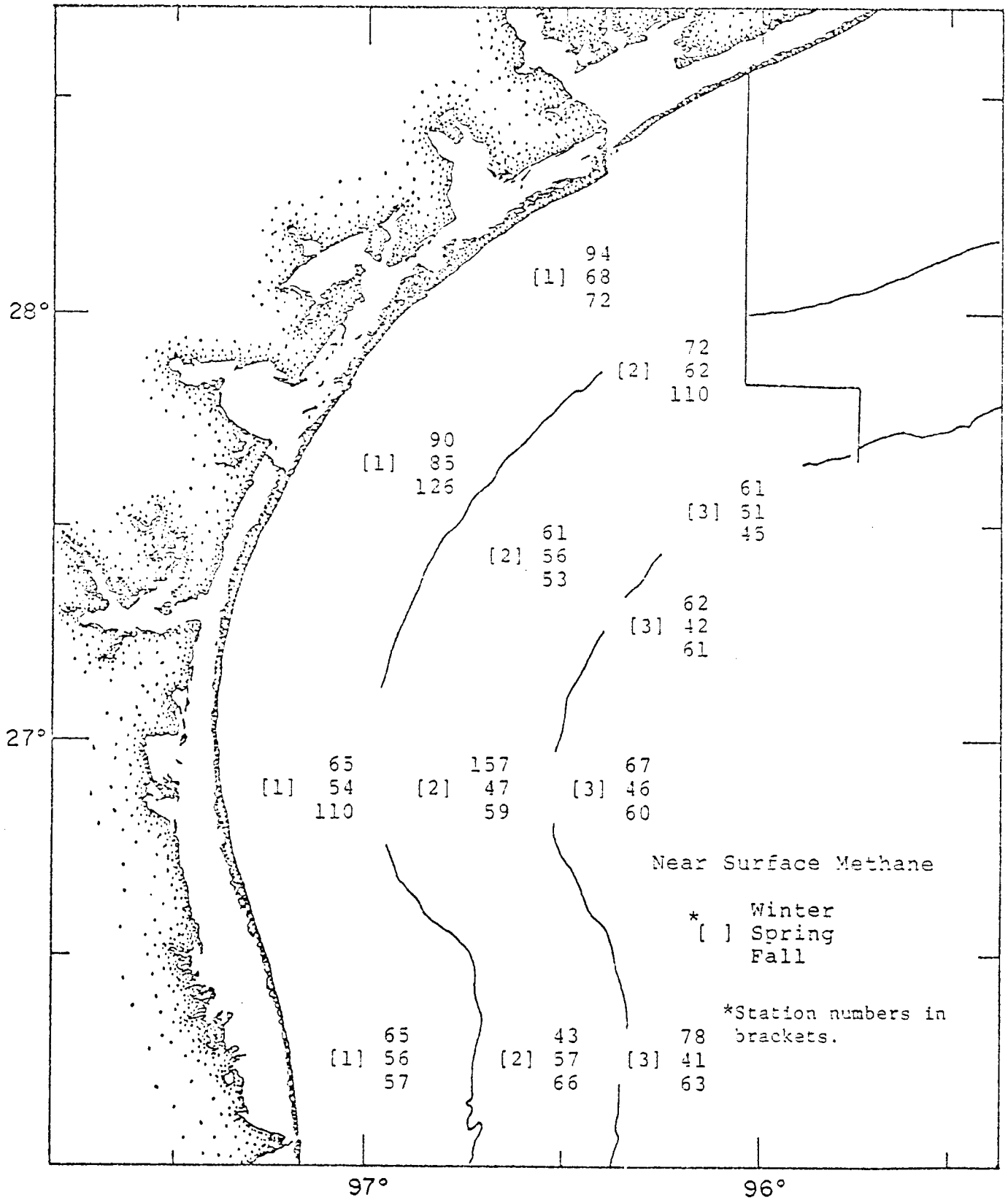

Figure 1. Near-Surface Methane Concentrations (nl/l) in the STOCS Area During the Seasonal Cruises in 1976.

known with any degree of certainty. Using this value and the Bunsen solubility coefficients reported by Yamamoto *et al.* (1976), the equilibrium solubility of methane in the water column at the existing temperature and salinity has been computed for each observation. Also computed for each observation is an R value, where R is the ratio between measured and equilibrium methane concentrations and R greater than 1.0 indicates supersaturation. The R values (Table 2) for methane ranged from 0.92 to 12.4 times equilibrium concentrations in the water column (mean = 2.4; 378 observations).

Figure 2 shows R values of near-surface methane concentrations during the seasonal cruise in the STOCS area. Although higher values were usually observed at near-shore stations, there were no seasonal or spatial patterns observed in these surface samples. There were 68 surface observations for methane in the STOCS area during 1976 which ranged from 1.04 to 3.73 times equilibrium concentrations and averaged 1.81. Thus the surface waters in the STOCS area showed a consistent surface supersaturation with respect to the partial pressure of methane in the atmosphere.

These observations agree with the reports of several other investigators (Lamontagne *et al.*, 1971; 1973; 1974; Brooks and Sackett, 1973; 1977; Swinnerton and Lamontagne, 1974; Brooks, 1975; and Scranton and Brewer, 1977) which showed that the mixed layer of the ocean was supersaturated with respect to the partial pressure of methane in the atmosphere. The supersaturation appears to be a permanent feature of the world ocean, except in regions of strong upwelling such as the Yucatan Shelf (Brooks *et al.*, 1973) and in some ice-covered areas of the Antarctic (Lamontagne *et al.*, 1974). Sources of excess methane in coastal water include offshore production operations, runoff, transportation activities and dif-

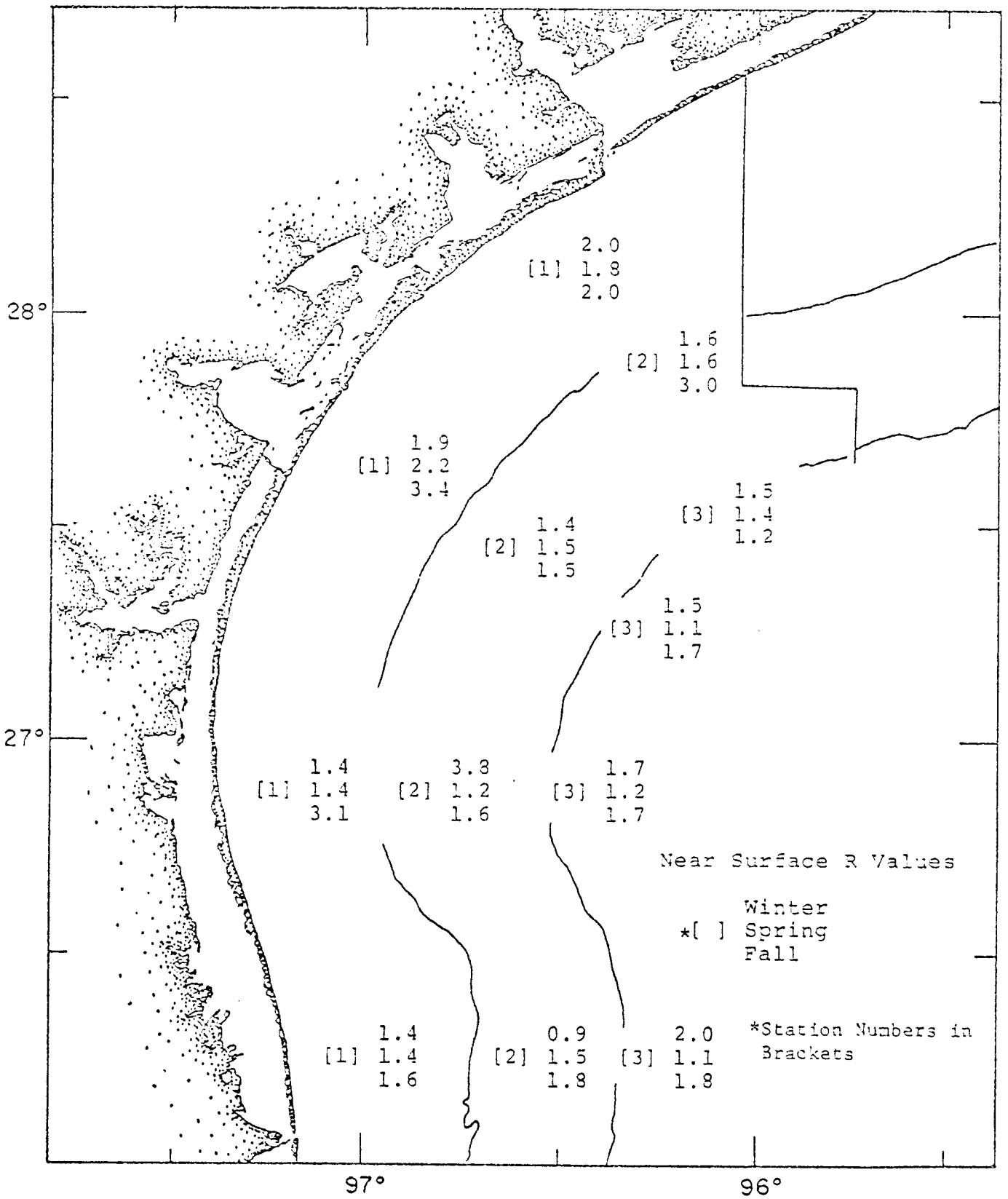

Figure 2. Near-Surface R (degree of saturation) Values for Methane in the STOCS Area During the Seasonal Cruises in 1976.

fusion from methane-rich sediments. The source of the excess methane in the open ocean has been attributed to *in situ* production in the water column (Lamontagne *et al.*, 1973; Brooks and Sackett, 1973; Seiler and Schmidt, 1974; and Scranton and Brewer, 1977). Since man-derived additions of LMWH on the South Texas shelf do not, at present, appear to be affecting LMWH levels, the excess methane on the STOCS must come from *in situ* production, diffusion out of methane-rich sediments, or gas seepage.

Vertical Methane Distribution

The vertical distribution of methane in the STOCS region exhibits both seasonal and spatial variations. Table 3 shows the number of observations, the average mean water column concentration, the average surface concentration, and the minimum and maximum values for each of the nine seasonal and monthly samplings. Table 4 shows the surface and near-bottom methane concentrations at STOCS stations along Transect II. There did not appear to be any discernible seasonal pattern in surface methane concentrations along Transect II, although higher surface methane concentrations were observed during the December sampling at all stations. Station 1/II generally had higher surface methane levels that the stations further offshore, no doubt due to the close proximity of the sediment-water interface and coastal contributions (*e.g.*, runoff). Higher surface methane concentrations were associated with Station 1, Transects I and II, probably due to more river and estuarine runoff into these near-shore areas.

Figure 3 shows the methane profiles along Transect I during the winter sampling period. These profiles are typical of the higher concentrations found throughout the water column at the inshore stations.

TABLE 3

SUMMARY OF NUMBER OF METHANE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (In nl/l) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	86	80	43	330	71
March	37	102	51	297	83
April	35	68	42	145	60
Spring	95	87	41	370	55
July	33	100	47	316	63
August	21	178	55	500	76
Topo High	16	102	50	192	58
Fall	28	137	42	450	73
November	20	130	70	449	83
December	29	101	71	141	110

TABLE 4

SURFACE AND NEAR-BOTTOM METHANE CONCENTRATIONS (nl/l) AT STOCS STATIONS ALONG TRANSECT II

Sampling Month	STATION 1/II	2/II	3/II	SB	HB
		Surface			
Winter	90	61	62	-	-
March	88	96	113	56	61
April	86	78	44	46	47
Spring	85	56	42	41	53
July	83	69	52	54	58
August	126	60	64	66	62
Fall	126	53	61	55	71
November	91	84	83	83	78
December	107	98	107	106	132
		Near-Bottom			
Winter	84	92	59	-	-
March	98	87	82	147	76
April	91	49	95	145	99
Spring	99	121	74	75	82
July	60	280	140	270	316
August	-	500	88	431	405
Fall	115	128	42	156	450
November	84	105	161	449	415
December	106	88	102	123	133

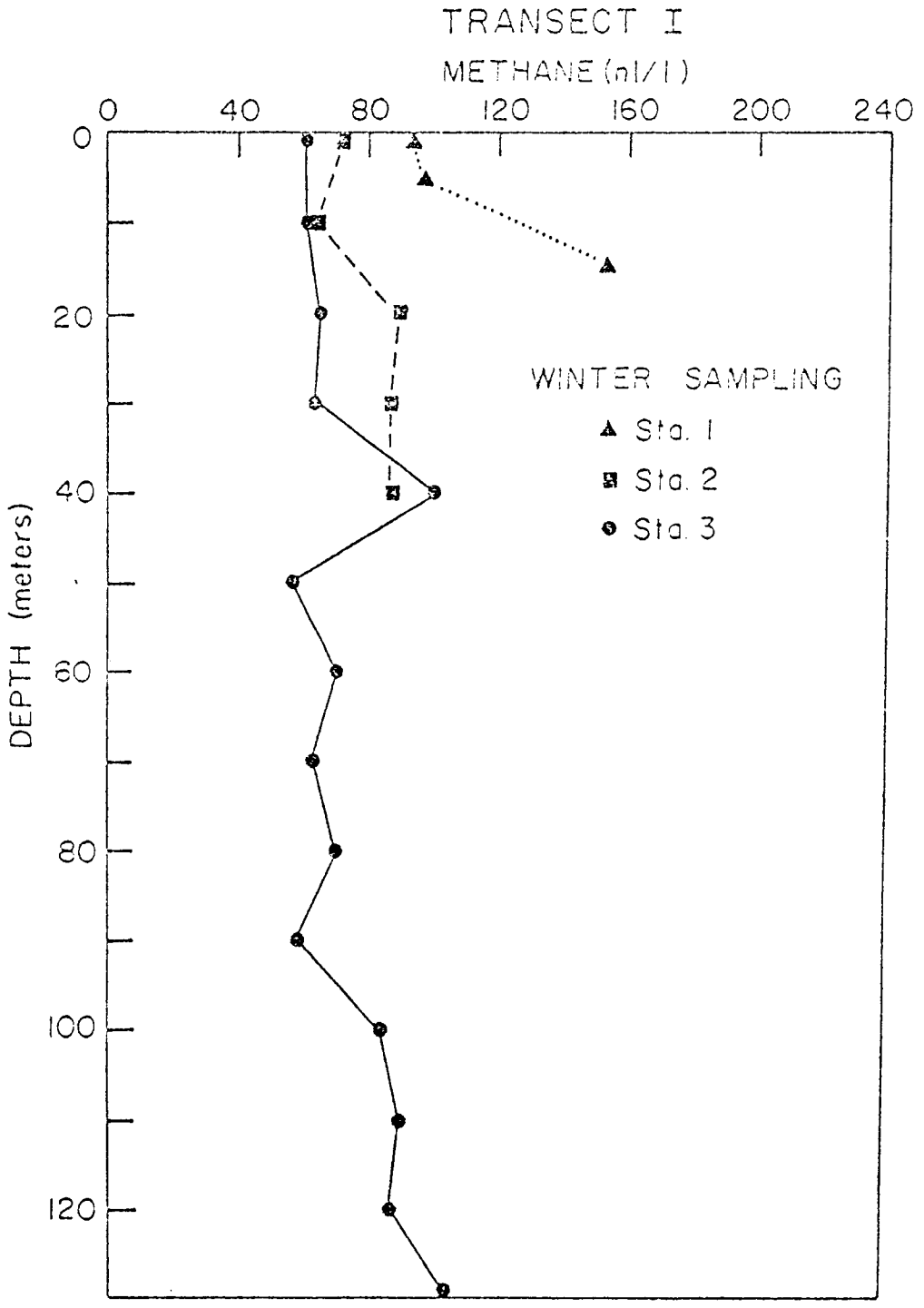

Figure 3. Vertical Methane Distribution at Stations Along Transect I During the Winter Sampling (1976).

Figures 4 through 8 show the vertical methane distribution at Stations 3/I, 3/II, 2/II, 3/III and 3/IV, respectively. These profiles indicate that the vertical methane distributions were similar during both the winter and spring samplings of Station 3, Transects I, II, III and IV. Only surface and near-bottom samples were obtained at these stations during the fall sampling. All nine samplings of Stations 3/II and 2/II are shown in Figures 5 and 6.

Of the twelve stations sampled, Station 3/IV is unique in that the near-bottom samples from this station (Figure 8) always showed very high methane concentrations. The concentrations were measured as high as 400 nl/l, with no seasonal influence. Methane concentrations typically remained above 100 nl/l, 20-40 meters above the bottom. These high concentrations measured continuously over a 2-year period are the result of natural gas seepage across the sea-sediment interface. Seeps appear to be a common phenomenon in the Gulf of Mexico. The Principal Investigators have detected many of these seeps with sonar equipment by the acoustical reflection produced by the rising bubble plume, or by hydrocarbon "sniffing" which identified dissolved hydrocarbon anomalies in the vicinity of seeps. Over 100 of these bubbling seeps have been detected in the waters of the Louisiana shelf (Tinkle *et al.*, 1973) and many are known to exist in the South Texas OCS region (Holmes, personal communications). Many of these seeps are associated with topographic highs, although many have been observed along flat bottoms such as at Station 3/IV. The Principal Investigators have collected over 25 seep gases in the Gulf of Mexico (Brooks *et al.*, 1974; Bernard *et al.*, 1976). The hydrocarbon patterns over these seeps are chiefly characterized by methane anomalies in near-bottom waters. All profiles over seeps show

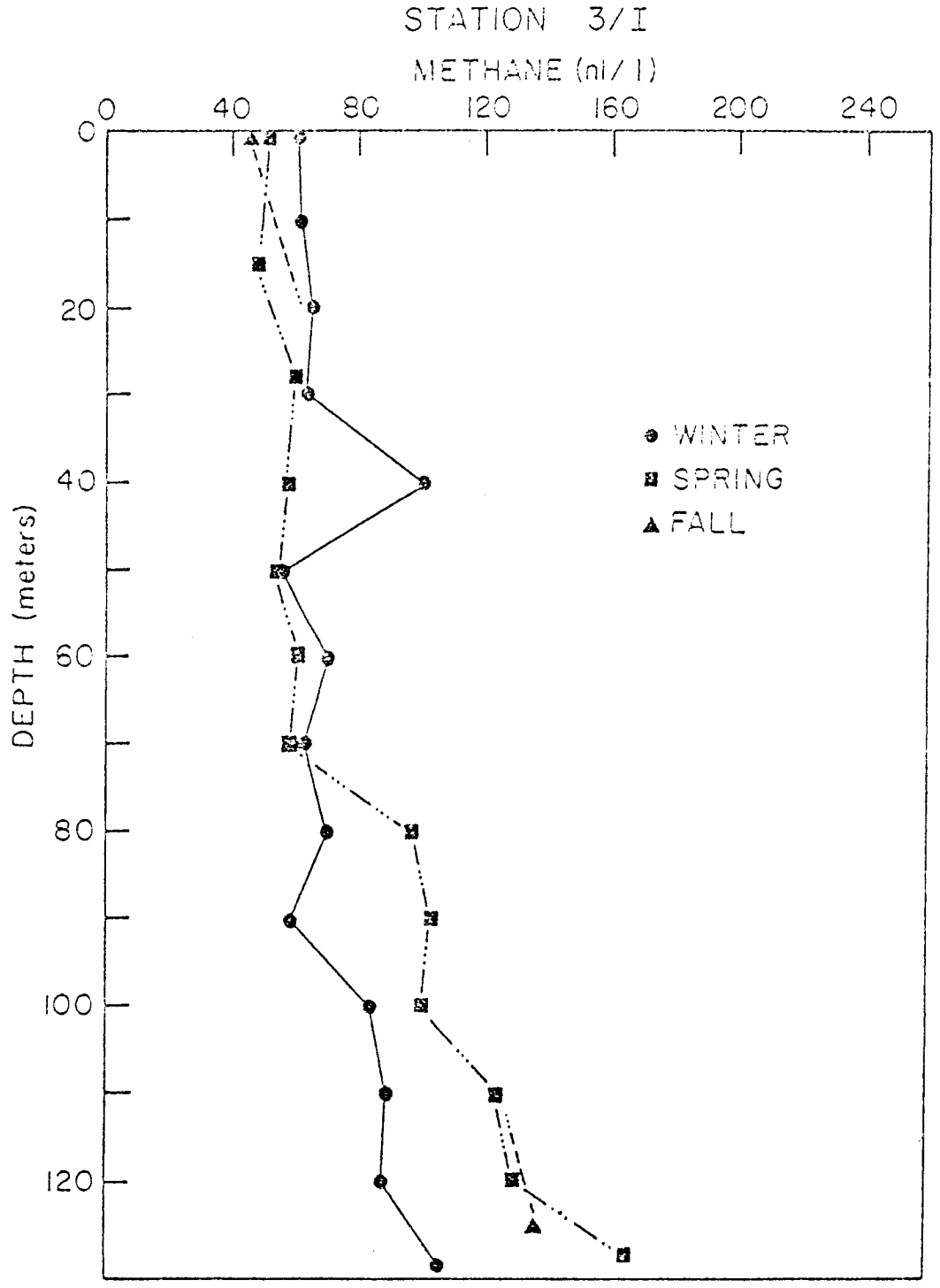

Figure 4. Vertical Methane Distribution at Station 3/I During the Three Seasonal Samplings (1976).

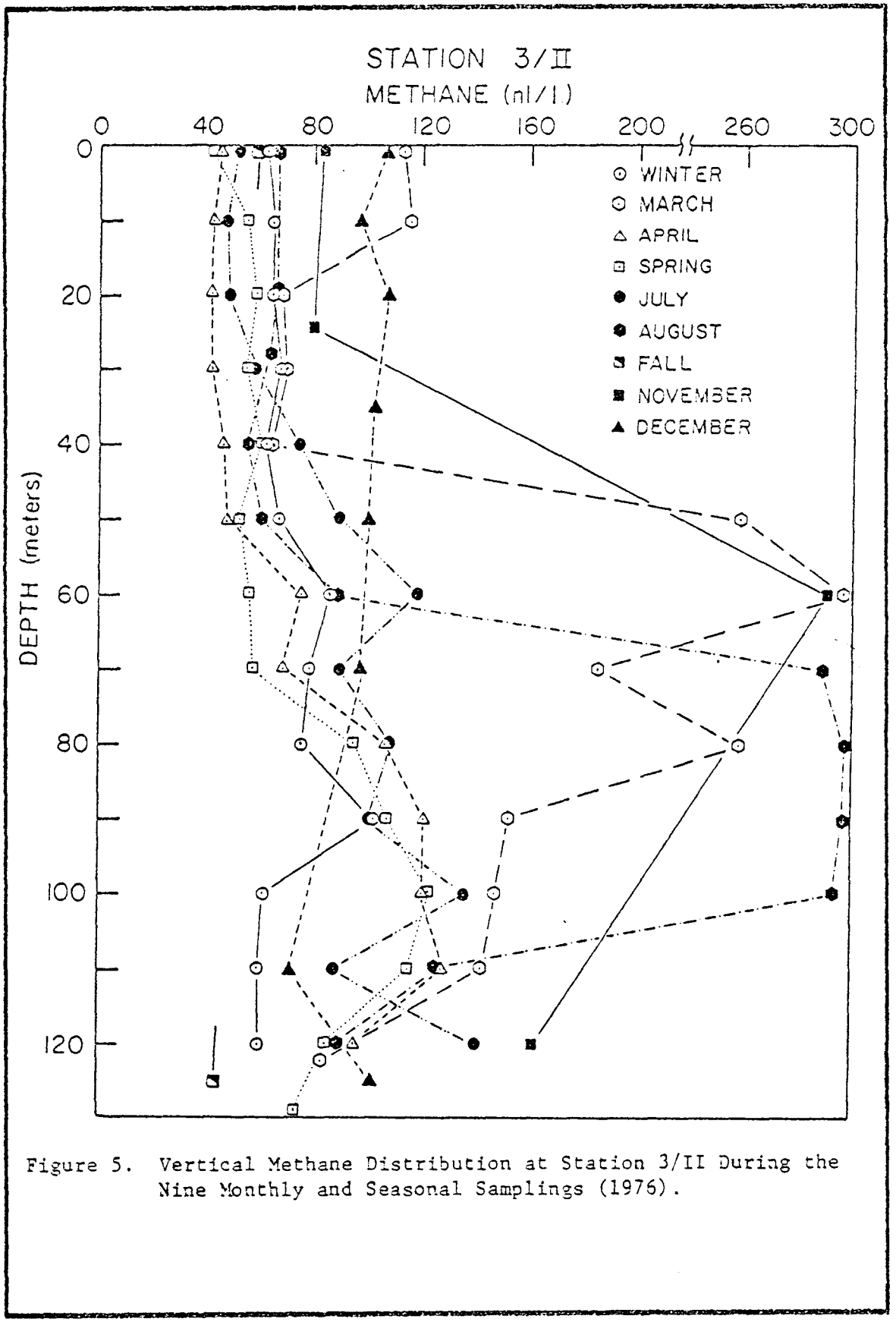

Figure 5. Vertical Methane Distribution at Station 3/II During the Nine Monthly and Seasonal Samplings (1976).

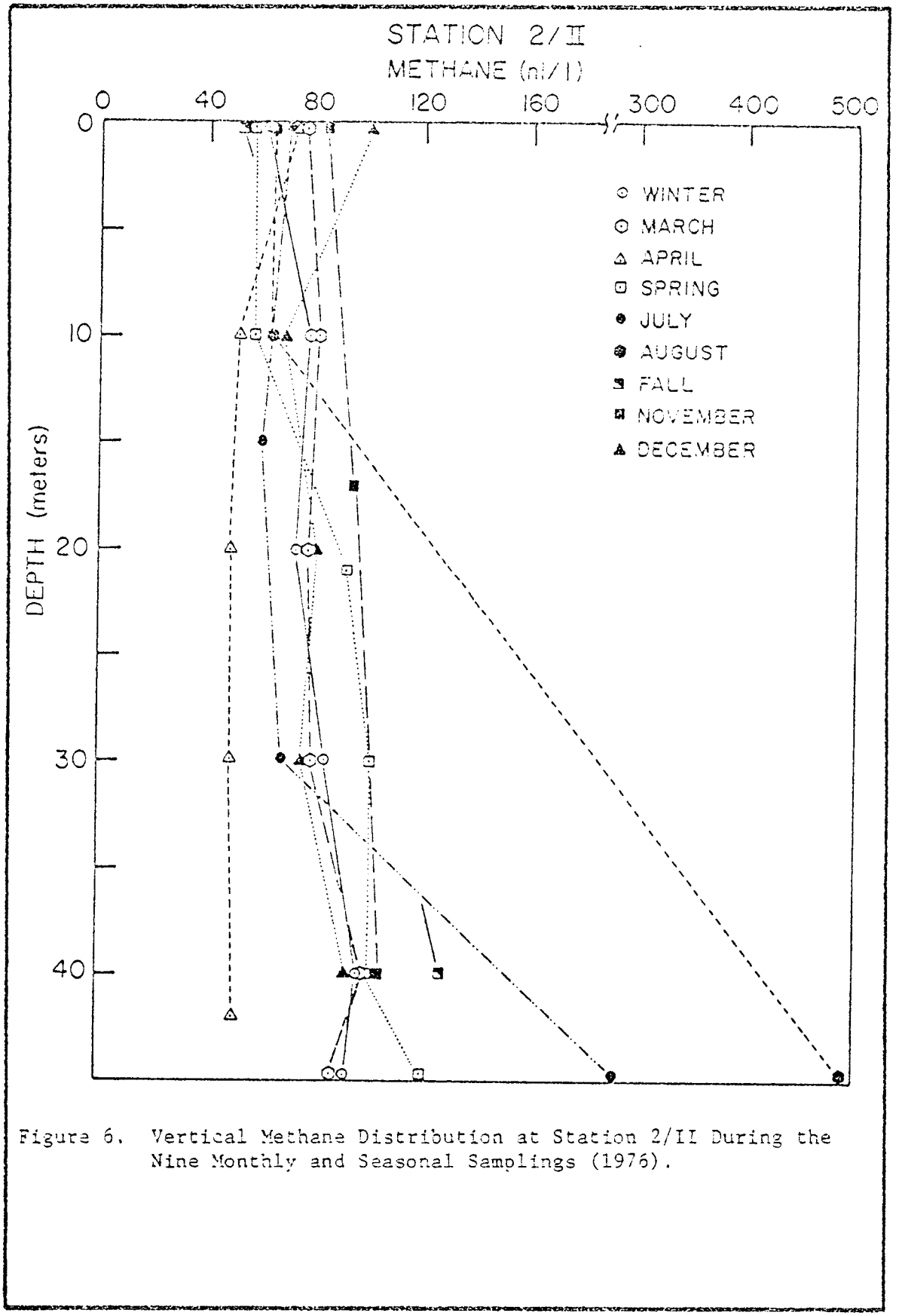

Figure 6. Vertical Methane Distribution at Station 2/II During the Nine Monthly and Seasonal Samplings (1976).

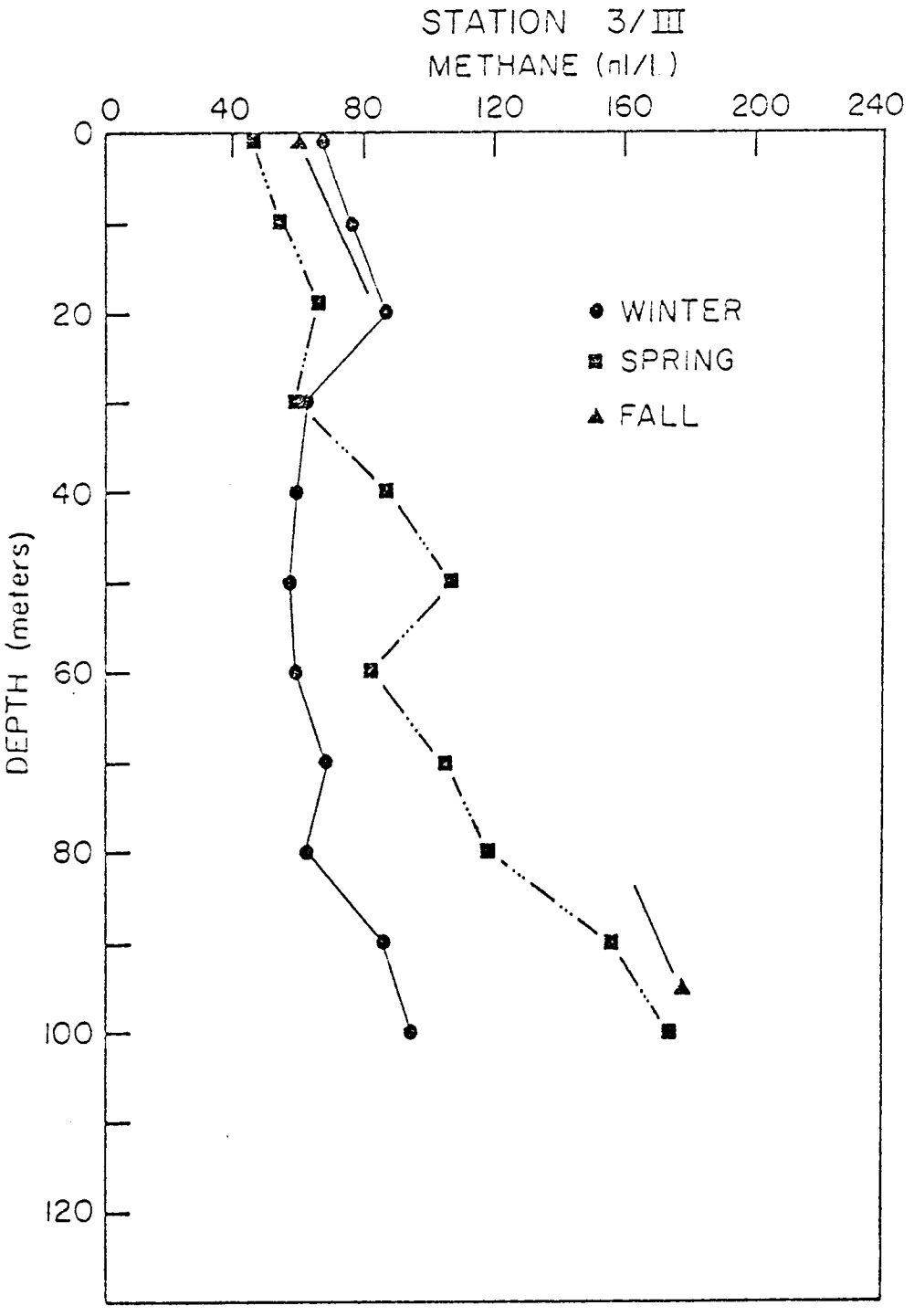

Figure 7. Vertical Methane Distribution at Station 3/III During the Three Seasonal Samplings (1976).

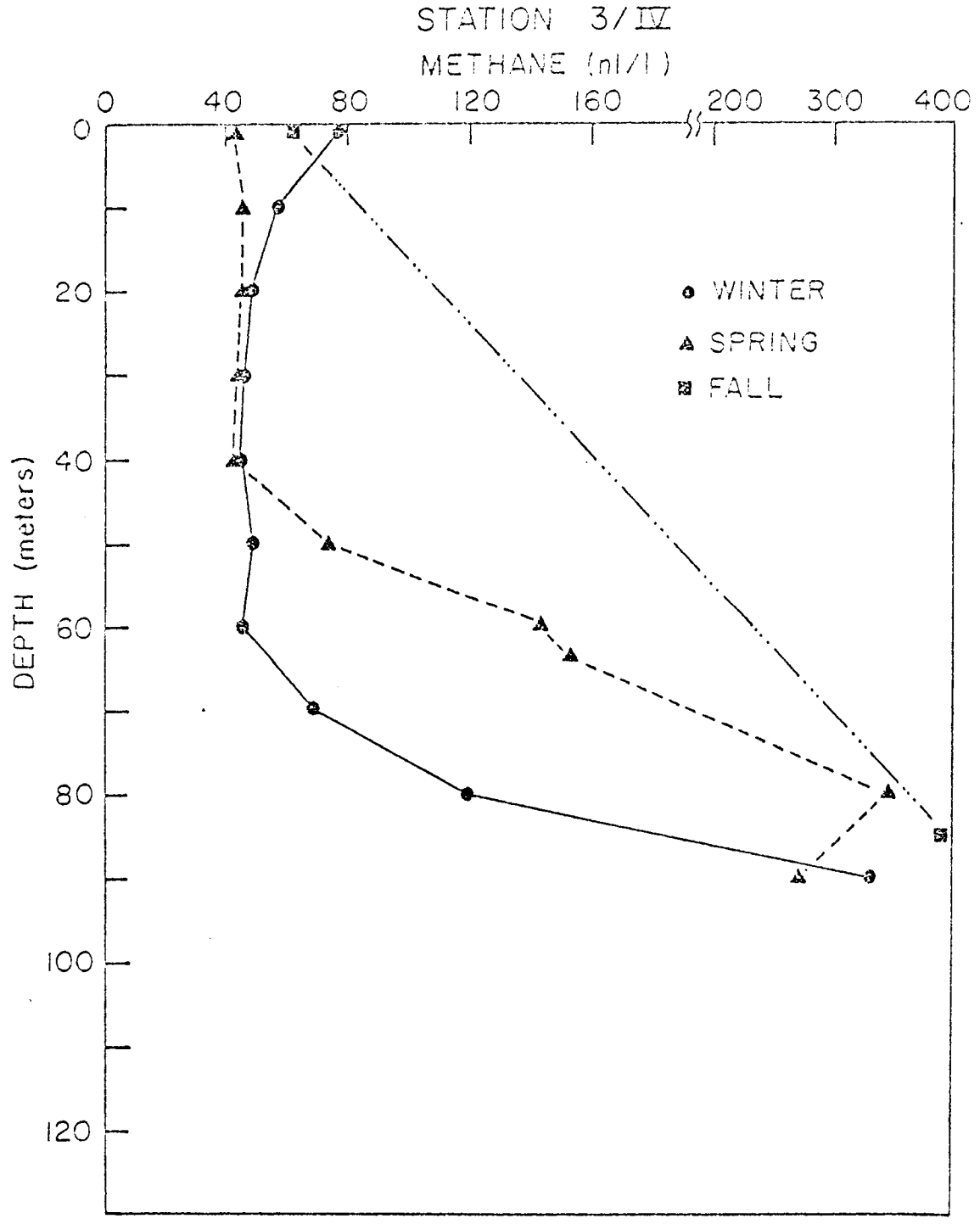

Figure 8. Vertical Methane Distribution at Station 3/IV During the Three Seasonal Samplings (1976).

a significant increase in dissolved methane with depth because of increasing solution of methane due to increased partial pressure in the bubbles with depth.

The gas emanating from one natural seep in the South Texas OCS area has been collected and analyzed in the laboratory. This gas collected during the Topographic Features Study cruise from Southern Bank consisted of 99.96 percent methane and 0.04 percent ethane. No higher hydrocarbons were detected in the gas. It had a $^{13}C/^{12}C$ ratio of -60.2 ppt relative to PDB (Peedee Belemnite), indicative a biogenic source. These seeps may explain some of the high nera-bottom concentrations found over Southern Bank (Table 4). Although not detected, similar seepage may be occurring at Hospital Rock.

The vertical distribution of methane shows large seasonal variations at intermediate depths in the water column (0 to 100 meters), observed in both the 1975 and 1976 sampling period. In 1975 the water column was fairly uniform with respect to methane during the winter sampling. A large maximum (up to 4,000 nl/l) was observed from the spring sampling at intermediate depths of several stations. This same maximum, although at much lower concentrations, was also observed from the fall sampling in 1975. In 1976, a similar but not identical pattern developed. No concentrations in the thousands of nl/l methane were observed in 1976 as was the case in 1975.

In 1976, the winter sampling of all deep stations (Stations 3/I, 3/II, 3/III and 3/IV) showed a fairly uniform methane distribution in the water column with the exception (as already noted) of Station 3/IV. The March sampling of Station 3/II (Figure 5) shows that a broad methane maximum had developed in the 50- to 80-m depth interval. This maximum was absent during the April, spring and July sampling of this station.

The maximum was again present during the August sampling at the 70- to 100-m depth interval. Although no mid-depth samples were taken during the fall sampling, near-bottom concentrations at Station 2/II during the fall (Table 4) seem to indicate the presence of the mid-depth maximum. It was also present during the November sampling, although insufficient sample depths were obtained to define its extent. In December, a uniform water column with respect to methane had again developed. This seasonal mid-depth maximum can also be seen at Station 2/II (Figure 6 and Table 4) in near-bottom samples.

The origin of the mid-depth maximum is only partially understood. It had been observed to be associated with the thermocline (Brooks *et al.*, 1977; Scranton and Brewer, 1977). One explanation for the maximum is advection of coastal water with high methane concentration off the shelf with subsequent loss of the methane in the mixed layer by air-sea exchange. However, it has recently been shown that advection off the shelf cannot account for this maximum in the open ocean (Scranton and Brewer, 1977). It is more probably due to *in situ* generation in the water column.

During the late spring, summer and fall months, stratification of the water column occurs, which restricts turbulent mixing of bottom and surface water masses. There subsequently develops an accumulation of suspended matter at this boundary because of the restriction of settling velocities across the density gradient. It is postulated that methane is formed in small micro-reducing environments from the organic matter in the suspended material. Another explanation is that the methane is formed in the reducing guts of zooplankters that are associated with the thermocline. Although the origin of the methane is uncertain, it appears to be associated with suspended material. In some of the data obtained

in 1976, there is a good visual correlation between methane and transmissometry (suspended matter).

The correlation of methane with other physical and biological parameters is shown in Figure 9. There is a good correlation in this figure between methane and ATP. The 50-m methane and ATP maximum is also associated with a suspended matter maximum (transmissometry). The ATP maxima are not associated with corresponding chlorophyll maxima, possibly indicating that these ATP maxima are bacterially derived. This figure seems to indicate that methane is being formed by bacteria in micro-reducing environments associated with the suspended material maximum.

Areal Distribution of Olefins

The concentrations of unsaturated hydrocarbons (*e.g.*, ethene and propene) were measured in surface and near-bottom samples at the twelve primary stations and two bank stations. Ethene was also measured at some intermediate depths by McAullife's (1971) method. The number of observations, mean, minimum, and maximum values for the olefins are shown in Table 2. The unsaturates dominate over their saturated analogs in most areas of the STOCS, with exceptions generally occurring in the deeper waters of Station 3 along the four transects. Ethene averaged 4.6 nl/l in the STOCS area, but concentrations as high as 25 nl/l were measured. Propene concentrations were almost always a factor of 5 lower than ethene concentrations, only averaging 0.9 nl/l in the STOCS area in 1976.

Unlike methane, the exchange of olefins across the air-sea interface in the STOCS region is more speculative. This is because there are no well-established atmospheric partial pressures for ethene and propene.

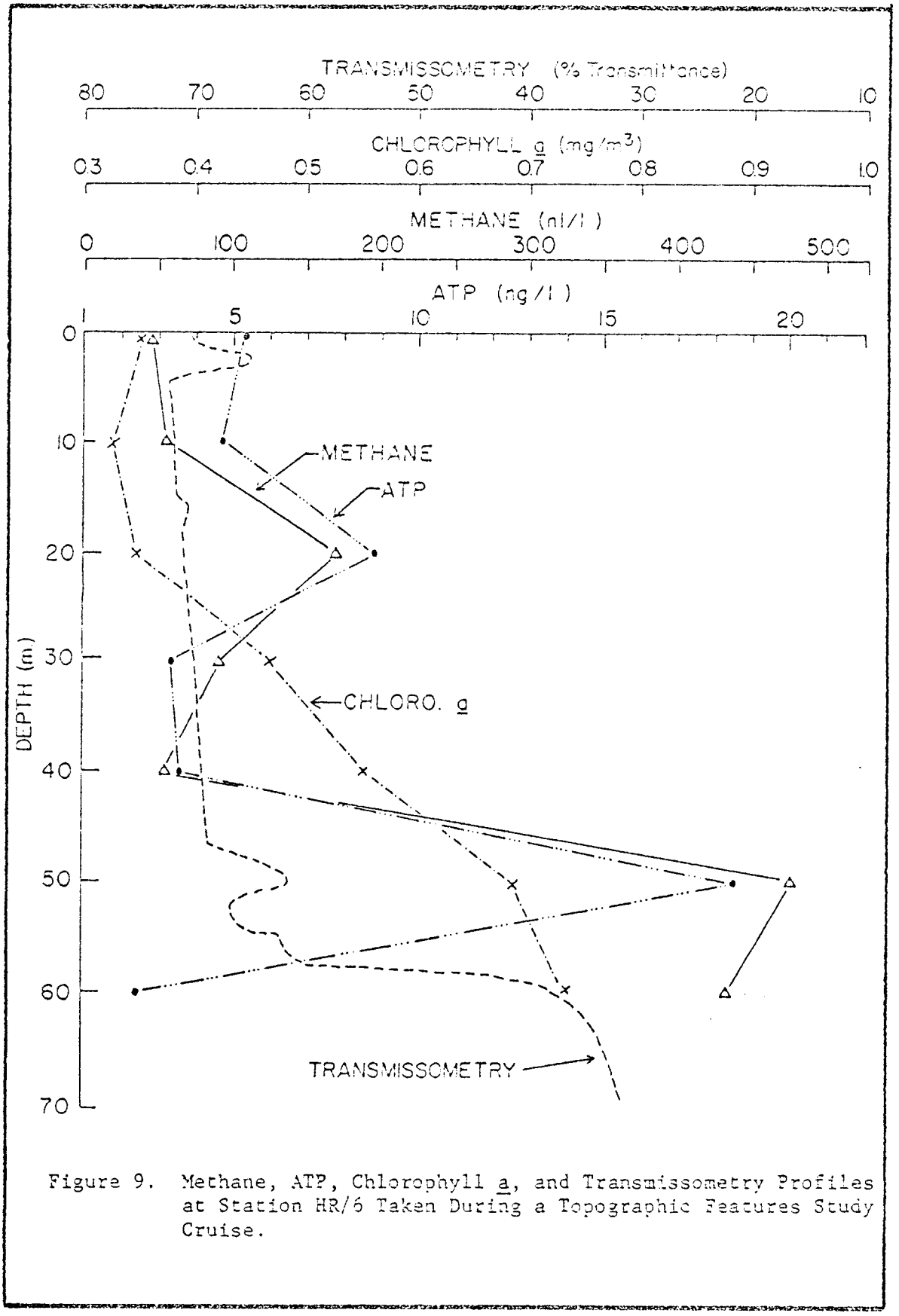

Figure 9. Methane, ATP, Chlorophyll a, and Transmissometry Profiles at Station HR/6 Taken During a Topographic Features Study Cruise.

Also, solubility data at oceanic temperatures and salinities do not exist in the literature. However, since oxidation of the olefins occurs within hours in the atmosphere, all the olefins found in the surface waters of the Gulf must be biologically derived and/or introduced through losses from refined products during transportation or manufacturing operations. It is therefore assumed that the direction of olefin exchange across the air-sea interface is from the ocean to the atmosphere.

Figures 10 and 11 show near-surface concentrations of ethene and propene, respectively, during the seasonal cruises in the STOCS area in 1976. These figures indicate that the highest concentrations of olefins occurred at inshore stations during the winter seasonal cruise. The opposite trend was observed during the spring and fall seasonal samplings; highest surface concentrations were observed at offshore stations. There did not appear to be any overall north-south trend in olefin levels in the STOCS area. The trends observed probably reflect biological productivity patterns, since olefins are known to be metabolic intermediates.

Tables 5 and 6 show the number of observations, the average mean water column concentrations, the average surface concentrations, and the minimum and maximum values found during each of the nine seasonal and monthly samplings for ethene and propene, respectively. The mean values in these tables show a general trend of low concentrations in the winter months with higher concentrations in the spring, summer and fall. These trends emulate seasonal productivity measurements.

Vertical Distribution of Olefins

Since the Bureau of Land Management contract specified only surface

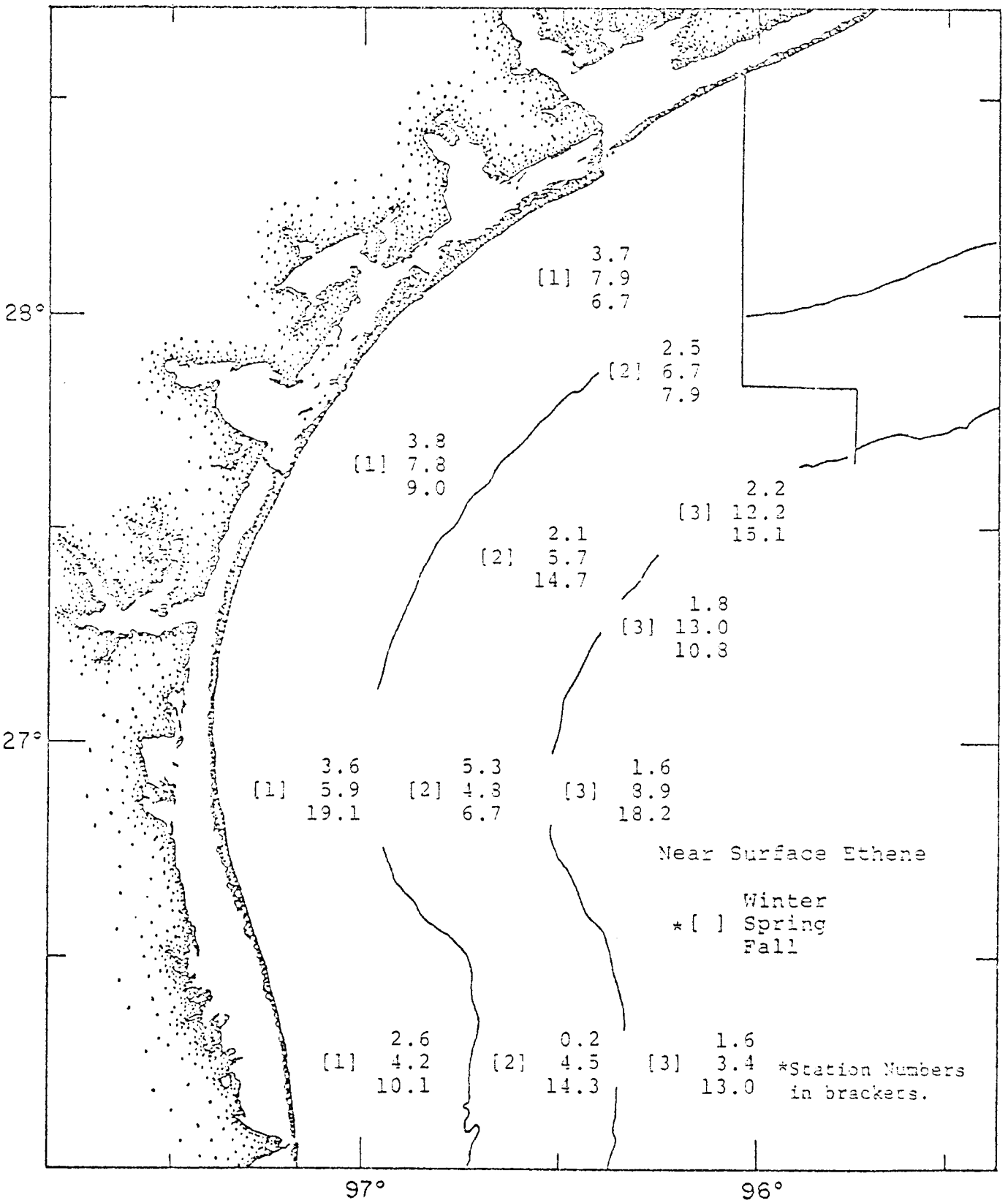

Figure 10. Near-Surface Ethene Concentrations (nl/l) in the STOCS Area During the Seasonal Cruises in 1976.

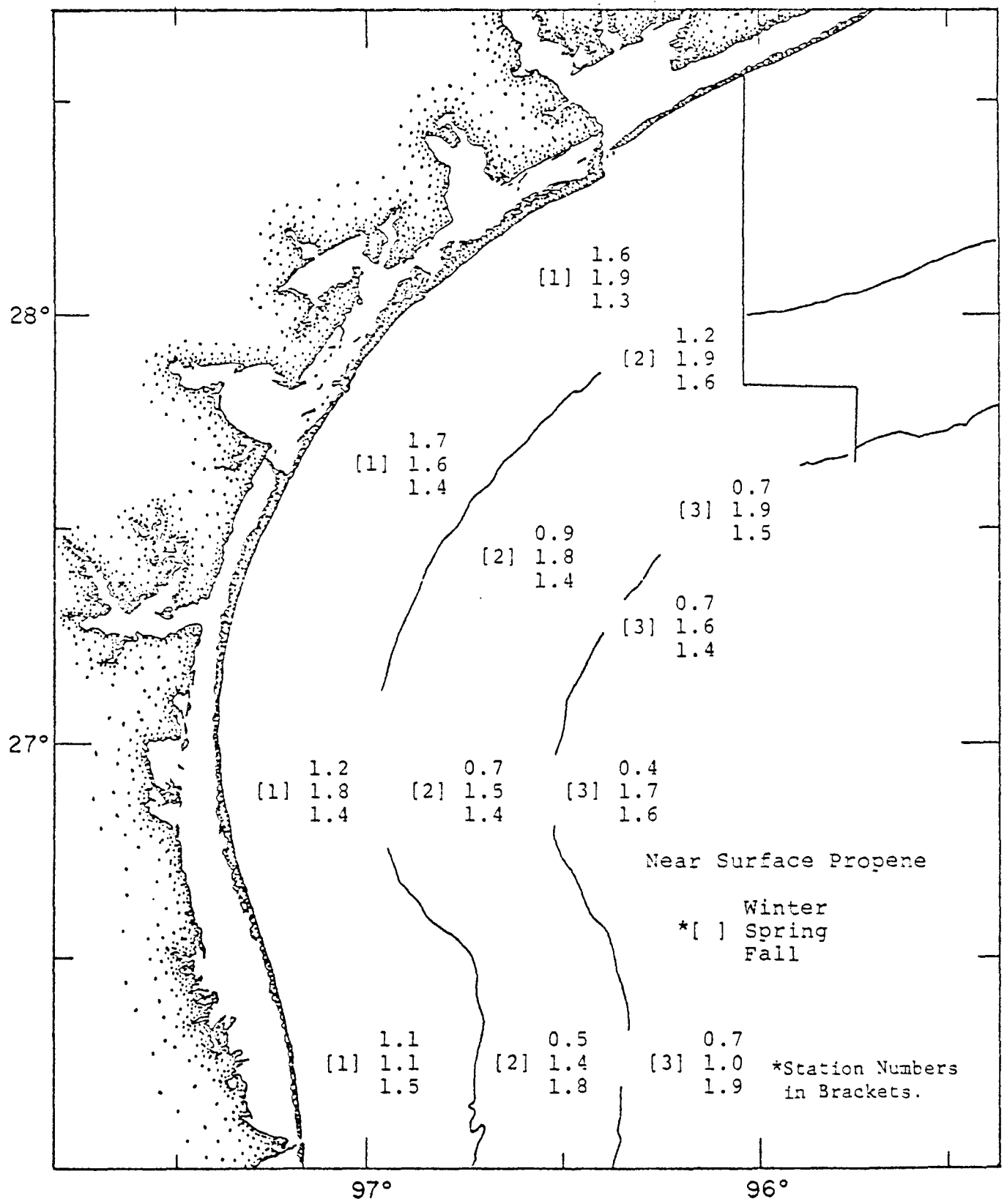

Figure 11. Near-Surface Propene Concentrations (nl/l) in the STOCS Area During the Seasonal Cruises in 1976.

TABLE 5

SUMMARY OF NUMBER OF ETHENE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/l) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	39	2.7	0.2	9.0	2.6
March	37	2.4	0.7	7.5	2.8
April	24	3.1	0.8	25.0	7.5
Spring	90	4.6	0.7	13.0	8.9
July	29	6.9	1.2	23.0	5.0
August	10	6.9	0.1	11.4	9.0
Topo High	16	5.3	2.8	8.4	7.9
Fall	28	8.8	1.0	19.1	10.4
November	10	3.6	0.6	6.0	4.5
December	10	2.2	0.6	2.6	2.4

TABLE 6

SUMMARY OF NUMBER OF PROPENE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/l) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	24	0.8	0.2	1.7	1.1
March	10	0.5	0.2	0.8	0.6
April	10	0.7	0.1	1.3	0.9
Spring	26	1.1	0.2	1.9	1.6
July	10	0.8	0.3	1.2	1.0
August	10	1.4	0.3	2.5	2.1
Topo High	16	1.1	0.4	1.9	1.4
Fall	28	1.1	0.3	1.9	1.4
November	10	0.8	0.2	1.5	1.0
December	10	0.5	0.2	0.7	0.5

and near-bottom sampling, the vertical distribution of ethene and propene cannot be well defined in the STOCS region. Tables 7 and 8 show surface and near-bottom concentrations of ethene and propene, respectively, for each of the nine seasonal and monthly samplings of Transect II. The near-bottom samples were almost always lower than surface samples, in agreement with observations that olefins are high in surface waters but decrease rapidly with depth. Olefins were found only in trace amounts below a few hundred meters in the water column. In the water column there was generally a subsurface maximum in ethene concentrations (see Appendix K, Tables 1 through 10). The maximum was generally shallower than the methane maximum and was probably associated with phytoplankton maxima in the water column.

Distribution of C_2-C_4 Saturated Hydrocarbons

Ethane and propane concentrations were measured at the twelve primary stations and two bank stations. The number of observations, mean, minimum and maximum values for these saturated compounds are shown in Table 2. The saturated hydrocarbon concentrations were generally lower than corresponding olefins in the coastal waters of the STOCS area. Ethane and propane showed the same mean value of 0.4 nl/l in the STOCS area in 1976. Figures 12 and 13 show surface ethane and propane concentrations, respectively, during the seasonal cruises in the STOCS area in 1976. Concentrations of these LMWH components were generally higher at inshore stations and decrease seaward. They showed little seasonal variation, as seen in Tables 9 and 10, which are monthly tabulations of number of observations, mean, minimum, and maximum concentrations for ethane and propane. Butane levels were almost always below detection limits in the STOCS area.

TABLE 7

SURFACE AND NEAR-BOTTOM ETHENE CONCENTRATIONS (nl/l) AT STOCS STATIONS ALONG TRANSECT II

Sampling Month	STATION 1/II	2/II	3/II	SB	HB
		Surface			
Winter	3.8	2.1	1.8	-	-
March	4.1	2.5	3.0	1.9	2.3
April	4.8	25.0	2.4	2.6	2.5
Spring	7.8	5.7	13.0	10.2	7.9
July	3.1	4.1	5.9	5.6	6.1
August	8.4	7.0	9.8	8.2	11.4
Fall	9.0	14.9	10.8	9.1	8.3
November	4.7	2.7	4.1	5.2	6.0
December	2.3	2.6	2.4	2.3	2.4
		Near-Bottom			
Winter	2.4	1.7	0.7	-	-
March	3.7	2.6	0.7	1.9	2.6
April	2.9	2.5	0.8	2.0	2.1
Spring	4.7	2.9	0.7	3.1	3.5
July	3.5	4.6	1.2	3.0	3.8
August	-	6.4	0.1	5.9	6.5
Fall	5.9	18.6	1.0	1.7	2.5
November	3.5	3.8	0.6	2.2	3.6
December	2.1	2.3	0.6	2.4	2.4

TABLE 8

SURFACE AND NEAR-BOTTOM PROPENE CONCENTRATIONS (nl/l) AT STOCS STATIONS ALONG TRANSECT II

Sampling Month	STATION 1/II	2/II	3/II	SB	HB
		Surface			
Winter	1.7	0.9	0.7	-	-
March	0.8	0.5	0.6	0.6	0.5
April	1.3	1.3	0.6	0.6	0.8
Spring	1.6	1.8	1.6	-	1.5
July	1.0	0.8	1.2	1.1	1.0
August	2.1	1.7	2.5	2.1	2.3
Fall	1.4	1.4	1.4	1.6	1.3
November	1.5	0.8	0.9	1.0	0.9
December	0.4	0.7	0.5	0.5	0.6
		Near-Bottom			
Winter	0.8	0.6	0.2	-	-
March	0.8	0.5	0.2	0.4	0.5
April	0.8	0.6	0.1	0.5	0.5
Spring	1.1	0.6	0.3	0.5	0.6
July	1.0	0.6	0.4	0.3	0.4
August	-	0.5	0.3	0.4	0.3
Fall	1.4	0.8	0.4	0.3	0.4
November	1.2	1.0	0.2	0.3	0.5
December	0.5	0.6	0.2	0.5	0.5

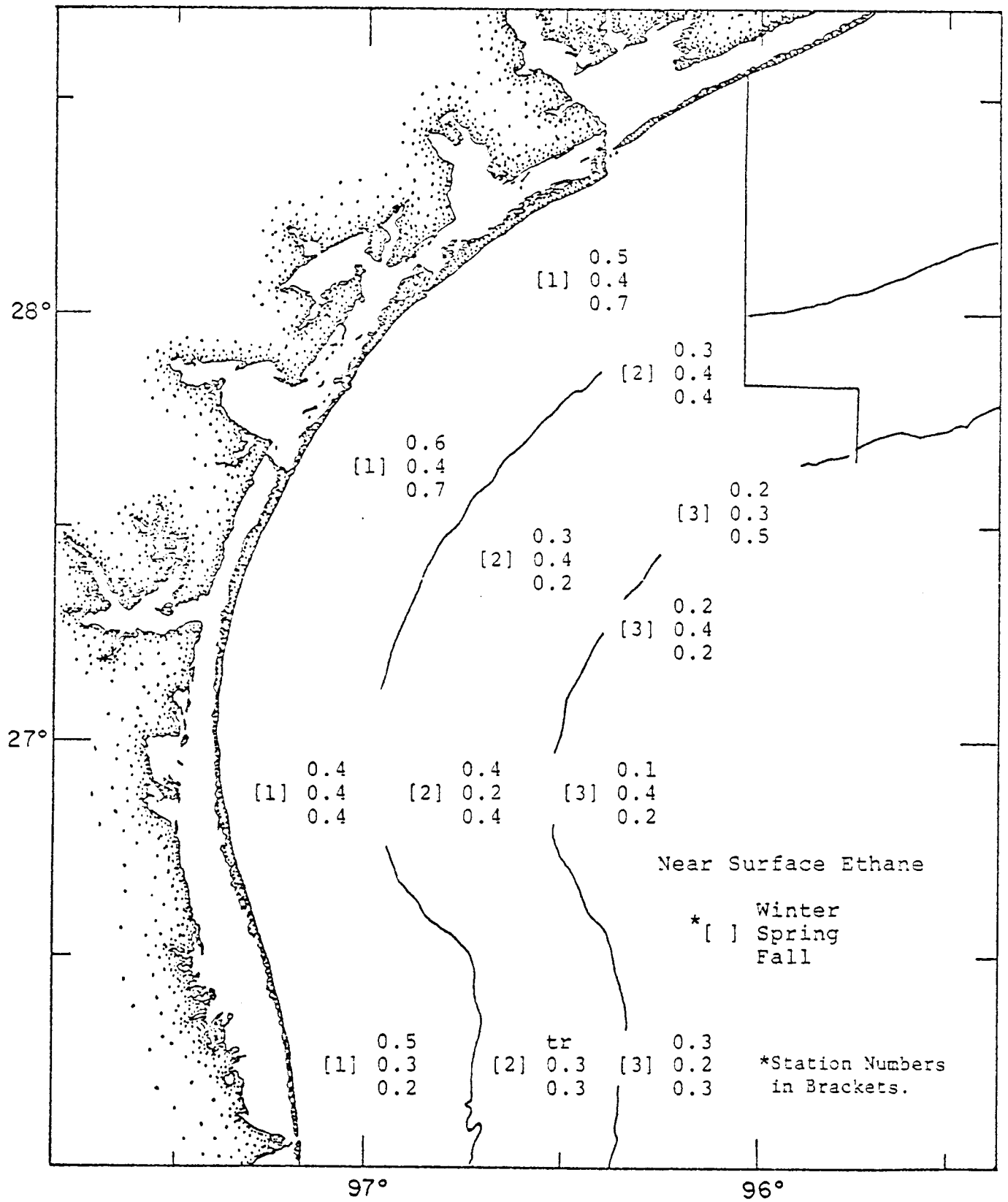

Figure 12. Near-Surface Ethane Concentrations (nl/l) in the STOCS Area During the Seasonal Cruises in 1976.

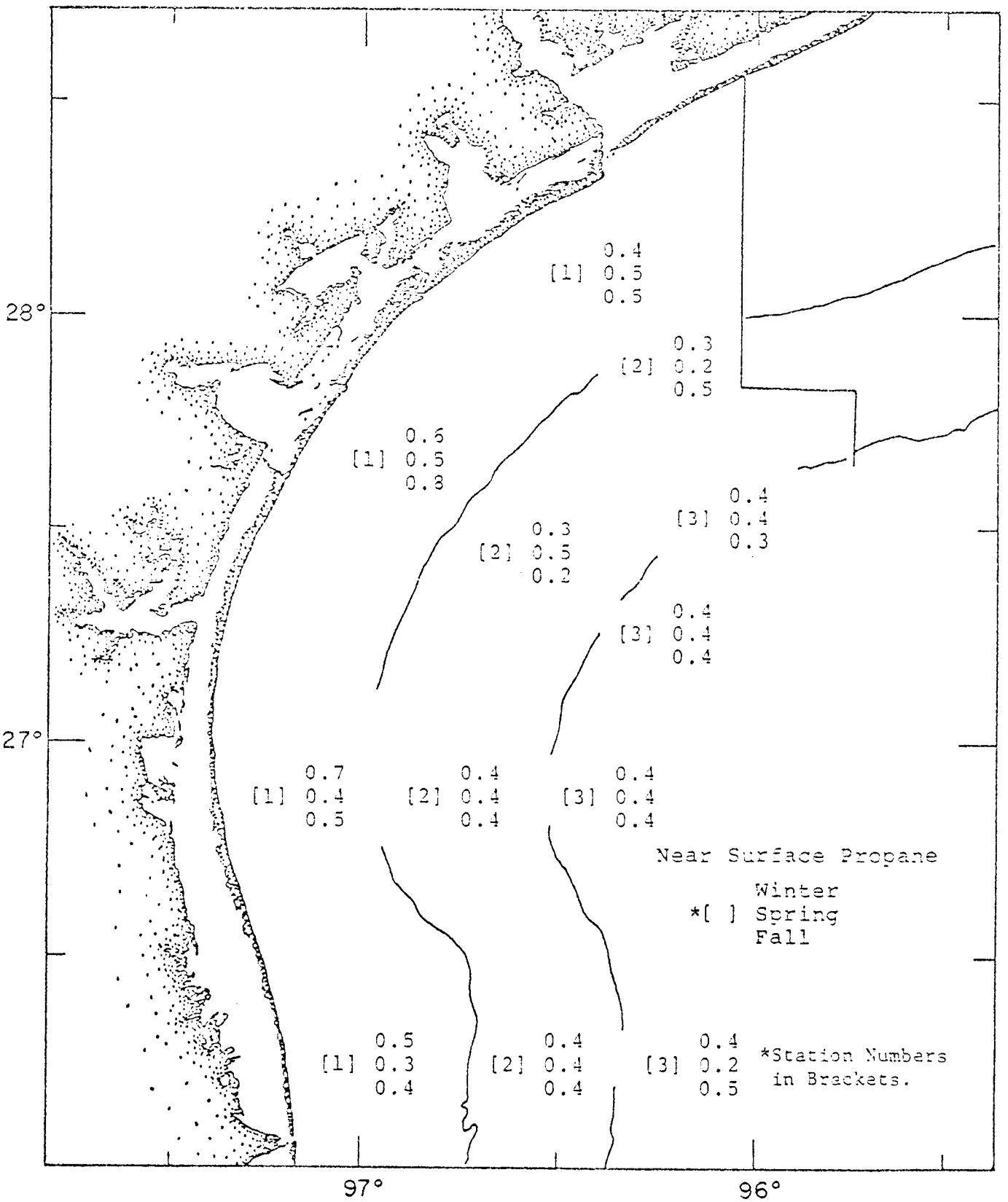

Figure 13. Near-Surface Propane Concentrations (nl/l) in the STOCS Area During the Seasonal Cruises in 1976.

TABLE 9

SUMMARY OF NUMBER OF ETHANE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/l) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	23	0.3	0.1	0.6	0.4
March	11	0.5	0.3	1.3	0.4
April	10	0.5	0.3	0.9	0.6
Spring	27	0.4	0.2	0.6	0.4
July	10	0.5	0.2	1.0	0.4
August	10	0.4	0.2	0.8	0.3
Topo High	16	0.4	0.2	0.7	0.2
Fall	28	0.4	0.2	0.9	0.3
November	10	0.5	0.4	0.6	0.4
December	10	0.5	0.4	0.8	0.6

TABLE 10

SUMMARY OF NUMBER OF PROPANE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in nl/l) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	24	0.5	0.3	0.7	0.4
March	10	0.3	0.3	0.5	0.3
April	10	0.4	0.2	0.6	0.4
Spring	26	0.4	0.2	0.7	0.4
July	10	0.5	0.3	0.8	0.4
August	10	0.5	0.3	0.6	0.4
Topo High	16	0.4	0.3	0.6	0.4
Fall	28	0.5	0.2	0.8	0.5
November	10	0.5	0.4	0.6	0.5
December	10	0.5	0.4	0.6	0.5

The saturated C_2-C_4 hydrocarbons have man-derived and natural sources similar to methane. They are derived from petroleum either from offshore platforms, transportation activities, or runoff. Although there is no evidence that they are produced *in situ* in the water column, they are found in trace amounts in gas seepage. Most biogenic gas contains small amounts (<0.5 percent) of ethene and part-per-million quantities of propane. It might be reasonable to assume that some of the long-lived anthropogenic hydrocarbons (*e.g.*, ethane and propane) found in marine atmospheres are deposited into the ocean either by rain-out or by air-sea exchange.

Tables 11 and 12 show surface and near-bottom ethane and propane concentrations along Transect II. There was no large variation in the vertical distribution of these components with depth, although bottom samples were generally higher than surface samples, probably reflecting diffusion or seepage out of the sediments.

Hydrocarbon Correlations

As mentioned in the earlier discussions, several LMWH show close correlations with other chemical and biological parameters. Table 13 shows correlations of the LMWH with each other and other selected chemical parameters measured in the study. Methane showed little correlation with other LMWH. There was a weak correlation between methane and ethane (Table 13) and little correlation between methane and propane as shown in the scatter plot in Figure 14. Since both ethene and propene are products of metabolic activities, they show a correlation coefficient of 0.65. Figure 15 shows a scatter plot of this correlation. Ethene and ethane show little correlation as shown by the scatter plot in Figure 16. Ethane and propane also show a correlation of 0.51 as shown

TABLE 11

SURFACE AND NEAR-BOTTOM ETHANE CONCENTRATIONS (nl/l) AT STOCS STATIONS ALONG TRANSECT II

Sampling Month	STATION 1/II	2/II	3/II	SB	HB
			Surface		
Winter	0.6	0.3	0.2	-	-
March	0.9	0.3	0.4	0.3	0.3
April	0.9	0.9	0.3	0.4	0.3
Spring	0.4	0.4	0.4	-	0.3
July	0.7	0.4	0.2	0.3	0.2
August	0.4	0.2	0.2	0.2	0.3
Fall	0.7	0.2	0.2	0.2	0.2
November	0.6	0.4	0.4	0.4	0.4
December	0.6	0.6	0.4	0.8	0.5
			Near-Bottom		
Winter	0.3	0.2	0.2	-	-
March	0.6	0.3	0.3	0.5	0.4
April	0.6	0.3	0.4	0.4	0.5
Spring	0.4	0.5	0.4	0.3	0.5
July	0.6	1.0	0.5	0.5	0.6
August	-	0.8	0.4	0.7	0.7
Fall	0.6	0.9	0.3	0.5	0.5
November	0.5	0.5	0.4	0.5	0.6
December	0.6	0.6	0.4	0.5	0.5

TABLE 12

SURFACE AND NEAR-BOTTOM PROPANE CONCENTRATIONS (nl/L) AT STOCS STATIONS ALONG TRANSECT II

Sampling Month	STATIONS 1/II	2/II	3/II	SB	HB
		Surface			
Winter	0.6	0.3	0.4	-	-
March	0.4	0.3	0.3	0.3	0.3
April	0.6	0.5	0.2	0.3	0.3
Spring	0.5	0.5	0.4	-	0.4
July	0.5	0.3	0.4	0.4	0.3
August	0.6	0.3	0.4	0.4	0.5
Fall	0.8	0.2	0.4	0.4	0.5
November	0.6	0.5	0.4	0.4	0.5
December	0.5	0.6	0.4	0.6	0.5
		Near-Bottom			
Winter	0.3	0.3	0.6	-	-
March	0.5	0.3	0.4	0.4	0.3
April	0.5	0.3	0.5	0.4	0.3
Spring	0.4	0.5	0.5	0.4	0.5
July	0.5	0.8	0.7	0.5	0.6
August	-	0.6	0.6	0.4	0.5
Fall	0.6	0.5	0.4	0.5	0.5
November	0.5	0.5	0.5	0.5	0.5
December	0.5	0.5	0.5	0.5	0.4

TABLE 13

CORRELATION COEFFICIENTS[+] ON STOCS 1976 DATA

Variable	Methane	Ethene	Ethane	Propene	Propane
Methane	1.0	-	0.426	-	-
Ethene		1.0	-	0.648	-
Ethane	0.426	-	1.0	-	0.510
Propene	-	0.648	-	1.0	-
Propane	-	-	0.510	-	1.0
Depth	-	-	-	-0.674	-
Temperature	-	0.600	-	0.659	-
Salinity	-	-	-	-0.490	-
Oxygen	-	-	-	0.401	-
Phosphate	-	-	-	-	-
Nitrate	-	-	-	0.443	-
Silicate	-	-	-	-	-

[+] Only correlations greater than 0.400 are tabulated.

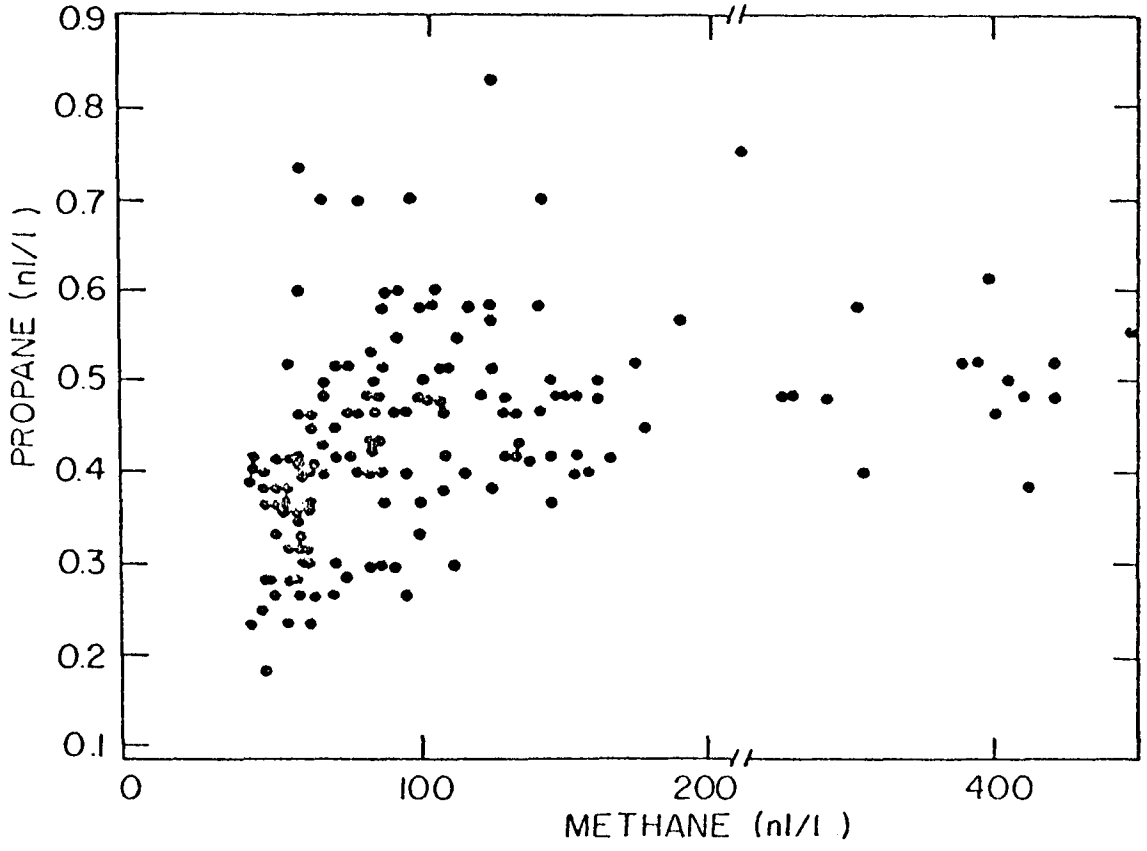

Figure 14. Scatter Plot of Propane Versus Methane for all Samples Analyzed in the STOCS Area in 1976.

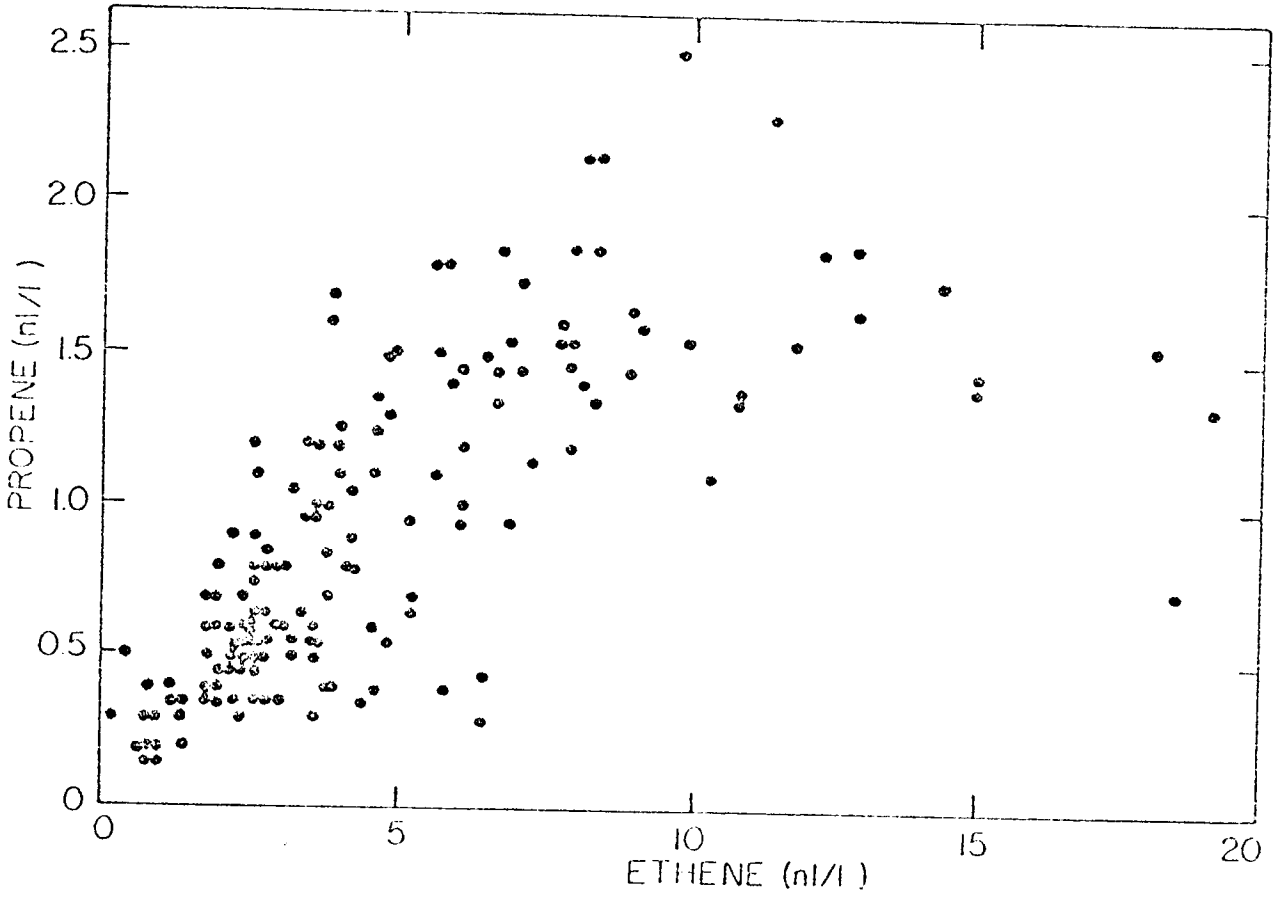

Figure 15. Scatter Plot of Propene Versus Ethene for all Samples Analyzed In the STOCS Area in 1976.

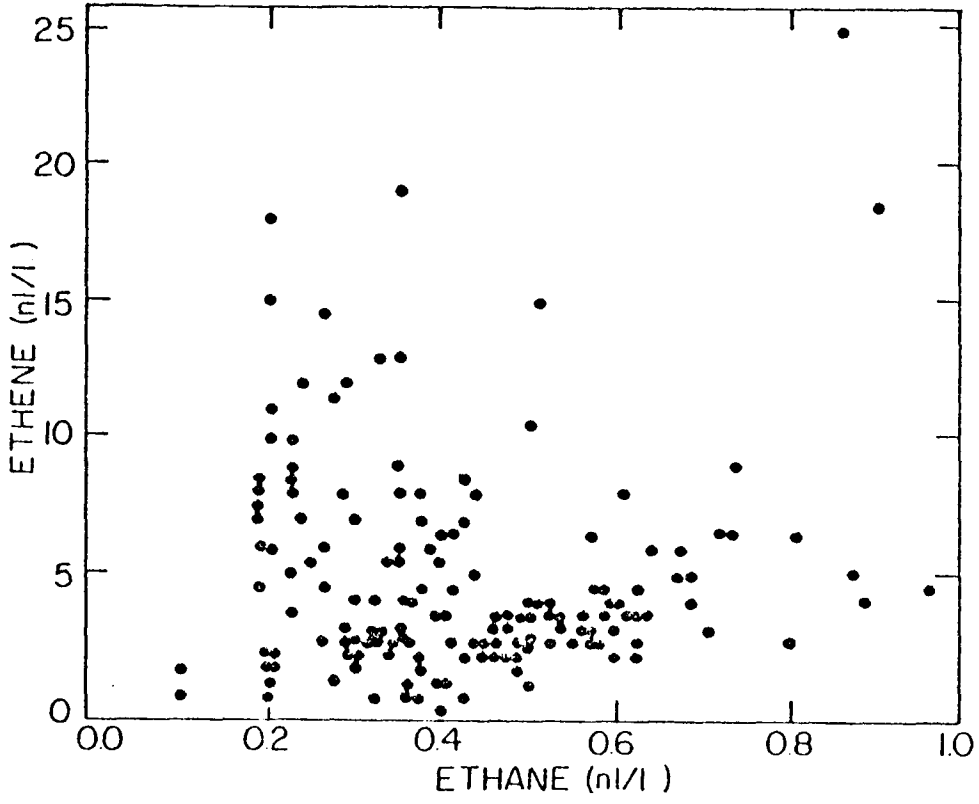

Figure 16. Scatter Plot of Ethene Versus Ethane for all Samples Analyzed in the STOCS Area in 1976.

by the scatter plot in Figure 17. Ethene and propene showed a strong correlation with temperature because biological processes are highly temperature and seasonally dependent. The strong negative correlation of propene with depth is indicative of its rapid disappearance with depth.

Although computer correlations with other biological and chemical parameters are not yet available for the 1976 data, initial computations indicate some strong correlations. Table 14 shows some LMWH correlations with productivity parameters using only winter, March and April data. Methane did not correlate with any of these productivity parameters. Ethene and ethane, however, showed some strong correlations with ATP, phytoplankton cell counts, and chlorophyll a. The correlations with propene were less significant. As mentioned earlier, methane showed strong visual correlations with transmissometry traces.

Dissolved Oxygen

Mean monthly oxygen concentrations, as well as minimum and maximum values observed in the STOCS area in 1976, are reported in Table 15. Table 16 lists surface and near-bottom dissolved oxygen values measured seasonally and monthly at the three primary stations on Transect II and the two banks. The surface concentrations at all stations were highest in the winter months and decreased in the summer, reflecting seasonal changes in surface temperature and salinities (Figures 18 and 19). Gas solubility in seawater increases with decreasing water temperature so surface oxygen concentrations are seasonally controlled, as further illustrated by surface measurements from the twelve primary stations in Figure 20. Not only were surface oxygen concentrations highest in the winter at all twelve stations, but near-shore stations which are subject

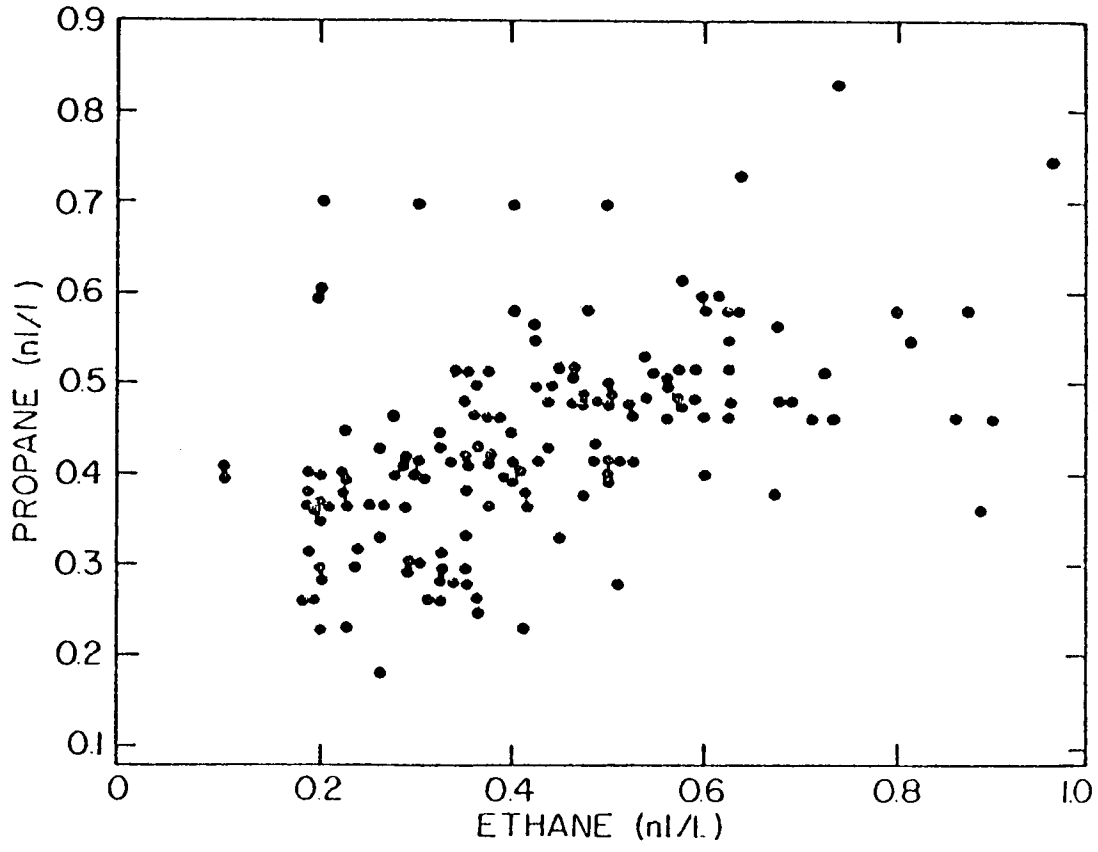

Figure 17. Scatter Plot of Propane Versus Ethane for all Samples Analyzed in the STOCS Area in 1976.

TABLE 1+

CORRELATION COEFFICIENTS[+] FOR WINTER, MARCH AND APRIL 1976 DATA

Variable	Phytoplankton Cell Counts	Chlorophyll a Net	Chlorophyll a Nanno	ATP
Methane	-	-	-	-
Ethene	0.8382	0.4631	-	-
Ethane	0.6550	0.6969	0.6618	0.7512
Propene	-	-	0.4054	0.5199
Propane	-	-	-	-

[+] Only correlations greater than 0.4000 are tabulated

TABLE 15

SUMMARY OF NUMBER OF OXYGEN OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (in ml/l) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	90	4.90	2.80	6.10	5.47
March	38	4.78	2.84	5.96	5.27
April	35	4.97	2.94	6.36	5.69
Spring	94	4.63	2.32	5.17	4.95
July	33	4.55	2.96	5.39	4.70
August	26	4.69	2.98	5.35	5.59
Topo High	24	4.44	2.88	4.92	4.69
Fall	82	4.52	2.50	5.52	4.68
November	20	5.03	2.98	5.77	5.24
December	30	4.72	2.76	5.57	4.89

TABLE 16

SURFACE AND NEAR-BOTTOM DISSOLVED OXYGEN CONCENTRATIONS (ml/L) AT SIOCS STATIONS ALONG TRANSECT II

Sampling Month	STATIONS 1/II	2/II	3/II	SB	HB
			Surface		
Winter	6.10	5.30	5.02	-	-
March	5.96	5.28	5.22	4.82	5.09
April	6.28	6.36	5.16	5.32	5.33
Spring	5.08	4.94	4.83	4.96	4.96
July	4.70	4.75	4.68	4.69	4.68
August	4.80	4.37	4.60	4.64	4.56
Fall	4.90	4.73	4.52	4.71	4.54
November	5.77	5.26	4.94	5.18	5.05
December	5.51	4.49	4.79	4.83	4.84
			Near-Bottom		
Winter	5.43	5.06	2.89	-	-
March	5.37	5.10	2.84	4.32	5.03
April	4.94	4.82	2.94	4.22	4.60
Spring	5.01	3.70	2.85	4.23	4.50
July	4.38	4.07	2.96	3.48	4.06
August	4.93	4.85	3.06	5.03	4.88
Fall	4.96	5.47	2.65	2.91	3.54
November	5.62	5.25	2.98	3.64	5.10
December	5.57	5.06	2.76	4.79	4.87

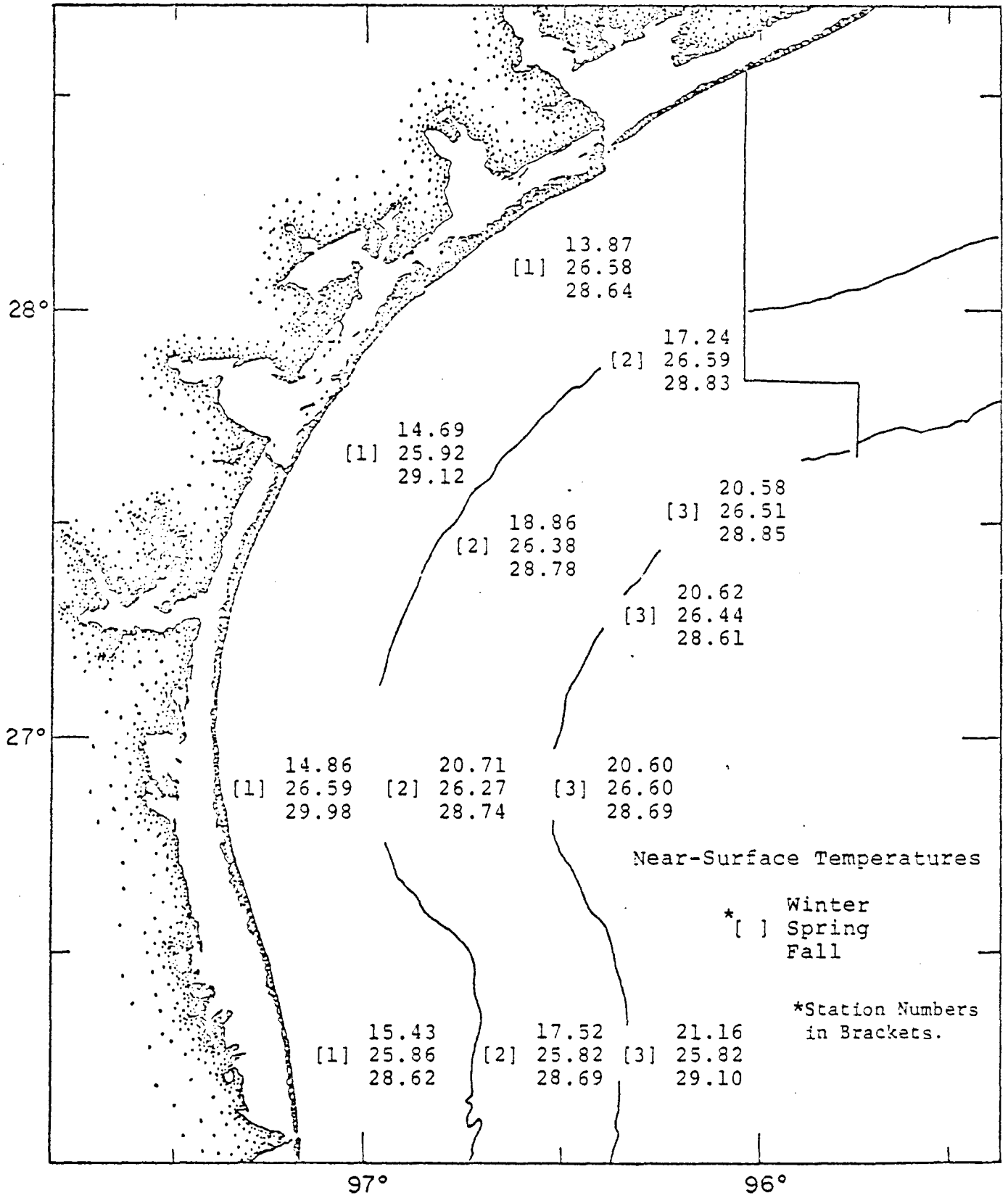

Figure 18. Near-Surface Temperatures (°C) in the STOCS Area During the Seasonal Cruises in 1976.

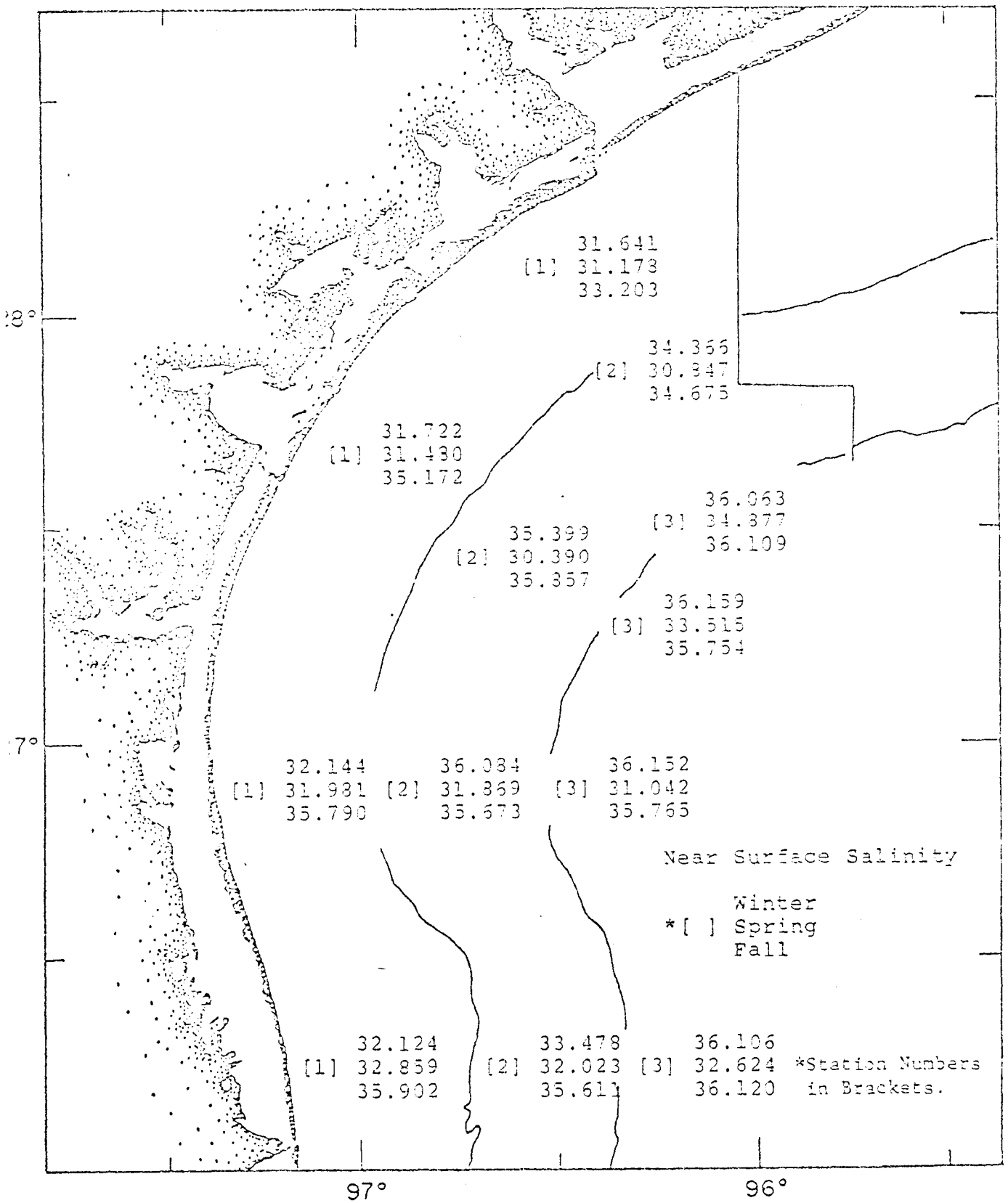

Figure 19. Near-Surface Salinities (ppt) in the STOCS Area During the Seasonal Cruises in 1976.

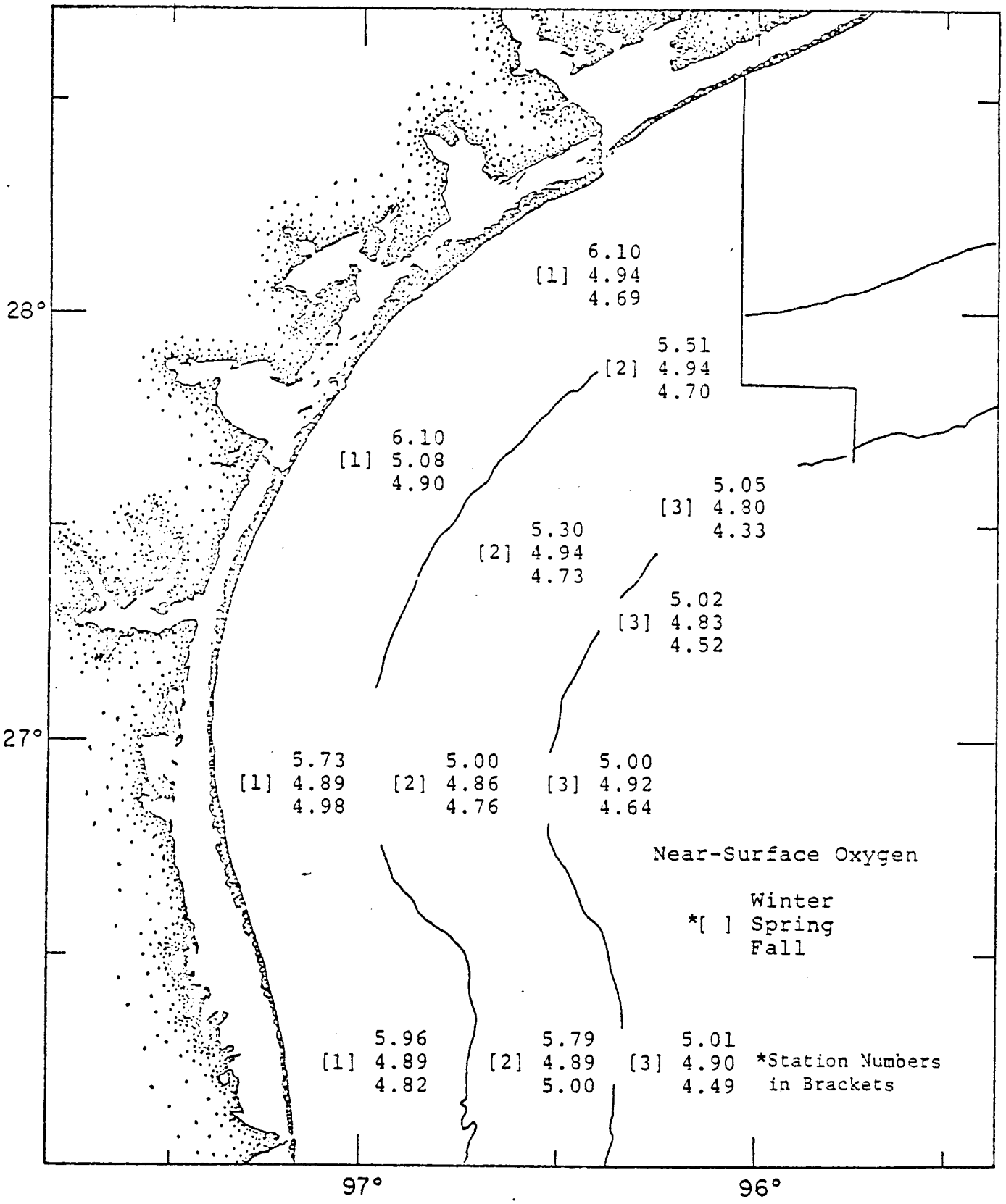

Figure 20. Near-Surface Dissolved Oxygen Concentrations (ml/l) in the STOCS Area During the Seasonal Cruises in 1976.

to more drastic seasonal temperature changes showed the greatest seasonal oxygen fluctuations. The influence of the land-mass climate on dissolved oxygen in near-shore waters is exemplified in Figure 21. Vertical oxygen profiles at three stations along Transect I show that surface water at Station 3 (furthest offshore) was relatively unaffected by winter temperatures. In contrast, Station 1, water (near-shore) was greatly affected and oxygen in surface water at Station 2 also increased. Figure 22 further illustrates that oxygen concentrations at Station 3 were relatively constant throughout the year, with a slight seasonal temperature influence on near-surface water.

Equilibrium oxygen solubilities can be calculated if the temperature and salinity of the water sample are known (Weiss, 1970). Calculated equilibrium values can be compared to measured oxygen concentrations to determine the relative influence of physical and biological processes. The ratios of measured surface oxygen values to calculated values are plotted seasonally for the twelve primary stations on Figure 23. Only small deviations from unity for all seasons indicate that fluctuations in surface oxygen concentrations were principally due to physical processes such as seasonal changes in water temperature and salinity.

Dissolved oxygen concentrations in the deeper waters (>100 m) at the outer stations ranged from 2.5 to 4.0 ml/l (Figures 21 and 22). This bottom water was characteristic of 200-300 m Western Gulf Water, with oxygen concentrations between 2.5 and 3.0 ml/l. This water was poorly mixed with the upper layer and dissolved oxygen had been gradually removed by biological processes.

Oxygen concentrations have been contoured in Figures 24 to 32 with cross-sectional maps of Transect II for each sampling period of 1976. The figures show the seasonal variations of the intrusion of oxygen-

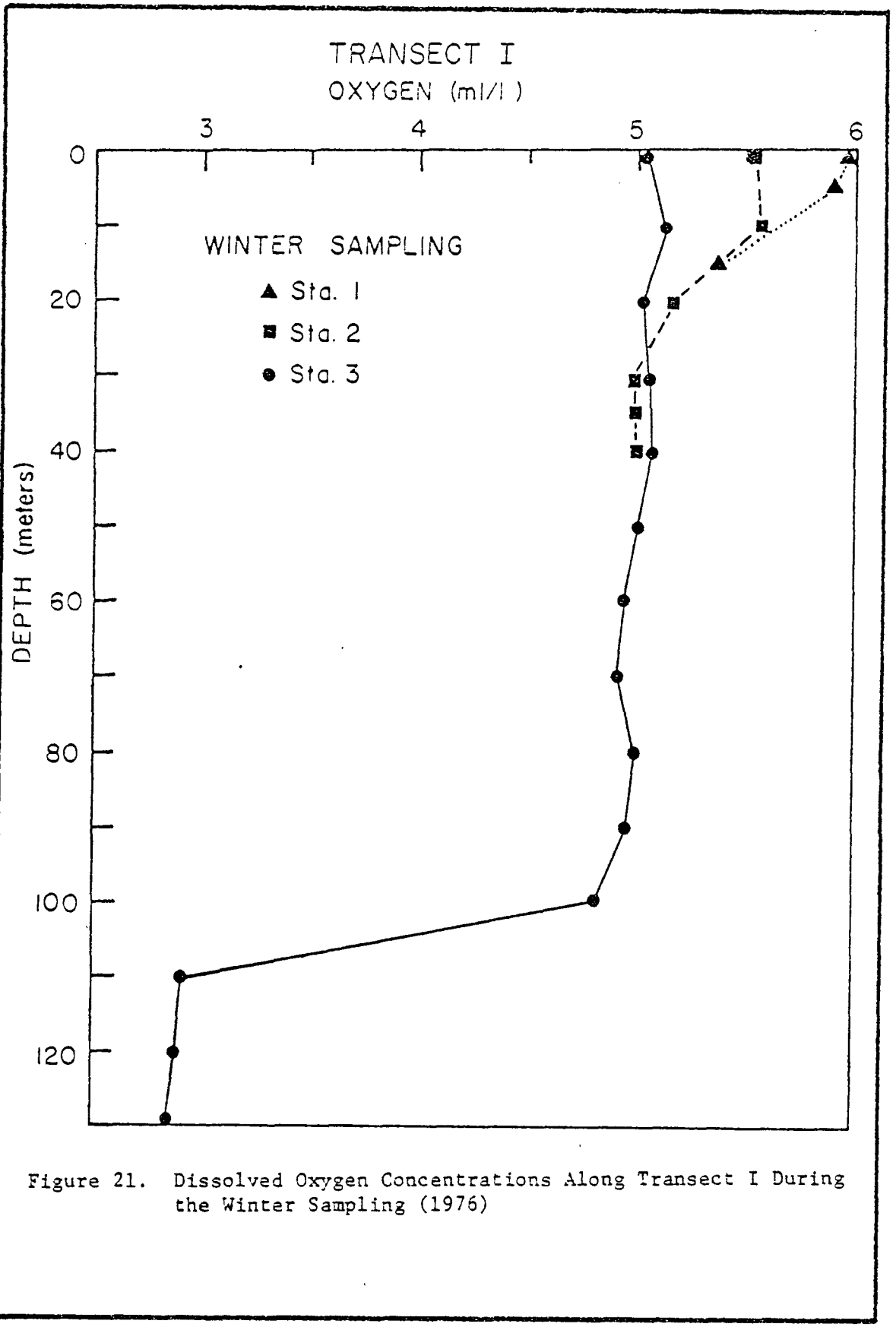

Figure 21. Dissolved Oxygen Concentrations Along Transect I During the Winter Sampling (1976)

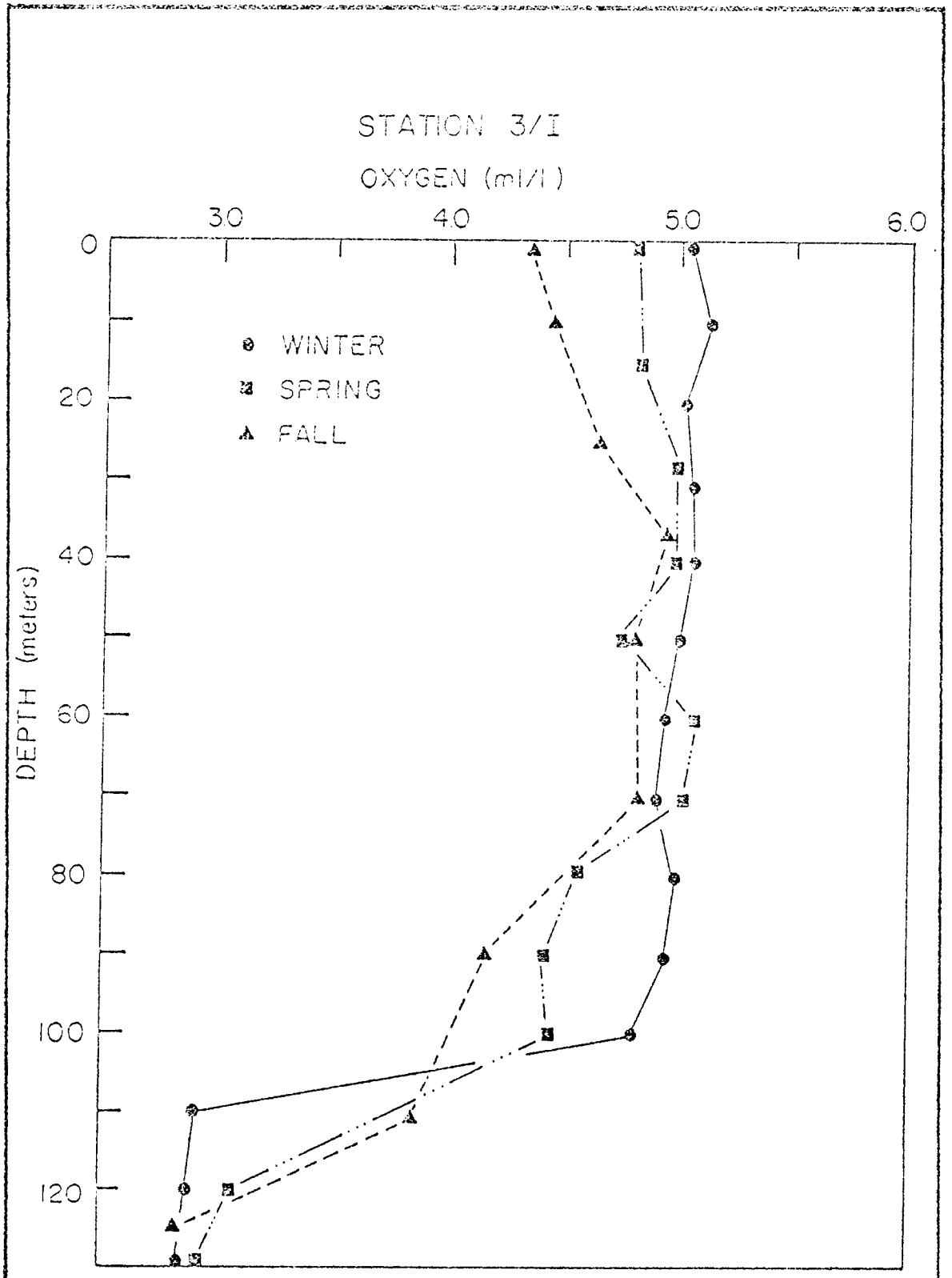

Figure 22. Dissolved Oxygen Concentrations at Station 3/I During the Three Seasonal Samplings (1976).

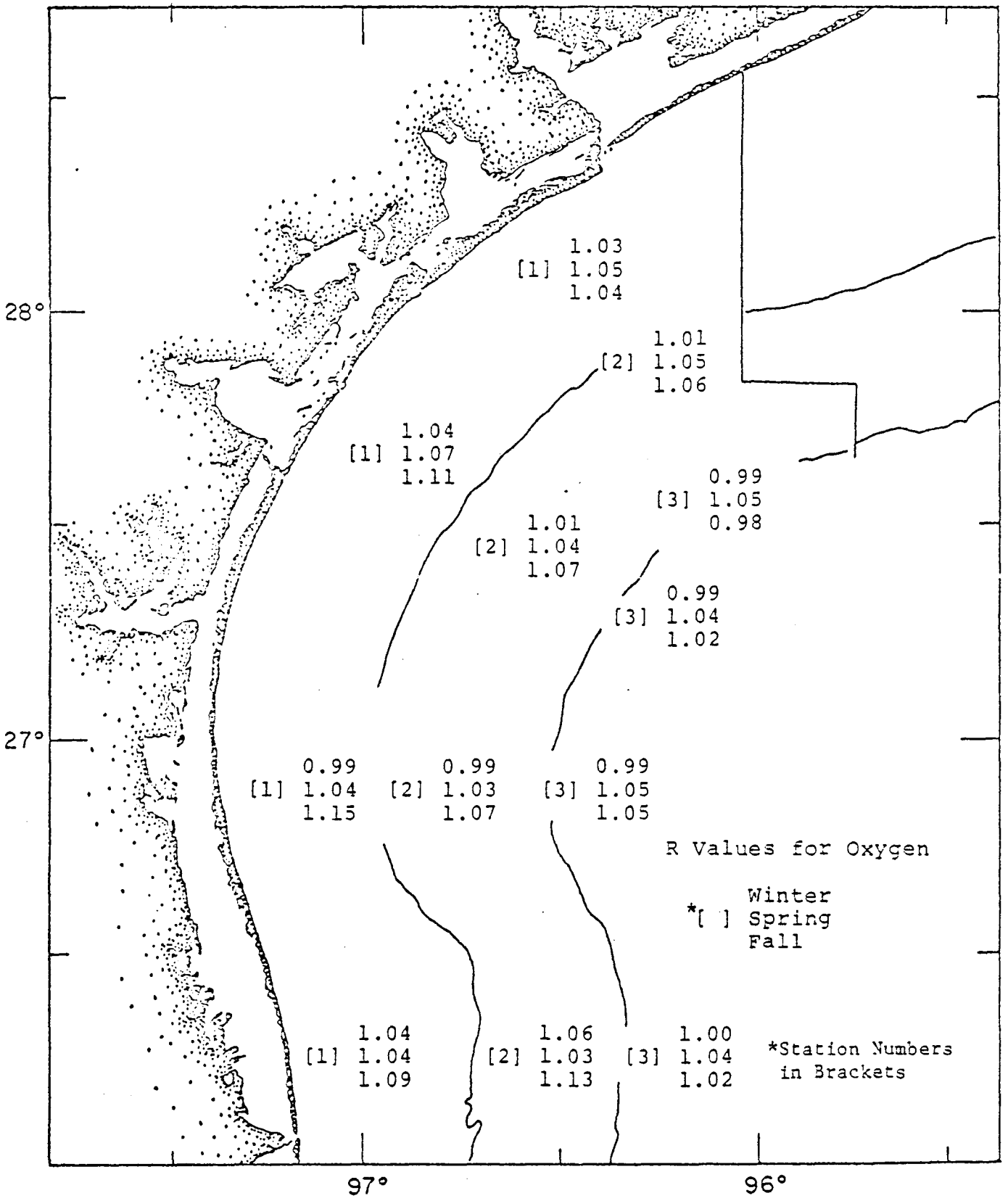

Figure 23. Near-Surface R (degree of saturation) Values for Dissolved Oxygen in the STOCS Area During the Seasonal Cruises.

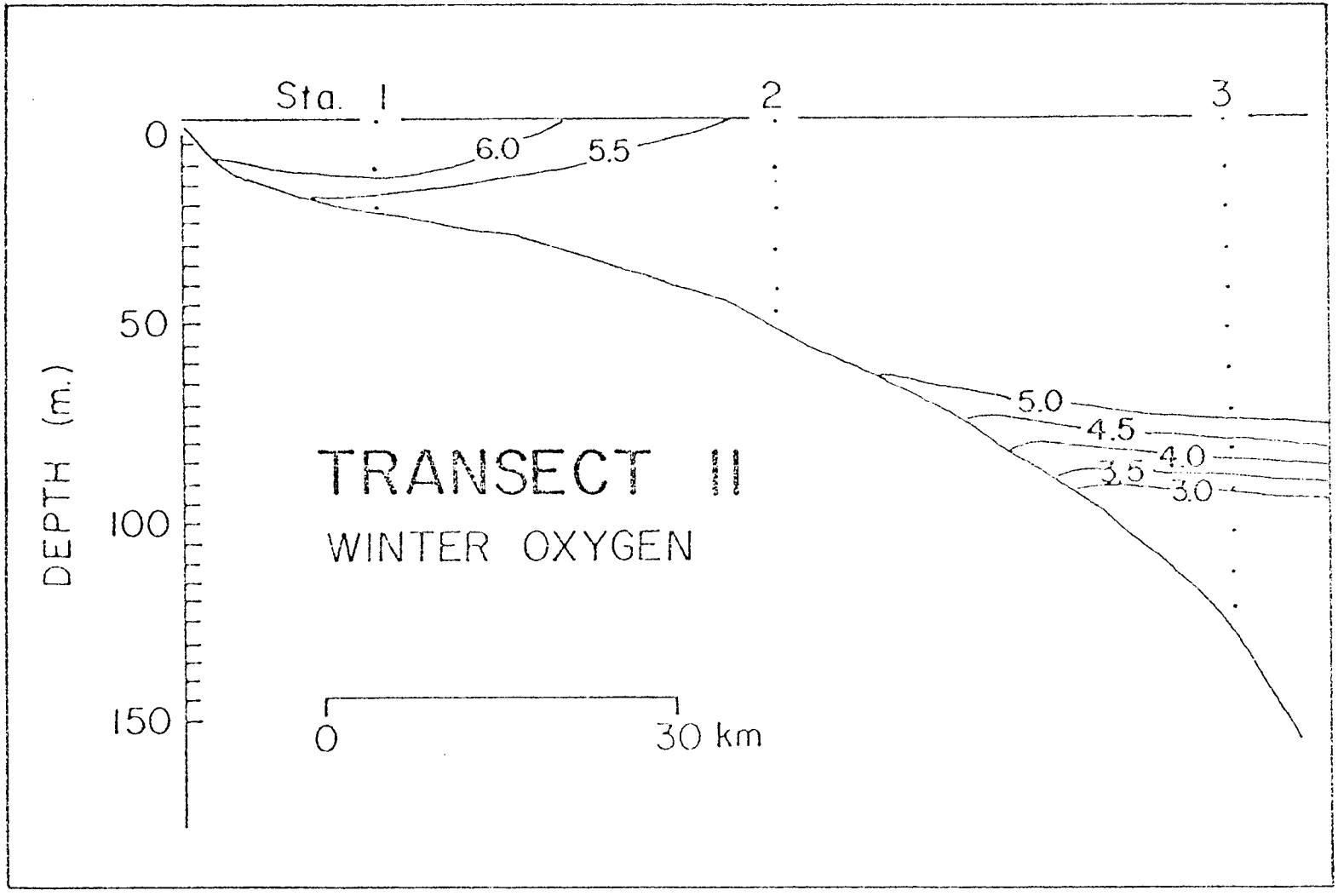

Figure 24. Dissolved Oxygen (ml/l) Cross-sectional Contours Along Transect II During the Winter Seasonal Sampling.

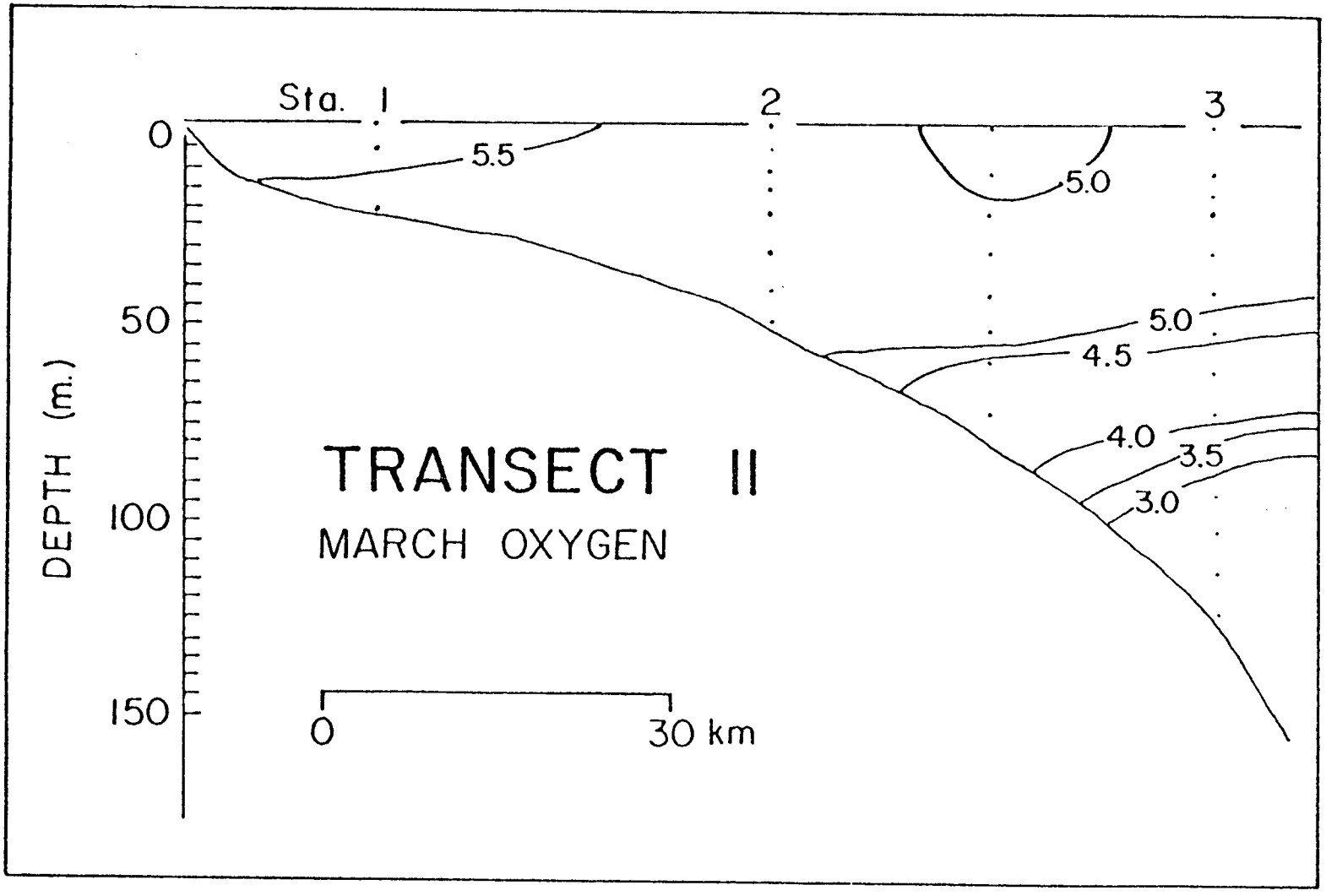

Figure 25. Dissolved Oxygen (ml/l) Cross-sectional Contours Along Transect II During the March Monthly Sampling.

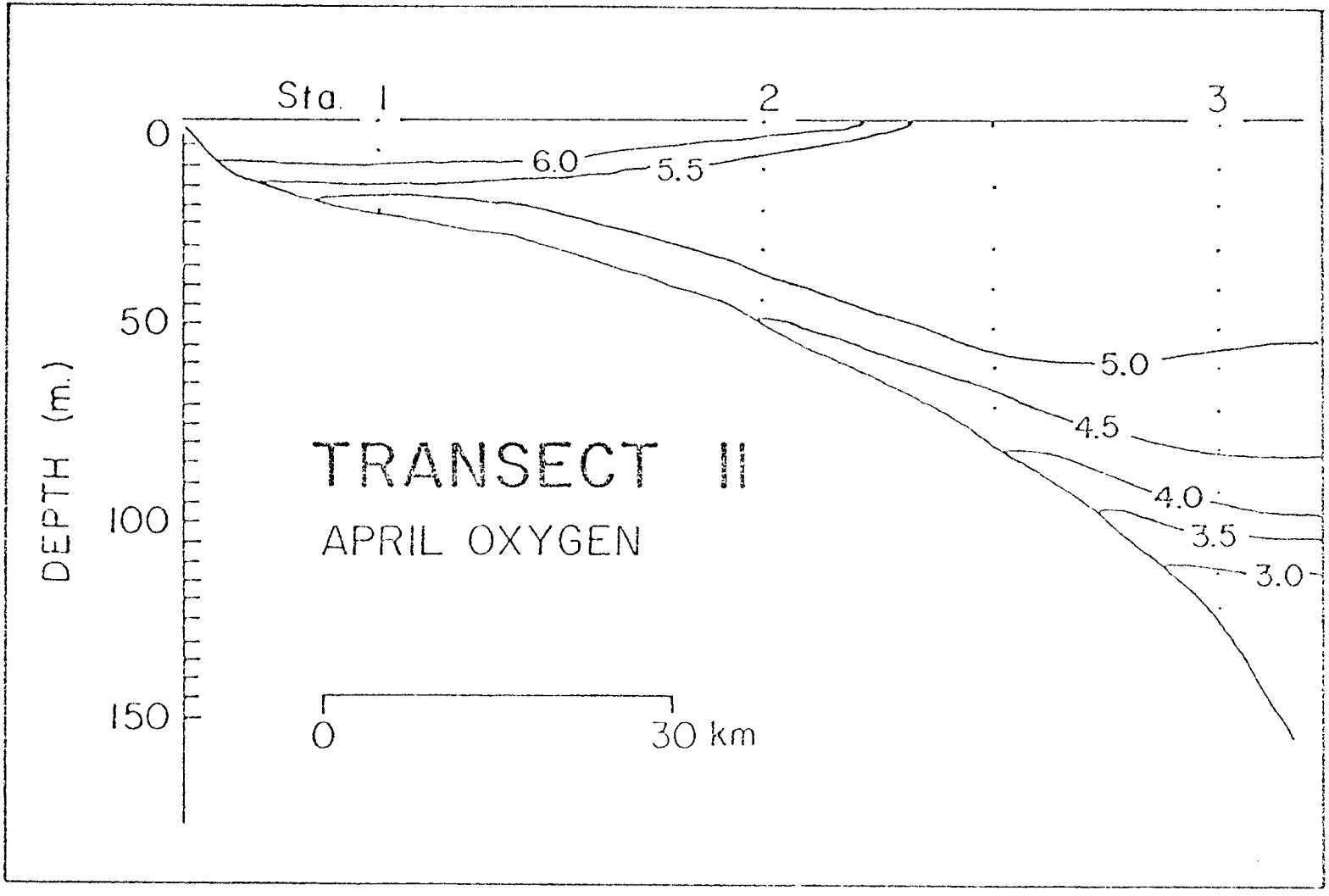

Figure 26. Dissolved Oxygen (ml/l) Cross-sectional Contours Along Transect II During the April Monthly Sampling.

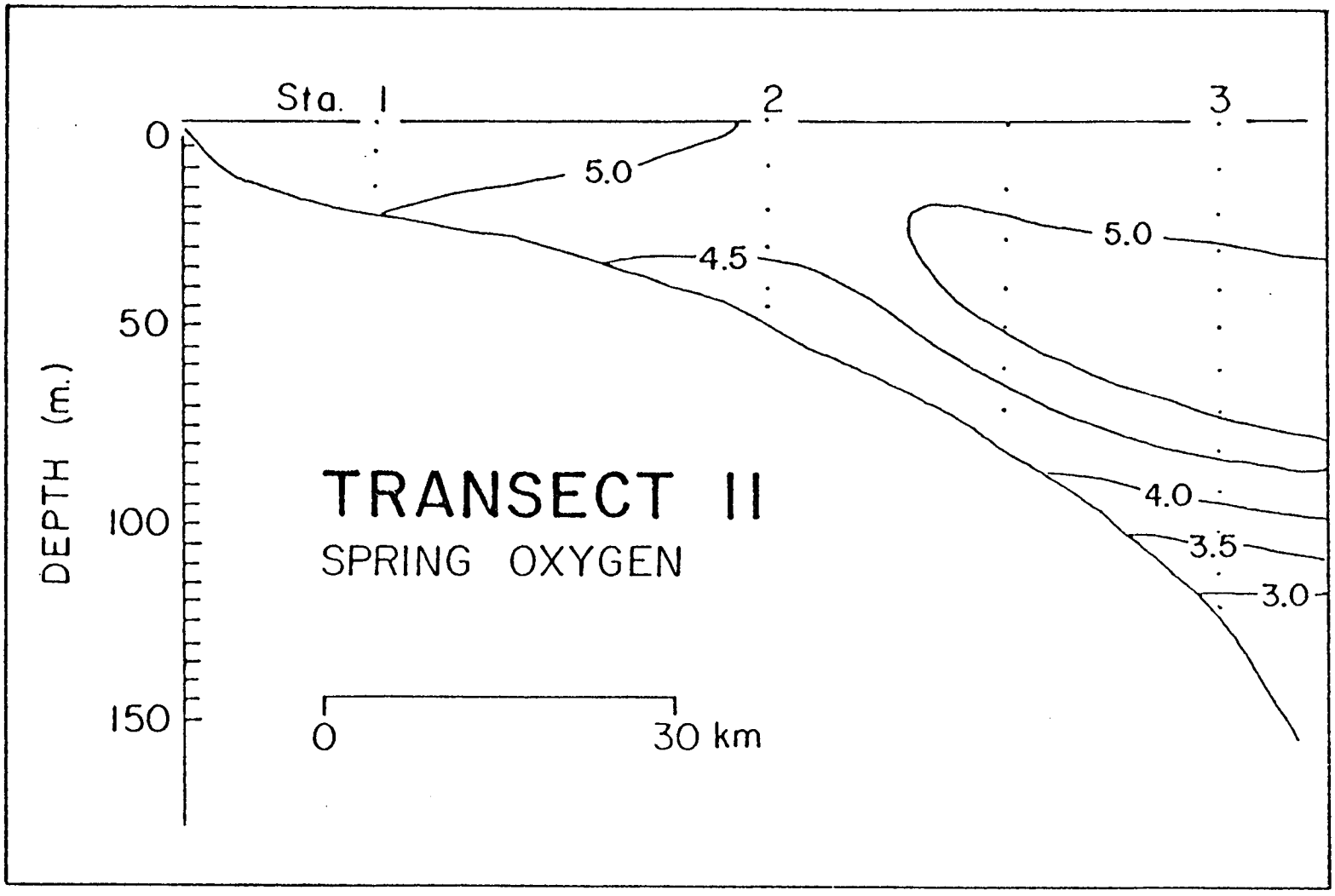

Figure 27. Dissolved Oxygen (ml/l) Cross-sectional Contours Along Transect II During the Spring Seasonal Sampling.

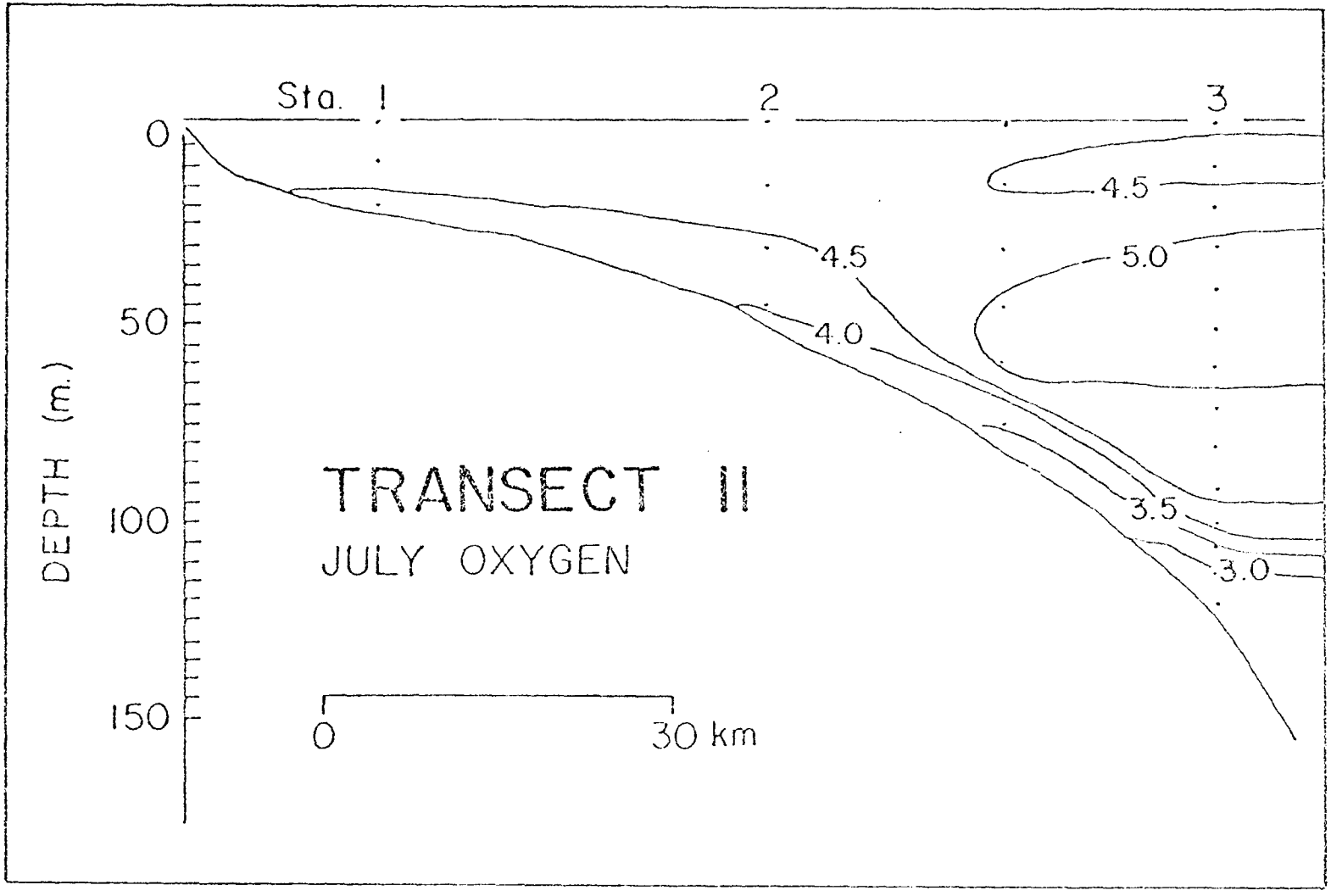

Figure 28. Dissolved Oxygen (ml/l)Cross-sectional Contours Along Transect II During the July Monthly Sampling.

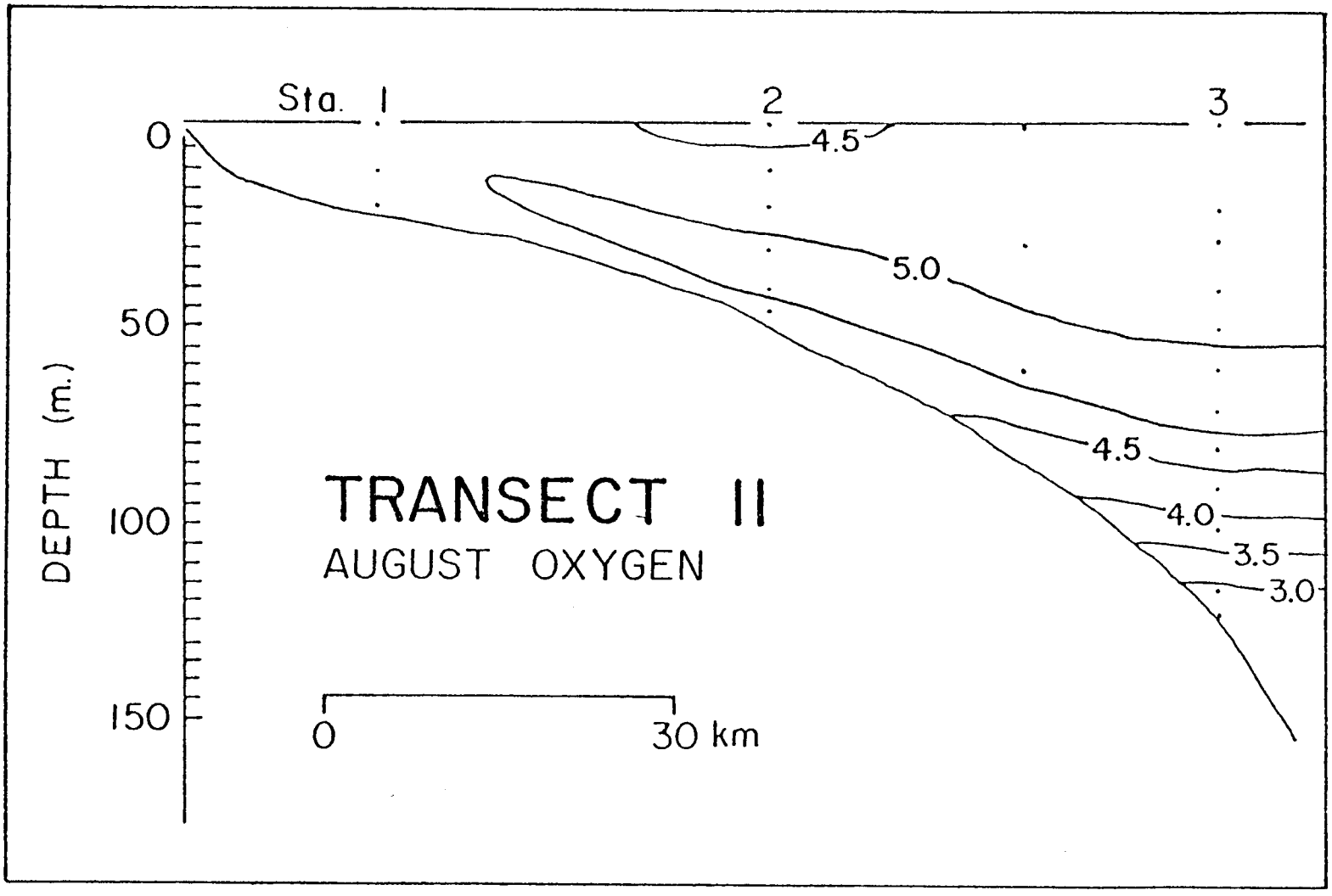

Figure 29. Dissolved Oxygen (ml/l) Cross-sectional Contours Along Transect II During the August Monthly Sampling.

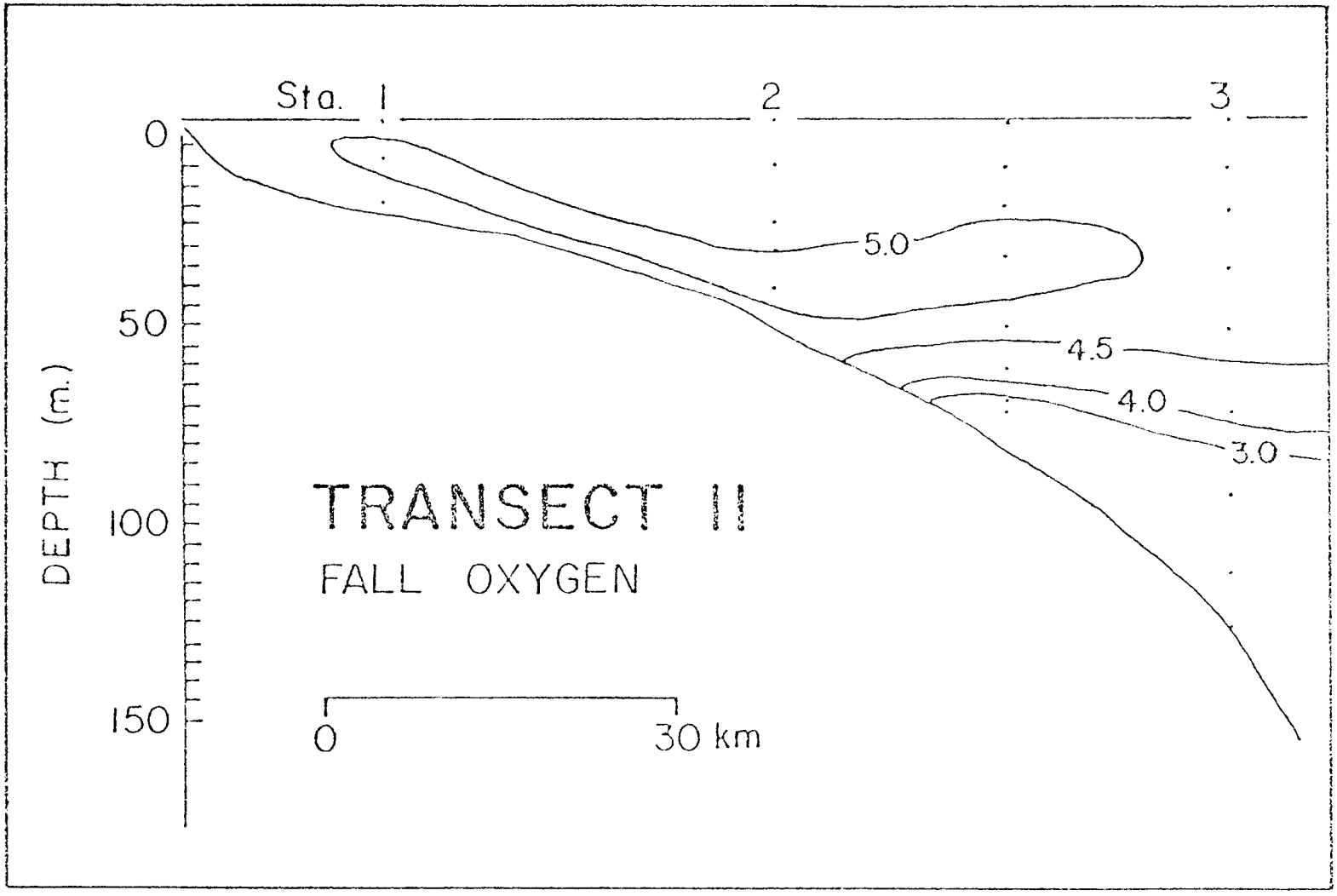

Figure 30. Dissolved Oxygen (ml/l) Cross-sectional Contours Along Transect II During the Fall Seasonal Sampling.

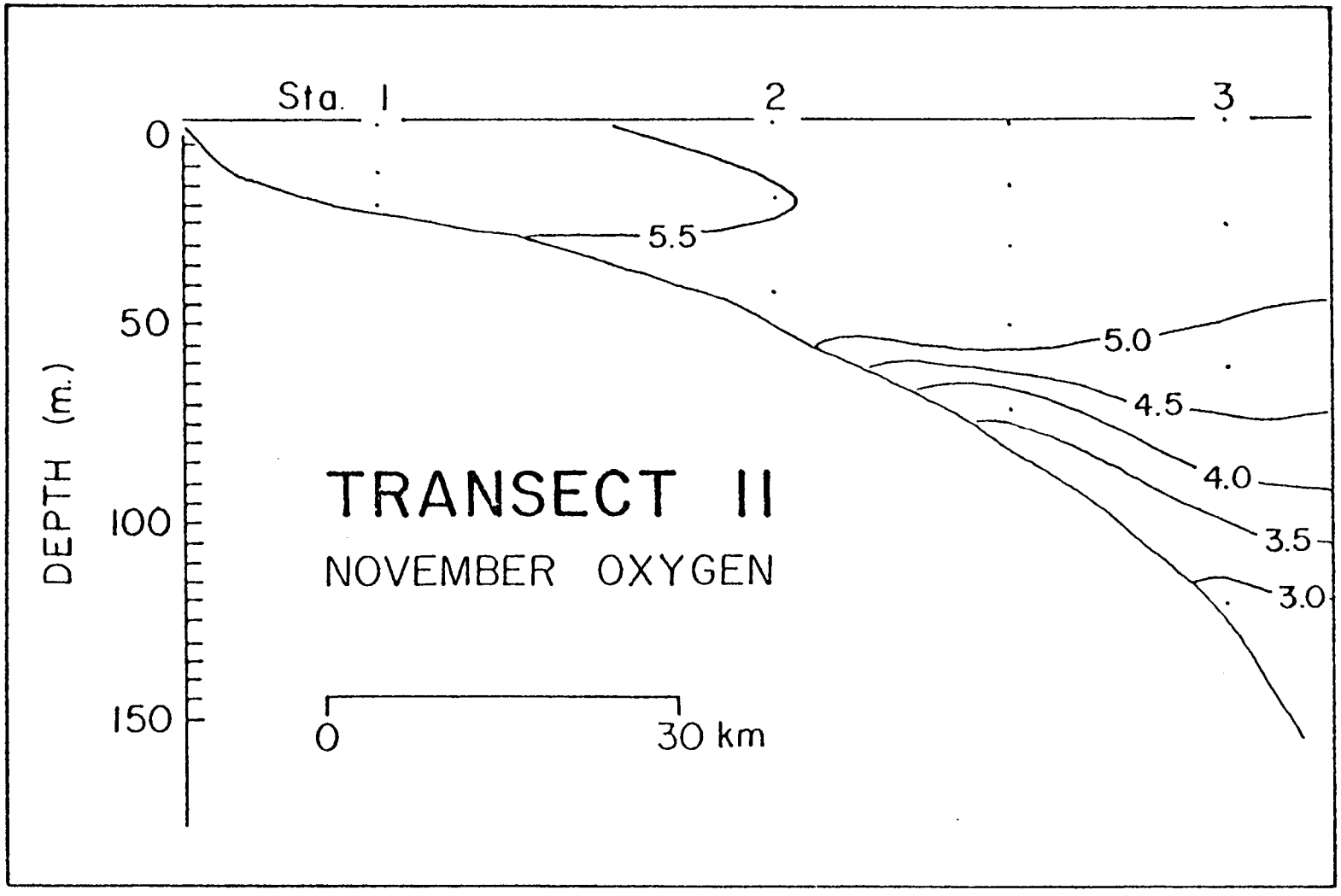

Figure 31. Dissolved Oxygen (ml/l) Cross-sectional Contours Along Transect II During the November Monthly Sampling.

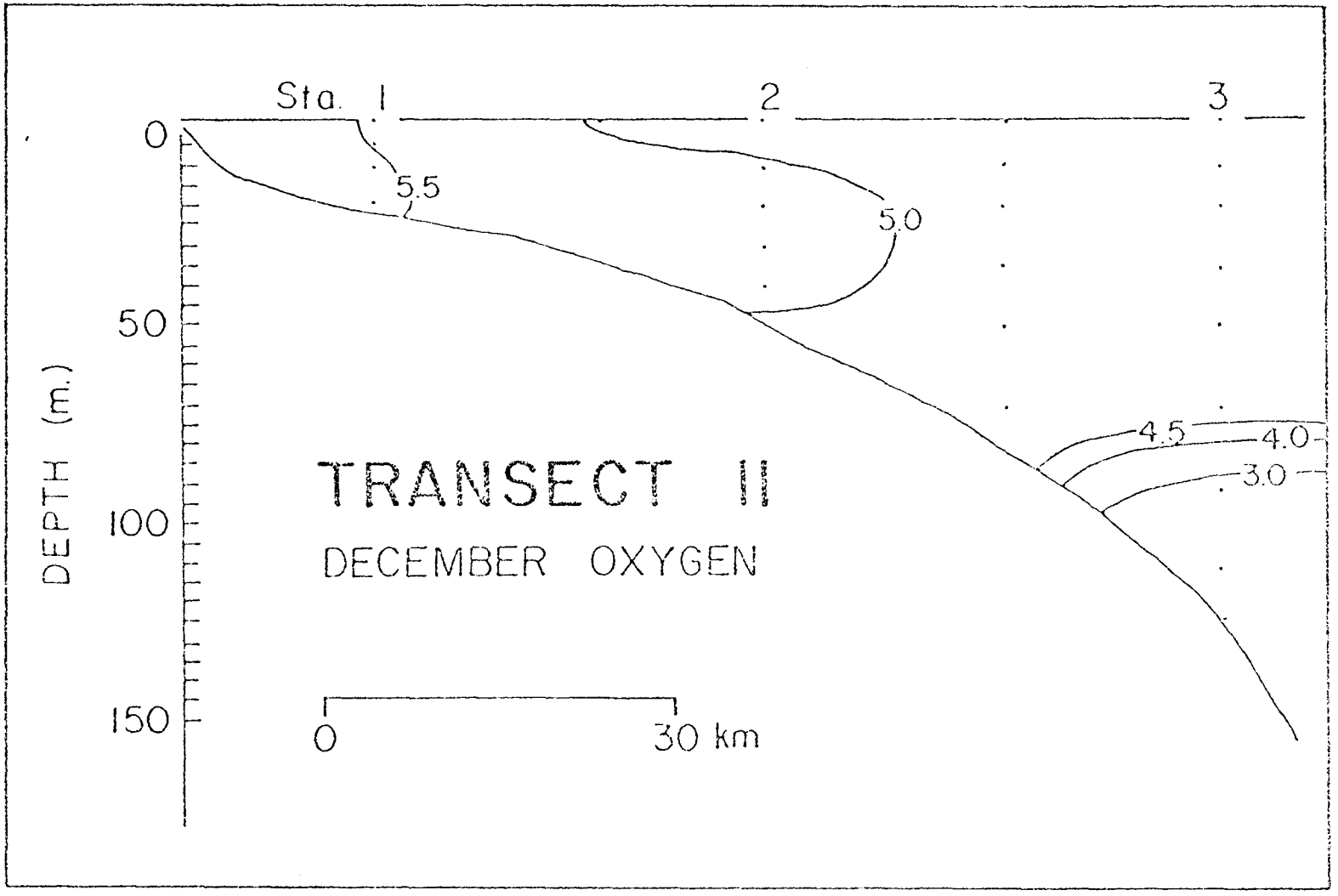

Figure 32. Dissolved Oyxgen (ml/l) Cross-sectional Contours Along Transect II During the December Monthly Sampling.

TABLE 17

SUMMARY OF NUMBER OF NITRATE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (μM) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	56	1.2	0.1	15.0	0.1
March	25	1.4	0.1	15.2	0.2
April	25	1.4	0.1	14.3	0.2
Spring	56	1.5	0.1	13.4	0.4
July	26	0.9	<0.1	9.2	0.1
August	24	1.5	<0.1	14.3	0.1
Topo High	24	1.3	<0.1	8.7	0.1
Fall	82	2.1	<0.1	22.1	<0.1
November	20	1.6	<0.1	17.1	0.2
December	30	1.9	0.3	15.6	0.5

depleted bottom water and the increasing stratification of the water column in summer. The highly-oxygenated winter surface water can also be traced as it is formed and gradually displaced from winter to summer.

Dissolved oxygen measurements performed in 1976 are tabulated in Appendix K, Tables 11-20. The degree of saturation for each sample was calculated by computer, and shows only slight deviations from unity for all samples except the oxygen-depleted bottom-water samples. Oxygen production from the phytoplankton blooms in spring and fall did not significantly raise oxygen values, indicating that oxygen levels in the STOCS area are controlled mainly by physical processes.

Nutrients

All nutrient concentration measurements performed in 1976 are reported, along with respective temperatures, salinities, and dissolved oxygen concentrations in Appendix K, Tables 11-20. Nutrient concentrations are reported in micromolar (μM) units which are equivalent to previously used microgram-atom units. The nutrients, nitrate, phosphate, and silicate, are discussed separately.

Nitrate

Table 17 lists minimum, maximum, and mean monthly nitrate concentrations in the STOCS area for 1976. Surface values were generally lower than 0.5 μM, being typical of Gulf of Mexico near-surface water. Monthly surface and near-bottom concentrations from primary stations along Transect II and the two banks are listed in Table 18 and surface values are plotted along with monthly surface means in Figure 33. Nitrate concentrations generally increased through the early spring until June, probably reflecting increased runoff from land during spring rainfall.

TABLE 18

SURFACE AND NEAR-BOTTOM NITRATE CONCENTRATIONS (μM) AT STOCS STATIONS ALONG TRANSECT II

Sampling Month	STATIONS 1/II	2/II	3/II	SB	HB
			Surface		
Winter	0.1	0.1	0.2	-	-
March	0.1	0.1	0.4	0.2	0.2
April	0.2	0.2	0.4	0.1	0.1
Spring	0.5	0.4	0.3	0.5	0.2
July	0.1	0.1	0.1	0.1	0.2
August	0.1	<0.1	<0.1	0.1	0.2
Fall	<0.1	<0.1	<0.1	<0.1	<0.1
November	0.1	0.3	0.1	0.2	0.1
December	0.5	0.4	0.5	0.5	0.4
			Near-Bottom		
Winter	0.3	0.2	5.6	-	-
March	0.4	0.4	15.2	0.5	0.3
April	0.4	0.4	14.3	0.6	0.4
Spring	0.3	0.3	11.2	1.2	0.9
July	0.1	0.8	9.2	3.9	2.4
August	<0.1	<0.1	14.0	0.2	0.2
Fall	<0.1	<0.1	22.1	17.5	5.6
November	0.3	0.3	17.1	8.1	3.8
December	0.3	0.4	15.6	0.7	0.3

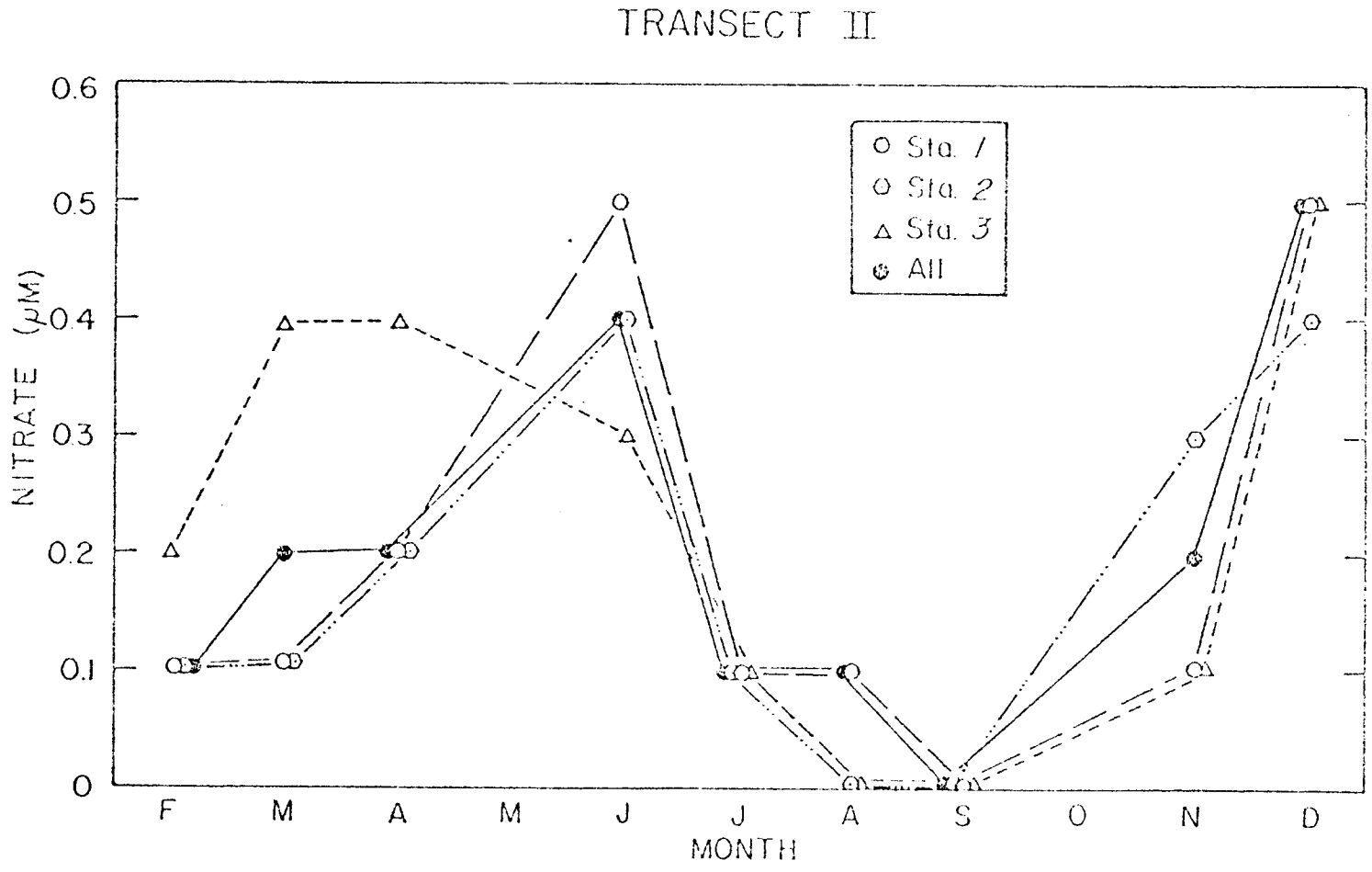

Figure 33. Monthly Variations in Nitrate Along Transect II in 1976.

Nitrate is the limiting nutrient for productivity in the Gulf, and concentrations drop to near-zero in the summer after major phytoplankton blooms in the spring. Nitrate concentrations remain low until the beginning of regeneration and/or destabilization of the water column with increased vertical mixing in November-December. The annual spring increase in surface nitrate is illustrated over the entire STOCS area in Figure 34. This figure of winter, spring and fall values at all twelve primary stations also shows that near-shore spring surface concentrations were generally highest due to inputs from land.

Vertical profiles of nitrate concentrations are illustrated in Figures 35 and 36. Figure 35 shows spatial variations of nitrate in winter at the primary stations of Transect I. Seasonal variations of nitrate in the water column at Station 3, Transect I, are shown in Figure 36. The figures display a marked increase in nitrate below 60 to 70 meters, indicative of nutrient regeneration and the influence of 200-300-m Western Gulf Water near the bottom. The intrusion of a tongue of 200-300-m Western Gulf Water was mentioned earlier in the discussion of dissolved oxygen.

Nitrate concentrations have been contoured in Figures 37 to 39 with cross-sectional maps of Transect II for each seasonal sampling period of 1976. Figure 37 indicates that winter nitrate was generally lower than 0.5 μM throughout the cross-section, increasing moderately below 60 meters. Spring runoff boosted near-shore and surface nitrate concentrations, as seen in Figure 38, leaving only a wedge of low-nitrate water between 10 and 40 meters. Also, the water column began to stratify with warming of the surface waters, hindering the mixing of deeper nutrient-rich water with shallower water. The high productivity in spring and

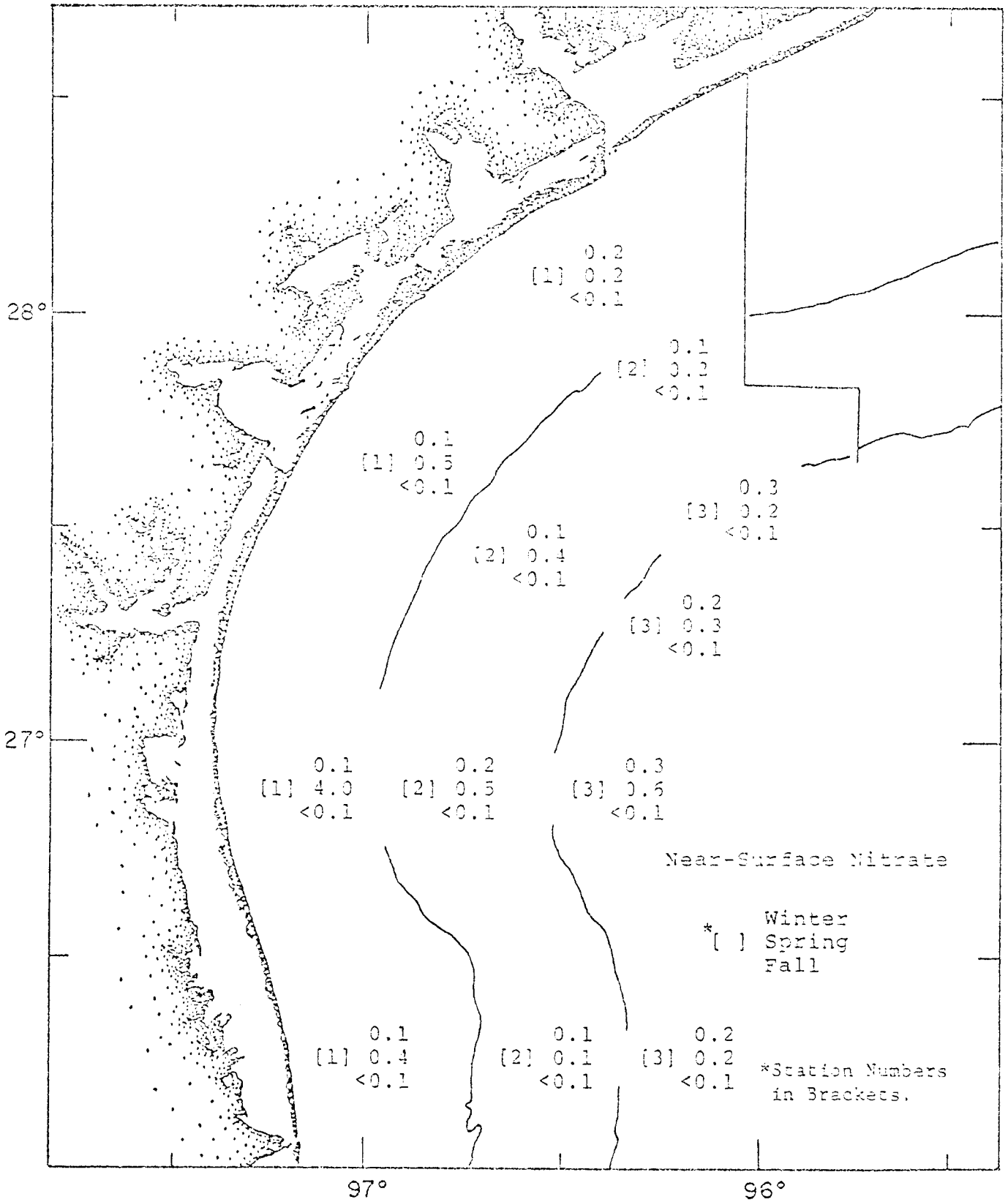

Figure 34. Near-Surface Nitrate Concentrations (uM) in the STOCS Area During the Seasonal Cruises in 1976.

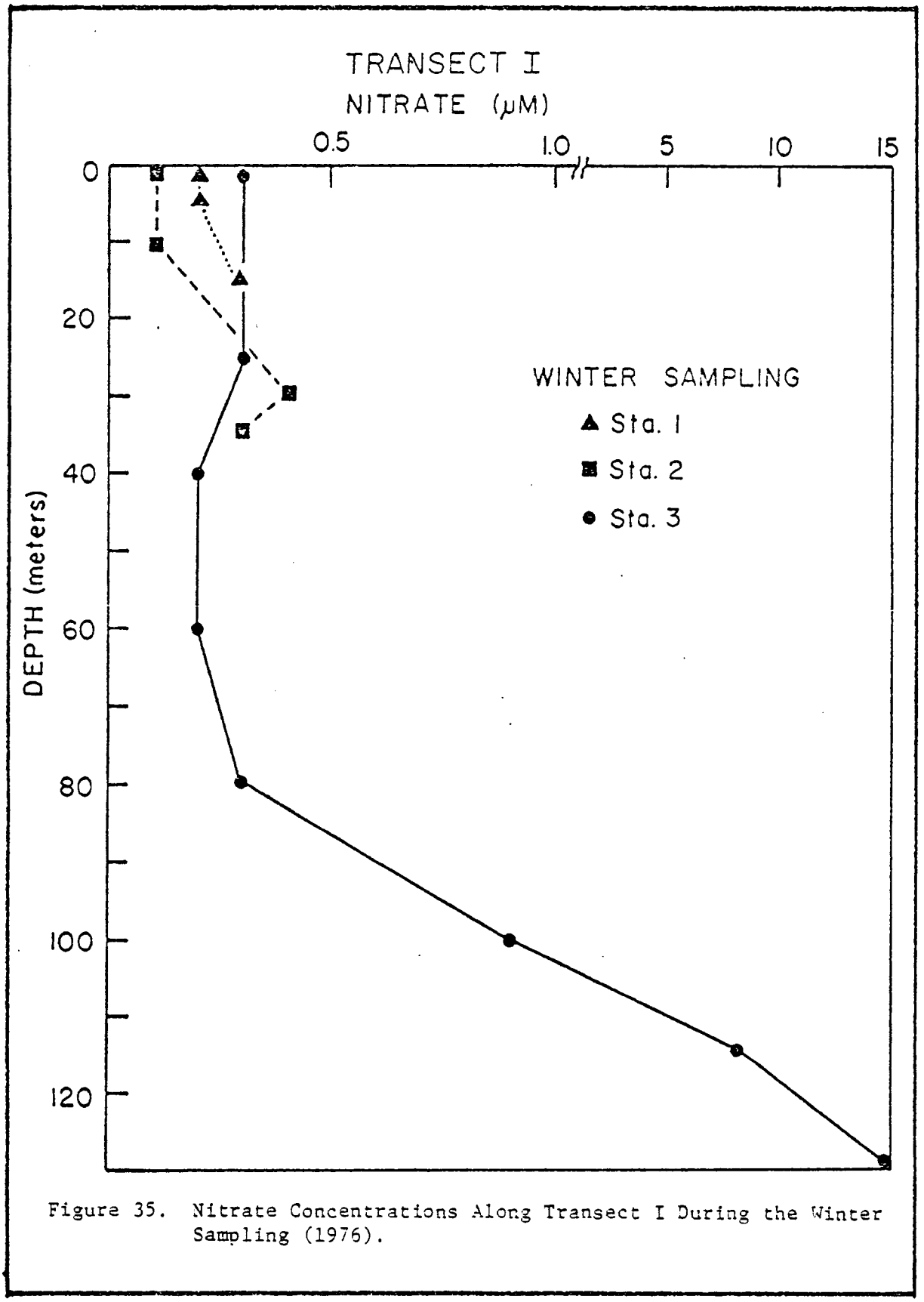

Figure 35. Nitrate Concentrations Along Transect I During the Winter Sampling (1976).

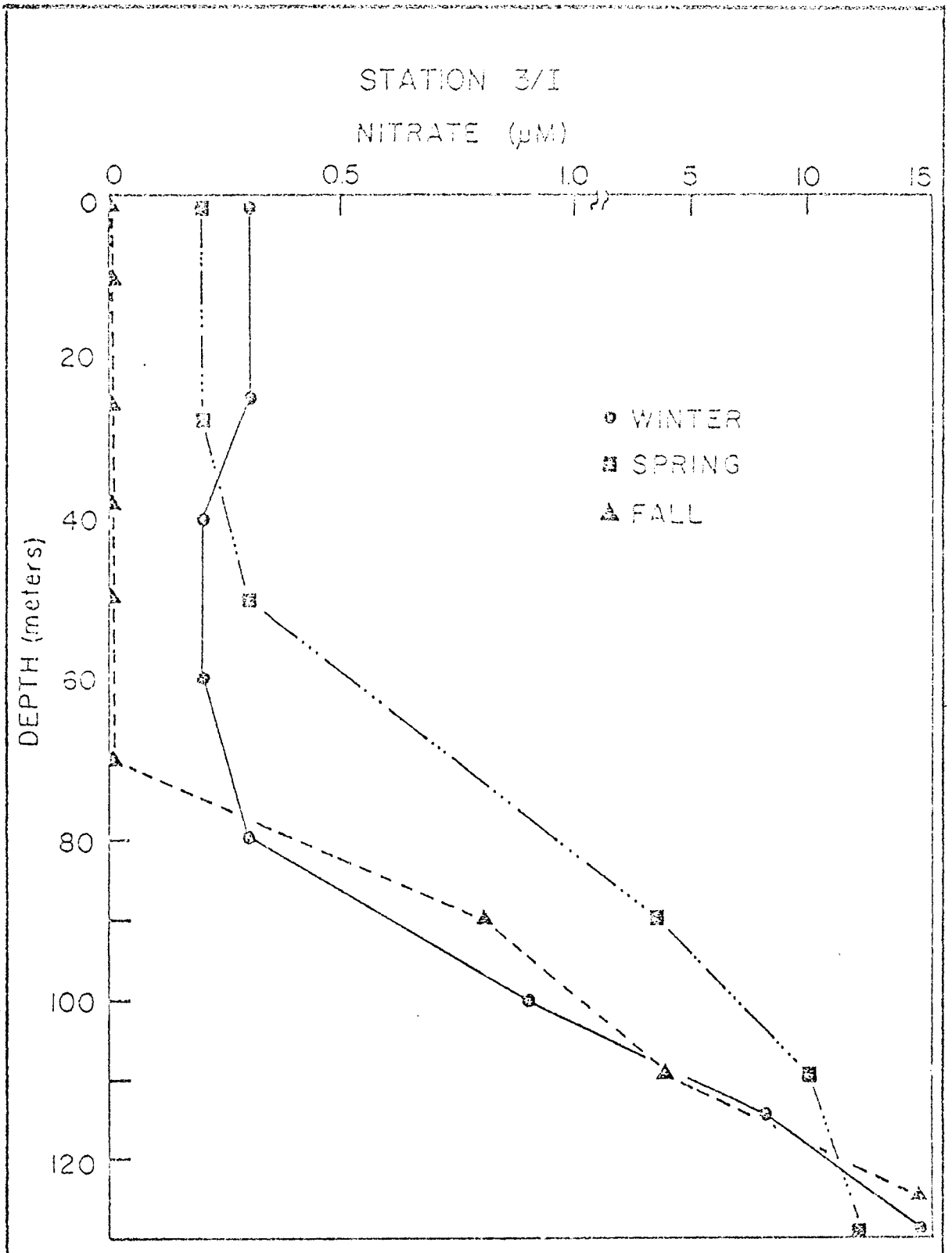

Figure 36. Nitrate Concentrations at Station 3/I During the Three Seasonal Samplings (1976).

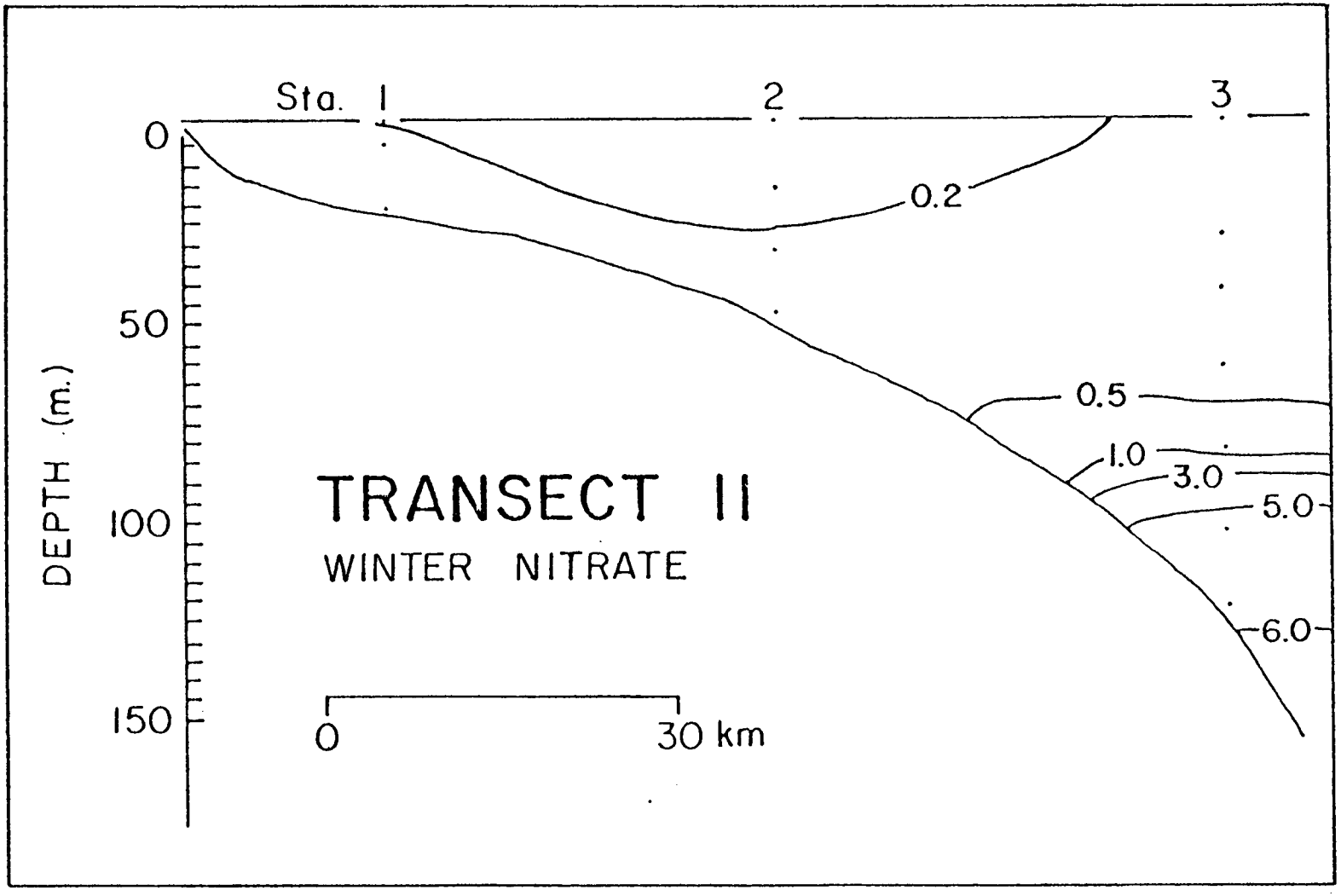

Figure 37. Nitrate (μM) Cross-sectional Contours Along Transect II During the Winter Seasonal Sampling (1976).

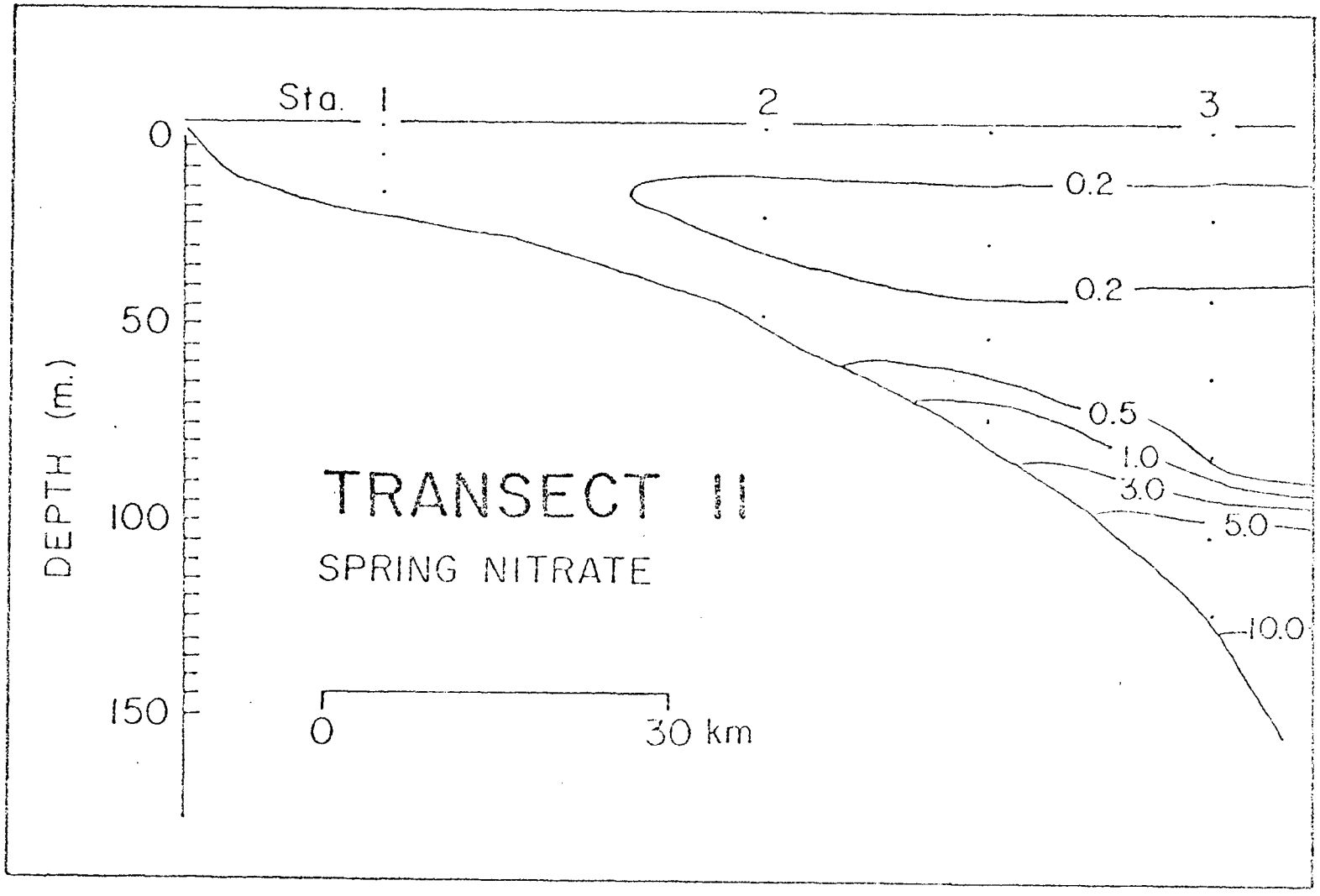

Figure 38. Nitrate (μM) Cross-sectional Contours Along Transect II During the Spring Seasonal Sampling (1976).

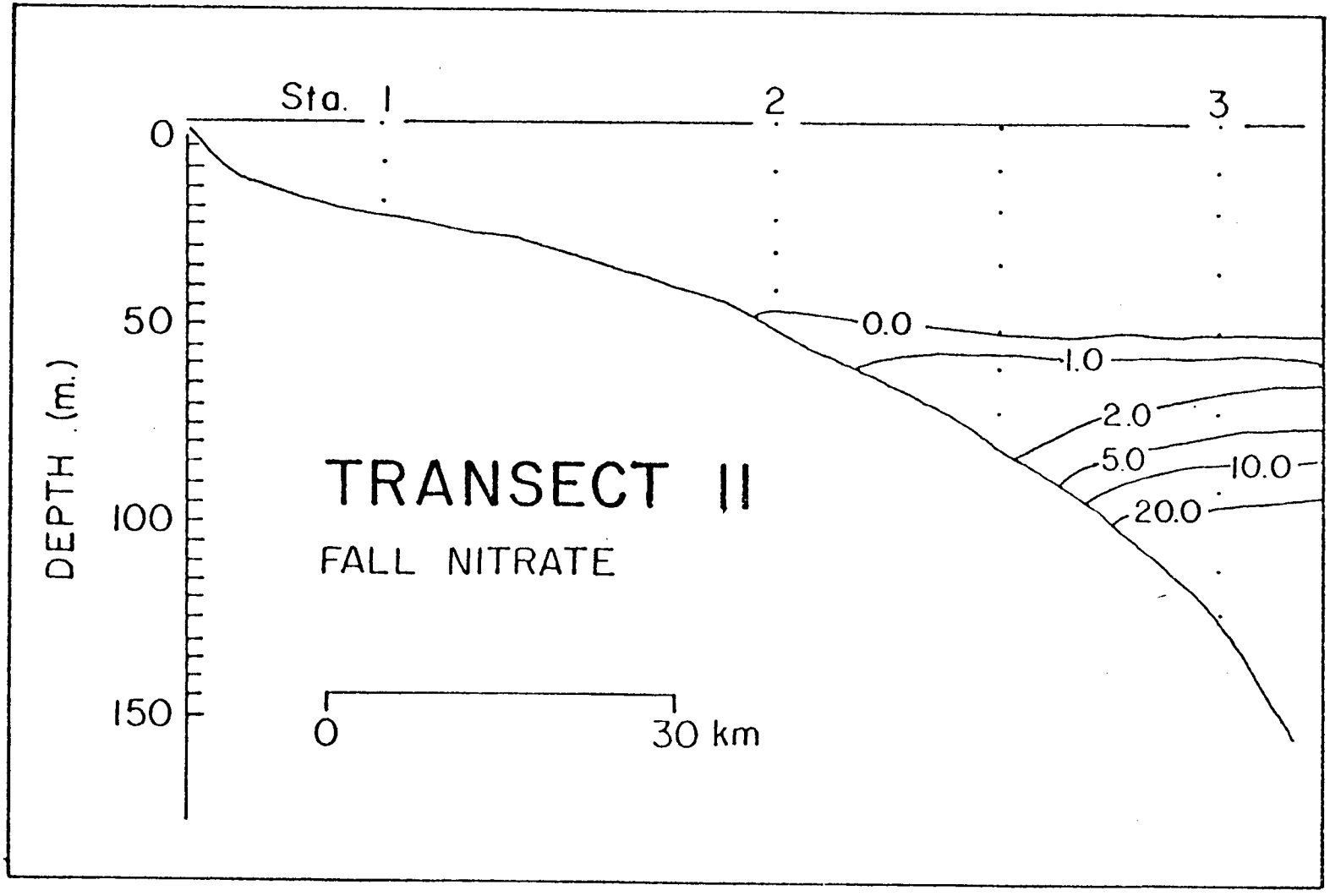

Figure 39. Nitrate (μM) Cross-sectional Contours Along Transect II During the Fall Seasonal Sampling (1976).

early summer stripped the water of the limiting nutrient, nitrate, and waters above 50 m were barren by fall (Figure 39). By this time the water column was highly stratified, inhibiting mixing of deep and shallow water, and nitrate concentrations rose rapidly to values as high as 20 µM in deeper waters. As winter temperatures cooled and destratified shelf waters, surface nitrate concentrations rose once again.

Phosphate

Table 19 lists minimum, maximum and mean monthly phosphate concentrations in the STOCS area in 1976. Surface and near-bottom phosphate levels for the primary station of Transect II and the two banks are listed on Table 20. These monthly Transect II surface variations are illustrated along with mean surface values in Figure 40. Concentrations were generally lower than 0.30 µM, being representative of Western Gulf surface water. Since phosphate is not the limiting nutrient, values seldom approach zero. Figure 40 does show the decrease in phosphate to a minimum in the summer after the major phytoplankton blooms, however, corresponding to the removal of nitrate discussed earlier. Since nitrate remains depleted through the summer and fall, productivity is restricted and phosphate is allowed to accumulate in the later months.

Seasonal surface phosphate variations over the entire STOCS area are illustrated in Figure 41. This table of winter, spring and fall phosphate levels indicates that the highest surface values were found near-shore, generally in the winter. A vertical profile of phosphate levels from the winter sampling of Transect I in Figure 42 further illustrates higher near-shore concentrations. This figure shows consistently higher phosphate levels down through the water column at Station 1 (near-shore), decreasing somewhat at Station 2 (midway), and consistently

TABLE 19

SUMMARY OF NUMBER OF PHOSPHATE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (μM) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	56	0.33	0.01	1.21	0.22
March	25	0.21	0.03	0.94	0.20
April	25	0.19	0.02	0.85	0.15
Spring	56	0.15	0.01	0.79	0.10
July	27	0.36	0.06	2.43	0.14
August	24	0.23	<0.01	1.14	0.16
Topo High	24	0.36	<0.01	1.35	0.20
Fall	82	0.31	<0.01	1.33	0.22
November	20	0.17	<0.01	0.96	0.10
December	30	0.17	0.02	1.02	0.12

TABLE 20

SURFACE AND NEAR-BOTTOM PHOSPHATE CONCENTRATIONS (uM) AT STOCS STATIONS ALONG TRANSECT II

Sampling Month	STATIONS 1/II	2/II	3/II	SB	HB
			Surface		
Winter	0.36	0.16	0.15	-	-
March	0.22	0.11	0.57	0.05	0.06
April	0.15	0.18	0.34	0.06	0.04
Spring	0.13	0.07	0.09	0.17	0.05
July	0.23	0.11	0.21	0.06	0.08
August	0.35	0.06	0.11	0.16	0.14
Fall	0.49	0.35	0.10	0.06	0.10
November	0.28	0.17	0.01	<0.01	0.02
December	0.44	0.05	0.02	0.06	0.05
			Near-Bottom		
Winter	0.30	0.48	1.04	-	-
March	0.20	0.06	0.94	0.25	0.07
April	0.37	0.37	0.85	0.24	0.19
Spring	0.15	0.38	0.55	0.13	0.13
July	0.60	2.43	0.82	0.70	0.52
August	0.63	0.19	1.14	0.08	0.06
Fall	0.75	0.03	1.33	1.06	0.54
November	0.33	0.04	0.96	0.51	0.27
December	0.27	0.13	1.02	0.12	0.07

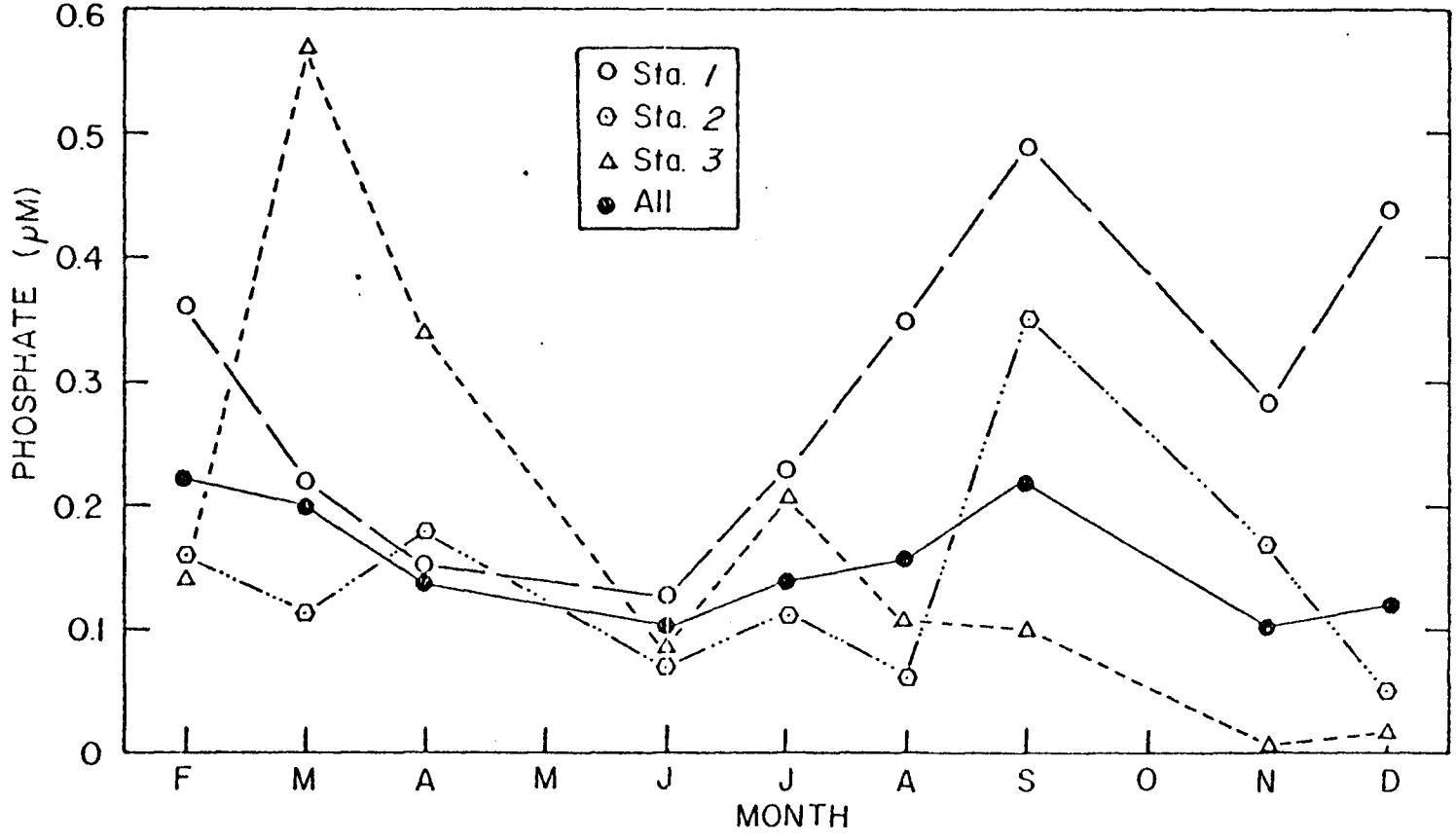

Figure 40. Monthly Variations in Phosphate Along Transect II in 1976.

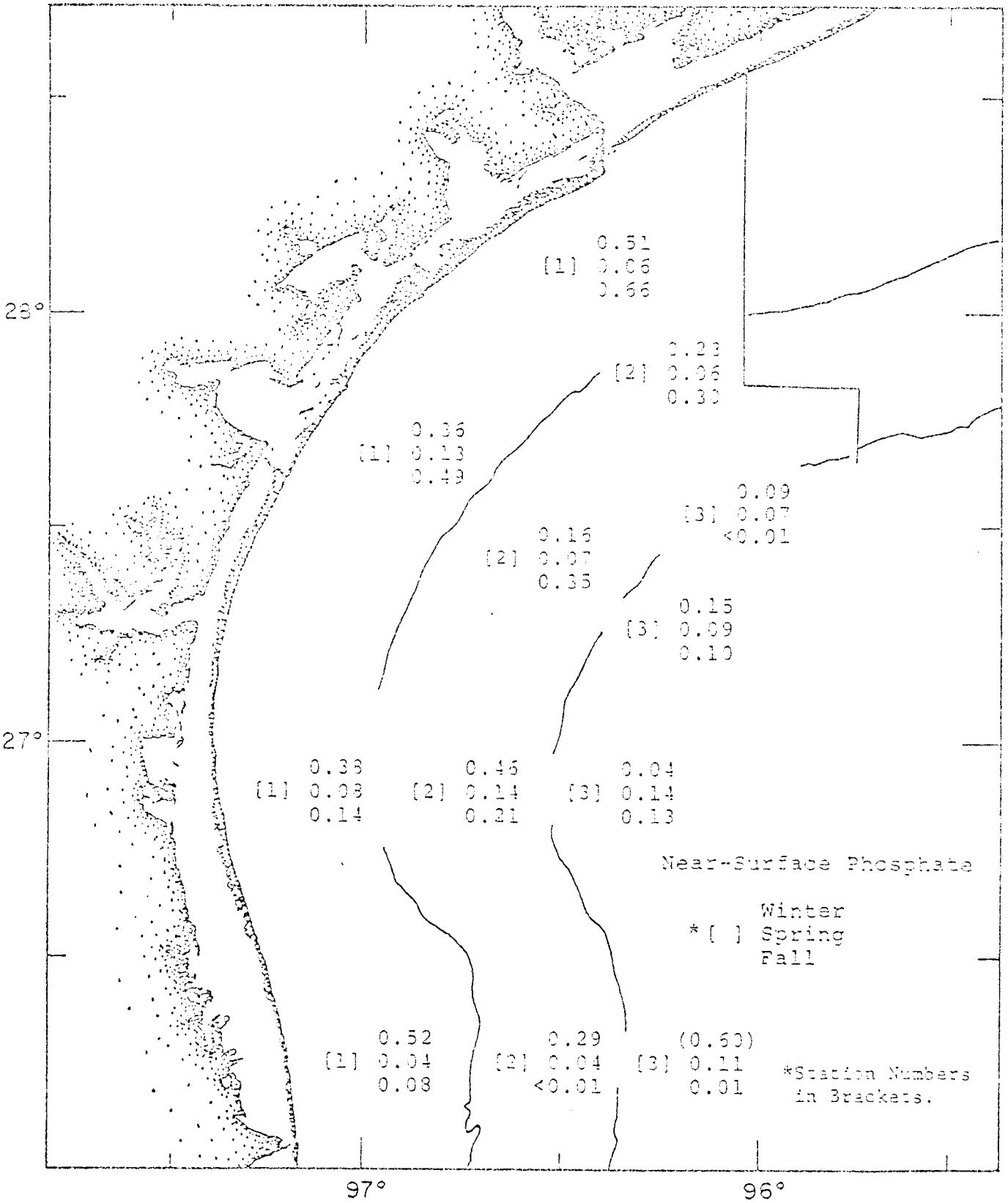

Figure 41. Near-Surface Phosphate Concentrations (µM) in the STOCS Area During the Seasonal Cruises in 1976.

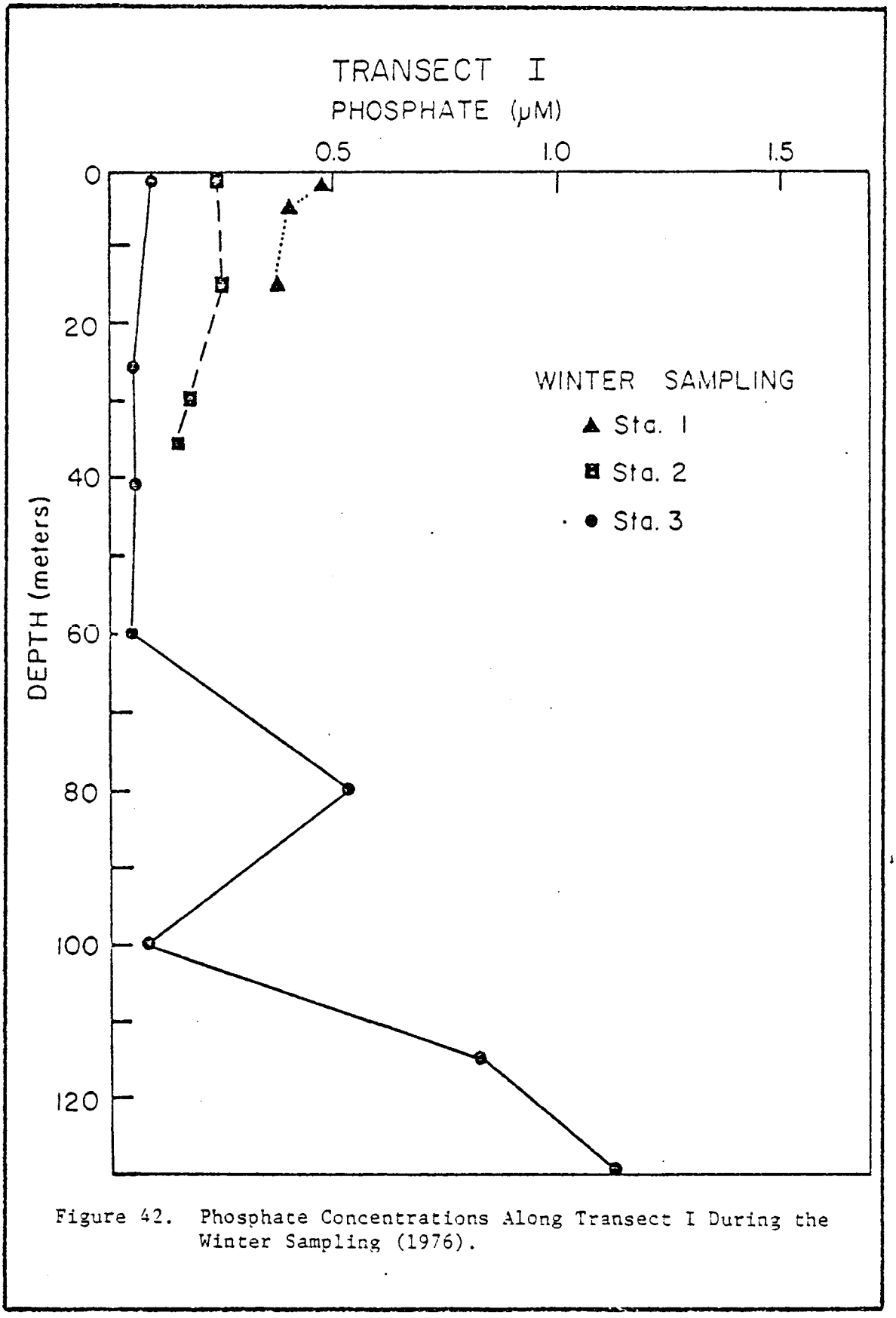

Figure 42. Phosphate Concentrations Along Transect I During the Winter Sampling (1976).

low levels to 60 m at Station 3 (offshore). Figure 43 shows seasonal phosphate variations throughout the water column of Station 3, Transect I. From this figure, winter and spring values are consistently higher in the upper 60 m, with concentrations of all seasons increasing markedly below 60 m. These general increases correspond to the same trends with depth for nitrate discussed earlier.

The phosphate gradient below 60 m is clearly seen from phosphate-level contours through cross-sections of Transect II in Figures 44 to 46. The three figures, drawn from winter, spring and fall sampling data, all exhibit high near-shore phosphate levels decreasing offshore laterally and increasing down-slope towards deeper water. The influence of the 200-300 m Western Gulf Water below 60 m is clearly seen. Since phosphate is not the limiting nutrient, seasonal trends of shallow offshore water are not as obvious as with nitrate.

Silicate

Table 21 lists minimum, maximum and mean monthly silicate concentrations of the STOCS region in 1976. Monthly surface and near-bottom concentrations are listed for the primary Transect II stations and the two banks in Table 22. These monthly surface levels are plotted along with mean surface values in Figure 47.

Seasonal silicate concentrations exhibit variations similar to those of nitrate and phosphate in the STOCS region. Silicate is not the productivity-limiting nutrient in this region so levels rarely approach zero, but seasonal trends can be distinguished. Figure 47 shows that silicate concentrations increased from moderate winter levels to levels as high as 7.0 uM near-shore and about half of that further offshore in early spring. These increases were due to increased spring rainfall and

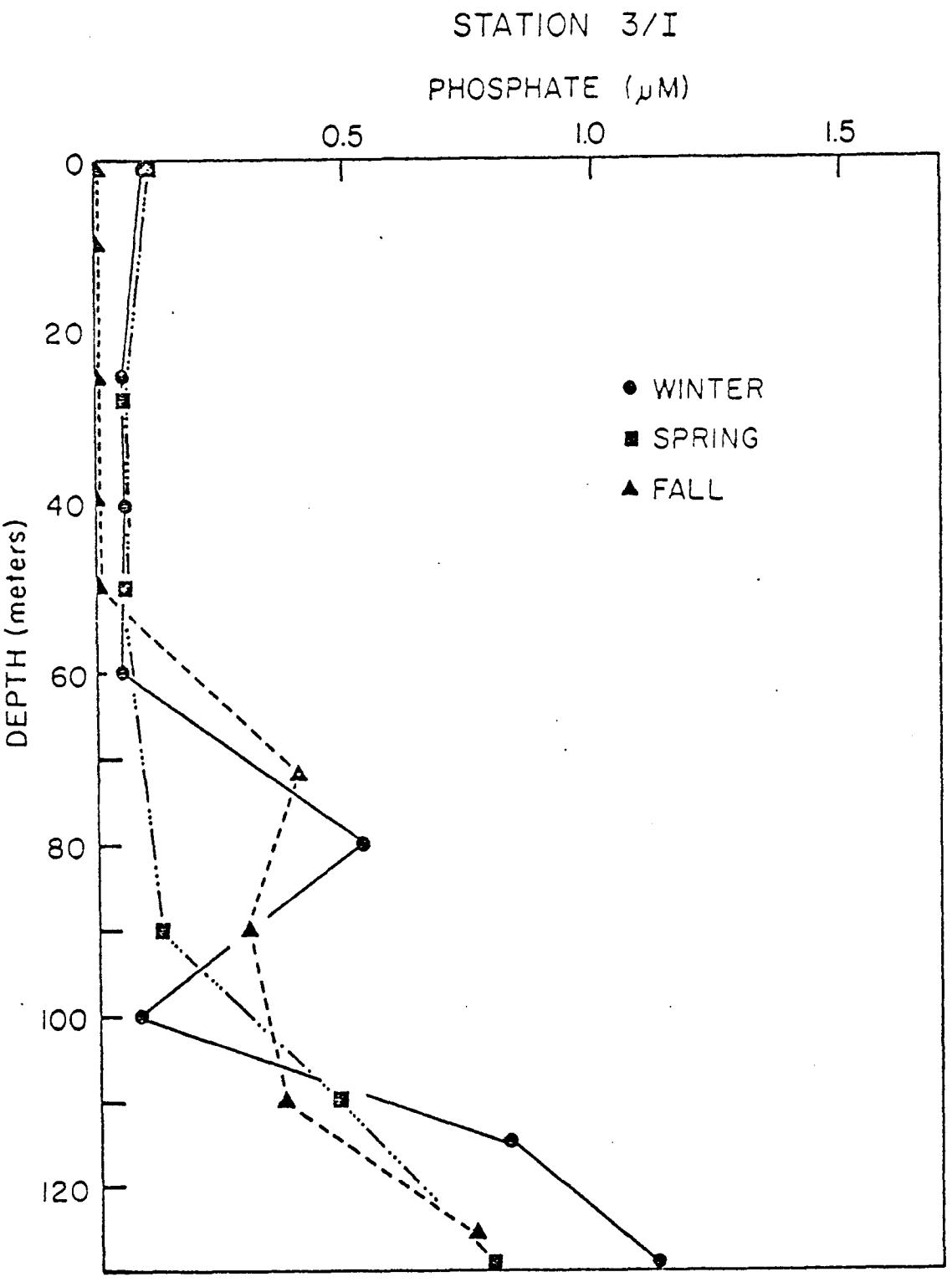

Figure 43. Phosphate Concentrations at Station 3/I During the Three Seasonal Sampling (1976).

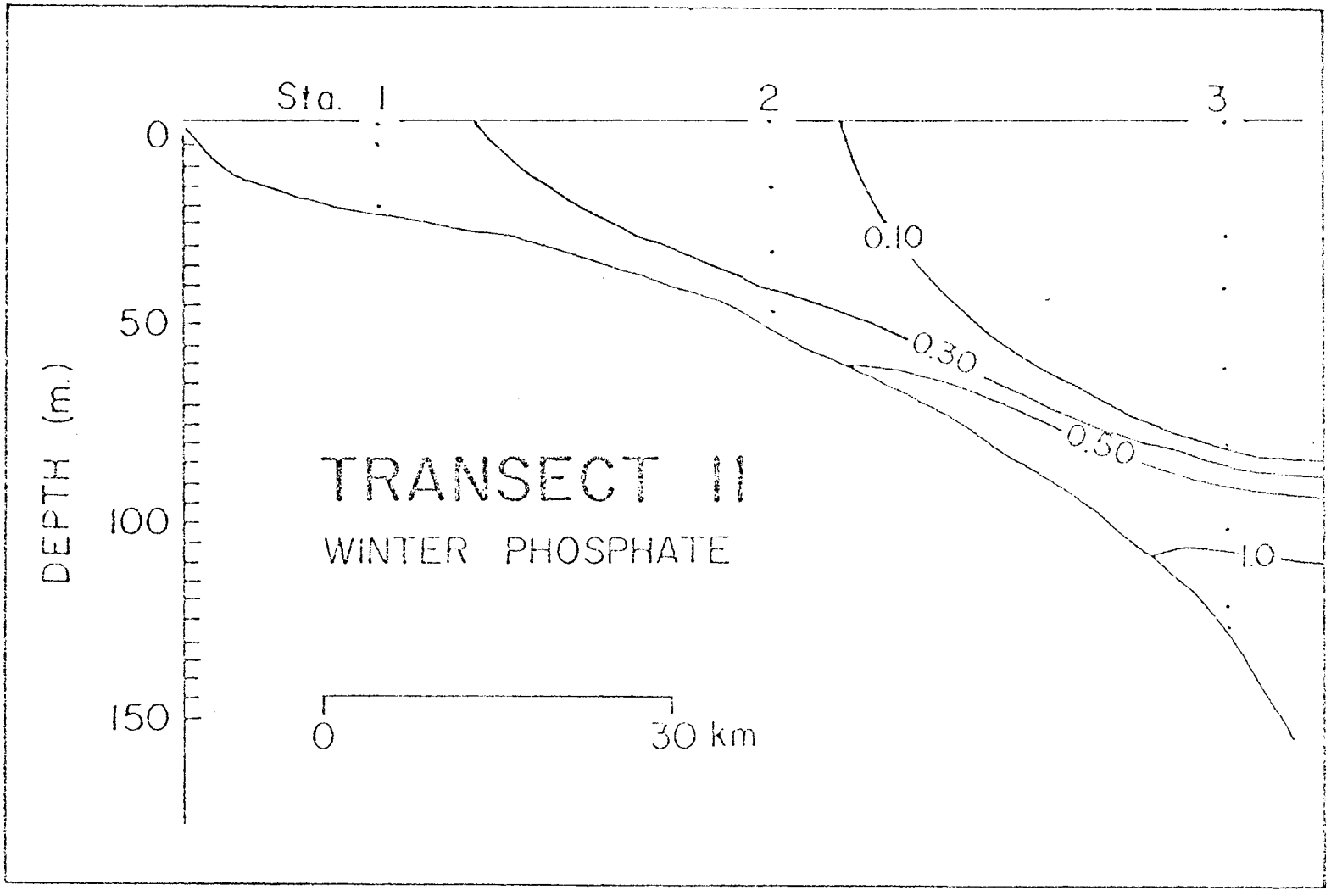

Figure 44. Phosphate (μM) Cross-sectional Contours Along Transect II During the Winter Seasonal Sampling (1976).

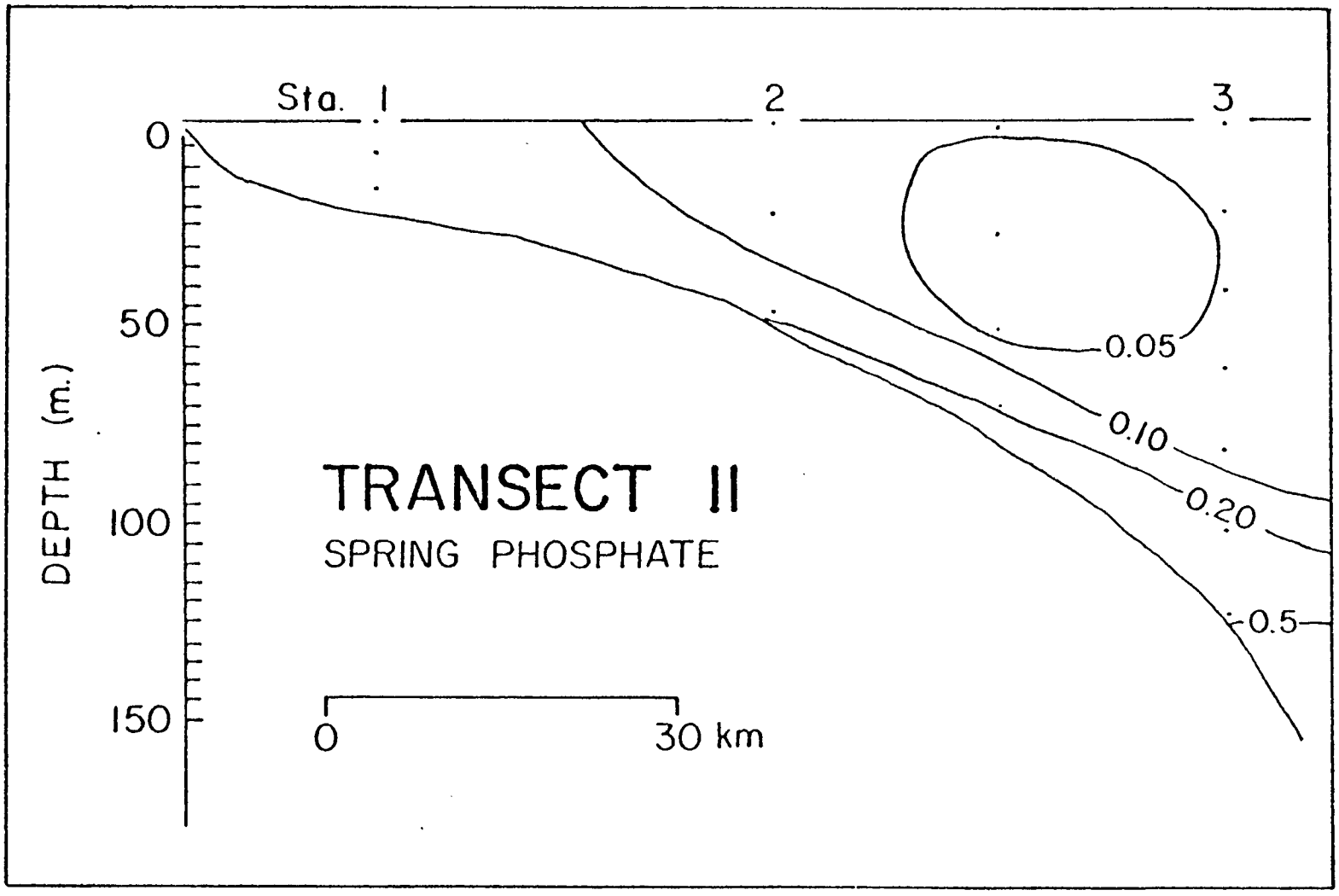

Figure 45. Phosphate (μM) Cross-sectional Contours Along Transect II During the Spring Seasonal Sampling (1976).

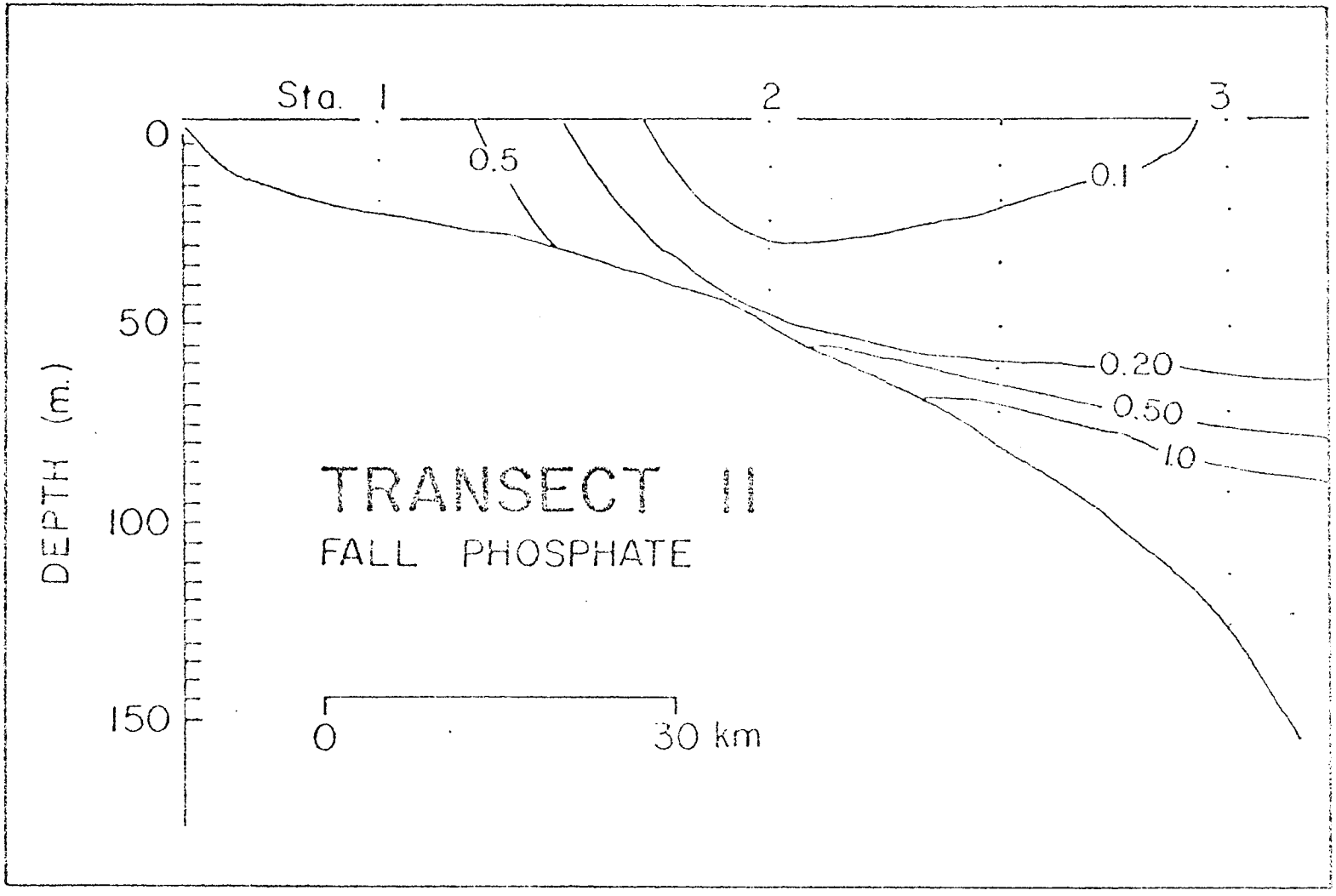

Figure 46. Phosphate (μM) Cross-sectional Contours Along Transect II During the Fall Seasonal Sampling (1976).

TABLE 21

SUMMARY OF NUMBER OF SILICATE OBSERVATIONS, MEAN, MINIMUM AND MAXIMUM CONCENTRATIONS (μM) OBTAINED SEASONALLY AND MONTHLY IN THE STOCS AREA (1976)

Season	Number of Observations	Mean	Minimum	Maximum	Surface Mean
Winter	56	2.4	0.8	7.8	2.3
March	25	3.4	1.4	8.5	3.6
April	25	1.8	0.1	6.6	0.6
Spring	56	3.2	0.8	15.8	1.7
July	27	2.1	0.7	11.4	1.4
August	24	1.9	0.6	5.0	2.4
Topo High	24	2.1	0.7	7.1	1.3
Fall	82	2.4	0.4	6.7	2.6
November	20	3.4	1.3	6.8	3.4
December	30	2.5	0.8	5.1	2.3

TABLE 11

SURFACE AND NEAR-BOTTOM SILICATE CONCENTRATIONS (UM) AT STING STATIONS ALONG TRANSECT II

Sampling Month	STATIONS 1 II	2 II	3 II	SB	HB
			Surface		
Winter	3.3	2.1	1.3	-	-
March	8.5	2.5	2.7	1.7	2.6
April	0.1	0.5	1.1	0.7	0.7
Spring	1.5	1.3	1.4	2.1	1.9
July	2.6	1.6	1.3	0.8	0.9
August	4.8	1.2	2.1	1.6	2.1
Fall	6.0	3.3	2.8	0.8	0.9
November	4.3	6.1	2.3	2.2	2.1
December	1.4	1.9	1.8	1.3	1.6
			Near-Bottom		
Winter	3.3	2.1	5.4	-	-
March	6.1	2.0	7.0	3.2	2.5
April	0.9	2.3	6.6	4.0	2.8
Spring	2.0	10.0	6.9	6.9	4.3
July	3.9	11.4	2.5	5.2	3.9
August	3.7	2.5	4.5	1.2	0.3
Fall	6.3	2.0	6.7	1.6	0.3
November	6.6	2.9	5.8	5.9	4.2
December	4.5	2.3	4.4	2.0	1.7

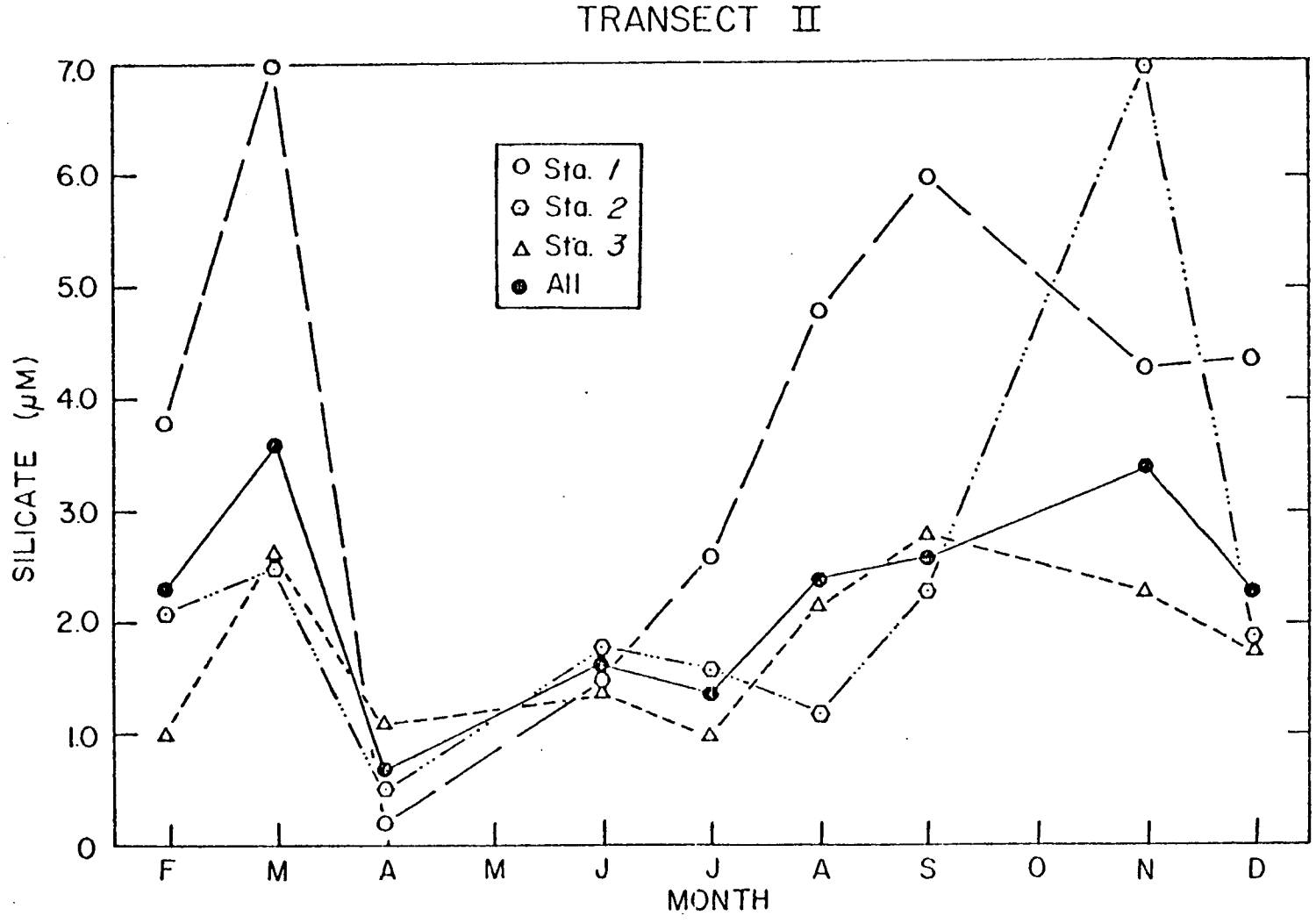

Figure 47. Monthly Variations in Silicate Along Transect II in 1976.

runoff from land, washing silicate-bearing minerals into the region. Levels dropped drastically with the increased productivity during March and April, and gradually increased through the summer and fall (similar to phosphate). Since silicate is not limiting to productivity, concentrations were not held near zero during the summer. Moderate silicate levels were again attained by December, completing the annual cycle.

Seasonal variations of near-surface silicate over the entire STOCS region are illustrated in Figure 48. Levels were typically highest near-shore and lowest during the spring increase in productivity.

Vertical profiles of silicate at Transect I are shown in Figures 49 and 50. In Figure 49, the nearshore winter levels (Station 1) are consistently higher than the stations further offshore, in agreement with surface values (Figure 48). The plot of seasonal variations through the water column at Station 3 in Figure 50 demonstrates the relatively small effects of silicate input from land and productivity on offshore stations. Vertical profiles in winter, spring and fall concentrations are nearly identical throughout the water column.

As with nitrate and phosphate, silicate concentrations increased below 60 m, reflecting the influence of nutrient regeneration and the presence of 200-300-m Western Gulf Waters on the deeper STOCS water. The silicate gradient with depth is clearly illustrated by contours of silicate concentration on cross-sectional maps of Transect II in Figures 51 to 53. Figure 51 shows high levels of near-shore silicate in the winter reflecting the nearby land influence, with levels decreasing laterally offshore. Increasing silicate with depths below 60 meters is evident. In the spring after the phytoplankton blooms, near-surface silicate was depleted, as seen in Figure 52. By fall, near-shore levels increased due to the land mass influence and since silicate is not the limiting

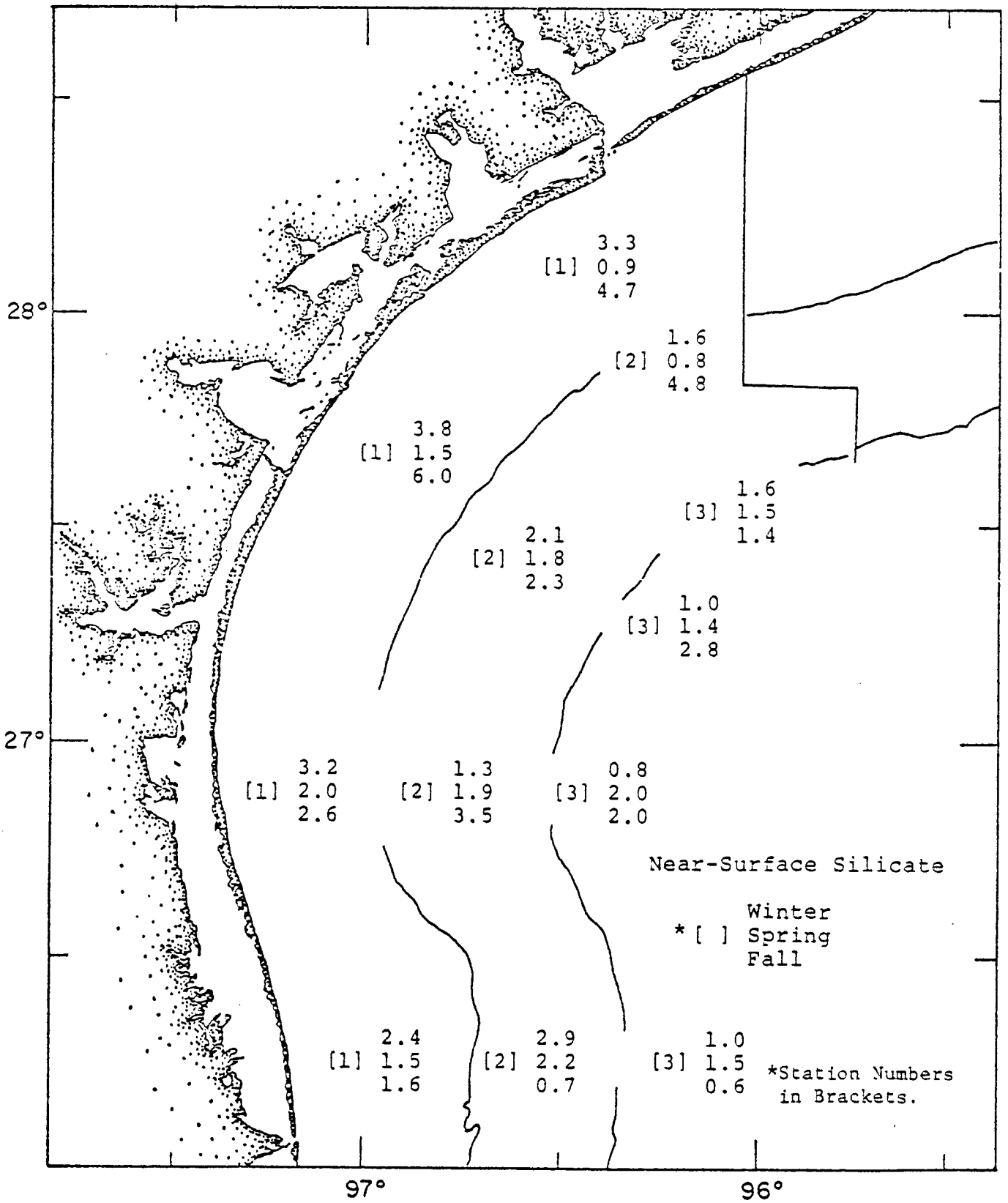

Figure 48. Near-Surface Silicate Concentrations (μM) in the STOCS Area During the Seasonal Cruises in 1976.

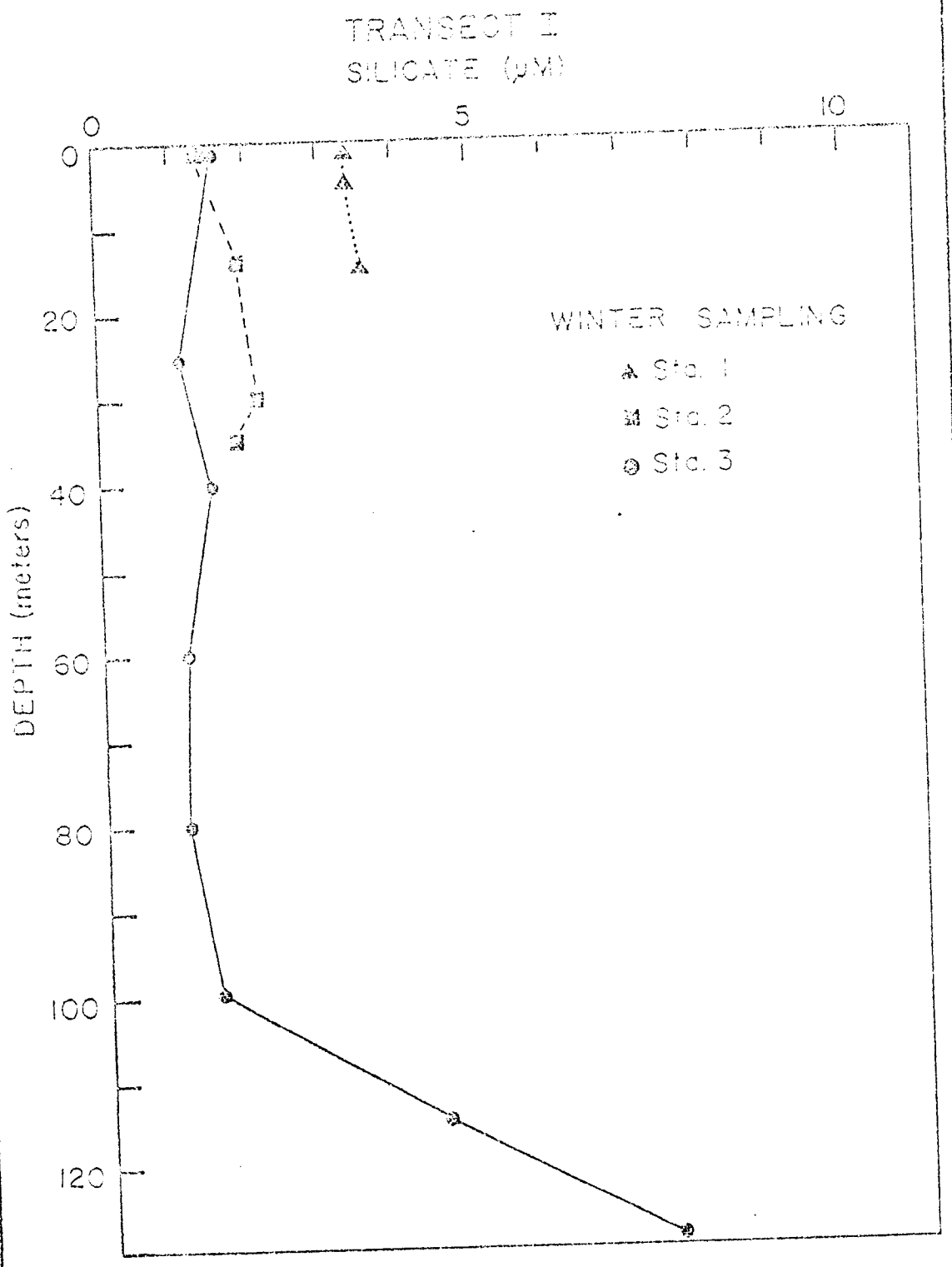

Figure 49. Silicate Concentrations Along Transect I During the Winter Sampling (1976).

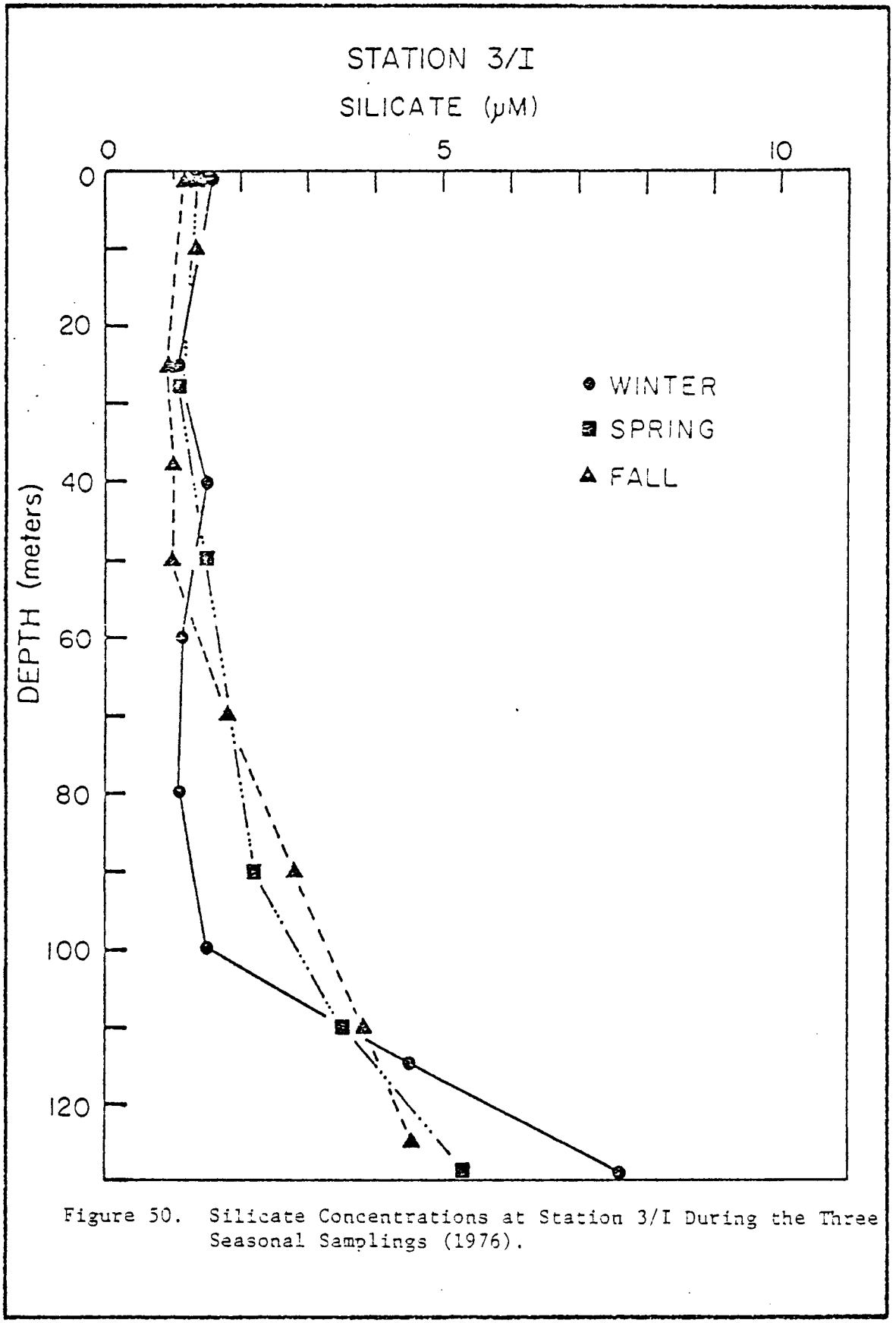

Figure 50. Silicate Concentrations at Station 3/I During the Three Seasonal Samplings (1976).

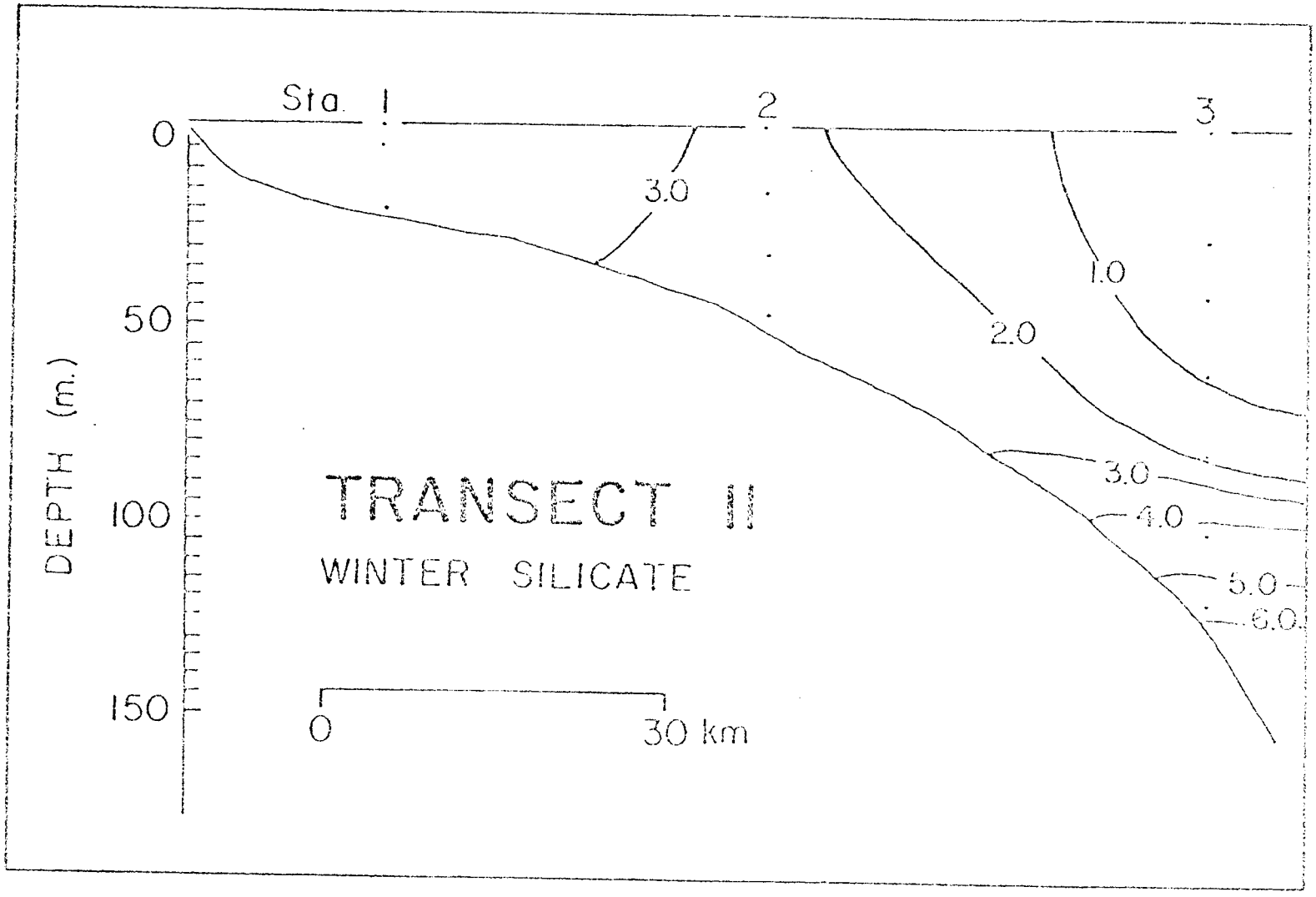

Figure 51. Silicate (μM) Cross-sectional Contours Along Transect II During the Winter Seasonal Sampling (1976).

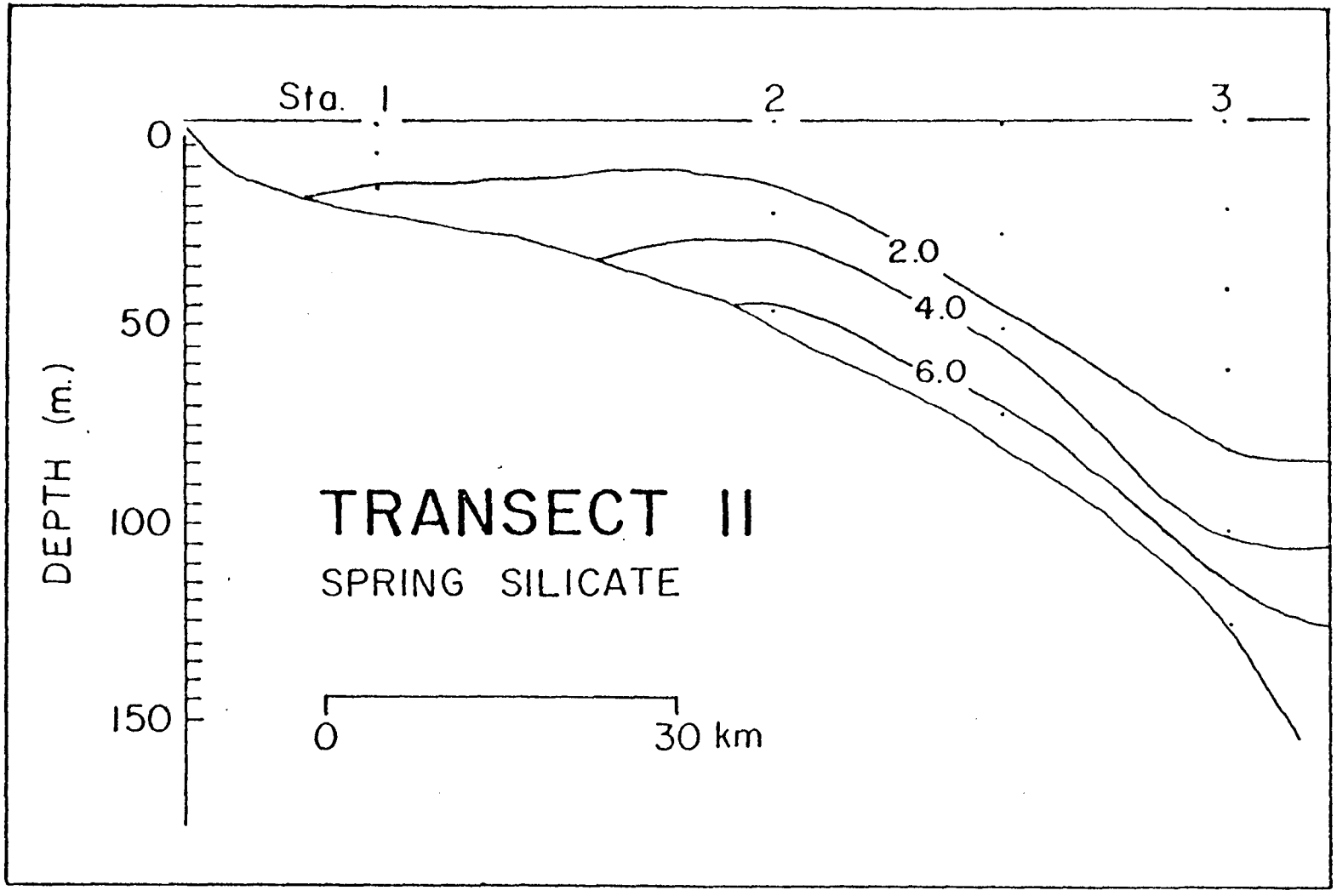

Figure 52. Silicate (μM) Cross-sectional Contours Along Transect II During the Spring Seasonal Sampling (1976).

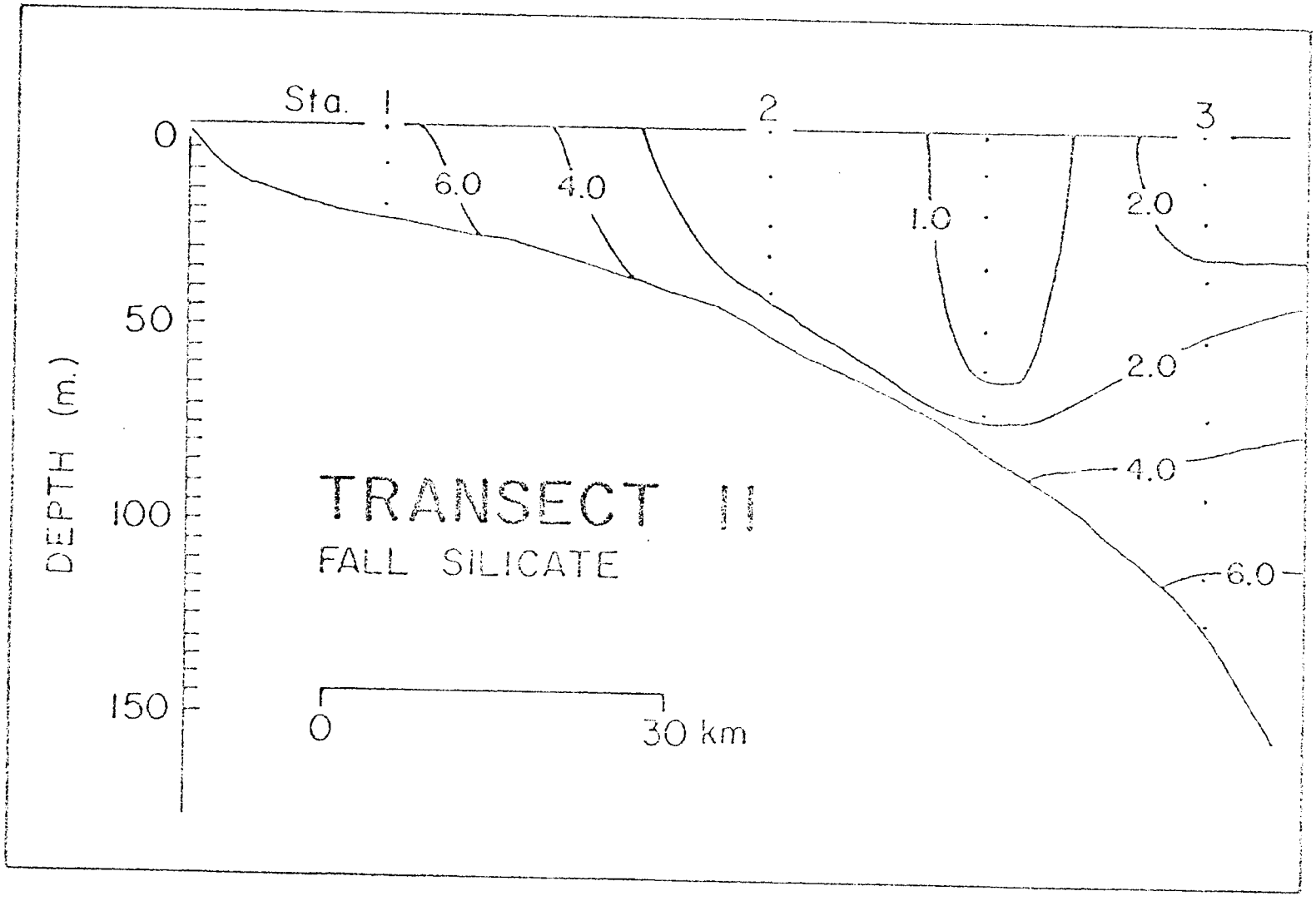

Figure 53. Silicate (μM) Cross-sectional Contours Along Transect II During the Fall Seasonal Sampling (1976).

nutrient, overall silicate concentrations were increased.

Oxygen and Nutrient Correlations

Correlation coefficients for dissolved oxygen, nutrients and LMW-hydrocarbons data are tabulated in Table 23. The table shows reasonable correlations of oxygen, phosphate, and nitrate with depth, due primarily to the intrusion of nutrient-rich, oxygen-poor water at depth in the STOCS region. Silicate did not correlate well with depth due to large surface influxes of silicate from continental runoff. For similar reasons, oxygen correlated with nitrate and phosphate, but not with silicate. As expected, the best correlation was nitrate with phosphate (0.67), since theoretically, these nutrients are assimilated by primary producers in stoichiometric proportions. Silicate correlated reasonably well with phosphate (0.55), but poorly with nitrate (0.40) because nitrate is the productivity limiting nutrient, whereas neither phosphate or silicate remain depleted during the summer and fall.

TABLE 22

CORRELATION COEFFICIENTS[1] ON STOCS 1974 DATA

Variable	Oxygen	Phosphate	Nitrate	Silicate
Methane	-	-	-	-
Ethene	-	-	-	-
Ethane	-	-	-	-
Propene	-	-	-	-
Propane	-	-	-	-
Depth	-0.6287	0.4872	0.6797	-
Temperature	-	-	-	-
Salinity	-	-	-	-
Oxygen	1.0	-0.4549	-0.6275	-
Phosphate	-0.4549	1.0	0.6710	0.5539
Nitrate	-0.6275	0.6710	1.0	0.4012
Silicate	-	0.5539	0.4012	1.0

[1] Only correlations greater than 0.4000 are tabulated

CONCLUSIONS

The lower Texas shelf is relatively "clean" with respect to hydrocarbons, as LMWH in the South Texas OCS area are chiefly derived from natural sources. The major source of methane appears to be *in situ* production in the water column. There appears to be a seasonal pattern to the vertical distribution of methane in the water column. In the winter, due to turbulent mixing, the water column is fairly uniform with respect to saturated LMWH. During the summer and fall, as stratification of the water column develops, a maximum in methane associated with the thermocline develops. This concentration maximum can be almost an order of magnitude higher than water above and below. The maximum probably results from accumulation of suspended matter on the stratification boundary due to restriction of settling velocities across the density gradient, with subsequent production of methane in small micro-reducing environments of suspended particles.

Gas seepage also accounts for some natural inputs of saturated LMWH. The increase in methane levels in bottom water at Station 3/IV indicates that seepage is occurring near this station. Gas seepage has also been observed and collected at Southern Bank. This gas was principally methane (99.96 percent) with only a small amount of ethane (0.04 percent). The molecular composition along with $^{13}C/^{12}C$ ratios on the methane (-60.2 ppt relative to PDB) indicates a biogenic source. Many other areas of gas seepage have been observed by acoustical reflections in the STOCS area.

The unsaturated hydrocarbons (*e.g.*, ethene and propene) generally follow productivity patterns, being low in winter with higher values in the spring, summer, and fall. Ethene and propene are known to be produced by biological processes, thus their strong correlations with phytoplankton productivity parameters are to be expected. Ethene also shows a shallow subsurface maximum associated with a productivity maximum. The unsaturates dominate over their saturated analogs in the STOCS area.

Oxygen concentrations in the upper 60 meters of the STOCS region varied seasonally, being generally highest at near-shore stations in the winter and lowest in the summer. Temperature and salinities were measured on subsamples of water taken for oxygen determinations so that equilibrium oxygen concentrations could be calculated and compared to measured values. Ratios of measured oxygen to equilibrium oxygen concentrations indicated that oxygen variations in the upper 60 meters are controlled by physical processes (seasonal changes in seawater temperature and salinity) rather than productivity fluctuations. The mass of highly-oxygenated water can be traced by cross-sectional concentration contours as it is formed near-shore in the winter and displaced by warming in the spring and summer. The intrusion of oxygen-depleted 200-300 m Western Gulf Water is evident year-round below approximately 70 meters in the STOCS region, and seasonal variations in stratification of the water column can be seen by the extent of vertical mixing with this bottom water.

Nutrient concentrations are representative of open Gulf

surface water in most of the water above 60 meters, but continental runoff influences near-shore concentrations, especially in the spring. Nitrate is the limiting nutrient to productivity, and disappears after the spring phytoplankton blooms through the summer and early fall. Phosphate and silicate are affected by the high spring productivity but are not completely removed. These nutrients are gradually replenished during the summer and fall to moderately high values by December. The intrusion of the nutrient-rich 200-300-m Western Gulf Water can again be clearly seen below 70 meters from nutrient concentration contours across cross-sectional diagrams of Transect II.

LITERATURE CITED

Atkinson, L. P., and F. A. Richards. 1967. The occurrence and distribution of CH_4 in the marine environment. Deep Sea Res. 14:673.

Atlas, E. ., S. E. Hager, L. I. Gordon, and P. K. Park. 1971. A practical manual for use of the Technicon Autoanalyzer in seawater nutrient analyses, revised. Technical Rep. 215, Nat. Mar. Fish. Sci.

Bernard, B. B., J. M. Brooks, and W. M. Sackett. 1976. Natural gas seepage in the Gulf of Mexico. Earth Planet. Sci. Lett. 31:48.

Brooks, J. M. 1975. Sources, sinks, concentrations, and sub-lethal effects of light aliphatic and aromatic hydrocarbons in the Gulf of Mexico. Ph. D. Dissertation, Texas A & M Univ., College Station, Texas.

_____. 1976. The flux of light hydrocarbons into the Gulf of Mexico via runoff. In H. L. Windom and R. A. Duce, eds. Marine pollutant transfer. D. E. Heath and Co., Lexington, Mass.

_____, A. D. Fredricks, W. M. Sackett, and J. W. Swinnerton. 1973. Baseline concentrations of light hydrocarbons in the Gulf of Mexico. Env. Sci. Technol. 7:633.

_____, J. R. Gormly, and W. M. Sackett. 1974. Molecular and isotopic composition of two seep gases from the Gulf of Mexico. Geophys. Res. Lett. 1:213.

_____, B. B. Bernard, and W. M. Sackett. In press. Input of low-molecular-weight hydrocarbons from petroleum operations into the Gulf of Mexico. Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms.

_____, and W. M. Sackett. 1973. Sources, sinks and concentrations of light hydrocarbons in the Gulf of Mexico. J. Geophys. Res. 78:5248.

_____. In Press. Significance of low-molecular-weight hydrocarbons in coastal waters. Advances in organic geochemistry, 1975.

Lamontagne, R. A., J. W. Swinnerton, and V. J. Linnenbom. 1971. Non-equilibrium of carbon monoxide and methane at the air-sea interface. J. Geophys. Res. 76:5117.

_____. 1974. C_1-C_4 hydrocarbons in the North and South Pacific. Tellus 26:71.

_____, and W. Smith. 1973. Methane concentrations in various marine environments. J. Geophys. Res. 78:5317.

MacDonald, R. W. 1976. Distribution of low-molecular-weight hydrocarbons in Southern Beaufort Sea. Env. Sci. Technol. 10:1241.

McAullife, C. 1971. Gas chromatographic determination of solutes by multiple phase equilibrium. Chem. Technol. 1:46.

Sackett, W. M., and J. M. Brooks. 1974. The use of low-molecular-weight hydrocarbon concentrations as indicators of marine pollution. NBS Spec. Publ. 409, marine pollution monitoring (petroleum) proceedings of a symposium and workshop held at NBS. 13-17. Gaithersburg, Md.

_____. 1975. Origin and distribution of low-molecular-weight hydrocarbons in Gulf of Mexico coastal waters. Pages 211-230 *in* T. M. Church, ed. Marine chemistry in the coastal environment. ACS symposium series 18. ACS Washington.

Scranton, M. I., and P. G. Brewer. In press. Occurrence of methane in the near-surface waters of the Western Subtropical North Atlantic. Deep Sea Res.

Seiler, W., and V. Schmidt. 1974. Dissolved non-conservative gases in seawater. *in* E. D. Goldberg, ed. The sea, Vol. V. Wiley Interscience, New York.

Strickland, J. D. H., and T. R. Parsons. 1972. A practical handbook of seawater analysis, bulletin 167. Fish. Res. Bd. Can., Ottawa.

Swinnerton, J. W., and R. A. Lamontagne. 1974. Oceanic distribution of low-molecular-weight hydrocarbons; baseline measurements. Env. Sci. Tech. 8:657-663.

_____, and V. J. Linnenbom. 1967. Gaseous hydrocarbons in seawater: determination. Science 156:1119.

_____, and C. H. Cheek. 1969. Distribution of methane and carbon monoxide between the atmosphere and natural waters. Env. Sci. Technol. 8:657.

Tinkle, A. R., J. W. Antoine, and R. Kuzeia. 1973. Detecting natural gas seeps at sea. Ocean Industry 8:139.

Weiss, R. F. 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep Sea Res. 17:721.

Yamamoto, S., J. B. Alcauskas, and T. E. Crozier. 1976. Solubility of methane in distilled water and seawater. J. Chem. Eng. Data 21:78.

CHAPTER SIXTEEN

HIGH-MOLECULAR-WEIGHT HYDROCARBONS IN ZOOPLANKTON, SEDIMENT AND WATER

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ABSTRACT

An extensive survey of the level of natural and petroleum type hydrocarbons in seawater, zooplankton and sediment was made. The presence or absence of indicator parameters including, n-paraffins, odd-even ratios, unresolved GLC humps, and GC/MS confirmation of aromatic hydrocarbons, were taken as a measure of petroleum contamination.

The zooplankton samples showed unambiguous and substantial petroleum contamination. Twenty-six of the 84 samples examined showed contamination. A similar observation was made during the 1975 STOCS study, and, in fact, there was a slight increase in the percent of samples contaminated. This contamination was probably due to micro-tar-balls in the zooplankton tows. Further study is recommended.

Dissolved and particulate hydrocarbons in seawater were determined in 73 and 70 samples, respectively. The data indicated that concentrations of dissolved and particulate hydrocarbons were similar in magnitude. Hydrocarbons in both fractions decreased in concentration with distance offshore. Seasonal trends in concentration were also indicated for both fractions but were difficult to interpret. The hydrocarbon composition of the two fractions were often similar with a slight odd carbon preference indicated among n-alkanes. Hydrocarbons in the C_{25} - C_{33} molecular weight range were generally the most abundant.

Analyses of 175 sediment samples from the open shelf revealed that sediment hydrocarbon chemistry is complex. The observed hydrocarbon patterns require multiple sources, including plankton, bacteria, infauna, and perhaps higher plant detritus. Detection of traces of petroleum hydrocarbon in this matrix will require detailed studies of sediment as an active sink for organic matter.

INTRODUCTION

These studies of high-molecular-weight hydrocarbon patterns and abundances in the water column, zooplankton and sediment were undertaken within a double conceptual framework. The driving force for the studies has been the urgent need for BLM, as the responsible federal agency, to provide a significant body of benchmark and descriptive data for environmental quality monitoring purposes. Overlapping this environmental quality concern of BLM and the scientific community, has been the wish of the participating scientists to extend our understanding of the organic geochemistry of continental margins. A sharing of these needs, environmetal and basic science, has resulted in a large body of useful chemical data.

Background

The study, and this report, deal with hydrocarbons in the molecular weight range of C-15 to C-36, saturated, unsaturated and aromatic, isolated from the water column, zooplankton tows and sediments. Our knowledge of dissolved and particulate organic compounds in seawater is meager, in part, because of the low levels of occurrences and, in part, because most studies described in the literature were generally designed for special purposes. For this reason, the large descriptive study of hydrocarbons in this study is a useful contribution to the field, although technical problems preclude sophisticated statistical inferences. The hydrocarbons of seawater are best understood if related to the general organic chemistry of seawater. Organic matter (OM) in seawater is derived from living material. For experimental and biological reasons, OM is generally classified as dissolved or particulate. The particulate material may be further sub-divided into living and non-living (detritus).

The study of these materials has received great impetus due to recent developments in analytical instrumentation. There is now a fairly clear picture of the level of total particulate organic matter (POC) and total dissolved organic matter (DOC) in various marine environments. Specific chemical compounds have been isolated and identified in a few cases. However, our knowledge at this level is so fragmentary that definition of the reactivity and function of POC and DOC cannot be made. Hydrocarbons, both natural and petroleum derived, constitute a fraction of both POC and DOC. The approximate levels of the various organics in the water column are given in Tables 1 and 2.

Several points should be noted from these data; the coastal seas are enriched in organic matter relative to the open ocean; only 10-20 percent of the total DOC can be accounted for as individual organic molecules (some workers speculate that the remainder may have reacted to form a high molecular weight polymer); and, that fraction of DOC composing hydrocarbons, natural or man-induced, is among the least abundant molecular types in the sea, being only slightly more abundant than vitamins. This low level suggests that oil pollution might be quickly obvious in seawater, but low level is also the reason that the measurement of oil baselines in the sea is so difficult. BLM programs are providing a DOC-HC baseline by virtue of the scale of the program.

The chemical composition of living matter, including the marine plankton, is well known, especially when compared to seawater and sediment. Biochemistry texts treat this topic in a general way. Generalizations regarding the chemical makeup of marine life which are useful for oil pollution research include the following; marine life contains essentially no aromatic hydrocarbons; in general, the normal paraffins of cells have

TABLE 1

ORGANIC MATTER IN SURFACE GULF OF MEXICO SEAWATER (mg/l)

CONSTITUENT	LOCATION	CONCENTRATION RANGE
DOC[1]	Open Gulf[3]	0.45 - 1.1
POC[2]	Open Gulf	0.02 - 0.13
DOC	N.W. Gulf OCS[4]	1.2 - 2.5
POC	N.W. Gulf OCS	0.1 - 0.4
DOC	N.W. Gulf, inner shelf[4]	1.0 - 4.0
POC	N.W. Gulf, inner shelf	0.2 - 2.5
DOC	N.W. Gulf estuaries[4]	3 -30
POC	N.W. Gulf estuaries	1 -30

[1]DOC = total dissolved organic matter
[2]POC = particulate organic matter
[3]from Fredericks and Sackett (1970)
[4]from Mauer and Parker (1972)

TABLE 2

SPECIFIC DISSOLVED ORGANIC COMPOUNDS IDENTIFIED IN SURFACE SEAWATER (μg/l)

SUBSTANCE	LOCATION	CONCENTRATION RANGE	REFERENCES
DOC[1]	Average value	1000	
Amino Acids total	North Atlantic	6 - 47	Pocklington (1971)
Alanine	"	1.7 - 15	"
Leucine	"	0.1 - 5.4	"
Total carbohydrates	Average	200 -600	Williams (1975)
Individual sugars	Average	0 - 20	"
Lipids			
Total Lipids	Gulf of Mexico	150 -310	Jeffrey (1970)
Total Fatty Acids	N. E. Pacific	1 - 9	Williams (1965)
Individual Fatty Acids	N. E. Pacific	0 - 2	Williams (1965)
Total n-Paraffins	Gulf of Mexico	0.8 - 1.0	Parker (1972)
Non-Volatile Hydrocarbons	Mediterranean	4	Monaghan (1973)
Vitamins	Average	0.1	Stumm (1975)

[1]DOC = total dissolved organic matter

an odd number of carbon atoms; and, hydrocarbons are a minor constituent of marine life, and even less concentrated in seawater (Clark and Blumer, 1967).

Useful environmental insights most likely result when the overall organic geochemistry of systems is considered. This is especially so for the sediment oil pollution problem since sediments are made complex by the long time interval of exposure. One must keep in mind that all petroleum had its origin in sediment much like that observed today. The organic geochemistry of sediments is well studied with regard to the occurrence and chemical transformations of fatty acids, amino acids, fatty alcohols, stable carbon isotopes and kerogen (Eglinton, 1969; Yen, 1977). Table 3 describes an idealized Gulf of Mexico shelf sediment based on data gathered in this laboratory over a 10 year period. As the case for seawater, the hydrocarbons constitute a minor faction of the total organic matter in surface sediment. The generalizations made for living matter apply somewhat to surface sediment, but exceptions are more frequent. In general, a suitable working hypothesis is that aromatic hydrocarbons are absent or very low and normal paraffins are mostly of odd carbon numbers. Hydrocarbon patterns are complex because bacteria and infauna add their own special hydrocarbon composition to sediment over the decades of deposition. Thus, sediment may be viewed as the time-integrated organic record. If oil pollution is added to sediment, the record will persist for some time, whether for days, weeks or months is not known. The sediment hydrocarbon data gathered under this BLM program is considered in this framework.

METHODS AND MATERIALS

The purpose of this study was to obtain a significant body of analy-

TABLE 3

MATERIAL BALANCE IN AN IDEALIZED GULF OF MEXICO SURFACE SEDIMENT[1]

Dry Weight	16 g
Total Organic Carbon	100 mg
Non-Lipid Carbon	95 mg
Total Lipid Carbon	5 mg
Total Non-Saponifiables	3 mg
Total Fatty Acids	0.4 mg
Total Sterols	0.1 mg
Total Fatty Alcohols	0.1 mg
Total Saturated Hydrocarbons	0.03 mg

[1]from Parker (1967), Parker (1969), and Sever and Parker (1969)

tical data on environmental hydrocarbons levels which could serve as a baseline against which future levels might be compared. An intense effort was made to identify hydrocarbon molecules which might serve as indicators of petroleum. The experimental elements were: careful collection, preservation and transportation of samples in the field; laboratory analyses using as nearly as possible standardized and modern techniques; and, central data storage and computer analysis.

Sample Collection

The sampling frequency, stations sampled, and number of samples collected was as shown in Table 4.

Zooplankton samples were collected with a 1-m net (250 μm NITEX mesh) which was towed obliquely from near-bottom to near-surface for 15 minutes. The net, dedicated to this use, was kept in a special clean box when not in use. This clean box was constructed of 3/4-in. plywood which was covered inside and outside with a layer of fiberglass cloth sealed with epoxy resin. The box was of sufficient size to contain the frame, net and all lines. The lid sealed so as to exclude dust. Samples were not "washed down" the net into the cod-end so as to avoid contamination from the ship's pumps. If the net needed cleaning, it was lowered to a depth of 20 m, raised, and lowered without a cod-end collecting jar. The samples were placed in precleaned glass jars with teflon lid liners and frozen on board ship. The jars were precleaned by washing with high phosphate detergent and hot water followed by rinsing with double distilled water then with methanol.

Seawater samples were collected from 10 m below the surface using a collection device which consisted of a glass carboy which could be opened and closed by means of a plug attached to a nylon line from the ship's deck. A minimum of 38 ℓ (two, 5-gallon carboys) was taken. The samples

TABLE 4

SUMMARY OF SAMPLING FREQUENCY, STATIONS SAMPLED AND NUMBER OF SAMPLES

Sample Type	Station/Transect	TIME	Number of Samples	
Water/dissolved	1-3 / I-IV	3 Seasons	36	
Water/dissolved	1-3 /II	6 Months	18	
Water/dissolved	20 replicate samples taken at random		20	
				74
Water/particulate	1-3 / I-IV	3 Seasons	36	
Water/particulate	1-3 /II	6 Months	18	
Water/particulate	20 replicate samples taken at random		20	
				74
Zooplankton	1-3 / I-IV	3 Seasons	36	
Zooplankton	1-3 /II	6 Months	18	
Zooplankton	30 replicate samples taken at random		30	
				84
Sediment, shelf	1-7 / I-IV 5 replicate samples taken at each station during one season	3 Seasons	175	
Sediment, Topographic Highs			18[1]	
				193
		Total for Analyses		425

[1] 24 samples were planned, but 18 were taken

were filtered through glass fiber filters which had been precleaned by reflux with chloroform. The nominal pore size of the filters is 1.2 μm. The pads containing the particulate organic matter (POC) were placed in small glass jars with teflon lids and frozen. The filtrate was poisoned with 50 ml of chloroform and processed as soon as possible on returning to the laboratory.

Sediment samples were obtained as subsamples of a Smith-MacIntyre grab. Each subsample, weighing approximately 1 kg, was taken from the top 5 cm of the grab. The samples were placed in precleaned glass jars with teflon lid liners, taking care not to fill each jar more than one-half full. The samples were frozen on board ship and kept frozen until analysis. Obvious marine animals were seldom encountered in the sediment, but were discarded when found. If the sample was a pooled sample, this operation was performed at sea prior to freezing. In cases where chemical analysis was possible within a few days after collection, the samples were maintained at 0°C to avoid the risk of the jar breaking due to freezing.

Laboratory Analysis

Throughout this study, purified solvents, inorganic chemicals and double distilled water were used. Control samples and blanks were used to insure that no gross contamination was present. The laboratory was in a new building and had never been used for any other purposes. At no time was severe contamination encountered. The most serious problem was the chemical purity of the organic solvents used. For example, some lots of hexane and heptane contained a variety of hexanes and heptanes.

Zooplankton Samples

Zooplankton hydrocarbons were isolated and purified using the method described in Attachment A (Contract AA550-CT6-17) which is detailed in

the following description. The samples were quickly thawed by standing the jars in warm water. Care was taken not to contaminate the rim of the jar. The sample was inspected for tar-balls greater than 1 mm and any present were discarded. Micro-tar balls were present in some samples and were taken as a valid part of the sample. The thawed sample (approximately 25 g wet weight) were poured into a pre-cleaned (by extraction) cellulose Soxhlet extraction thimble (Whatman, single thickness, 33 x 80 mm) and allowed to drain. If the seawater filtrate showed color, it was extracted with a few ml of toluene which was added to the Soxhlet extractor.

The thimble was placed in a Soxhlet extractor, heated on a steam table, and continuously extracted for 12 hours using a solvent charge of 125 ml of the methanol-toluene (7:3) azeotrope. The extraction was repeated with fresh solvent and the extracts combined. The solid residue in the thimble was dried at 80°C and weighed. The extracts were taken to dryness on a roto-vap at 45°C with 1/2 an atmosphere of vacuum.

The lipid recovered by Soxhlet extraction was saponified by refluxing (6 hours) with a 0.5 KOH-methanol solution. This reflux solution had been pre-purified by extraction with toluene. In cases where GLC indicated that methyl esters were present, the sample was re-saponified. In general, ester formation did not occur if a few milliliters of distilled water were added to the saponification solution an hour prior to reflux termination. When saponification was complete the mixture was transferred while warm to 2 separatory funnel and enough saturated NaCl solution was added to cause two phases to form when 15 ml of n-hexane was added. The non-saponifiable lipids, which included hydrocarbons, were extracted into three 15 ml portions of n-hexane. The saponifiable fraction was discarded, and the non-saponifiable extracts combined and set aside for purification by column chromatography.

Column chromatography was carried out according to Attachment A (Contract AA550-CT6-17) to purify the total hydrocarbon extract and to separate it into two chemical fractions, saturated hydrocarbons and the non-saturated hydrocarbons which include both olefins and aromatic molecules. This method is described in the following paragraphs.

A large batch of one part alumina plus two parts silica gel, both Activity I, was prepared using hydrocarbon-free chemicals. Column chromatography was carried out using this material with a sample to packing ratio of 1:300 in a glass column with a teflon stopcock and with a length to inside diameter ratio of about 20:1. As standard procedure, the packed column was washed with two column volumes of hexane prior to sample loading.

The sample from the saponification procedure which had been reduced to a small volume (a few ml) using an all glass flash evaporator was transferred to the top of the column using a transfer pipette. The saturated hydrocarbons eluted with two column volumes of hexane were set aside for gas chromatography (GLC) and gas chromatography-mass spectrometry (GC/MS). Hexane insoluble material not previously added to the column was washed onto the column with a few milliliters of benzene. The nonsaturated, so called "aromatic", fraction was eluted with two column volumes of benzene. The benzene in this fraction was gradually replaced by adding hexane and evaporating under a stream of nitrogen, taking care not to let the sample go dry at any time, yielding a final volume of 1 ml or less. The hexane eluate was reduced in volume in the same way. The weight, when enough material was present to weigh, of each fraction was determined on an aliquot of the eluates. The samples were set aside and later submitted to GLC and, if appropriate, to GC/MS.

The GLC and GC/MS techniques used are described later in this section of the report.

A tissue sample spiked with n-C_{32} and phenanthrene was taken through the procedures decribed above and gave 95 and 98% recovery, respectively.

Water-Particulate High-Molecular-Weight Hydrocarbons

The frozen filters containing particulate hydrocarbons were thawed, placed in a 50 ml flask and extracted with 15 ml hexane on a hot plate at 50°C for at least three hours. The hexane was decanted, replaced with an equal volume of chloroform and the extraction was repeated for an additional three hours at 50°C. The extracts were combined and reduced to near dryness under a nitrogen stream. A small amount of hexane was added continuously to replace the chloroform phase. The hexane was evaporated to about 0.1 ml under a stream of purified nitrogen at room temperature. The sample was transferred to a micro silica-gel-alumina column (0.4 cm x 8 cm). Another 0.4 ml portion of hexane was used to rinse the vial in which the sample was evaporated and this hexane was added to the column. Hexane was used to elute a 0.2 ml initial fraction which was discarded, following which a 2 ml hexane fraction was collected. The non-saturates were eluted with 2 ml of benzene. Hexane and benzene eluates were evaporated under nitrogen to a volume of about 100 µl. The samples were tightly sealed in a vial with Teflon-lined caps and further concentrated to a volume of about 25 µl (exact volume was measured with a 50 µl syringe) just prior to gas chromatographic analysis.

Water-Dissolved High-Molecular-Weight Hydrocarbons

Samples were extracted with chloroform in a continuous flow extractor as shown in Figure 1. The 38 l sample was passed through the apparatus at a flow rate of about 26 ml/min. (38 l in 24 hours). The extraction efficiency of the method was tested at several flow rates. The results of these tests (Table 5) indicated an efficiency of greater than 95 percent when the flow rate was less than 38 l per 18 hours.

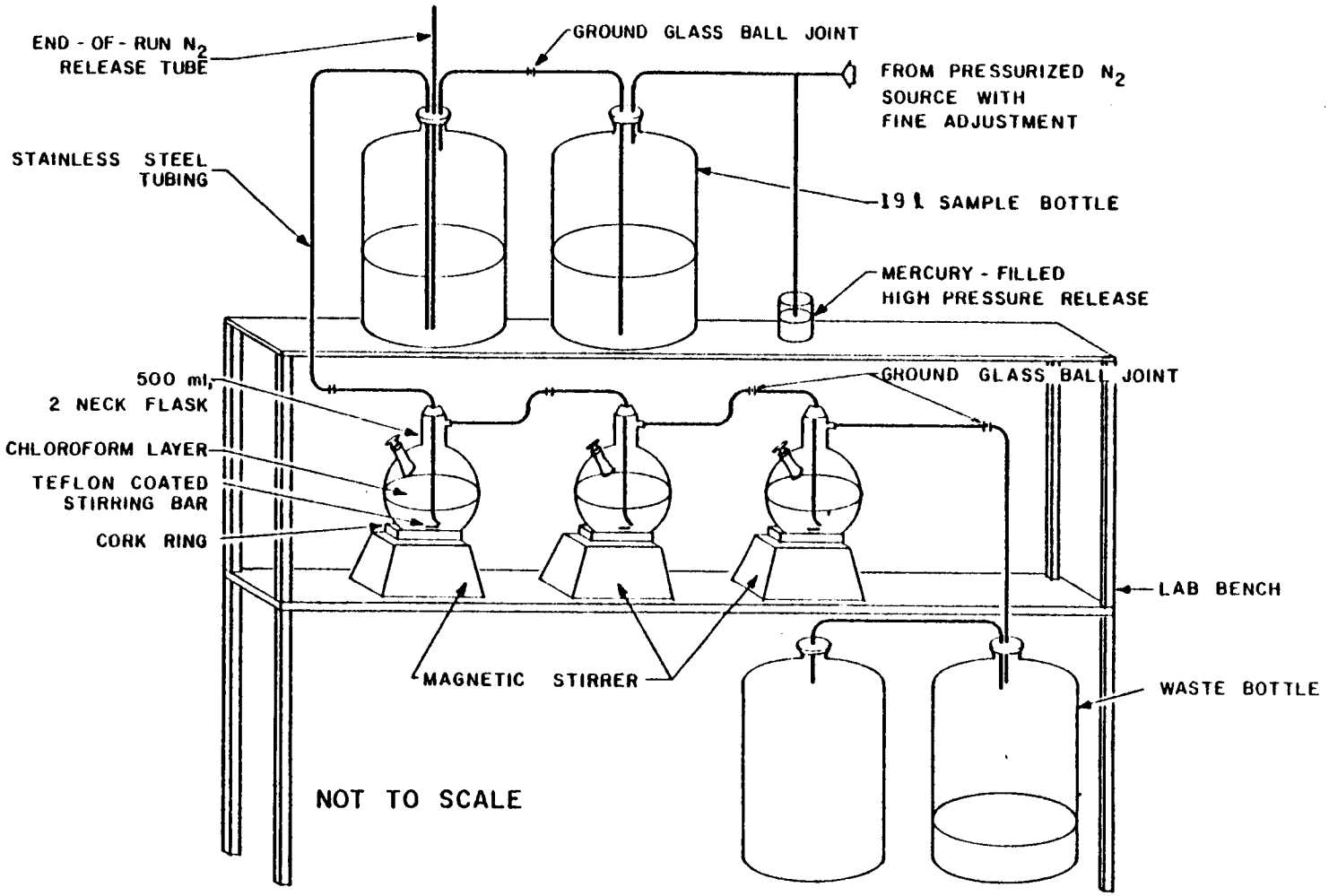

Figure 1. Apparatus for the Extraction of Seawater With Chloroform.

TABLE 5

EXTRACTION EFFICIENCY TEST RECOVERY OF n-C_{21} PARAFFIN ADDED TO 38-l OF FILTERED, EXTRACTED SEAWATER. CONCENTRATION: 0.13 µg/l

	#1	#2	#3	Av.	Percent Recovery
Standard	1742[1]	1747	1700	1730	100%
Standard Diluted, Evaporated	1727	1837	1682	1749	101%
Test 5 24 hr Extraction	1701	1639	1661	1667	96%
Test 6 24 hr Extraction	1779	1757	1638	1725	100%
Test 7 <18 hr Extraction	1649	1812 (1811)	1648	1702	98%
Test 8 <15 hr Extraction	1487	1506	1546	1513	87%

[1]Numbers represent electronic integration values

After the water had been extracted, chloroform from the extraction chambers was transferred through glass tubing to a flask in which the pressure had been reduced by means of a small diaphragm pump. Chloroform from the carboys was poured into the same flask. The sides of the extraction chambers and the collection carboys were rinsed with fresh chloroform. The chloroform extracts were then combined and reduced to a volume of 5 ml by distilling off the chloroform at reduced pressure through a Kuderna-Danish column. The sample was then further concentrated under nitrogen and transferred to a microsilica-gel-alumina column as described for the particulate hydrocarbon samples. The final hexane and benzene eluates were held for GLC and GC/MS analysis.

Sediment

The sediment samples were freeze-dried as the first step of analysis. This was accomplished by spreading the sample as a thin paste on stainless steel trays. The freeze drier used in the early part of the study was a small unit (Virtis Model 10-PR) which was fitted with an oversized vacuum pump and a double cold trap so as to accelerate drying. Later, a Virtis Model 25 SRC was used as factory installed.

Hydrocarbons were isolated from sediments and purified using the method described in Attachment A (Contract AA550-CT6-17) with the reflux option. This procedure is as follows: The freeze-dried sediment sample (200-300 g) was placed in a large round-bottomed flask (1-l) and covered (approximately 200 ml) with the toluene-methanol azeotrope (3:7) using care that the flask was not more than one-half full so as to avoid severe bumping on the steambath. The flask was placed in reflux on a steam bath for 14 hours using a Friedrichs condenser with standard taper joints and a drip tip. The solvent, while warm, was decanted through a prewashed filter (Whatman 541) and set aside for later analysis. Fresh solvent and

any sediment on the filter paper were added to the flask and the reflux extraction repeated for 7-10 hours. Finally, the sediment was filtered onto a Buchner funnel, the sediment washed with warm hexane and all extracts combined. The sediment was dried at 45°C and weighed.

The combined extracts were taken to just dryness on a roto-vap and taken up in hot KOH-methanol (0.5 N) for saponification. Saponification was carried out according to the procedure described above for zooplankton. No severe problem was encountered with the formation of methyl esters, but in cases where GLC or GC/MS indicated ester formation, the samples were resaponified. Texas coastal sediment is not high in organic matter relative to California basin sediments for which reason elemental sulfur was not indicated to be a problem in this study. The non-saponifiable fraction obtained in the hexane extract of the saponification mixture was taken to dryness and weighed, yielding the weight of non-saponifiable lipids.

The non-saponifiable lipids were submitted to silica-gel-alumina column chromatography according to the scheme described for zooplankton. Several times during the column chromatography of sediments it appeared that the saturated hydrocarbons were not completely eluted with the two column volumes of hexane. It was necessary to repeat the column chromatography. This problem deserves special care because often the hexane used was rich in cyclohexane which, being more polar, should cause even faster elution. Other workers may wish to use more solvent for this elution.

The two hydrocarbon fractions isolated from sediments and purified, saturated and non-saturated, were finally taken up in a small volume of hexane (0.05 to 0.5 ml) and both for GLC and GC/MS analysis.

Instrumentation

Gas Chromatographic Analyses

The primary tool for component identification and quantification used in this project was the gas chromatograph (GLC). Identification by GLC is accomplished by comparison of the relative retention times of the unknown compounds with those of selected known standard compounds. Such identification techniques are reasonably valid if the mixtures are not complex and expected components are encountered.

The GLC instruments used in this study were a Perkin-Elmer[1] Model 900 and a Varian Model 3700. Both instruments were equipped with dual column flame ionization detectors and electronic integration of peaks. An Infotronics Model CRS-204 and Columbia Scientific Industries Model CSI-38 digital integrators were used to quantify the GLC separate components. However, due to the complex nature of the GLC patterns and the lack of sophistication of the integrators, it was necessary to hand check each GLC.

Both GLC instruments used 0.32 cm (1/8 in.) by 183 cm (6 ft) dual, packed columns to effect the separation. The column packing material was 60-80 mesh Gaschrom Q (acid-washed) with a 5 percent by weight loading of FFAP (product of Varian Corp) as the stationary liquid-phase. Generally, the operating conditions were as given in Table 6. These columns and conditions were used for virtually all analyses of STOCS samples. On occasion, lower initial temperatures and longer initial or final hold times were used.

The high temperature to which these columns were subjected was higher

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[1]Use of brand names does not constitute an endorsement but is included for descriptive purposes only.

TABLE 6

OPERATING CONDITIONS FOR GLC ANALYSIS

Carrier Gas	Helium
Carrier flow rate	30 ml/min.
Flame detector gas flow rates	
Hydrogen	30 ml/min.
Air	300 ml/min.
Temperature programming	
Initial temperature	70°C
Initial temperature hold time	6 min.
Program rate of rise	6°C/min.
Final temperature	270°C
Final temperature hold time	24 min.

than that recommended by the liquid-phase manufacturer. For this reason, the columns had a large amount of column "bleed" at the high temperature which shortened the useful life. Approximately 100 samples could be analyzed before the resolution was considered too poor to permit further analysis.

Instrument sensitivity and resolution were checked daily by running a standard mixture of components. When the resolution fell below that recommended in Attachment A (Contract AA550-CT6-17), the GLC columns were replaced. The daily standard check was used to establish the sensitivity of the instrumentation to allow quantification of the GLC peak data.

GLC peak data for each sample are presented in Appendix L. The data consist of a listing of peak retention index and concentrations in the sample for each of the two analyzed fractions: hexane eluate and benzene eluate from liquid column chromatography. The retention index used is normalized to the relative retention times of the n-alkanes. Thus, for example, the hydrocarbon n-hexadecane has a relative retention index equal to 1600, n-heptadecane equal to 1700, etc. Hydrocarbons having intermediate retention times between n-alkanes are assigned interpolated retention indices; for example, pristane (19 carbon atoms) has a retention index of 1670 and phytane (20 carbon atoms) a retention index of 1780 in as much as their peaks are eluted prior to elution of n-heptadecane and n-octadecane, respectively, on the columns in this study. Retention indices depend upon the nature and molecular size of the component being eluted. Thus, on FFAP, branched chain hydrocarbons elute earlier than the straight chain homologs of the same molecular weight while unsaturation of carbon to carbon bonds will cause the component to elute later than the saturated compound having the same number of carbon atoms.

Gas Chromatography-Mass Spectrometer-Computer Analyses

Where complex component mixtures are to be analyzed it is necessary to augment the chromatographic technique with other ogranic compound identification methods. One of the more powerful methods is mass spectrometry. Gas chromatography combined with mass spectrometry GC/MS was applied to many of the samples also characterized by gas chromatography alone. A computerized data system was used to assist with data acquisition and data analysis.

The 1976 contract called for GC/MS analysis of 10% of the sample fractions generated in the study. In all, 84 zooplankton, 74 water filtrates, 74 water particulates and 175 sediment extracts were generated with two fractions for each sample. In addition, 18 sediment samples were processed in conjunction with the Topographic Features Study. Thus, 425 samples or 850 fractions were processed. Because the necessary data acquisition equipment was not available until the fourth quarter of study, data from only a limited number of the 85 contracted analyses were available for inclusion in this report. The remaining analyses and data are reported in an addendum to this report.

The instrument used was a DuPont Instruments Model 21-49 GC/MS with a DuPont Instruments Model 21-094B MS Data System. The chromatograph associated with this instrument was a Varian-Aerograph Model 2700 modified DuPont for this service. The effluent from the single chromatographic column was split 9:1 with the major portion of the sample going to the mass spectrometer and the minor portion to a flame ionization detector.

The chromatographic column and conditions used for GC/MS analysis were identical to those used in standard GLC techniques.

It was recognized that for this column these conditions were not necessarily the best for general GC/MS work and that column "bleed" above 220°C was high for GC/MS analyses. However, these parameters were the same as those used in the standard GLC analyses of the samples and, thus, the interpretation of the data was enhanced by direct comparison of the two data sets.

The mass spectrometer was operated with a source temperature of 200°C, electron accelerating potential of 70 volts and an ion accelerating potential of approximately 1400 volts. The mass range from above m/e = 500 to below m/e = 40 was continuously scanned.

The instrument is capable of unit resolution at m/e $\gtrsim$ 1100 but slits and focussing parameters were adjusted for maximum sensitivity at m/e $\sim$ 600. Sensitivity was estimated at better than 1.5 ng hydrocarbon at molecular weight 282 in the reconstructed chromatogram. Specific ion mass-chromatograms effectively allowed even better sensitivity.

Samples for GC/MS analysis were not selected randomly, but rather were selected to provide information about peaks which consistently were found prominent in many samples. The retention index - concentration data for all analyses were manipulated by a computer program to sort out those chromatographic peaks of "importance" and to flag those samples which could be used to characterize the peak by GC/MS analyses. Such lists of "important" peaks were prepared for each sample type (zooplankton, sediment, water) and each fraction type (saturated, non-saturated). These lists are given in Tables 7-10. Included in these tables are the peak identifications as determined (or confirmed) by GC/MS analysis. Not all peaks were identified. Not included in these tables are the most commonly encountered and easily identified n-alkanes and pristane.

TABLE 7

GAS CHROMATOGRAPHIC PEAKS OF SIGNIFICANCE
IN HEXANE ELUATES OF ZOOPLANKTON EXTRACTS[1]

Retention Index	Formula	Identification by GC/MS
1645	$C_{16}H_{32}$	C_{16}:1 hydrocarbon
1847	$C_{18}H_{36}$	C_{18}:1 hydrocarbon
1923	$C_{20}H_{40}$ or $C_{20}H_{38}$	C_{20}:1 or C_{20}:2 hydrocarbon
1965	$C_{20}H_{38}$	Phytadienes
2049	$C_{20}H_{40}$	C_{20}:1 hydrocarbon
2141	$C_{25}H_{46}$	C_{25}:3 highly branched hydrocarbon
2250	$C_{22}H_{44}$	C_{22}:1 hydrocarbon

[1]The frequently occurring n-alkanes and isoprenoids are omitted from this table.

TABLE 8

GAS CHROMATOGRAPHIC PEAKS OF SIGNIFICANCE
IN BENZENE ELUATES OF ZOOPLANKTON EXTRACTS

Retention Index	Formula	Identification by GC/MS
1923	$C_{15}H_{23}OH$	di-t-butyl creson (preservative)
1953	$C_{20}H_{38}$	phytadienes C_{20}:2 hydrocarbon
1984	$C_{20}H_{38}$	phytadienes C_{20}:2 hydrocarbon
2010	?	?
2036	?	?
2129	$C_{21}H_{38}$	C_{21}:3 hydrocarbon (branched)
2146	$C_{31}H_{31}COOCH_3$	Methyl ester of C_{16}:0 fatty acid
2188	$C_{21}H_{38}$	C_{21}:3 hydrocarbon (branched)
2221	?	?
2252	?	?
2281	$C_{19}H_{30}$	C_{19}:5 hydrocarbon
2332	?	?
2412	$C_{20}H_{39}COOCH_3$	Methyl ester of C_{21}:1 fatty acid
2441	$C_{25}H_{46}$	C_{25}:3 hydrocarbon (branched)
2515	$C_{25}H_{44}$	C_{25}:4 hydrocarbon (branched)
2669	?	phthalate ester
2740	$C_{25}H_{40}$	C_{25}:4 hydrocarbon
2759	$C_{20}H_{34}$	C_{20}:4 hydrocarbon
2799	$C_{20}H_{34}$	C_{20}:4 hydrocarbon
3027	$C_{30}H_{50}$	Squalene isomer
3056	$C_{30}H_{50}$	Squalene isomer
3148	$C_{24}H_{38}O_4$(?)	phthalate ester (dioctyl?)

TABLE 9

GAS CHROMATOGRAPHIC PEAKS OF SIGNIFICANCE
IN HEXANE ELUATES OF SEDIMENT EXTRACTS[1]

Retention Index	Formula	Identification by GC/MS
1444	$C_{14}H_{28}$	Tetradecene (probable straight chain)
1647	$C_{16}H_{32}$	Hexadecene (probable straight chain)
1742	$C_{17}H_{34}$(?)	Heptadecene (?)(probable straight chain)
1848	$C_{18}H_{36}$	Octadecene (probable straight chain)
1955	$C_{18}H_{37}OH$	Octadecanol
1972	?	Unknown
2054	?	Unknown
2147	$C_{21}H_{42}$	Heneicosene (probable straight chain)
2241	?	Unknown
3054	$C_{30}H_{50}$	Squalene

[1]The frequently occurring n-alkanes and isoprenoids are omitted from this table.

TABLE 10

GAS CHROMATOGRAPHIC PEAKS OF SIGNIFICANCE
IN BENZENE ELUATES OF SEDIMENT EXTRACTS[1]

Retention Index	Formula	Identification by GC/MS
1833	?	Unknown
2008	?	Branched ? - Unsaturated ?
2041	$C_{14}H_{29}COOCH_3$	Methyl ester of C_{15} fatty acid
2067	$C_{22}H_{46}$	Branched (isoprenoid ?)
2107	$C_{16}H_{29}OH$(?)	Possible C_{16}:2 alcohol
2150	?	Branched, unsaturated
2205	$C_{18}H_{35}OH$(?)	Possible C_{18}:1 alcohol
2321	$C_{19}H_{38}$	Zamenes
2422	$C_{19}H_{35}OH$(?)	Possible C_{19}:2 alcohol
2618	$C_{18}H_{33}COOCH_3$	Methyl ester of C_{19}:2 fatty acid
2643	$C_{30}H_{52}$(?)	Highly branched (dihydro squalene?)
2830	$C_{24}H_{38}$ or $C_{21}H_{39}OH$	C_{24}:6 hydrocarbon or possible C_{21}:2 alcohol
2862	$C_{20}H_{30}$(?)	C_{20}:6 hydrocarbon (?)
3010	$C_{23}H_{47}COOCH_3$	Methyl ester of C_{24} fatty acid
3044	$C_{25}H_{38}$ (?)	C_{25}:7 hydrocargon (?)
3222	$C_{20}H_{37}COOCH_3$	Methyl ester of C_{21}:2 fatty acid

[1]Fatty acid methyl esters are generally artifacts of the saponification procedure and many of the more commonly occurring esters are omitted from this list.

Peak identifications were made from analysis of the mass spectrum of the component. Interpretive techniques such as those given in McLafferty (1973) were used. Various "libraries" of mass spectral data were used to assist in interpretation of spectra. Computer searches were frequently made of three separate data bases: (1) Atlas of Mass Spectral Data-Purchased from DuPont Instruments, Inc. including 7054 mass spectra, most of which are from the Atlas published by John Wiley and Sons, Inc.; (2) MSSS (Mass Spectral Search System), a data base maintained by the Environmental Protection Agency, National Institutes of Health and National Bureau of Standards which contains over 30,000 mass spectra and which is made available through the commercial time sharing computer company - Cybernetics Division of ADP Network Services; and (3) a library and search algarithm maintained by Dr. Conrad Cone, Chemistry Department, University of Texas at Austin containing over 6000 mass spectra and available through the UT-Taurus interactive computer system. In addition, frequent use was made of the four volume Registry by Stenhagen *et al.* (1974).

The GC/MS analysis techniques were used primarily to attempt to identify specific compounds which occurred with some frequency in various samples. The results of such studies are reported in Appendix L, Figures 1.1 through 1.64. In some cases the data analysis was carried much further as given in the following example.

Figure 2 shows a total ion reconstructed gas chromatogram of the benzene eluted fraction of sediment sample AMCL. This may be compared with the flame ionization record for this same analysis (Figure 3). At each of the scan points shown in Figure 2, a mass spectrum was obtained.

The GC/MS "software" was used to search the mass spectra for mass fragment ions of "significance". Such a search is reported for this

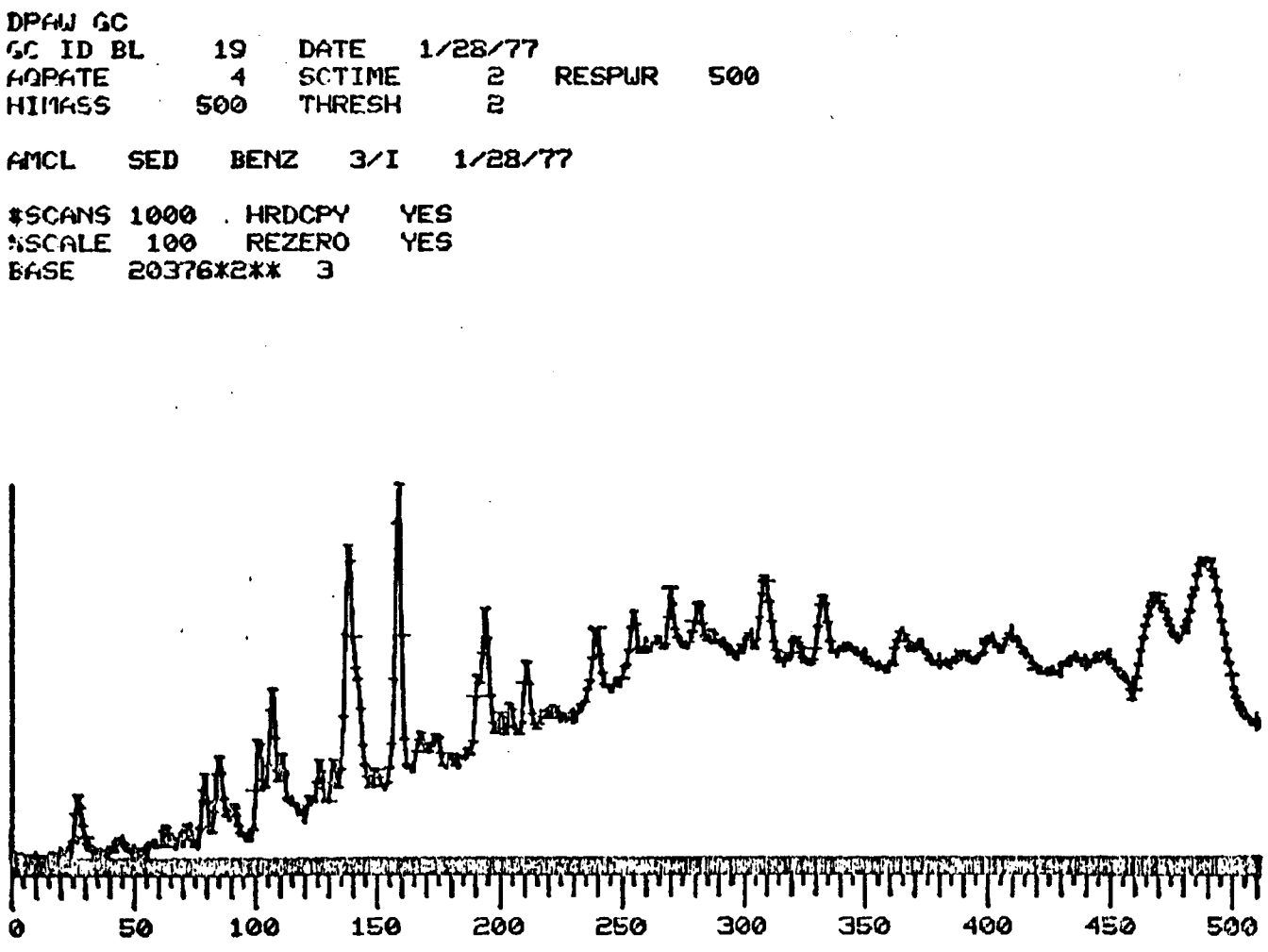


Figure 2. Reconstructed total ion gas chromatogram for sample AMCL benzene eluate.

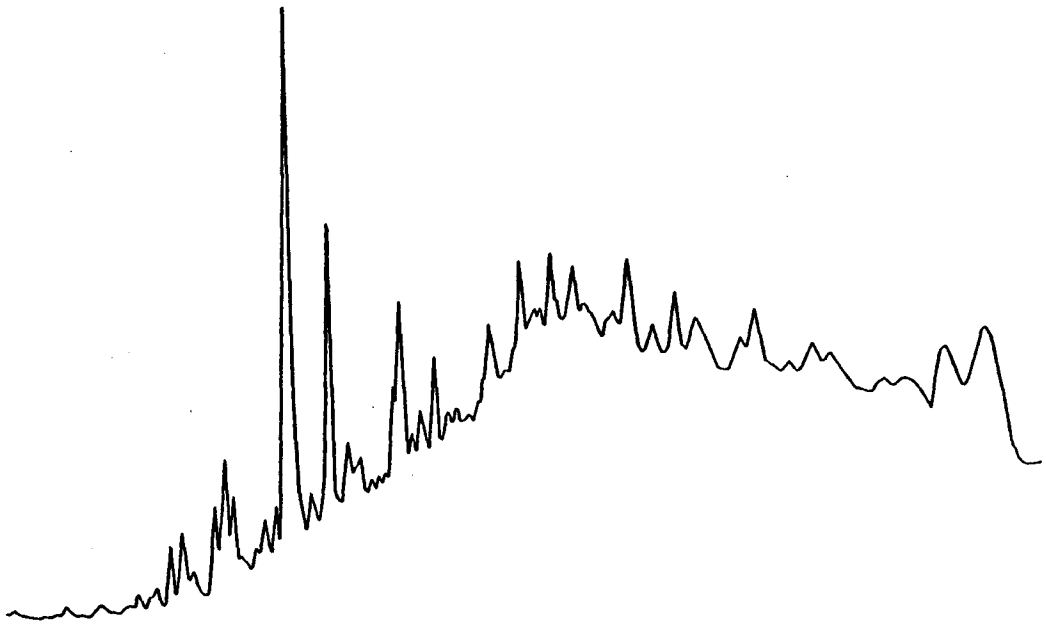


Figure 3. Flame-ionization Record for GC/MS Run of Sample AMCL Benzene Eluate.

example in Figure 4. The most significant ions given in Figure 2 are those frequently encountered in hydrocarbon samples (*e.g.*, 41, 43, 55, 57, 69, 83, etc.) which represent fragment ions of alkyl chains.

By constructing a chromatogram for a given ion, certain compounds can be emphasized in the total chromatogram. Such a mass chromatogram of mass 205, for example, is given in Figure 5. A prominent peak at scan number 111 can be seen to be insignificant in the total ion chromatogram. The mass spectrum of this compound is given in Figure 6. The compound has a molecular weight of 220, readily loses a methyl group (m/e 220-15 = 205), could be aromatic (relatively strong molecular ion), and has an alkyl group of about four carbons (m/e 57). A computer comparison of this mass spectrum with a library of 7054 mass spectra (Figure 7) shows a reasonable "goodness of fit" for the hindered phenol-ionol. This compound is commonly used as a preservative in solvents, foods and packaging items and probably represents an artifact of laboratory contamination.

A mass chromatogram at Mass 156 pinpoints a major peak at scan number 139 as shown in Figure 8. This peak is attributed to a dimethylnaphthalene used as a "spike" in this sample. The mass spectrum for this compound (Figure 9) confirms the identification.

Figure 10 shows a mass chromatogram of mass 149. This mass fragment is characteristic of phthalate esters which are universally employed as plasticizers and are frequently found as contaminants in laboratory processed samples. These compounds are easily recognized, difficult to avoid, and, in our laboratory, we have learned to recognize and ignore them.

The mass spectrum of the largest peak in the sample is given in Figure 11. The prominance of the fragments at m/e = 41, 55, 69, 83 and 97 strongly emphasize the unsaturated character of this compound. A

```
SIGNFPK
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

IGNORE        0,     0,     0,     0
MILOUT   500  HRDCPY     NO

  MASS    MAX    FIRST    SUM
         INTN    OCCUR   IONS *2** 6

    41    1000        5   17792
    43    1000        2   25640
    45    1000      380   14920
    55    1000      158   17447
    57    1000        4   13748
    69    1000       25   14067
    83    1000       89    7793
    91    1000      142    8300
   105    1000        1    5450
   152    1000      168    1825
   202    1000      343    2056
   156    1000      138    2809
   149    1000      333    2201
   178    1000      254    1754
   205    1000      110    1946
   218     992      492    2117
    81     970      118    8813
```

Figure 4. Significant Peak Index for Sample AMCL Benzene Eluate.

```
DRAW MC
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2   RESPWR   500
HIMASS      500    THRESH      8

AMCL   SED   BENZ   3/I   1/28/77

MASSES  205,     0,     0,     0
#SCANS  500    HRDCPY    NO
%SCALE  100    REZERO    YES
BASE    6900*2**  0
```

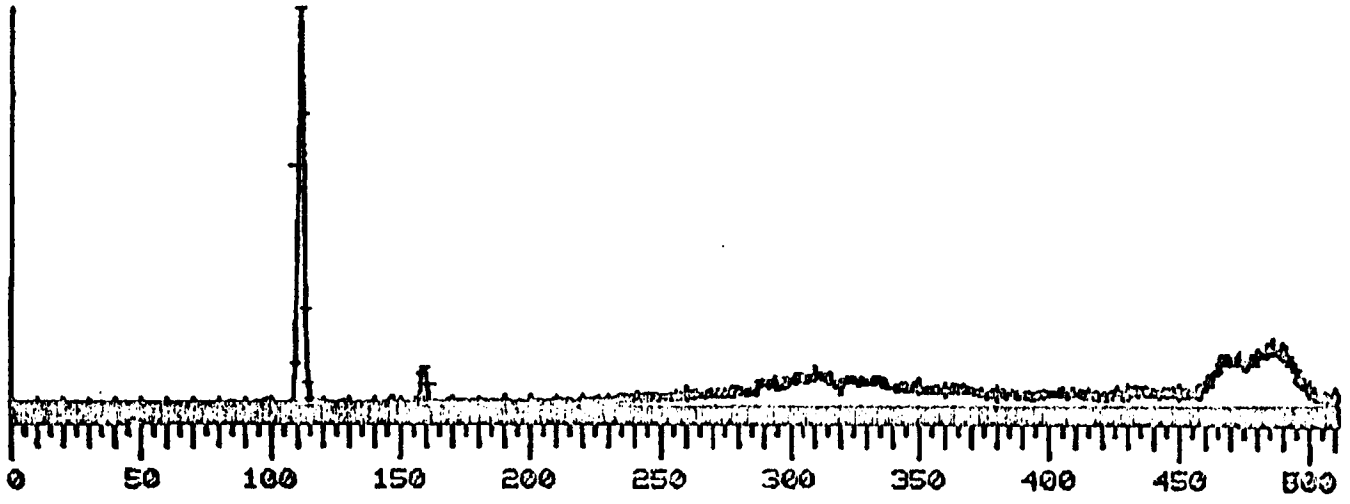


Figure 5. Mass Chromatogram at m/e = 205 for Sample AMCL Benzene Eluate.

```
DPAW MS
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

IGNORE     0,     0,     0,     0
%SCALE   100    #AMU'S   250      HRDCPY      NO
SUBTR      0    BASEPK     0      SCAN #    111
BKGRND   113
BASE    5184 *2** 0    % TOTAL IONIZ.      27
```

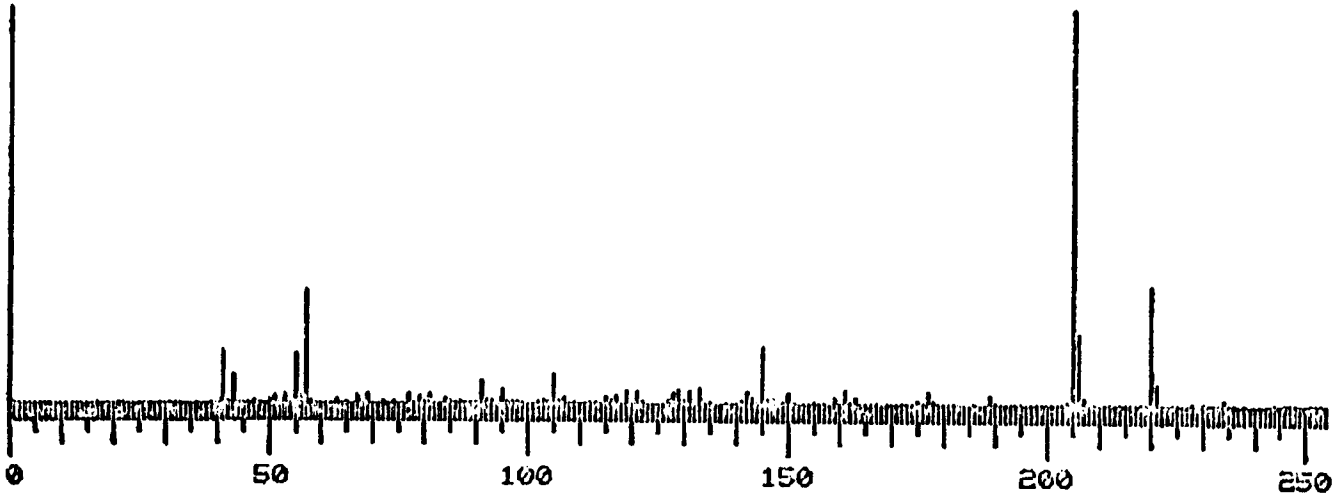


Figure 6. Mass Spectrum of Scan #11 for Sample AMCL Benzene Eluate.

```
LIBRARY SEARCH

GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

IGNORE     0,     0,     0,     0     HRDCPY    YES
INDEX    SCAN   BACKGR    SUBTR     BASE

     0     111      113         0         0

LAST INDEX PROCESSED  0



SCAN #      111     BASE      205
GOODNESS  ID. NO.  PAGE
   666  DOW-1598 1564 2,6-DITERTIARY BUTYL-P-CRESOL (IONOL)
   600  DOT-0069 1563 (2,6-DI-TERT-BUTYL-4-METHYL PHENOL)
   317  DOW-1517 1659
   315  DOW-1467 1566
   247  API-1888 1558
```

Figure 7. Library Search of Scan #111 for Sample AMCL Benzene Eluate.

```
DRAW MC
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

MASSES  156.     0.     0.     0
#SCANS  500    HRDCPY    NO
%SCALE  100    REZERO    YES
BASE    15697*2**  0
```

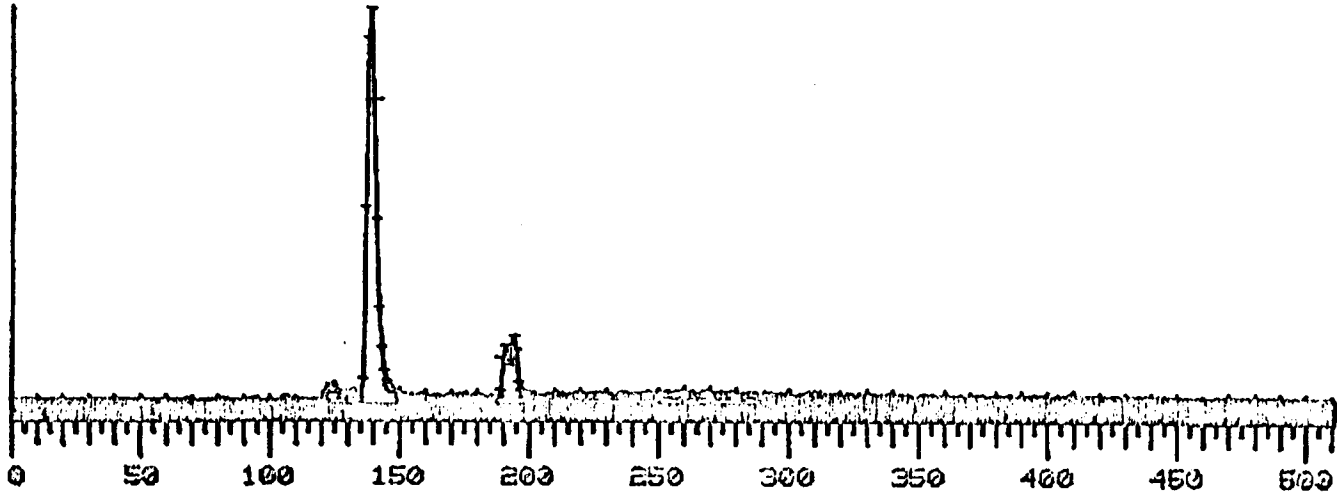


Figure 8. Mass Chromatogram at m/e = 156 for Sample AMCL Benzene Eluate.

```
DRAW MS
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL   SED   BENZ   3/I   1/28/77

IGNORE     0,     0,     0,     0
%SCALE   100    $AMU'S    204       HRDCPY      NO
SUBTR      0    BASEPK      0       SCAN #    139
BKGRND   136
BASE   14642 *2** 0    % TOTAL IONIZ.      17
```

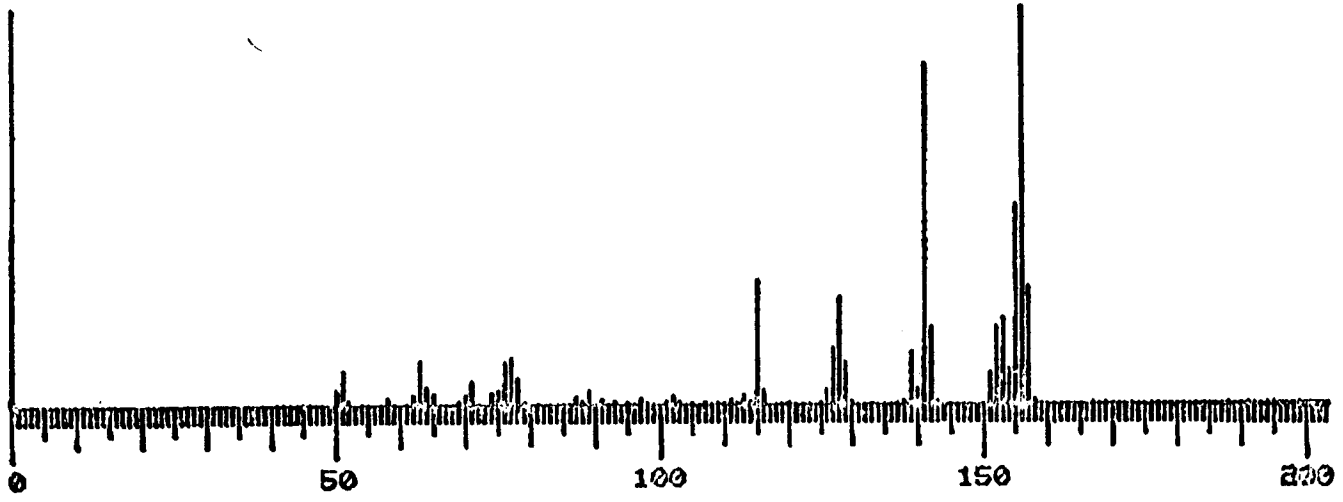


Figure 9. Mass Spectrum of Scan #139 for Sample AMCL Benzene Eluate.

```
DPAW MC
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

MASSES  149,     0,     0,     0
#SCANS  500    HRDCPY    NO
%SCALE  100    REZERO    YES
BASE    5740*2** 0
```

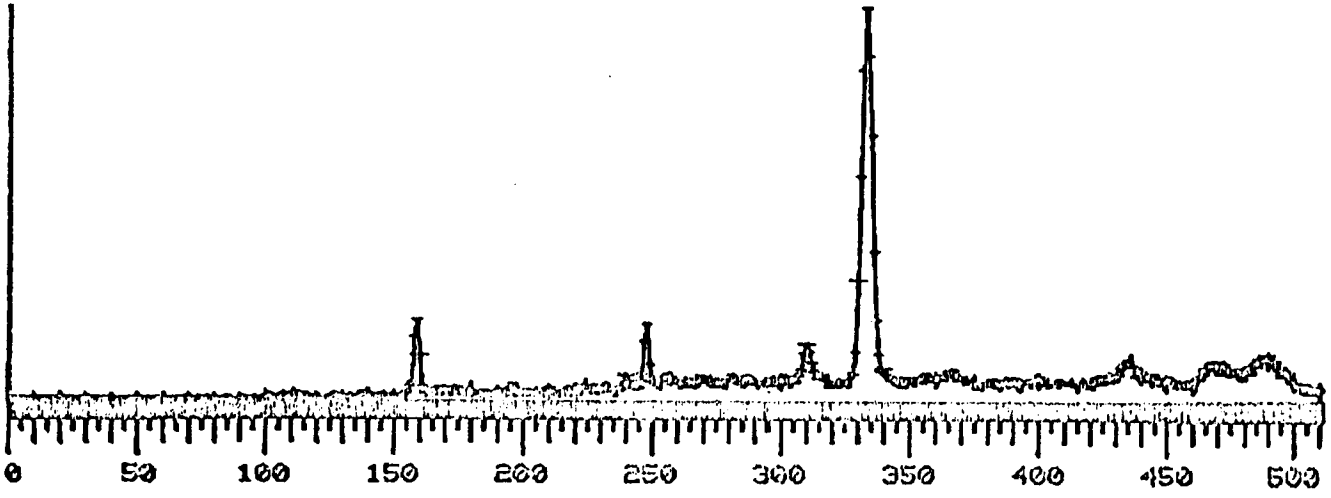


Figure 10. Mass Chromatogram at m/e = 149 for Sample AMCL Benzene Eluate.

IGNORE	0,	0,	0,	0			
%SCALE	100	#AMU'S	344		HRDCPY		NO
SUBTR	0	BASEPK	0		SCAN #		159
BKGRND	162						
BASE	6041	%2** 0	% TOTAL	IONIZ.			5

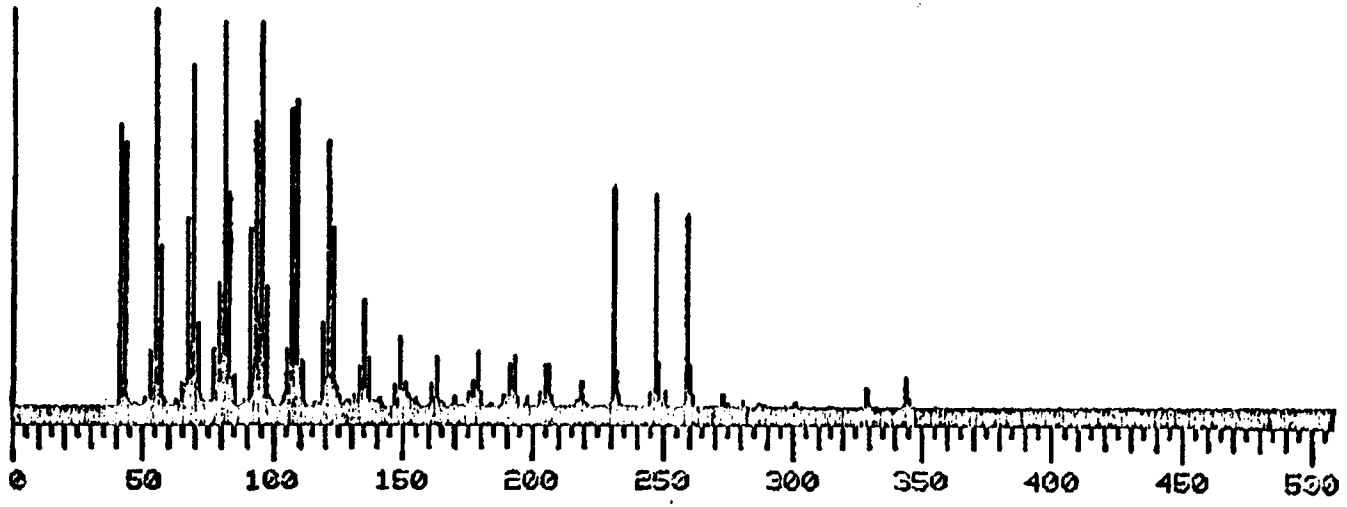


Figure 11. Mass Spectrum of Scan #159 for Sample AMCL Benzene Eluate.

molecular weight of 344 shows the compound has 25 carbon atoms and four sites of unsaturation. Polyolefins of this nature are common in marine organisms and can be readily expected in marine sediments. The non-uniform distribution of mass fragments suggests that branching occurs.

A mass chromatogram at m/e = 192 (Figure 12) reveals a pair of peaks at scan 273 and 281. A similar distribution of peaks is encountered in petroleums and is associated with methylphenanthrene isomers. The mass spectra at these scan numbers (Figures 13 and 14) show the very simple spectra of the polynuclear aromatics with a molecular weight 192 consistent with the empirical formula, $C_{15}H_{12}$. A similar mass chromatogram from the next higher homolog, $C_{16}H_{14}$, is given in Figure 15. The mass spectrum of Figure 16 is consistent with the interpretation as dimethylnaphthalene.

Continuing the search for compounds of polynuclear aromatic character, a mass chromatogram at m/e = 198 (Figure 17) suggests a possibility of peaks in the region of scans 250 to 280. The mass spectrum of scan number 260 (Figure 18) is very weak but suggests a compound of molecular weight 198 with aromatic character (strong molecular ion peak). This may represent a methyldibenzothiophene isomer. Comparison of Figures 2 and 17 shows the component is not discernable in the total chromatogram.

A suite of compounds in the early part of the chromatogram are emphasized by the mass chromatogram at m/e = 85 (Figure 19). Most of these compounds have nearly identical, very simple mass spectra such as that given in Figure 20. The molecular weight 234 is found for the first six of these peaks, suggesting a suite of isomeric compounds. Computer search of the mass spectral library was of no help, but application of the Mass Spectral Search System of the National Institutes of Health (using the Cybernet time-sharing computer system) reavealed di-n-hexyl-disulfide as a good possibility. Comparison of the mass spectrum with the MSSS spectrum of this compound suggests that these compounds are indeed

```
DRAW MC
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

MASSES  192.     0.     0.     0
#SCANS  500    HRDCPY    NO
%SCALE  100    REZERO    YES
BASE    1982*2**  0
```

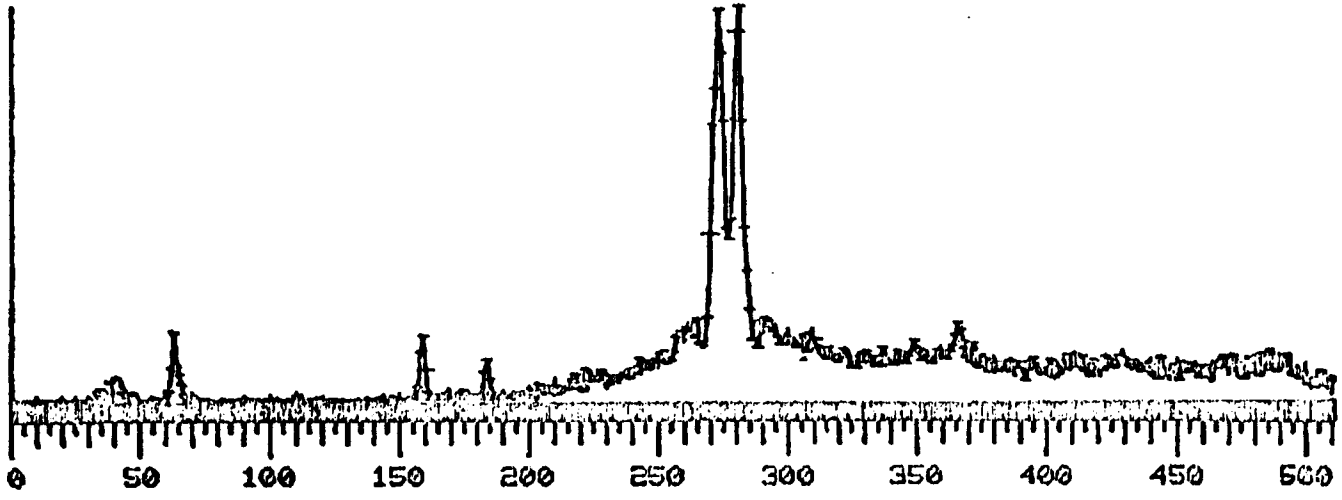


Figure 12. Mass Chromatogram at m/e = 192 for Sample AMCL Benzene Eluate.

```
IGNORE     0,     0,     0,     0
%SCALE   100    #AMU'S   204      HRDCPY      NO
SUBTR    276    BASEPK    81      SCAN #    273
BKGRND     0
BASE       319 *2** 0    % TOTAL IONIZ.      24
```

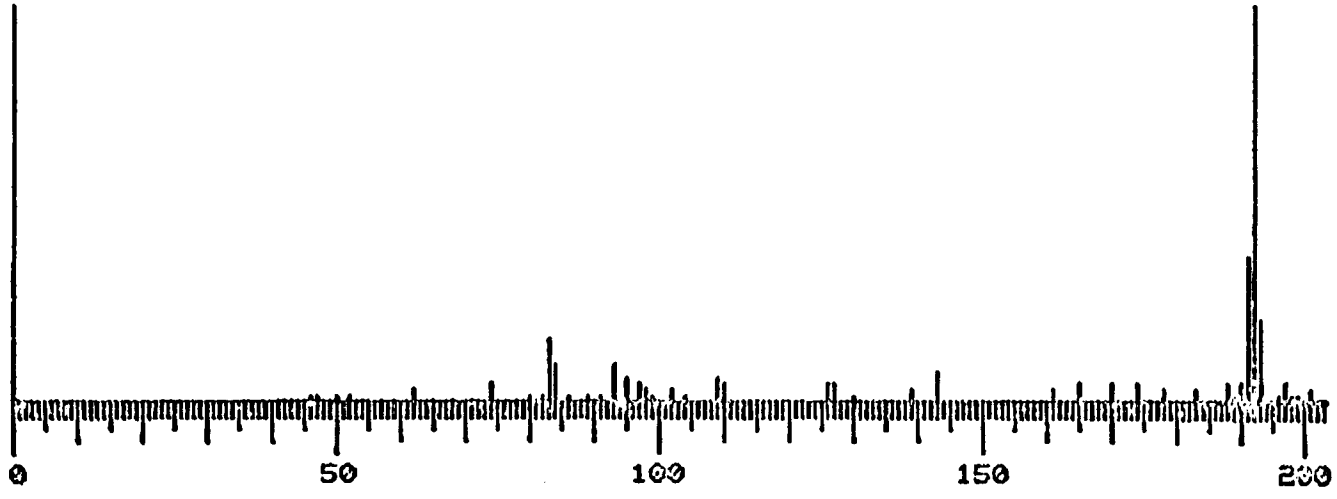


Figure 13. Mass Spectrum of Scan #273 for Sample AMCL Benzene Eluate.

IGNORE	0,	0,	0,	0			
%SCALE	100	#AMU'S	204		HRDCPY	NO	
SUBTR	283	BASEPK	85		SCAN #	281	
BKGRND	0						
BASE	271	*2** 0	% TOTAL	IONIZ.		33	

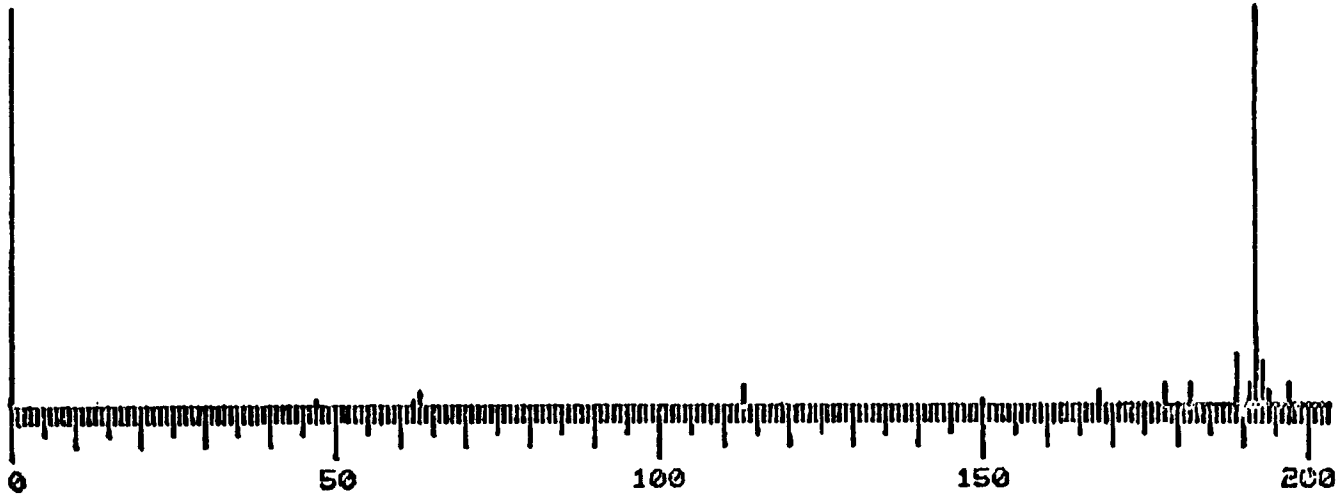


Figure 14. Mass Spectrum of Scan #281 for Sample AMCL Benzene Eluate.

```
DPAW MC
GC ID BL     19    DATE    1/28/77
AQRATE        4    SCTIME      2    RESPWR   500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

MASSES  206,     0,     0,     0
#SCANS  500    HRDCPY    NO
%SCALE  100    REZERO    YES
BASE    1627*2**  0
```

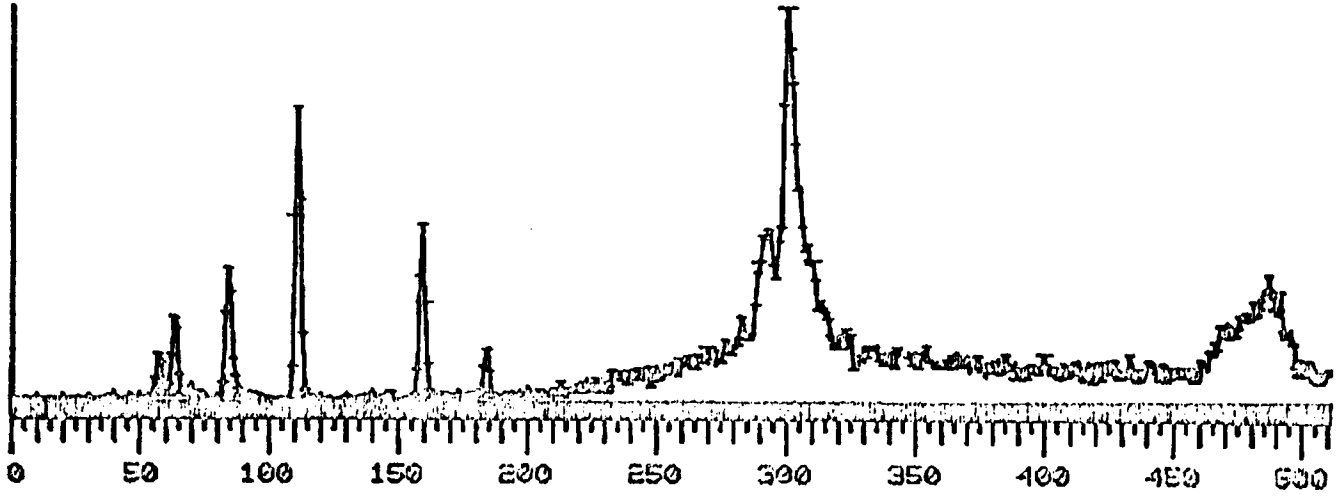


Figure 15. Mass Chromatogram at m/e 206 for Sample AMCL Benzene Eluate.

IGNORE	0,	0,	0,	0			
%SCALE	100	#AMU'S	255		HRDCPY	NO	
SUBTR	304	BASEPK	85		SCAN #	300	
BKGRND	0						
BASE	494	*2** 0	% TOTAL	IONIZ.		26	

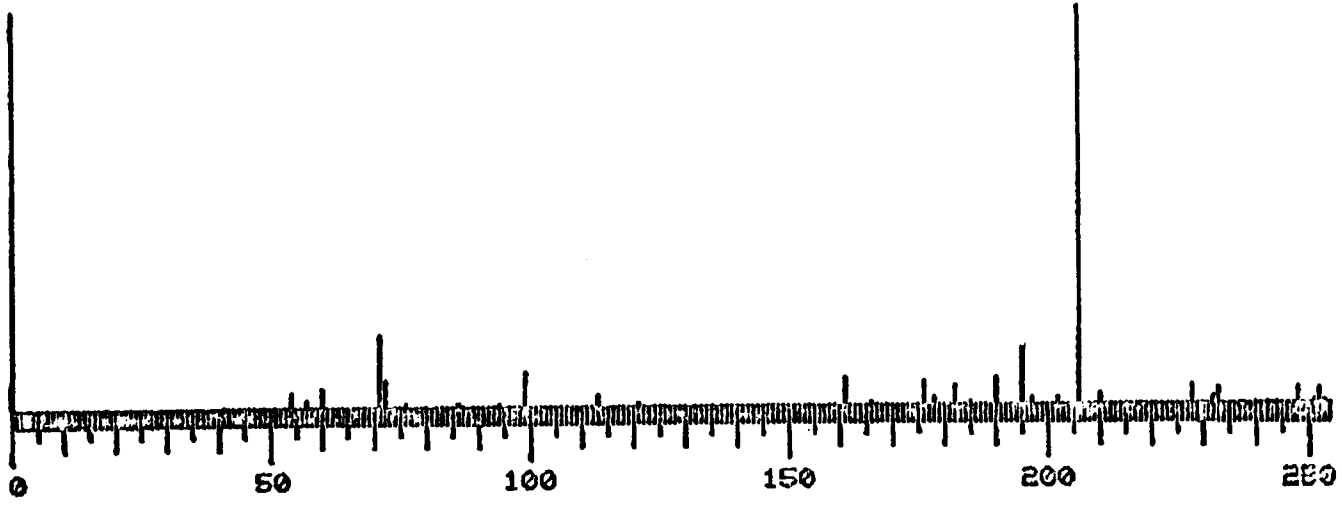


Figure 16. Mass Spectrum of Scan #300 for Sample AMCL Benzene Eluate.

```
DRAW MC
GC ID BL     19    DATE    1/28/77
AORATE        4    SCTIME      2   RESPWR   500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

MASSES  198,     0,     0,     0
#SCANS  500    HRDCPY    NO
%SCALE  100    REZERO    YES
BASE      534*2** 0
```

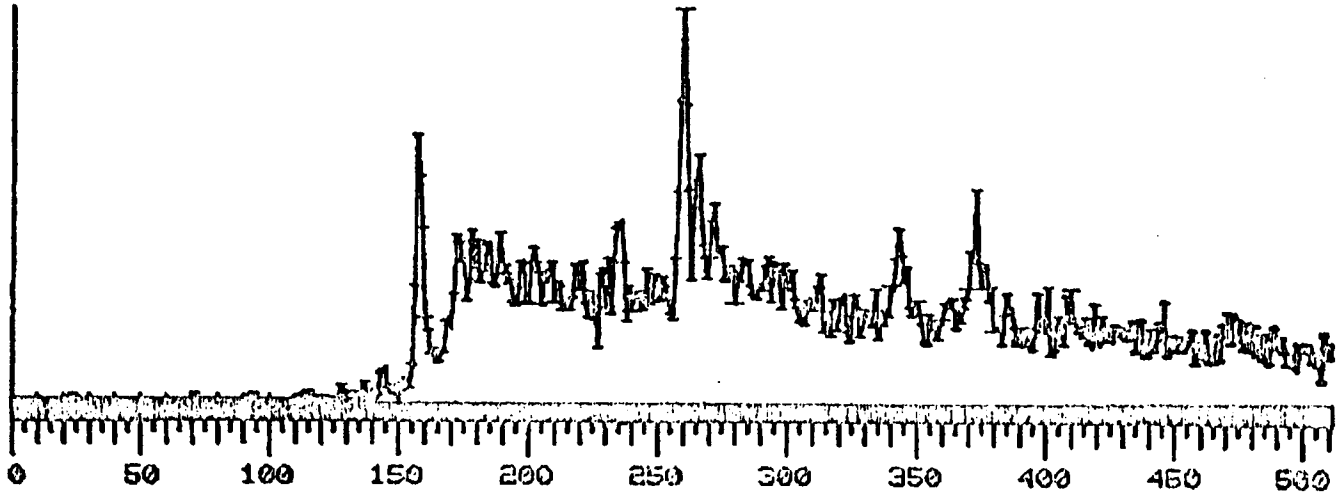


Figure 17. Mass Chromatogram at m/e = 198 for Sample AMCL Benzene Eluate.

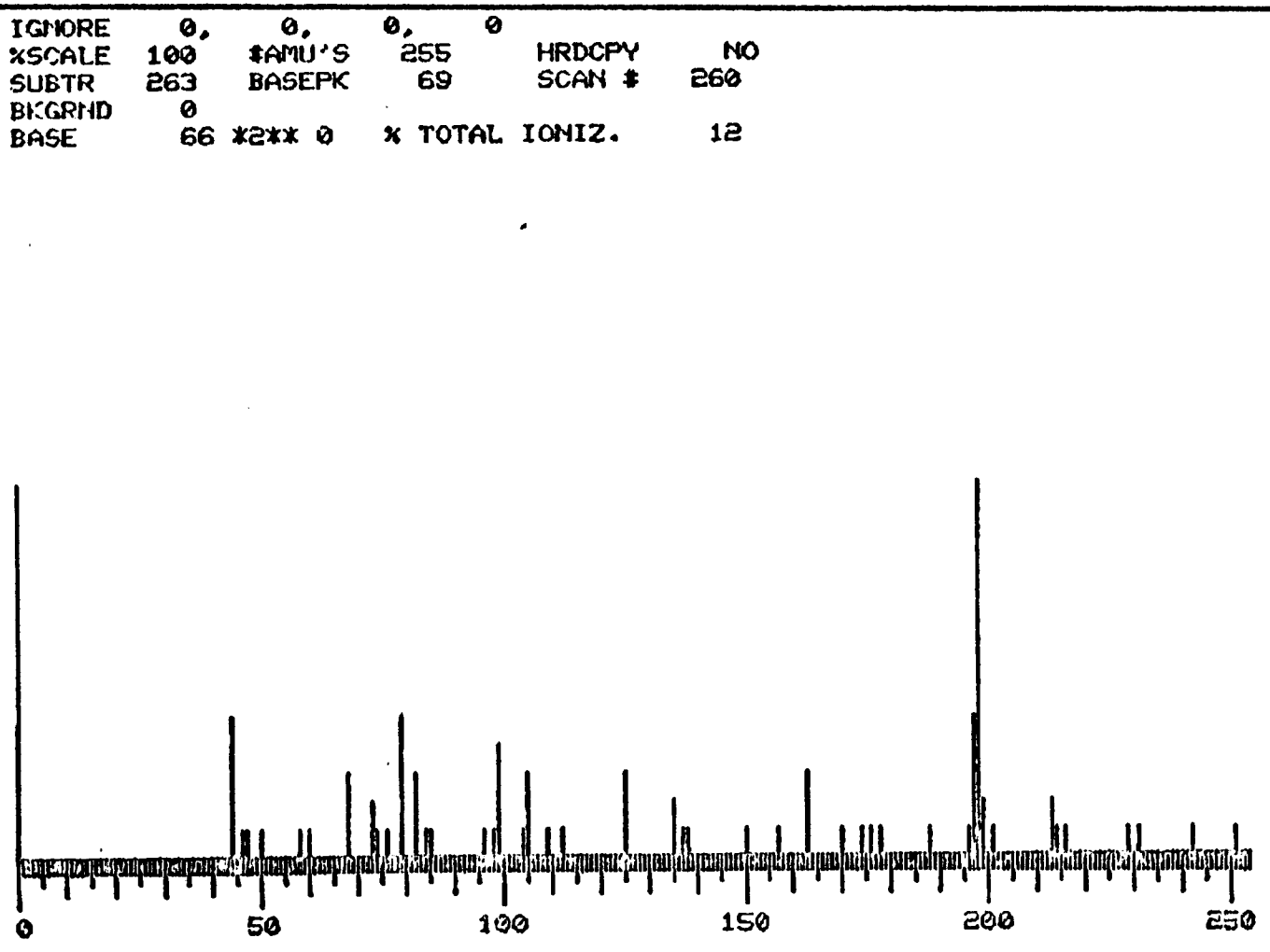


Figure 18. Mass Spectrum of Scan #260 for Sample AMCL Benzene Eluate.

```
DRAW MC
GC ID BL     19    DATE    1/28/77
AORATE        4    SCTIME      2    RESPWR    500
HIMASS      500    THRESH      2

AMCL    SED    BENZ    3/I    1/28/77

MASSES    85,     0,     0,     0
#SCANS   500    HRDCPY    NO
%SCALE   100    REZERO    YES
BASE     6988*2**  0
```

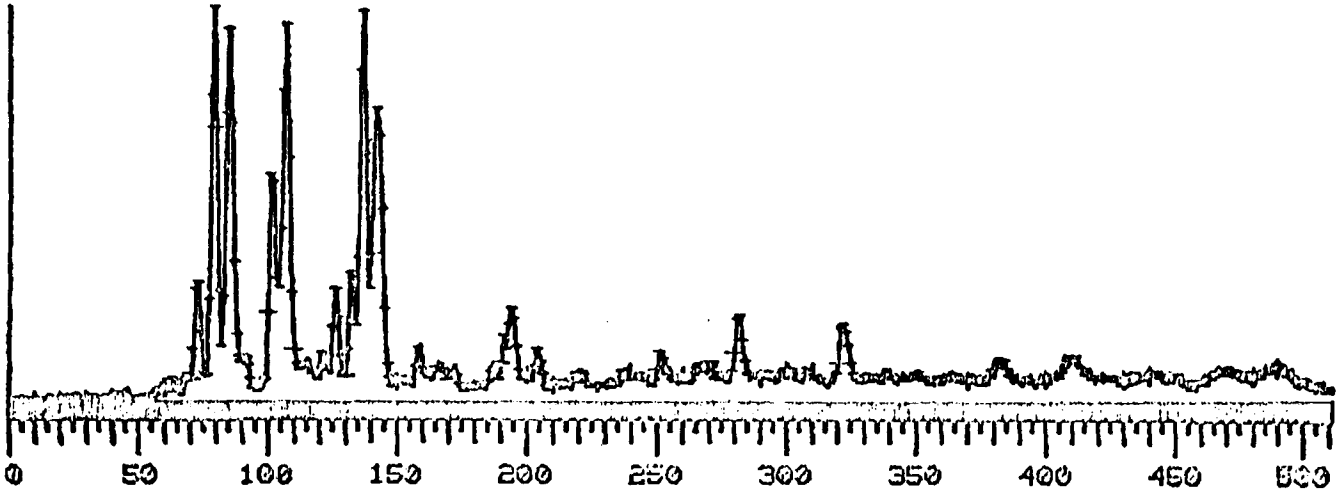


Figure 19. Mass Chromatogram at m/e = 85 for Sample AMCL Benzene Eluate.

IGNORE	0,	0,	0,	0	
XSCALE	100	#AMU'S	250	HRDCPY	NO
SUBTR	0	BASEPK	0	SCAN #	85
BKGRND	88				
BASE	6635	*2** 0	% TOTAL IONIZ.		25

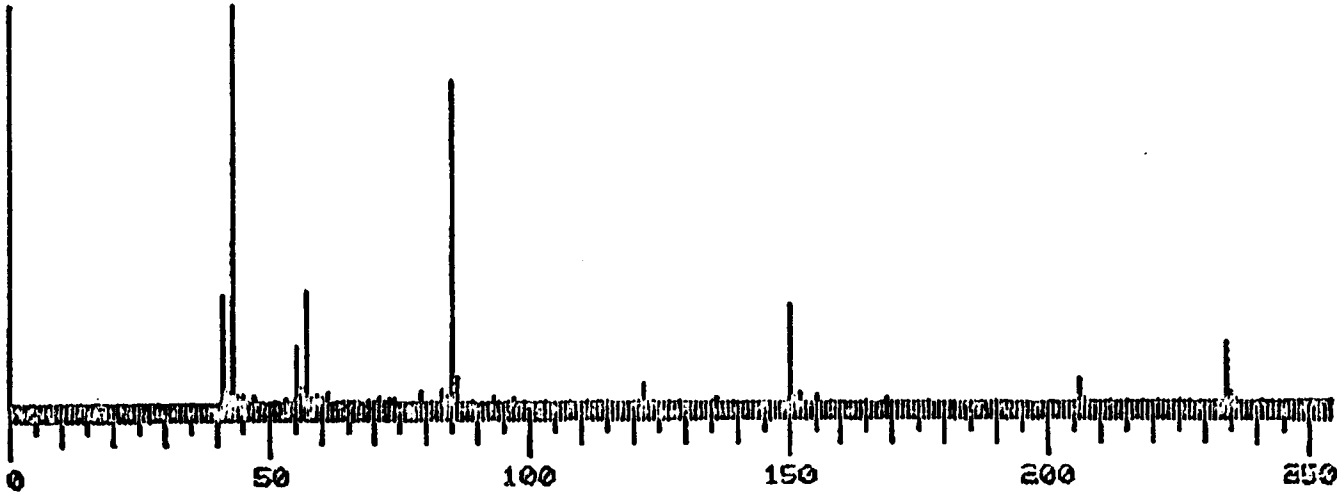


Figure 20. Mass Spectrum of Scan #85 for Sample AMCL Benzene Eluate.

disulfide isomers.

Similar GC/MS runs have been made on other samples in this study. The reconstructed total ion chromatograms and significant peak indices for these runs are given in Appendix L, Figures 1.1 through 1.64.

RESULTS AND DISCUSSION

Zooplankton

Hydrocarbon contents of marine zooplankton are relatively unstudied, especially in the STOCS area. Calder has reported on extensive studies of HMW-Hydrocarbons in the eastern Gulf of Mexico, MAFLA area (Calder, 1976). The papers of Blumer *et al.* (1963) and Blumer and Thomas (1965a and b) relate some of the first work of significance in this field. Other pertinent works are Gelpi *et al.* (1968), Winters *et al.* (1969), Han and Calvin (1969), Blumer *et al.* (1970; 1964), Blumer *et al.* (1971) and Youngblood *et al.* (1971).

These works indicate that marine organisms do not generally contain large quantities of hydrocarbons relative to amino acids, fatty acids, etc. The small quantities of hydrocarbons which occur naturally do not usually constitute a very complex mixture, although individual molecules can be complex. Two hydrocarbons, pristane and n-heptadecane, are generally dominant in marine zooplankton, but branched chain and polyunsaturated hydrocarbons also occur.

The biological origin of these hydrocarbons is not certain. Normal heptadecane is reported to be the primary hydrocarbon among photosynthetic microorganisms (Han and Calvin, 1969). However, this has not been found generally true as some coccoid blue-green algae have a nineteen carbon atom mono-olefin as the primary hydrocarbon (Winters *et al.*, 1969). Also, Blumer *et al.* (1971) found a polyunsaturated (C_{21}:6) hydrocarbon as the dominant hydrocarbon in cultured marine phytoplankton.

The physiological function of hydrocarbons remain unknown. Zooplankton

probably accumulate hydrocarbons in lipid tissues by assimilation through their diet. There seems to be some evidence of phytadienes being a product of zooplankton metabolism of phytol (Blumer and Thomas, 1965a).

In general, the quantities of hydrocarbons in zooplankton are low with estimates ranging from 10^{-5} to 1 percent of animal lipid fraction. Thus, qualitatively and quantitatively, natural hydrocarbons in zooplankton differ from the hydrocarbon of petroleum origin. In petroleum, the hydrocarbon fraction of the total soluble organic matter may be from 25 to 100 percent and is usually an extremely complex mixture of compounds in which olefins are seldom found. Most petroleums contain a full suite of saturated n-alkanes from hexane and lighter to pentatriacontane (C_{35}) and heavier in which the distribution of alkanes does not exhibit any predominance of carbon chain lengths having odd numbers of carbon atoms. Some of these comparative characteristics of petroleum and biogenic organic matter useful for the STOCS studies are discussed in Farrington *et al.* (1976).

The objective of this study was to measure the distributions of hydrocarbons in the STOCS zooplankton samples to determine baseline levels and to evaluate such measurements for use as a monitoring tool to detect petroleum influx into the STOCS area which might result from petroleum exploration and production.

The data from GLC analysis of 84 zooplankton samples are presented in Appendix L, Table 1.1 through 1.84. Illustrations of the n-alkanes distributions and OEP curves are given in Appendix L, Table 2, and Appendix L, Figures 2.1 through 2.69. Figures for some samples are omitted due to inadequate data for significant plots.

Inspection of these data show that those STOCS area samples supposed to be close replicates (*i.e.* collected on the same date and location and closely spaced in time) were not analytical replicates. This is illustrated by Figure 21 where samples from Station 1/L, fall season, showed a heavy

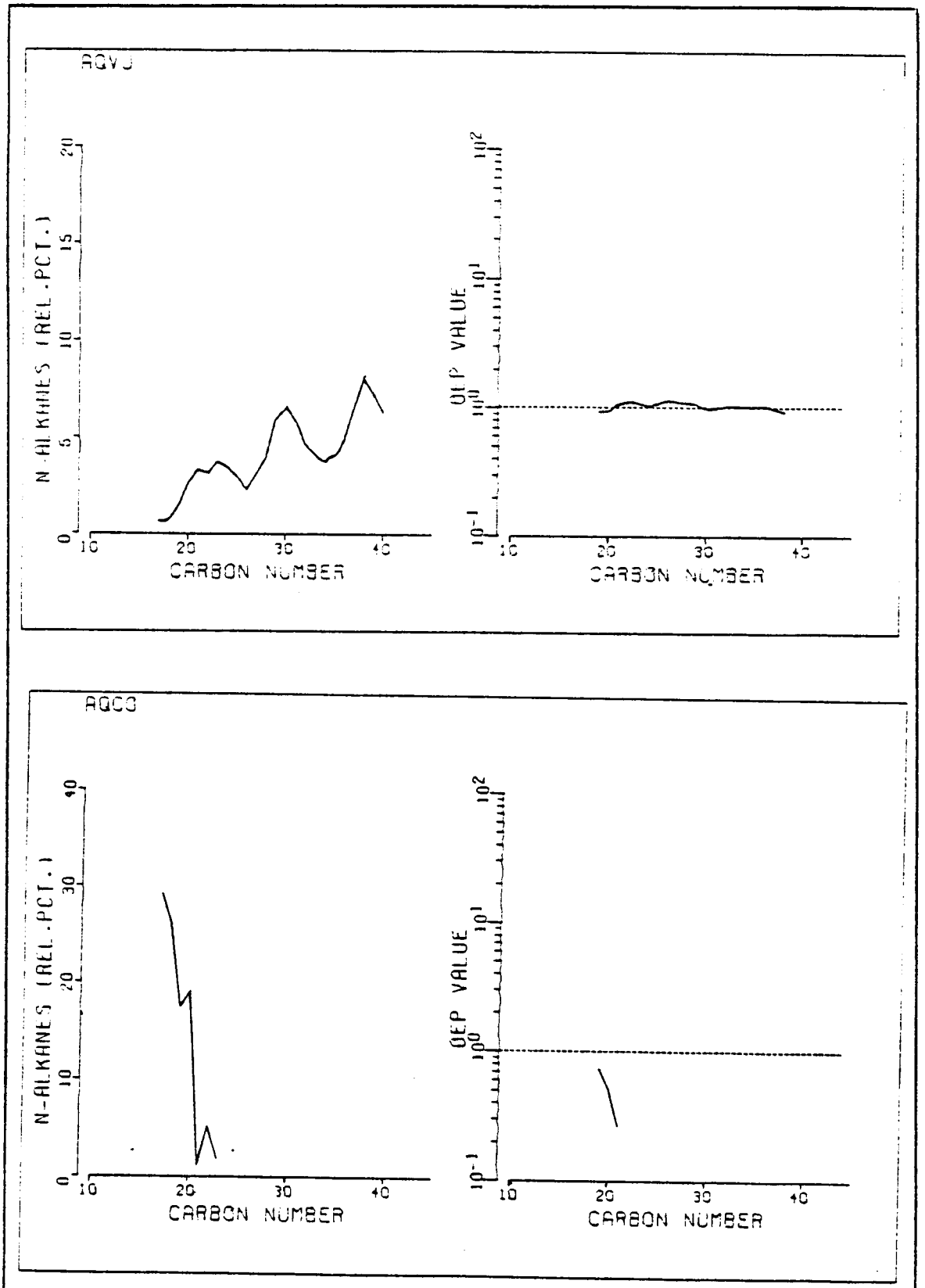


Figure 21. Comparison of n-alkanes Distributions for "Replicate" Samples.

contamination with petroleum-like hydrocarbons (sample AQVJ) while sample AQCO showed no such contamination.

The 1976 zooplankton samples generally showed more contamination with petroleum-like hydrocarbons than was observed in the 1975 study. Twenty-six samples (31%) showed contamination. This was due to the presumed presence of microscopic "tar balls" in the samples. The origin of this material is unknown. Pristane and n-haptadecane were still the most dominant hydrocarbons, even in many samples that showed petroleum-like contamination. Generally, the unsaturated hydrocarbons fraction (benzene eluate) was about equivalent to the saturated fraction in concentration. In some instances, however, the concentrations of the non-saturates were inordinately large. This may have been due to the presence of lipids which escaped saponification or, in some instances, might reflect large quantities of fatty alcohols in the sample.

In summary, the zooplankton showed substantial petroleum contamination. Representative chromatograms of normal and polluted zooplankton samples are shown in Figures 22 and 23, respectively. Zooplankton contamination may serve as an excellent pollution indicator. The high levels of C_{17} and pristane were also reflected in the macroepifauna and macronekton in Chapter 17 by Drs. Giam and Chan.

Water-Dissolved and Particulate High-Molecular-Weight Hydrocarbons

As discussed in the Introduction, the level of total dissolved and particulate organic matter in Gulf of Mexico seawater is in the 0.1 - 10 ug/l range. These low concentrations precluded routine measurements of the temporal and spatial variations of the concentrations of any specific chemical molecules. The present national BLM-OCS program is the first attempt to establish concentration ranges for hydrocarbon with respect to season and location. Recognizing the technical problems and the lack of published

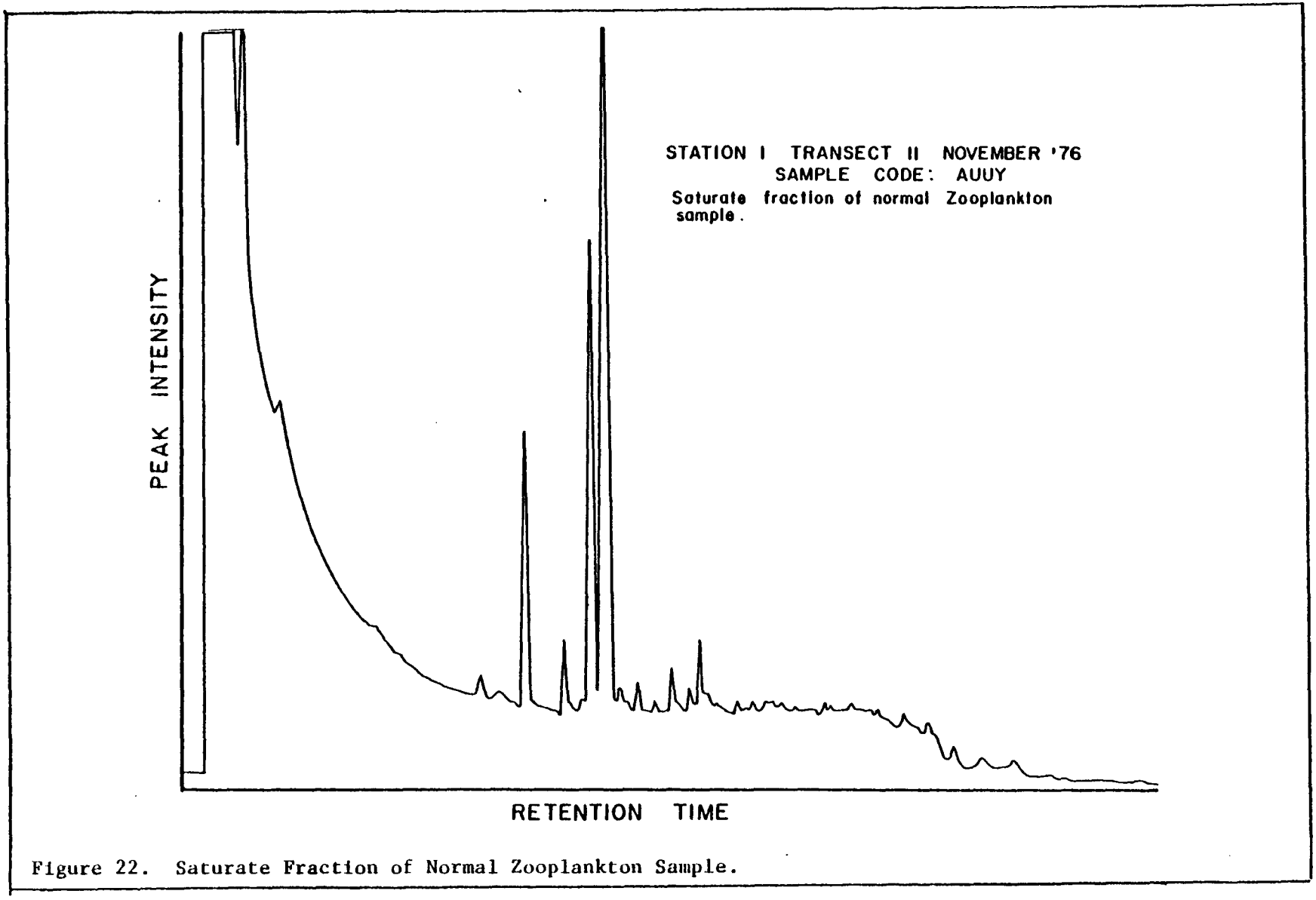


Figure 22. Saturate Fraction of Normal Zooplankton Sample.

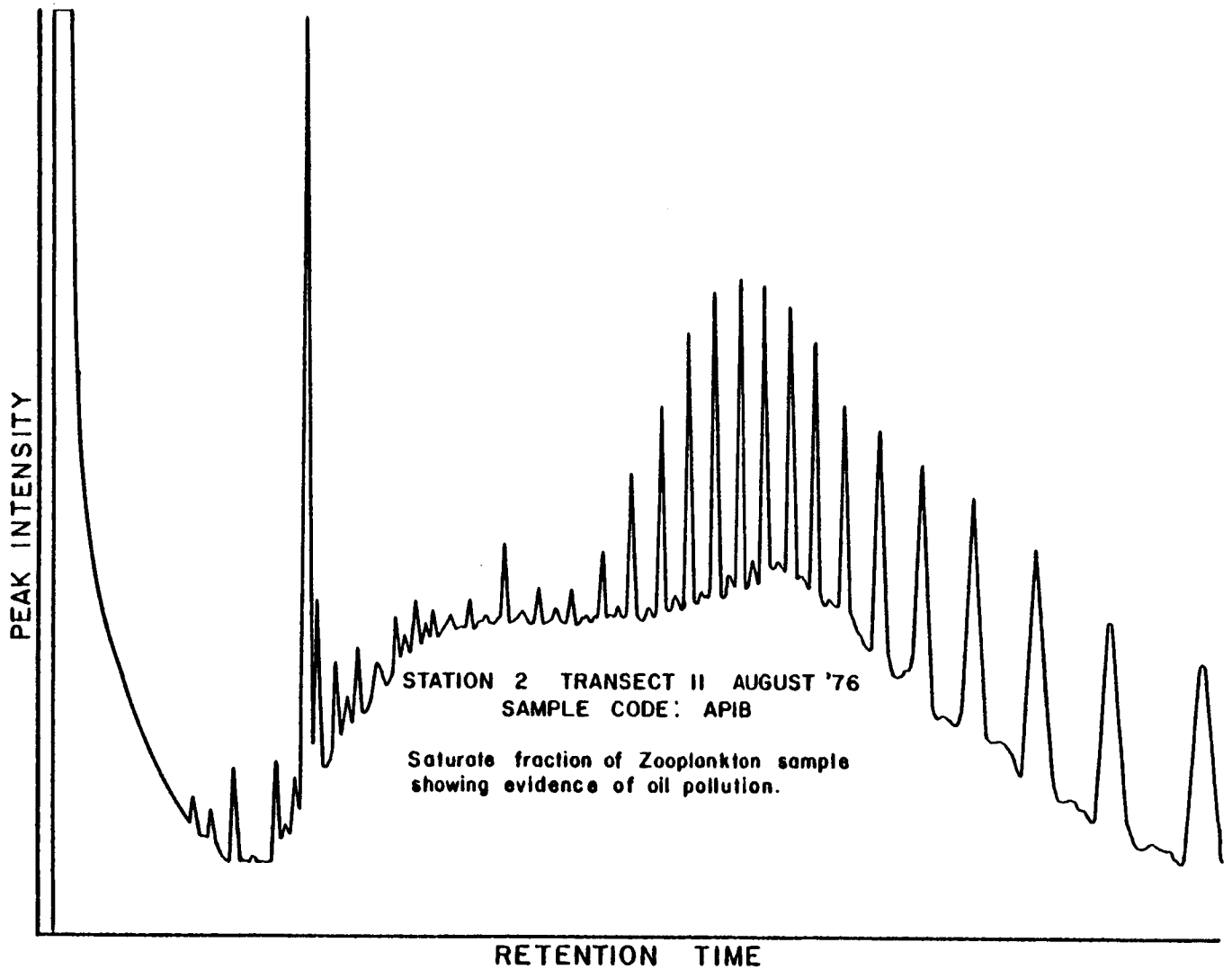


Figure 23. Saturate Fraction of Zooplankton Sample Showing Evidence of Oil Pollution.

background data, we approached these studies with the hope of not establishing an inflow-outflow transport model, but of simply affirming or denying certain statements that can be made about hydrocarbons in seawater. These include the following:

-what are the average levels of n-paraffins and non-paraffins in DOC and POC?

-are seasonal trends indicated?

-are location trends indicated?

-can the observed hydrocarbon patterns be accounted for as biological in origin or is petroleum contamination indicated?

-what additional studies are needed to bring our understanding of the hydrocarbon cycle in the sea to a useful and predictive status?

In trying to answer these questions, we relied on the published literature, other BLM studies, our own data, and on assumptions of organic geochemistry.

In a recent review of published data on the concentration of hydrocarbons in the marine environment, McAullife (1976) tabulated most pertinent data prior to 1976. Papers by Brown and Searl (1976) and Koons (1977) discussed hydrocarbon concentrations in the Pacific Ocean. BLM-sponsored studies have recently reported hydrocarbon concentrations in the MAFLA (Calder, personal communications, 1975) and STOCS (Parker *et al.*, 1976) areas of the Gulf of Mexico. The data indicate:

1. The highest concentration of hydrocarbon is located in the surface microlayer where concentrations range from about 10 µg/l in the open ocean to greater than 100 µg/l in coastal waters.

2. Concentrations of hydrocarbons decrease rapidly within the first 10 m of depth with an average concentration at 1 m of about 1 µg/l.

In addition to concentration changes with depth, seasonal changes were reported. Calder (personal communications, 1975) reported highest concentrations in February and lowest concentrations in fall. Parker *et al.* (1976) report highest values in April and lowest values in January. Both studies reported intermediate values in summer and each indicated a trend of higher con-

centrations inshore than offshore. Calder also consistently found higher values closer to the Mississippi River. No north-south trend was apparent in the STOCS study area.

Contract AA550-CT6-17 called for the analysis of 74 dissolved and 74 particulate samples. Results of 73 dissolved samples are reported in Appendix L, Tables 3.1 through 3.72. Sample APHY exploded during preparation and was lost..

Results of 70 particulate samples are reported in Appendix L, Tables 4.1 through 4.72. The four particulate samples not analyzed (three collected in the spring and one in July) were replicate samples which were sent to the quality control laboratory.

Particulate Hydrocarbon Hexane Eluate

Total particulate hydrocarbon data for the three seasonal sampling periods are presented in Figure 24. The data appeared to show a trend of higher concentrations at Station 1 of each transect with little difference in concentration between Stations 2 and 3. An exception to this trend was found in the winter values for Transects I, II and IV. A possible explanation lies in the zooplankton biomass data reported for these stations by Dr. Park. Dr. Park reports (Chapter 6) that the zooplankton biomass was much higher at Station 2 than Station 1 on Transects. I and II during this season.

Average concentrations of all 12 stations were calculated by season. Fall and winter values were 0.31 µg/l and the spring was 0.12 µg/l. The low spring values are difficult to interpret. Data on particulate hydrocarbons taken during the monthly samples are given in Appendix L, Table 4. Highest values along the transect were in March and July and the lowest during the spring and December sampling periods. Two anomalously high values were encountered. The first value, 1.69 µg/l (sample AUXU, 3/II Nov.) was explained by the presence of two small fish in the sample. The sample was processed

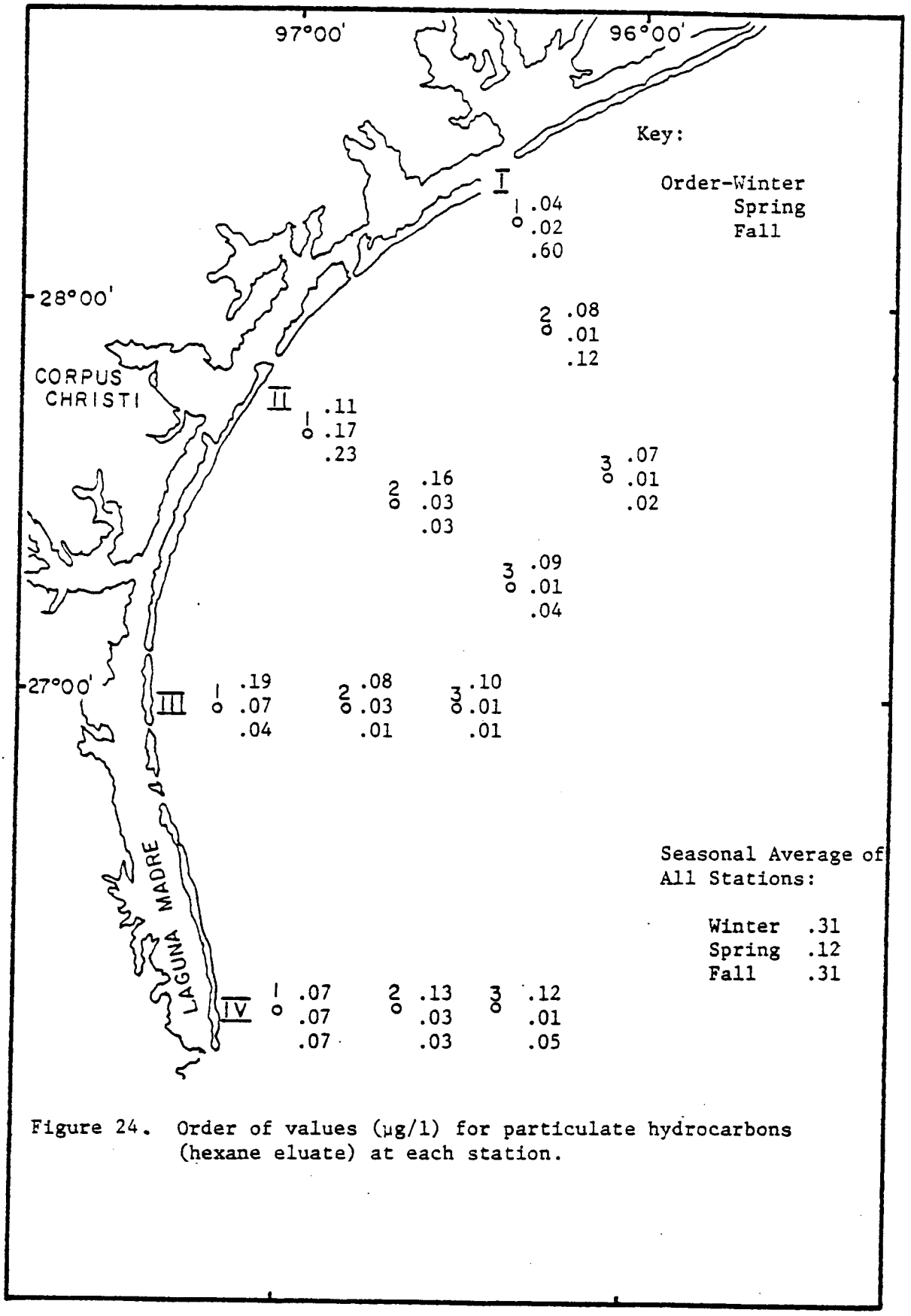


Figure 24. Order of values (µg/l) for particulate hydrocarbons (hexane eluate) at each station.

by the usual method. The other large value, sample AVTR, 2/II, December, 1.52 µg/l had no apparent explanation.

Percentage composition data show that the most abundant hydrocarbon in the hexane eluate was either a compound with a retention index of 2200 or an odd carbon number alkane between C_{25} and C_{33}. These results were similar to those found in the STOCS area in 1975. The 2200 compound, unidentified at present, was the most abundant hydrocarbon in the particulate fraction.

Mass spectral data (GC/MS) on the 2200 compound is inconclusive at this time but the major component of this peak is not n-C_{22}. Ratios of individual hydrocarbons in the particulate fraction and average OEP values are given in Appendix L, Table 5. Odd-even preference indices (OEP) values are plotted in Appendix L, Figures 3.1 through 3.60. A representative chromatogram of the hexane eluate is presented in Figure 25.

Particulate Hydrocarbon Benzene Eluate

The quantity of material in the particulate fraction which eluted with benzene ranged from less than 0.01 to 420 µg/l during the study period. About 70 percent of the samples contained between 0.01 and 0.3 µg/l. In one-half of the sampling periods there appeared to be a trend toward higher concentrations at Station 1. The yearly averages, however, did not reflect this trend due to several high values at Station 3.

Work is underway to obtain mass spectral data on larger components of the benzene fraction which were recurrent in several samples. This data will be included in the supplemental report.

Dissolved Hydrocarbon Hexane Eluate

Total hydrocarbon data from the hexane eluate of dissolved samples taken during the three seasonal cruises are given in Figure 26. The trend toward higher values at the inshore stations seen with particulate samples

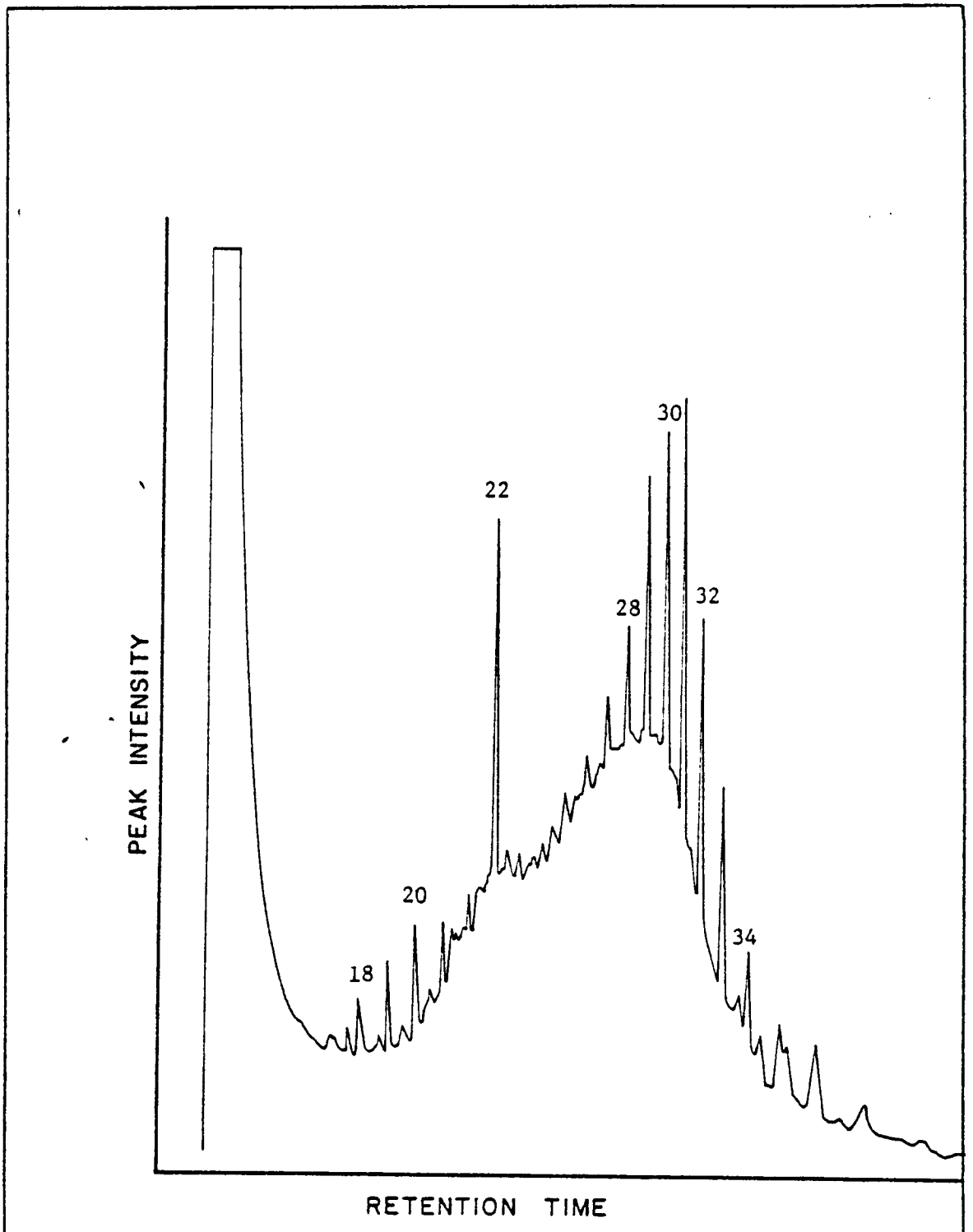


Figure 25. N-Paraffins in Particulate Matter for Sample AGXA at Station 1/III, Winter 1976.

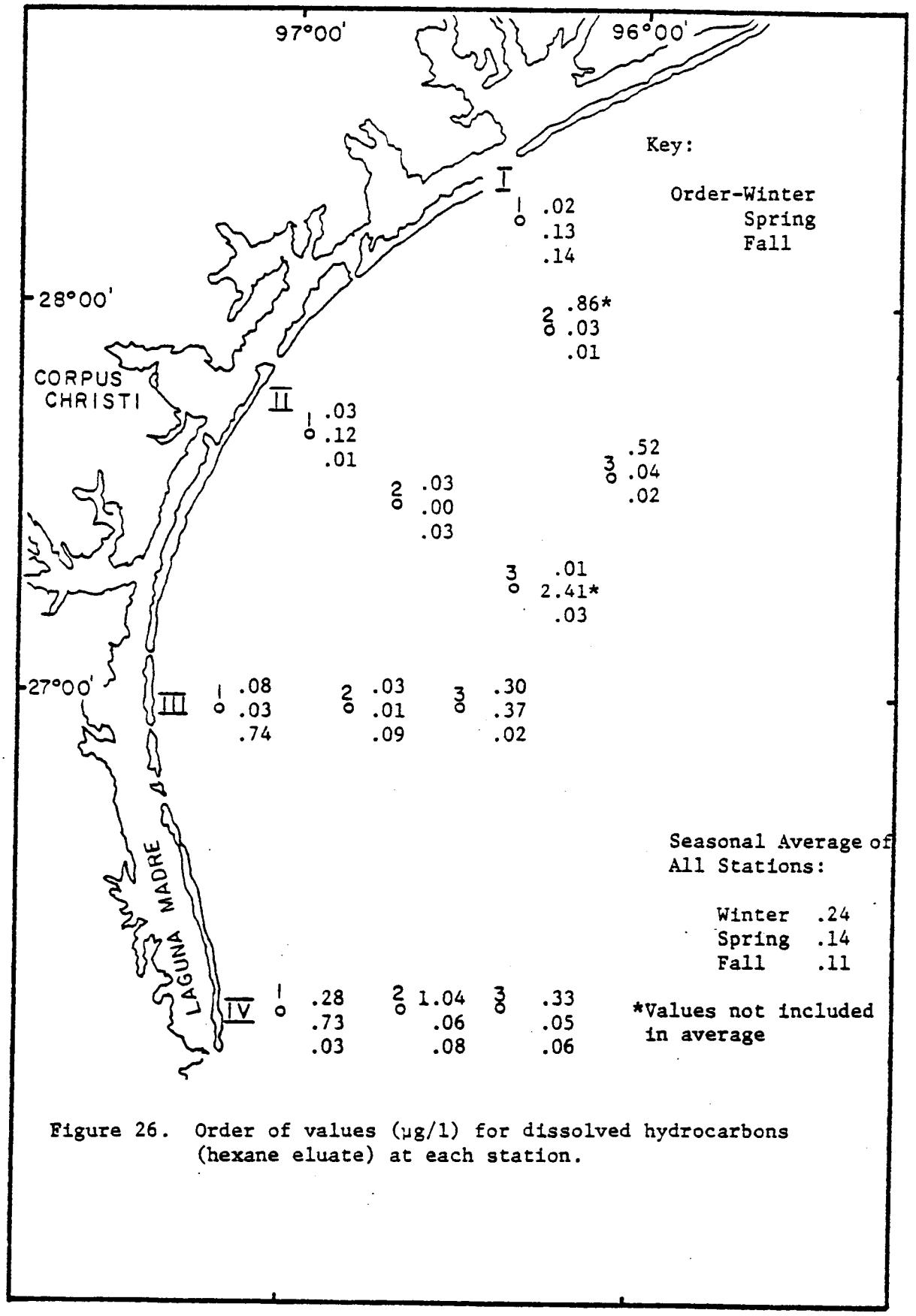


Figure 26. Order of values (µg/l) for dissolved hydrocarbons (hexane eluate) at each station.

was not as obvious but held true for about one-half of the transects.

Highest hydrocarbon concentrations were found in winter with a 0.24 µg/l average for all stations; the lowest values were in the fall, 0.11 µg/l. Calder previously reported in the MAFLA study area that highest concentration of hydrocarbons were in winter. STOCS winter samples from 1975 (Berryhill, 1977) had a winter average of all stations of only 0.13 µg/l, even though the water was not filtered.

The composition of the hexane eluate from dissolved samples was similar to that from particulate samples. Again, the compound with a retention index of 2200 or an odd carbon number n-alkane between C_{25} and C_{33} was usually the most abundant hydrocarbon.

Ratios of individual hydrocarbons in the dissolved fraction and average OEP values are given in Appendix L, Table 6. Odd-even preference indices (OEP) values are plotted in Appendix L, Figures 4.1 through 4.60. A representative chromatogram of the hexane eluate is presented in Figure 27.

Dissolved Hydrocarbon Benzene Eluate

As with particulate samples, dissolved hydrocarbon which eluted with the silica gel-alumina column covered a broad range of concentrations. About 46 percent of the samples had concentrations which were less than 0.3 µg/l. Another 26 percent of the samples had concentrations between 1 µg/l and 10 µg/l; 12 percent had concentrations 10-50 µg/l. Only 2 samples, less than 3 percent, had concentrations greater than 50 µg/l.

In summary, hydrocarbon concentrations in the water column during 1976 were similar to those reported for the STOCS area in 1975 and to those reported in other Gulf of Mexico studies (Parker *et al.*, 1972; Calder, personal communcation, 1977).

The particulate hydrocarbon concentrations indicated a trend toward higher concentrations at Station 1, as was the case with 1975 samples.

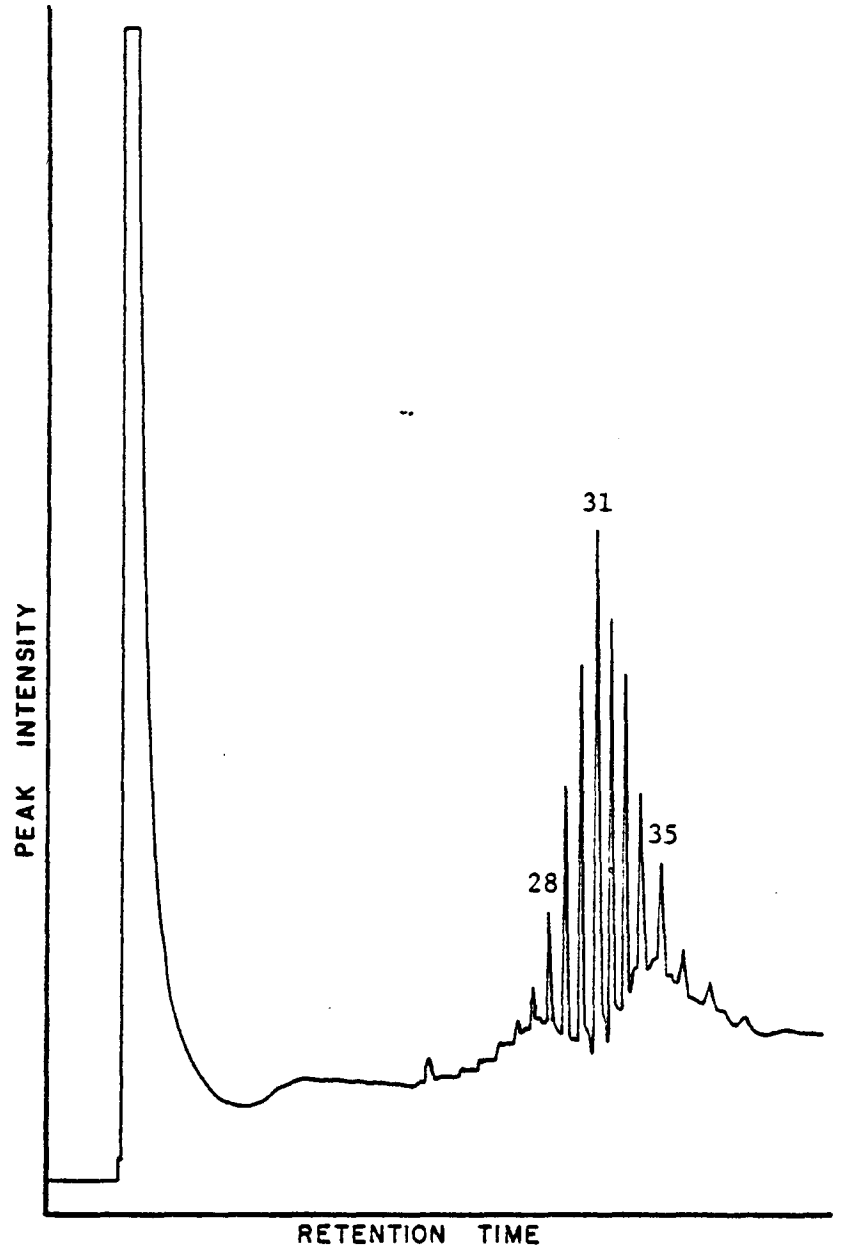


Figure 27. N-Paraffins in Dissolved Matter for Sample AVVO at Station 3/II, March 1976.

Both dissolved and particulate hydrocarbon concentrations indicated seasonal variations with highest values in winter samples.

The percentage composition of the hexane eluates of dissolved and particulate samples were similar to each other and to the composition reported in 1975. A compound with a retention index of 2200 or an odd carbon number n-alkane between C_{25} and C_{33} was usually the single most abundant hydrocarbon present.

Sediment

In the Introduction it was established that hydrocarbons constitute a small but ubiquitous fraction of the organic matter of sediments. Hydrocarbon geochemistry has received a good deal of attention but of a somewhat uneven nature. Two approaches have been taken: one with the purpose of characterizing petroleum with the hope of learning the factors which control its distribution; and, a second approach based on the model of biological (bio-lipids) molecules going into and surviving in sediments for geological time periods. Biological molecules (geo-lipids) are of interest to those concerned with the record of life (paleo-bio-geochemistry). A number of excellent studies have been made in these two areas and specific situations are well-understood, however, no general picture exists for hydrocarbon geochemistry, and especially not on the broad geographical scale needed by BLM (Eglinton, 1969).

By combining these two approaches, as well as borrowing their techniques, a conceptual model useful for environmental quality considerations can be stated. The essential elements of the model are: (1) Organic matter including hydrocarbon is being continuously supplied to recent sediment from the biota in the water column. The sedimentary hydrocarbon pattern might be expected to mirror the biota hydrocarbon. If so, n-C-17 and pristane would greatly dominate, but the observed pattern is much more

complex (Blumer, Guillard and Chase, 1971); (2) Therefore, other hydrocarbons are being generated. It is established that bacteria and infauna synthesize new hydrocarbons, thus adding to the complexity of the hydrocarbon pattern. Bacteria can add methyl branched and high molecular weight hydrocarbon (Davis, 1968; Tornabene and Markey, 1971); (3) Other sources of hydrocarbon for nearshore sediments are higher plants, seagrasses and benthic algae. These plants add C_{-20} to C_{-36} alkanes to sediment (Youngblood *et al.*, 1967); (4) In the case of BLM studies, petroleum must be recognized as another potential source of sedimentary hydrocarbon. While a large data base on the composition of petroleum is lacking, the general picture is known (Whitehead, 1963). Petroleum usually contains a full suite of n-paraffins, iso- and anteiso-paraffins, cycloalkanes, isoprenoids, and aromatic hydrocarbon, plus heteroatom compounds. The problem of recognizing petroleum in recent sediment is to determine whether these substances are present and to decide whether the biotic sources could supply them (Meinschein, 1961). (5) In this study, a decision was made to establish the general hydrocarbon composition of a large number of samples rather than to study a small suite of samples in great detail. This was necessary to provide a baseline at the actual sites where petroleum production is expected.

With this background information, several parameters were selected which might allow a decision as to the probability that a sample or group of sediment samples are petroleum contaminated. These parameters were:

The concentration of total saturated hydrocarbon;

The even-odd ratio of n-paraffins;

The concentration of non-saturated hydrocarbon;

The presence of specific aromatic hydrocarbon;

The presence of the isoprenoids, pristane and phytane and their ratios to n-hydrocarbons.

These parameters for the sedimentary hydrocarbons are discussed, evaluated and compared to similar studies in sub-sections below.

The complete hydrocarbon analyses for sediments are given in Appendix L, Table 7.1 through 7.190. Since it is difficult to make use of such large data sets, an attempt was made to further reduce the analytical data. The odd-even preference index (OEP) method has been generally useful (Scalan and Smith, 1969). OEP values are given in Appendix L, Table 8 and Figures 5.1 through 5.187 for those samples for which the data were suitable for such treatment.

The level of n-paraffins based on quantitative GLC ranged between 0.001 and 6 ppm, but most samples were between 0.1 and 2.0 ppm. This was similar to the values (3-12 ppm) reported by Gearing *et al.* (1976) for northeast Gulf shelf sediments, but not as high (100-200 ppm) as reported for California basin sediments (Hoering, 1968; Emery, 1960; Reed *et al.*, 1977).

The paraffin patterns in all samples were complex but most showed strong odd carbon numbers dominant in the C_{25} and C_{33} region. A typical GLC pattern is shown in Figure 28. This may be taken as evidence that petroleum derived material was very low. In addition, a few samples showed a bimodal carbon number distribution (Figure 29). This pattern has been reported for many sediment studies (Clark and Blumer, 1967; Meinschein, 1961; Gearing *et al.*, 1976).

The hydrocarbon patterns in sediment enabled the cautious formulation of the following interpretations:

-the paraffins showed little if any petroleum contribution;

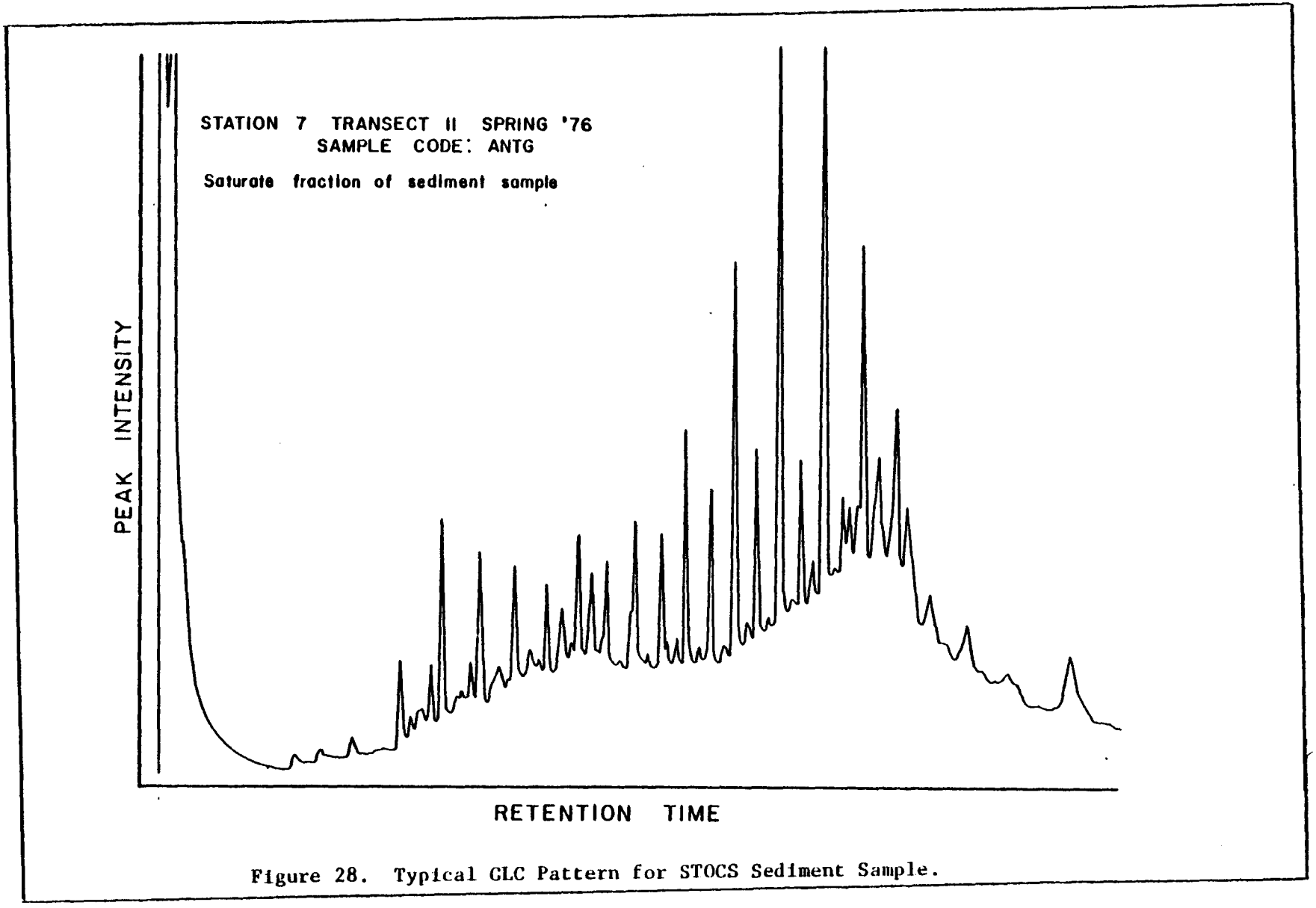


Figure 28. Typical GLC Pattern for STOCS Sediment Sample.

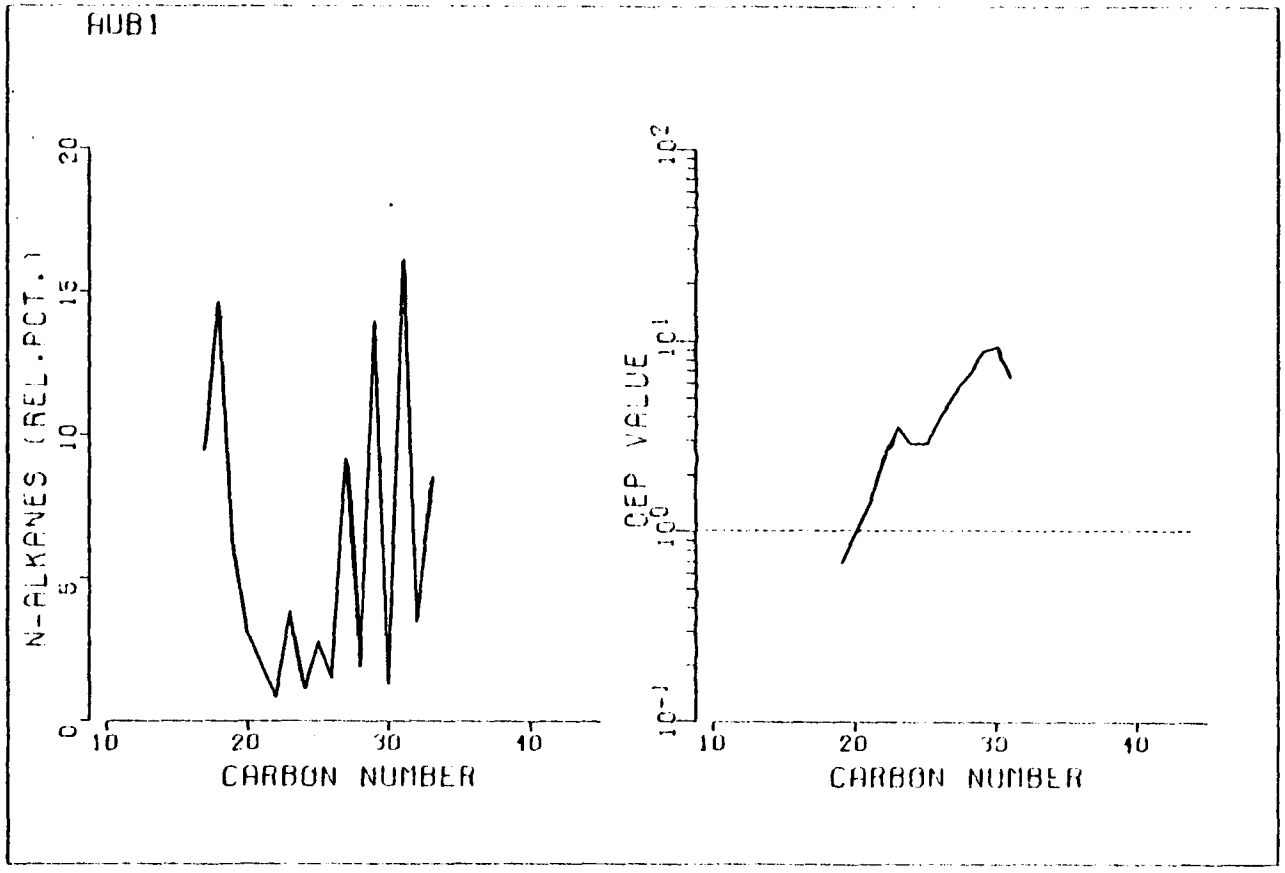


Figure 29. N-alkanes Distribution for Sediment Sample AUB1.

-the observed hydrocarbon patterns required multiple sources (plankton, bacteria, infauna, and perhaps higher plant detritus);

-isoprenoid hydrocarbon levels, pristane and phytane were low, confirming a low petroleum input but leaving unexplained the fate of the high levels of zooplankton pristane which must reach the sediment.

-the sediment was indicated as a final sink for marine hydrocarbon, but an active one which contributes new hydrocarbon to the deposit.

Intercalibration, Blanks and Controls

Throughout the course of this study of hydrocarbons from sediment, zooplankton and seawater, experimental care to prevent contamination was an overriding concern. As mentioned earlier, a previously unused suite of laboratories in a new building was dedicated to the study. The technical personnel were experienced in low-level organic work and care was taken to insure clean glassware and use of pure chemicals, and distilled solvents.

Quality control with respect to blanks was assumed by two approaches. First, blanks on solvents, critical operations and the total analytical scheme were run. At no time was a severe blank problem encountered, although the presence of cyclohexane in hexane was confirmed and the random presence of benzene was suspected. The second approach used to minimize error was to use the same laboratory, including glassware, chemicals, solvents and GLC for all analyses. Thus, seawater hydrocarbons at the sub-part-per-billion level served as control experiments for sediment and zooplankton hydrocarbons which were at the part-per-million level. The group of blank and control experimental gas chromatograms shown in Figures 30 and 31 include ones for solvent methanol, a freeze drier blank using silica gel, a test of a teflon wash bottle, a hexane blank, a hexane extract of fired sand and a hexane extract of freeze-dried, fired sand.

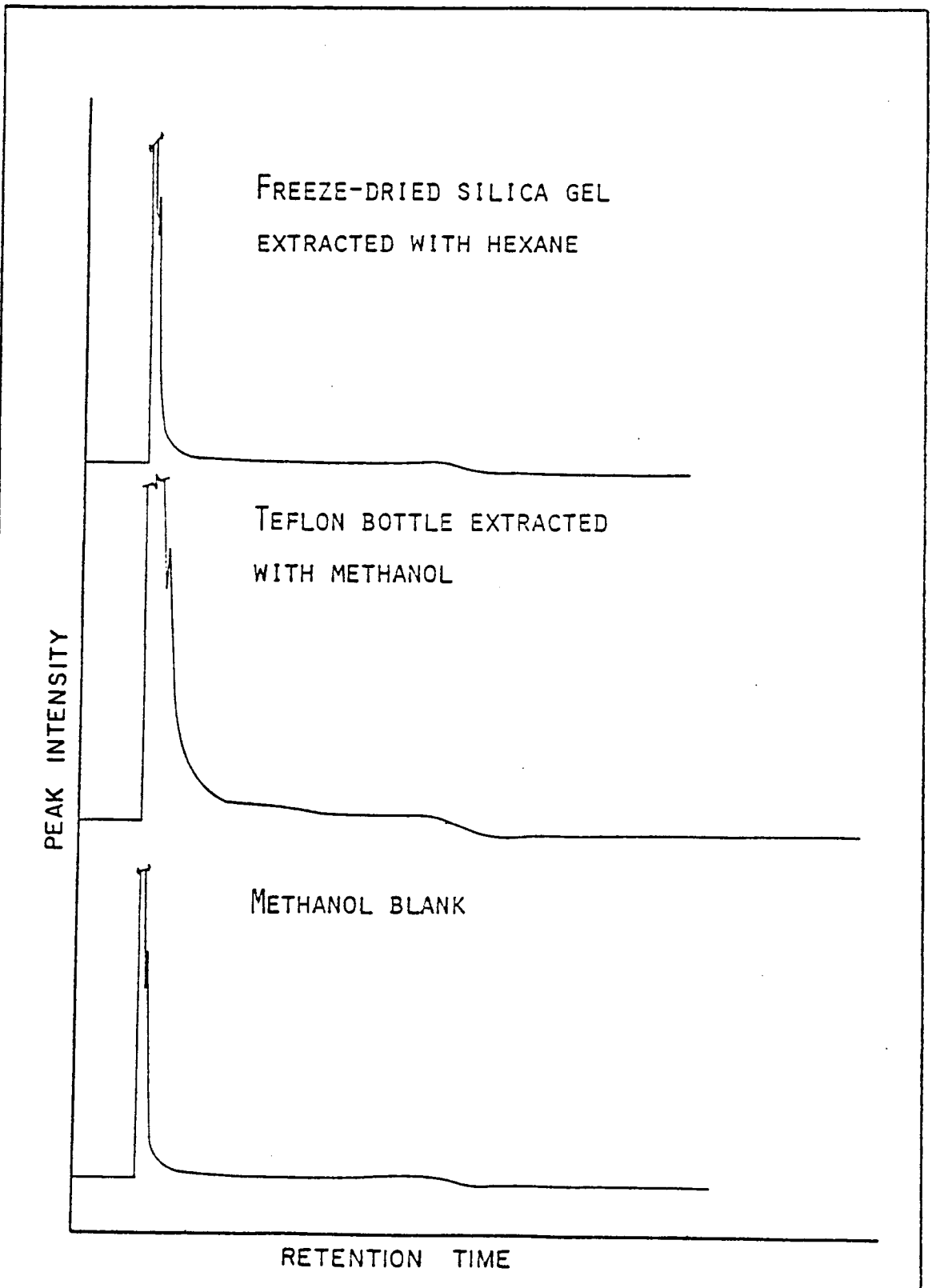


Figure 30. Gas Chromatograms of Procedural "Blanks" and "Tests".

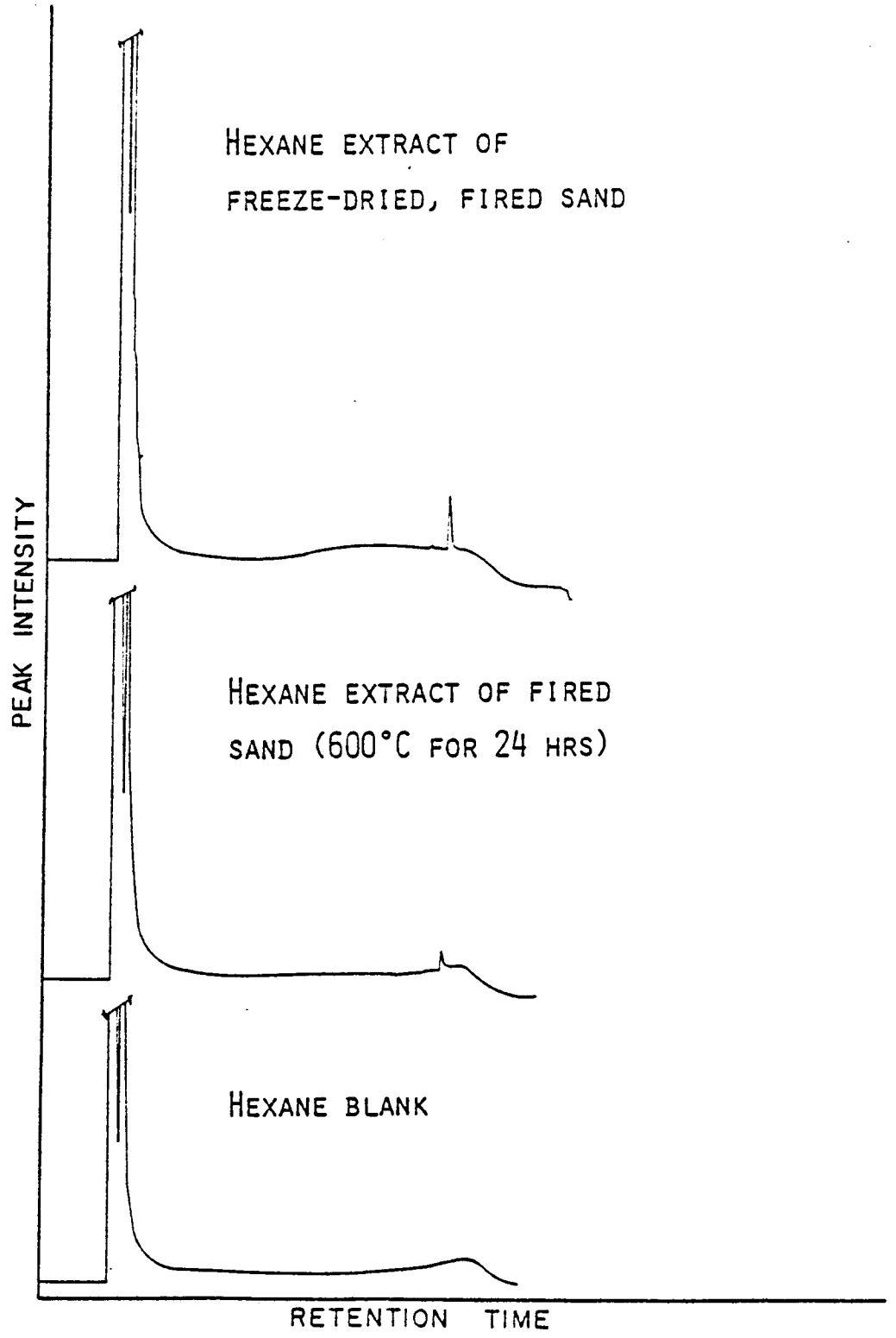


Figure 31. Gas Chromatograms of Procedural "Blanks" and "Tests".

To provide background data for an assessment of the level of contamination which might have occurred on shipboard during collection and processing, ships' fuel oil, lube oil and oily bilge water were characterized. The gas chromatographic patterns of hydrocarbon constituents of these are given in Figures 32, 33 and 34. Since these are petroleum products they are rich in hydrocarbons. However, no such GLC patterns routinely appeared in the sample analyses so that systematic contamination by the ship was judged to be absent.

A series of control experiments were conducted on sediments to test the recovery of added (spike) hydrocarbons. Fifty-one wet sediment samples were spiked with approximately 30 µg of n-C_{24}, the normal paraffin with 24 carbon atoms. Recoveries ranged from 2 to 173 percent, based on GLC analyses of the sediment using the methods described (Table 11). The average recovery was 34 percent. Fifty percent of the samples had yields between 30 and 90 percent. These data at face value indicate that recoveries were incomplete with, on the average, about one-third of n-C_{24} being recovered. That was probably the case for the spiked n-C_{24}, however, the recovery of the spike should not be viewed as the extraction efficiency of hydrocarbons native to the sample. Spike recoveries are generally low in this type experiment, as judged by previous experience on fatty acids. The reason relates to the fact that the spike cannot be added so as to be naturally present in the sediment matrix. The data for hydrocarbons in sediments in this report are estimated to represent 50 percent of the sediment hydrocarbons. If more severe extraction techniques such as HF decomposition were used, this probably could be raised but would also degrade the hydrocarbons so that some structural information would be lost. It has also been demonstrated that new hydrocarbons can be generated from kerogen, fatty acids and fatty alcohols under relatively mild conditions (Hoering,

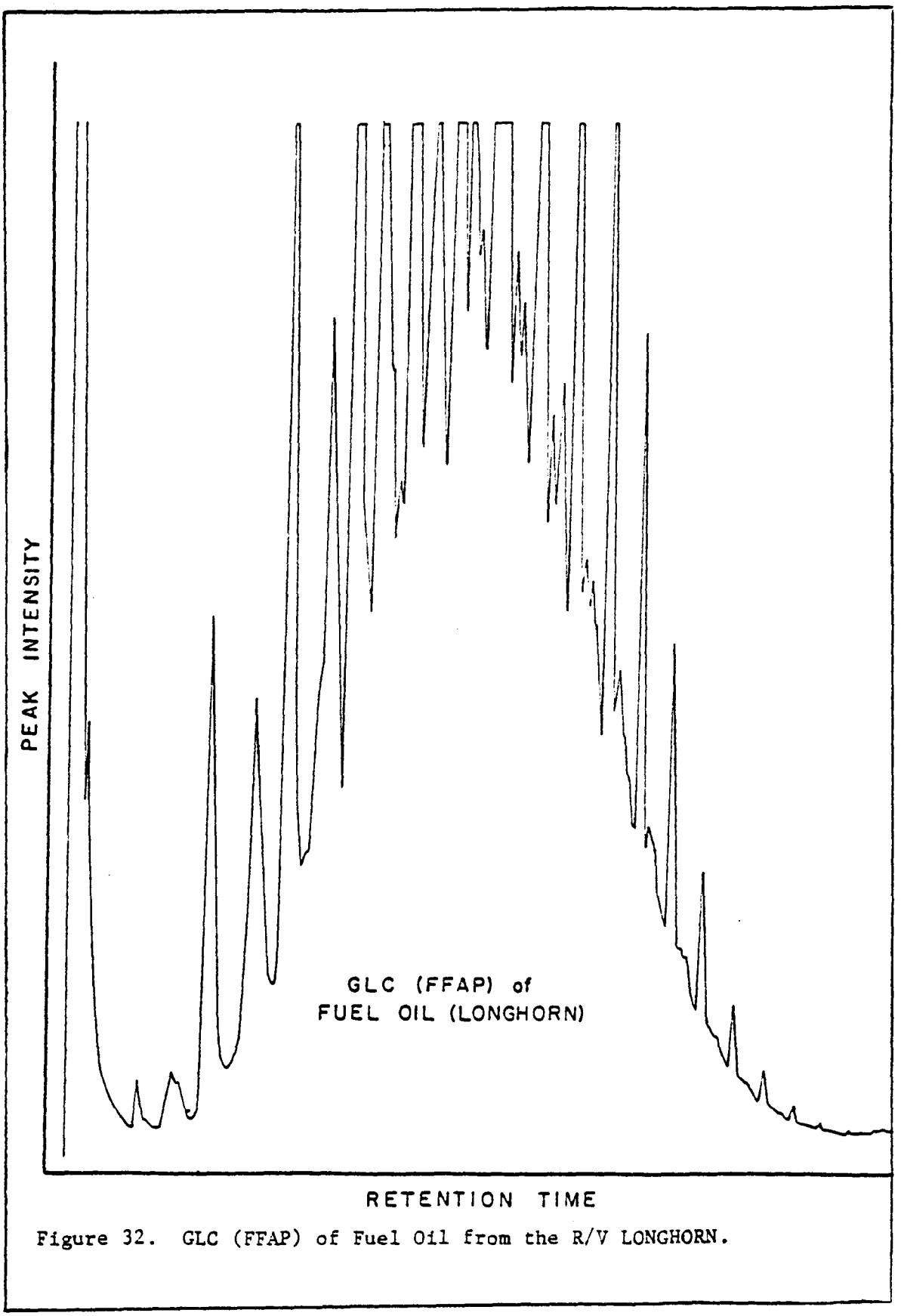


Figure 32. GLC (FFAP) of Fuel Oil from the R/V LONGHORN.

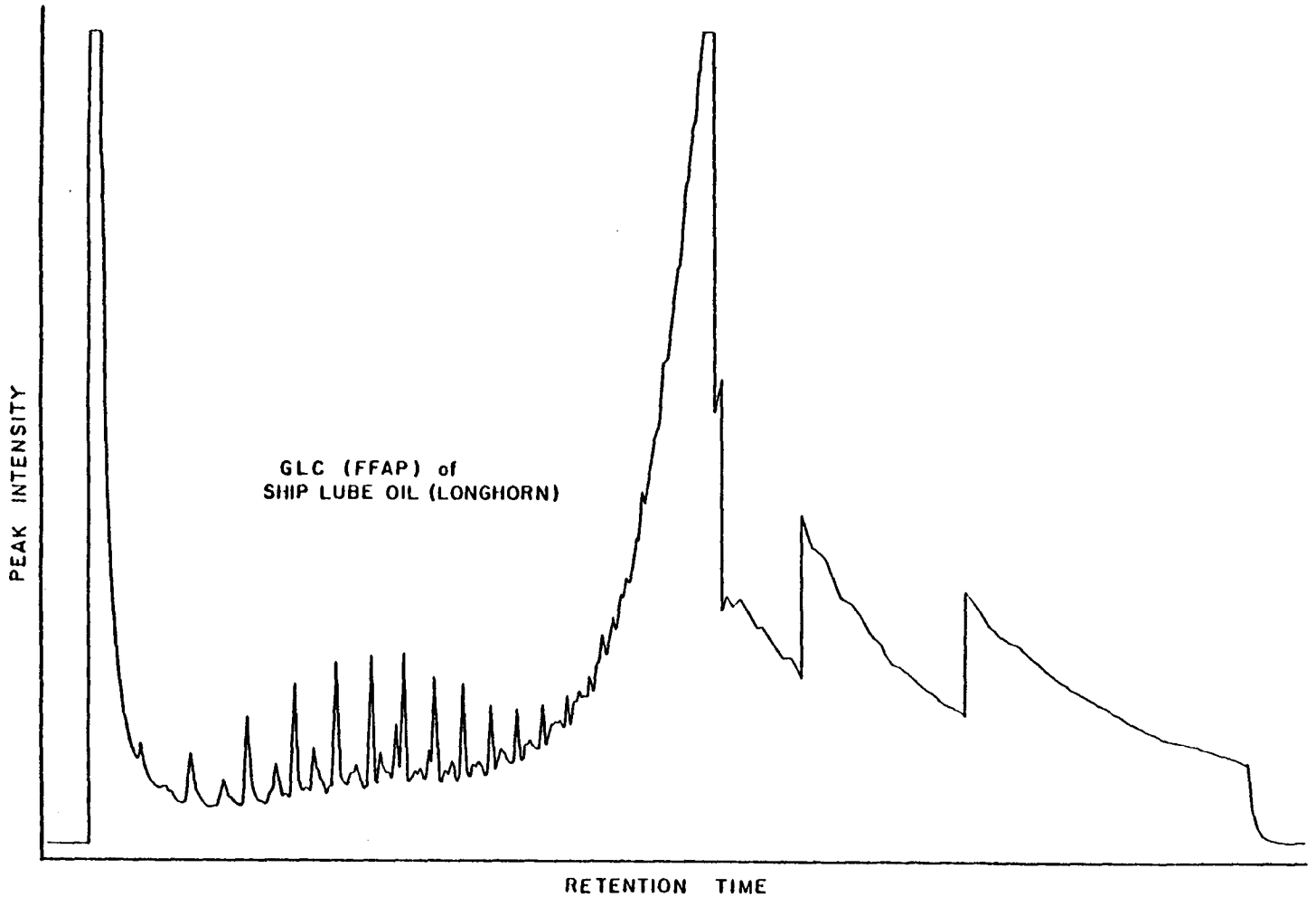


Figure 33. GLC (FFAP) of Ship's Lube Oil from the R/V LONGHORN.

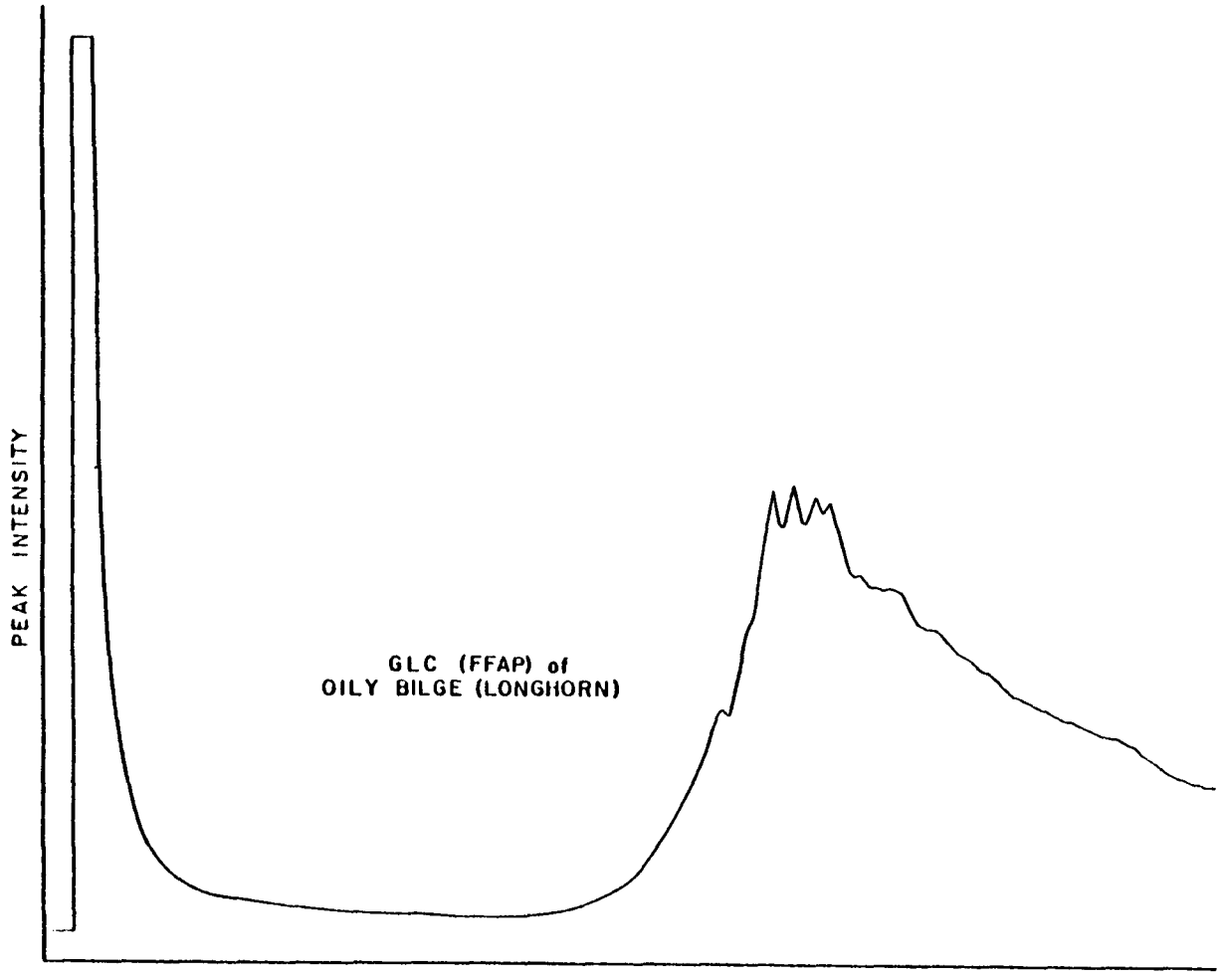


Figure 34. GLC (FFAP) of Oily Bilge Water from the R/V LONGHORN.

TABLE 11

RECOVERY OF SPIKE ($n-C_{24}$) FROM SEDIMENT

Sample Code	Recovery Percent	Sample Code	Recovery Percent
MCJ	16	NPW	13
MCL	22	NPX	70
MCM	32	NPY	173
MCN	11	NRM	5
MCO	6	NRN	33
MNP	46	NRP	32
MNR	30	NRQ	2
MSW	59	NTF	6
MXQ	12	NTH	38
MXR	6	NTI	14
MXS	16	RCT	69
MZQ	33	RGB	30
NBM	14	RHS	53
NDF	14	RLT	50
NDG	15	RPI	48
NDI	28	RQR	59
NEZ	77	RSB	64
NFA	40	RTM	23
NFB	42	SDF	19
NIO	18	SIS	41
NKL	57	OB1	63
NKO	45	OB2	10
NMI	43	GB3	3
NML	26	GB4	12
NOE	17		
NPU	8		
NPV	92		

Average 34%

1972). A suitable compromise for BLM objectives seems to have been reached.

Several formal and informal inter-laboratory comparisons were made during the course of this project. Useful information was gleaned from each one, however, they all suffered from the unfortunate fact that no certified standard material for petroleum hydrocarbons were available. Such materials are available for trace metals and for low levels of radionuclides. Thus, the inter-laboratory experiments were comparisons which served to improve our techniques, not to test them.

Three inter-laboratory comparison studies were undertaken:

-hydrocarbon in Alaskan sediment-National Bureau of Standards;

-hydrocarbon in spiked California sediment-BLM via Dr. I. Kaplan, UCLA;

-hydrocarbon in Texas Oysters-Exchanged with Dr. C. S. Giam, TAMU and Dr. J. Farrington, WHOI.

In addition to the above, a special comparative study of the extraction efficiency of chloroform and toluene for the water soluble components of No. 2 fuel oil was made. This report (Appendix L, Report 1) suggested that both solvents are satisfactory.

The Alaskan sediment supplied to several BLM contractors was a natural sediment which was thought to have been contaminated by natural oil seeps. The hydrocarbon levels were low, so the main value of the exercise was to test the techniques for low level sediment contamination. The results and methods are reported in Appendix L, Report 2.

A sample of Santa Barbara sediment spiked with South Louisiana crude was supplied to several BLM contractor labs for inter-laboratory comparison. The sample was treated essentially as a BLM sediment except that it was not freeze-dried, methanol drying being substituted. The recovery of n-paraffins, based on our knowledge of the South Louisiana crude was

low, but satisfactory. The main loss was due to the fact that South Louisiana crude is rich in light hydrocarbons and C_{-10} and C_{-15}, and our methods exclude most of these. A large suite of aromatic hydrocarbons were recovered, including C_2 and C_3 ring compounds. One interesting side light was that a high level of P, PDDE [*i.e.*, 1, 1-dichloro-2, 2-bis(p-chlorophenyl) ethylene], a chlorinated hydrocarbon was identified. The complete report on this study is given in Appendix L, Report 3.

The Harbor Island oyster sample was analyzed by GC/MS and shown to contain several classes of petroleum-derived aromatic compounds (Appendix L, Report 4). Classes of aromatics identified included alkyl naphthalenes, phenanthrenes and dibenzothiophenes.

In summary, a substantial effort was made to produce high quality hydrocarbon analyses through blanks, controls, inter-laboratory comparisons and especially experience. One of us (PLP) has served on the BLM Hydrocarbon Methodology Review Group which has served as a useful forum to help resolve some of the technique problems which have arisen and to accelerate communication among chemists dealing with BLM samples.

LITERATURE CITED

Attaway, D. H., P. L. Parker, and J. A. Mears. 1970. Normal alkanes of five coastal spermatophytes. Contr. Mar. Sci. 15.

Berryhill, H. L., Jr. (Ed.) 1977. Environmental studies, South Texas Outer Continental Shelf, 1975. An atlas and integrated summary prepared for the U.S. Bureau of Land Management.

Blumer, M., R. R. L. Guillard, and T. Chase. 1971. Hydrocarbons of marine phytoplankton. Interntl. Jour. on Life in Oceans and Coastal Waters, March, 8(3):183-189.

_____, M. M. Mullin, and D. W. Thomas. 1964. Pristane in the marine environment. Helgolaender Wiss. Meeresuntersuch. 10:187.

_____, M. M. Mullin, and D. W. Thomas. 1963. Pristane in zooplankton. Sci. 140:974.

_____, M. M. Mullin, and R. R. L. Guillard. 1970. A polyunsaturated hydrocarbon (3, 6, 9, 12, 15, 18-heneicosahexane) in the marine food web. Mar. Biol. 6:226-235.

_____, and D. W. Thomas. 1965a. Phytadienes in zooplankton. Sci. 147:1148-1149.

_____, and D. W. Thomas. 1965b. Zamene, isomeric C_{19} monoolefinis from marine zooplankton, fishes, and mammals. Sci. 148:370-371.

Brown, R. A., and T. D. Searl. 1976. "Non-volatile hydrocarbons in the Pacific Ocean" sources, effects and sinks of hydrocarbons in the aquatic environment. Proc. of the Symp., Aug 9-11, Washington, D. C.

Calder, J. A. 1976. Hydrocarbons from zooplankton of the eastern Gulf of Mexico *In* Sources, effects, and sinks of hydrocarbons in the aquatic environment. AIBS Symposium Volume Washington, D. C.

Clark, R. C., Jr., and M. Blumer. 1967. Distribution of paraffins in marine organisms and sediment. Limnol. Oceang. 12:79.

Davis, J. B. 1968. Paraffinic hydrocarbons in the sulfate-reducing bacterium Desulfovibrio Desulfuricans. Chem. Geol. 3:155-160.

Eglinton, G., and M. T. J. Murphy. (Eds.) 1969. Organic Geochemistry.

Emery, K. O. 1960. The sea off Southern California. John Wiley and Sons, Inc. New York.

Farrington, J. W., J. M. Teal, and P. L. Parker. 1976. Petroleum hydrocarbons. *In* Strategies for Marine Pollution Monitoring. E. D. Goldberg (Ed.), John Wiley and Sons, New York.

Fredericks, A. D., and W. M. Sackett. 1970. Organic carbon in the Gulf of Mexico. Jour. of Geophysical Res., Oceans and Atmospheres, 75(12): 2199-2206.

Gearing, P., J. N. Gearing, T. F. Lytle, and J. S. Lytle. 1976. Hydrocarbons in 60 northeast Gulf of Mexico shelf sediments: a preliminary survey. Geochimica et Cosmochimica Acta. 40:1005-1017.

Gelpi, E., J. Oro, H. J. Schneider, and E. O. Bennett. 1968. Olefins of high molecular weight in two microscopic algae. Sci. 161:700-702.

Han, J., and M. Calvin. 1969. Hydrocarbon distribution of algae and bacteria, and microbiological activity in sediments. Prac. Nat. Acad. Sci. 64:436-443.

Hoering, T. C. 1972. Annual report to the Director, Geophysical Laboratory, Carnegie Institution. Publication #1615.

_____. and R. M. Mitterer. 1968. Production of hydrocarbons from the organic matter in recent sediment. Annual Report to the Director, Geophysical Laboratory, reprinted from Carnegie Institution Year book 66.

Jeffrey, L. M. 1970. *In* Symposium on organic matter in natural waters. D. W. Hood (ed.) Univ. of Alaska. pp. 55-71.

Koons, C. B. 1977. Distribution of volatile hydrocarbons in some Pacific Ocean waters. Proc. from the 1977 Oil Spill Conference, March 8-10, New Orleans.

Mauer, L. G., and P. L. Parker. 1972. The distribution of dissolved organic matter in the near-shore waters of the Texas coast. Contr. in Mar. Sci. 16.

McAuliffe, C. D. 1976. Surveillance of the marine environment for hydrocarbons. Mar. Sci. Communications, 2(1):13-42.

McLafferty, F. W. 1973. Interpretation of mass spectra. 2nd Edition, W. A. Benjamin, Inc., Reading, Mass.

Meinschein, W. G. 1961. Significance of hydrocarbons in sediments and petroleum. Geochimica et Cosmochimica Acta, 22(1):58-64.

Monaghan, P. H., J. H. Seelinger, and R. A. Brown. 1973. The persistent hydrocarbon content of the sea along certain tanker routes. A preliminary report. American Petroleum Institute Report. 18th Annual Tanker Conference. Hilton Head Island, South Carolina. 298pp.

P. L. Parker. 1967. Fatty acids in recent sediment. Contr. in Mar. Sci. 12:135.

_____. 1969. Fatty acids and alcohols. *In* Organic Geochemistry, Methods and Results. Springer-Verlag, New York.

_____., J. K. Winters, and J. Morgan. 1972. *In* Baseline studies of pollutants in the marine environment and research recommendations. pp. 555-582. IDOE Baseline Conference, May 24-26, New York.

_____., R. S. Scalan, and J. K. Winters. 1976. Heavy hydrocarbons project-water, zooplankton, neuston and sediment. *In* Environmental assessment of the South Texas outer continental shelf chemical and biological survey component. pp. 443-530. Rept. to the Bureau of Land Management, Contract 08550-CT5-16. 598pp.

Pocklington, R. 1971. Free amino-acids dissolved in North Atlantic Ocean Waters. Nat. 230. April 9.

Reed, W. E., I. R. Kaplan, M. Sandstrom and P. Mankiewicz. 1977. Petroleum and anthropogenic influence of the composition of sediments from the Southern California bight. Proc. of 1977 Oil Spill Conference, March 8-10, New Orleans.

Scalan, R. S., and J. E. Smith. 1969. An improved measure of the odd-even predominance in the normal alkanes of sediment extracts and petroleum. Geochimica et Cosmochimica Acta. 34:611-620.

Sever, J., and P. L. Parker. 1969. Fatty acids (normal and isoprenoid) in sediments. Sci. 164:1052.

Stenhagen, E., S. Abrahamsson, and F. W. McLafferty. 1974. Registry of mass spectral data. John Wiley and Sons, New York.

Stransky, K., M. Striebl, and V. Herout. 1967. Distribution of wax hydrocarbons in plants at different evolutionary levels. Collection Czechoslov. Commun. 32.

Stumm, W., and P. A. Brauner. 1975. Chemical speciation. *In* Chemical Oceanography. Vol. 1.

Tornabene, T. G., and S. P. Markey. 1971. Characterization of branched monounsaturated hydrocarbons in *Sarcina lutea* and *Sarcina flava*. Lipids 6(3).

Whitehead, W. L., and I. A. Breger. 1963. Geochemistry of petroleum. *In* Organic geochemistry. Monograph No. 16-Earth Science Series. MacMillan Co. New York.

Williams, P. J. 1975. Biological and chemical aspects of dissolved organic material in sea water. *In* Chemical Oceanography. Vol. 2.

Williams, P. M. 1965. Jour. Fish Res. Bd. Canada. 22:1107.

Winters, J. K., P. L. Parker, and C. Van Baalen. 1969. Hydrocarbons of blue-green algae: Geochemical significance. Sci. 163:467-468.

Yen, T. F. (ed.) 1977. Chemistry of Marine Sediments. Ann Arbor Sci. Publ. Inc., Ann Arbor, Mich.

Youngblood, W. W., M. Blumer, R. R. L. Guillard and F. Fiore. 1971. Saturated and unsaturated hydrocarbons in marine benthic algae. Mar. Biol. 8:190-201.

ADDENDUM

GC/MS ANALYSIS OF PARTICULATE AND DISSOLVED WATER EXTRACTS

There were 22 fractions from the water analyses which were analyzed using the GC/MS. Their reconstructed total ion chromatograms and significant peak indices are given in Figures 1 through 22. Generally, the hexane fractions contained the normal aliphatics which could be identified with considerable confidence using retention times from the gas chromatograph. The GC/MS verified the aliphatic patterns found in the hexane fractions. Occasionally the series of aliphatics were also seen in the benzene fraction.

Phthalates could be seen in both the hexane and benzene fractions. It must be assumed that most phthalate is introduced into the sample from sources inherent in sampling and analysis. Of the 22 fractions analyzed with the GC/MS, phthalates were found in nine of the benzene fractions and five of the hexane fractions.

Squalene is commonly seen in zooplankton samples and also in some water samples. Of the fractions run on the GC/MS, squalene was observable in only trace amounts.

Three of the particulate benzene fractions (each from Station 1, Transect II) contained fatty acid methyl esters. Presumably, they were present in the plankton which are most abundant at the inshore stations. Long chained wax esters were found in one benzene fraction. These were tentatively identified as esters of C_{14}:1 and C_{16}:1 fatty alcohols and C_{14}:1 and C_{16}:1 fatty acids.

In one benzene fraction a series of compounds was found that showed strong evidence of silicon containing compounds, possibly made up of $(CH_3)_3Si$ and $(CH_3)_2Si\,O$ units. These compounds are presumed to be contaminants introduced into the sample.

Four of the benzene fractions analyzed by GC/MS showed chlorinated hydrocarbons as the major constituents. These are believed to be formed

```
SEQUEN     48     PAGE      1

SIGNFPK
GC ID BP    120    DATE    9/28/77
AGRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      4

BLM WATER PART. WINTER, 2/IV,AGGK, HEXANE

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY    YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 4

     41    1000     137    8797
     43    1000      87   14631
     45    1000     196   18901
     69    1000       1   10784
    149    1000     149     996
     57     976      78    7504
     55     941     104    5267
```

Figure 1. Total Ion Chromatogram and Significant Peak Indices for Water Sample AGGK, Collected During the Winter Seasonal Sampling from Station 2/IV.

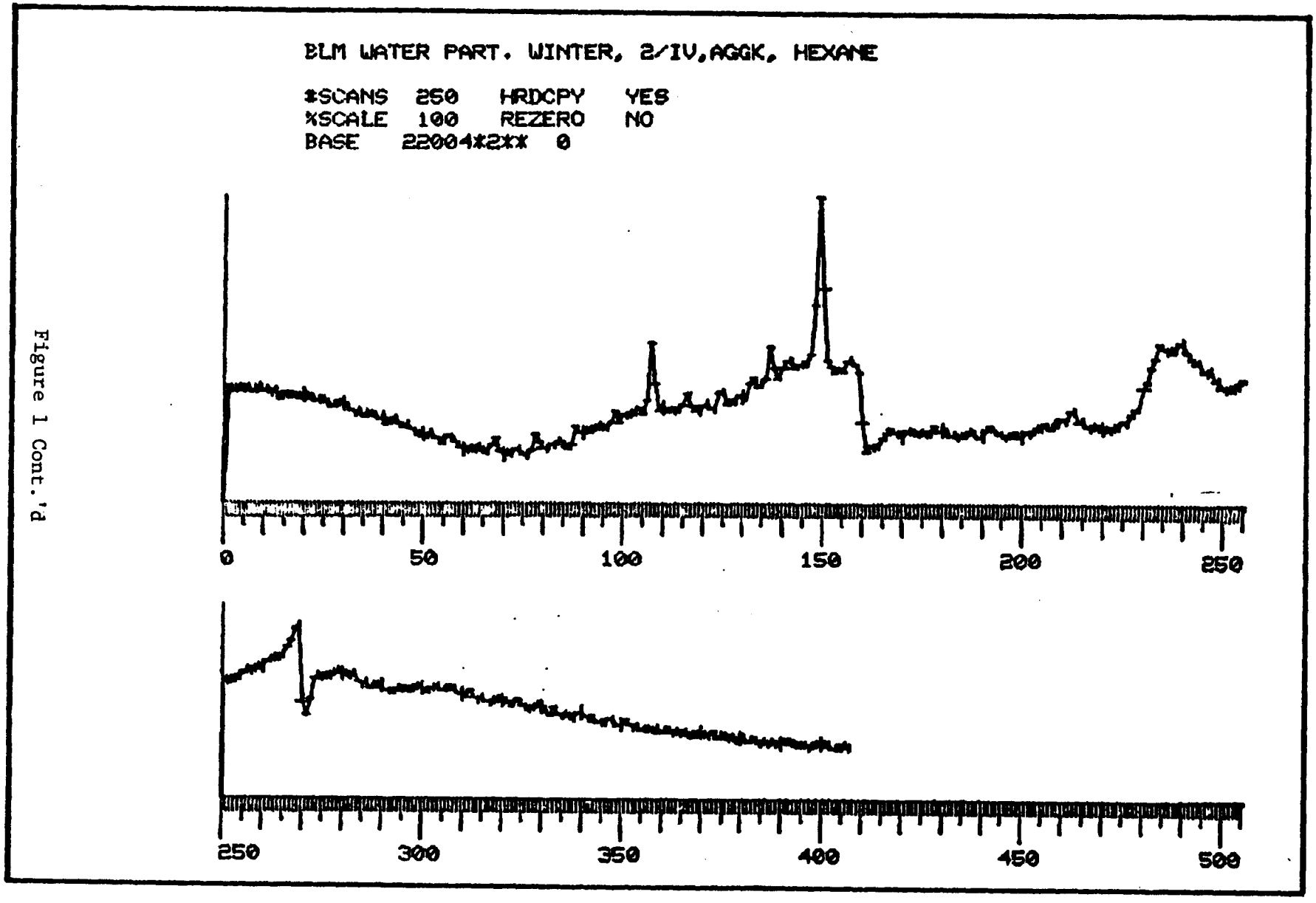


Figure 1 Cont.'d

```
SIGNFPK
GC ID BD     11    DATE    2/ 7/78
AQRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      8

AGIB-WAT-BZ 3/IV  '76 2-7-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

  MASS    MAX    FIRST    SUM
         INTN    OCCUR   IONS X2XX 7

    43    1000       9    7020
    45    1000     175   19320
    57    1000      36    4476
    77    1000      18   10652
    83    1000      41    2407
    91    1000      43     941
   109    1000     167    4578
   112    1000       1   13337
    95    1000      48    3241
   147    1000     109    3088
   149    1000     181    3111
   128    1000      87    3646
   125    1000      70    1270
   163    1000     159    1572
   131    1000      59    1283
   146    1000      28    5563
   180    1000      62    1541
   162    1000     118    3284
   177    1000      79     782
   121    1000     165    1498
   196    1000     143    2077
   198    1000     124    1546
   219    1000     166    1537
   242    1000     156    1092
   199     998     147    1999
   183     982     165    1547
    94     979     153    4837
   111     960     165    5648
   208     957     133     981
   130     955      48    1667
   133     950      60    2813
   154     946     107     997
   199     938     160     983
   132     927      48    1702
   201     914     160     790
   182     907      62     920
    41     899      37    3078
   217     865     166    1154
   176     853      78    1646
    99     851     159    3064
```

Figure 2. Total Ion Chromatogram and Significant Peak Indices for Water Sample AGIB, Collected During the Winter Seasonal Sampling from Station 3/IV.

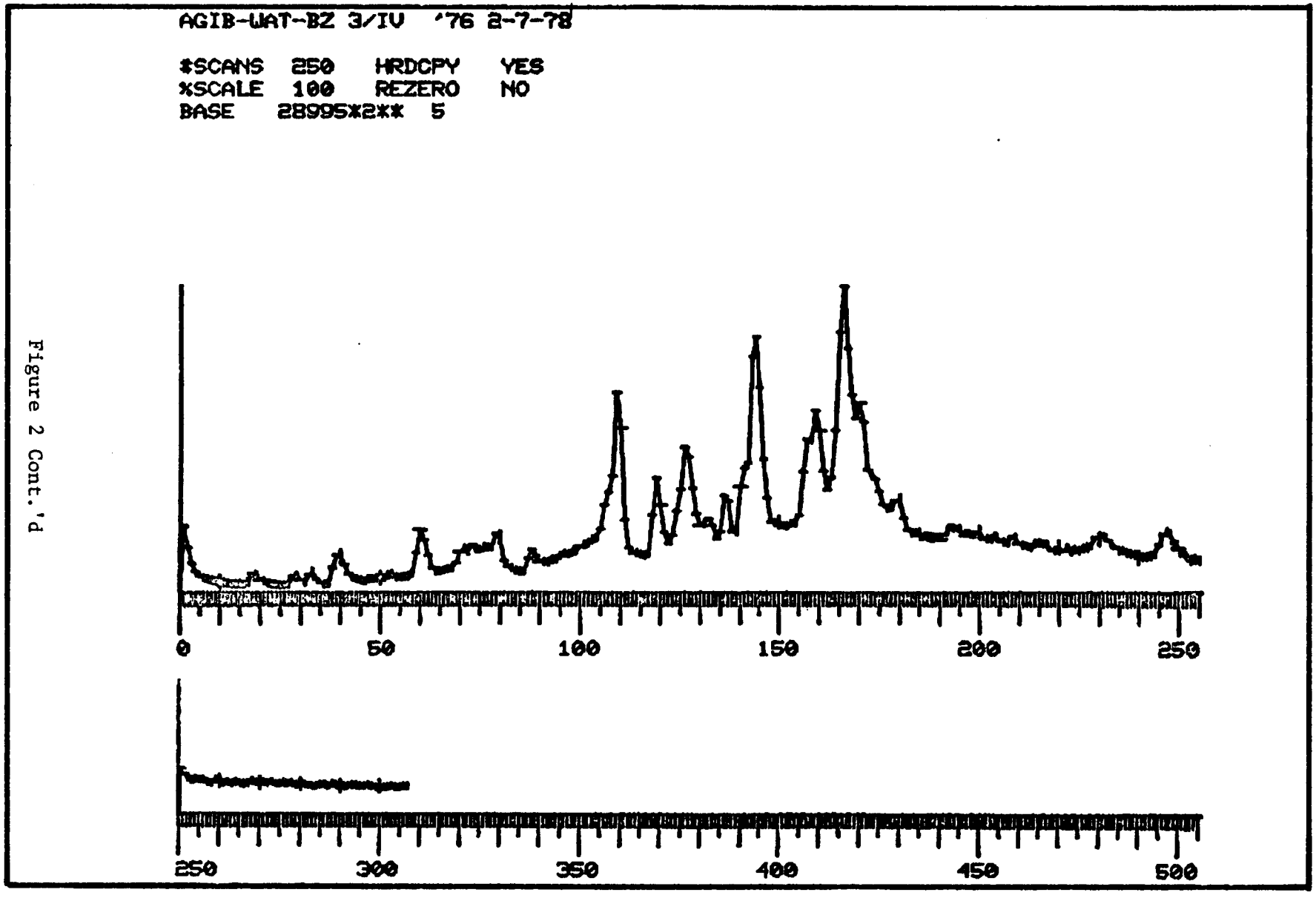


Figure 2 Cont.'d

```
:EGMEN     36     PAGE      1

SIGNIFPK
GC ID BJ      11    DATE    2/ 8/78
AQRATE         2    SCTIME      4    RESPWR    500
HIMASS       500    THRESH      8

AGUW-WAT-BEN  3/II '76  2-8-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS X2XX 6

     43    1000        2    6740
     45    1000      128   16988
     57    1000        1    4021
    253    1000       95      43
    149    1000      131    2513
     41     953       33    3224
     56     937        1    1751
```

Figure 3. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AGUW, Collected During the Winter Seasonal Sampling from Station 3/II.

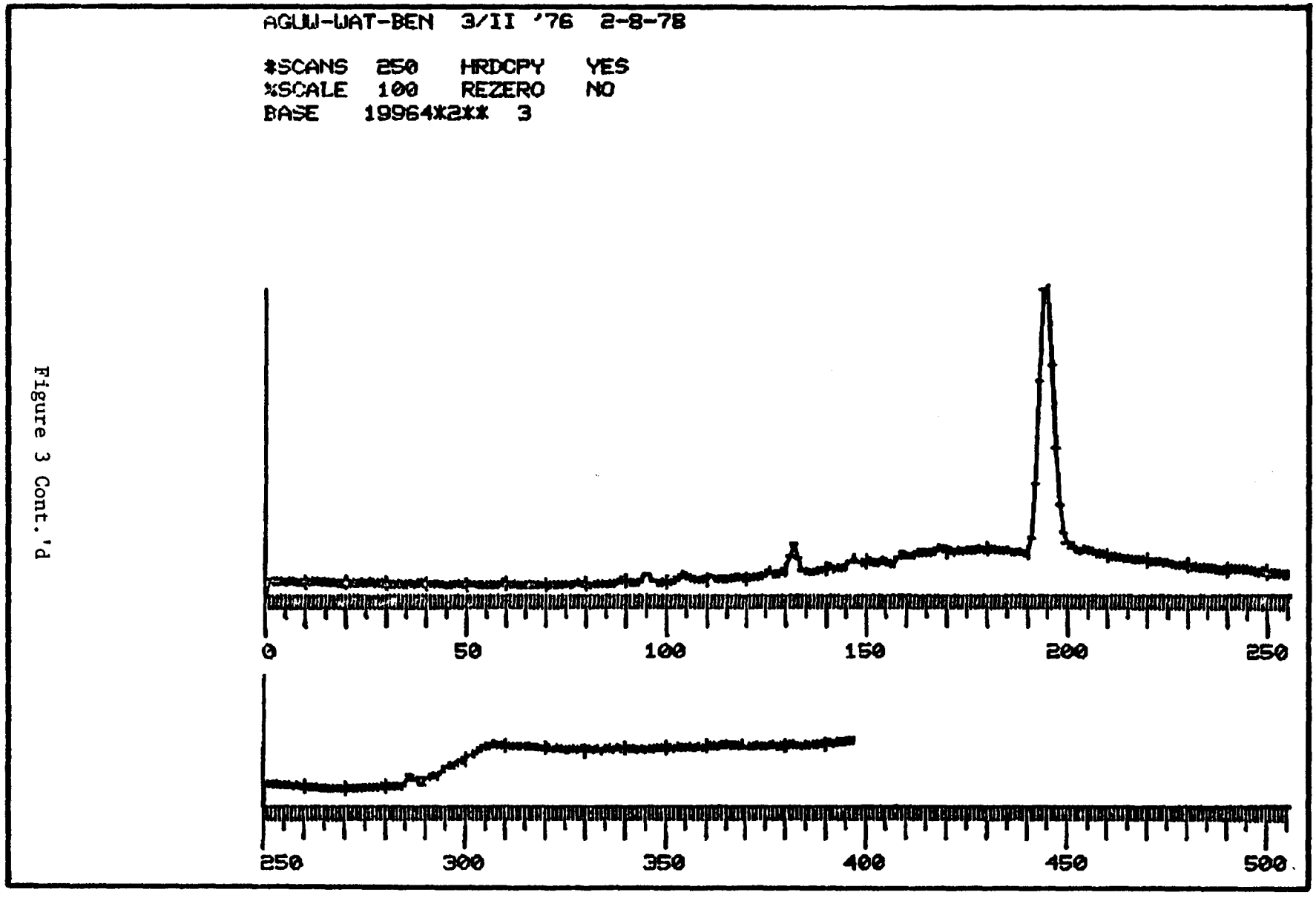


Figure 3 Cont.'d

```
SEQUEN     38     PAGE      1

SIGNFPK
GC ID BP    114    DATE    9/28/77
AGRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      4

BLM WATER WINTER DIS 2/III,AGYQ,BENZENE

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 6

     43    1000      77   19836
     45    1000      98   23593
     57    1000      61   16077
     78    1000       1   17489
    149    1000      80   10472
```

Figure 4. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AGYQ, Collected During the Winter Seasonal Sampling from Station 2/III.

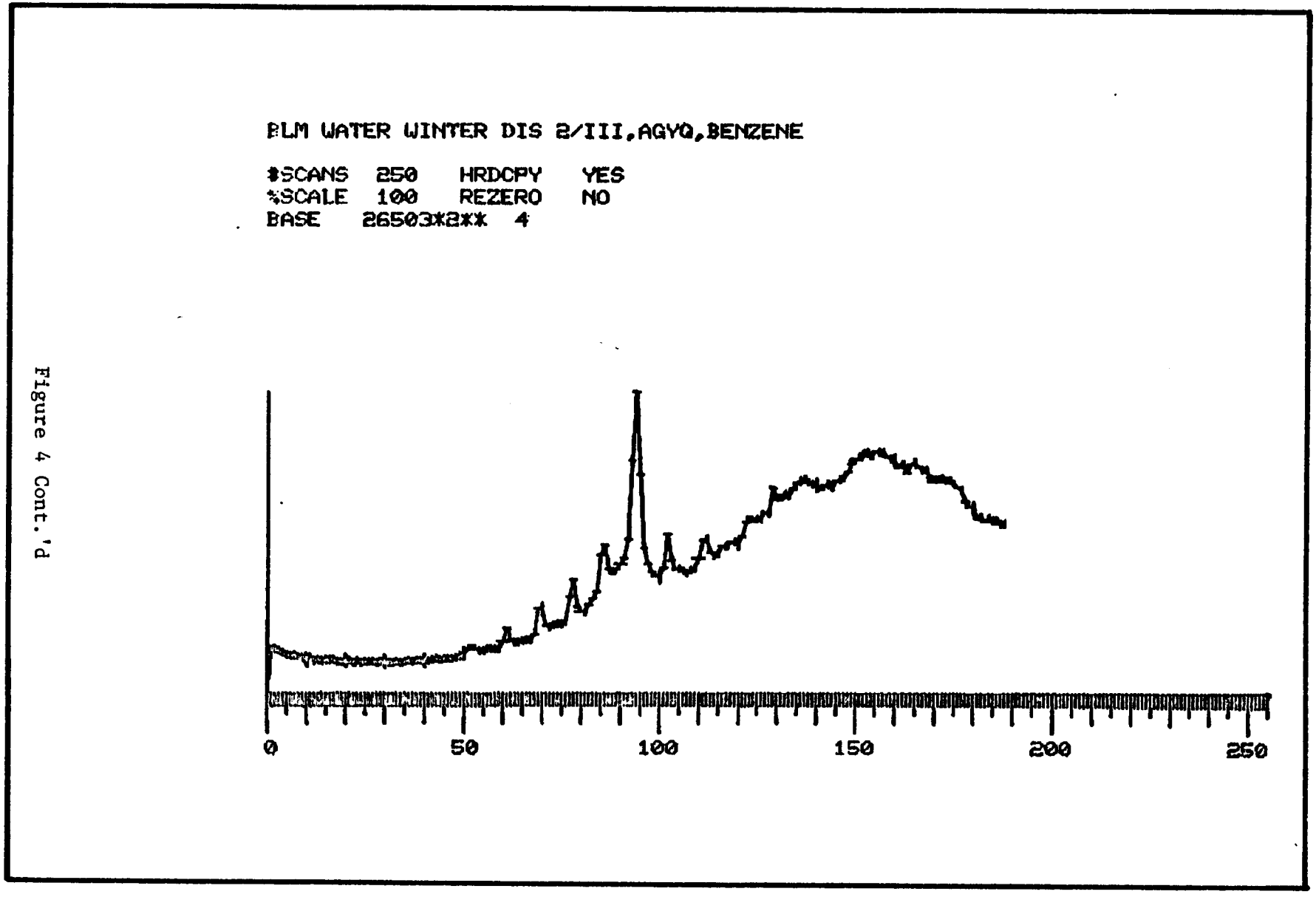


Figure 4 Cont.'d

```
:IGNFPK
GC ID BD      8    DATE   2/ 6/78
AVRATE        2    SCTIME     4    RESPWR   500
HIMASS      500    THRESH     8

HKDF-WAT-HX  1/II  '76  2-6-78

IGNORE       0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 5

     43    1000       1   15924
     45    1000     175   24123
     57    1000       4   14043
    149    1000     163    3724
     56     975      54    7064
     41     961     115    8821
```

Figure 5. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AKDF, Collected During the April Monthly Sampling from Station 1/II.

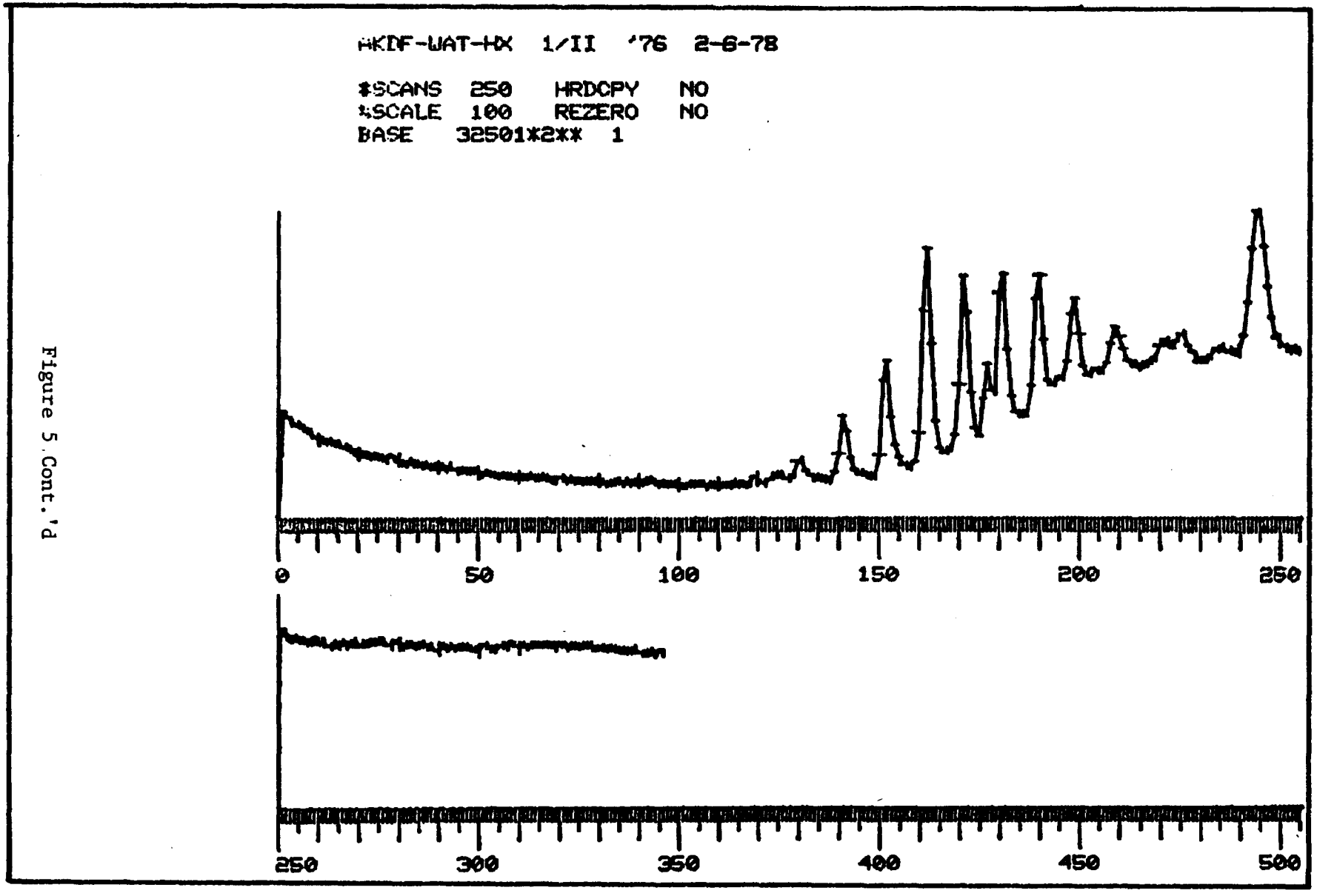


Figure 5 Cont.'d

```
:!GMFPK
GC ID BD      6    DATE    2/ 6/78
MAPNTE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      8

HKDF-WAT-BZ  1/II '76 BD006 2-6-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES
```

MASS	MAX INTN	FIRST OCCUR	SUM IONS *2XX* 6
43	1000	51	7426
45	1000	186	17796
57	1000	1	5218
97	1000	149	4166
109	1000	70	1545
141	1000	118	561
149	1000	182	1868
128	1000	88	10270
178	1000	200	1575
162	1000	120	19394
154	1000	108	282
196	1000	150	3883
198	1000	163	3755
120	962	83	135
176	959	119	311
41	957	72	4166
63	929	126	14177
77	918	118	1512
164	908	120	14494

Figure 6. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AKDF, Collected During the April Monthly Sampling from Station 1/II.

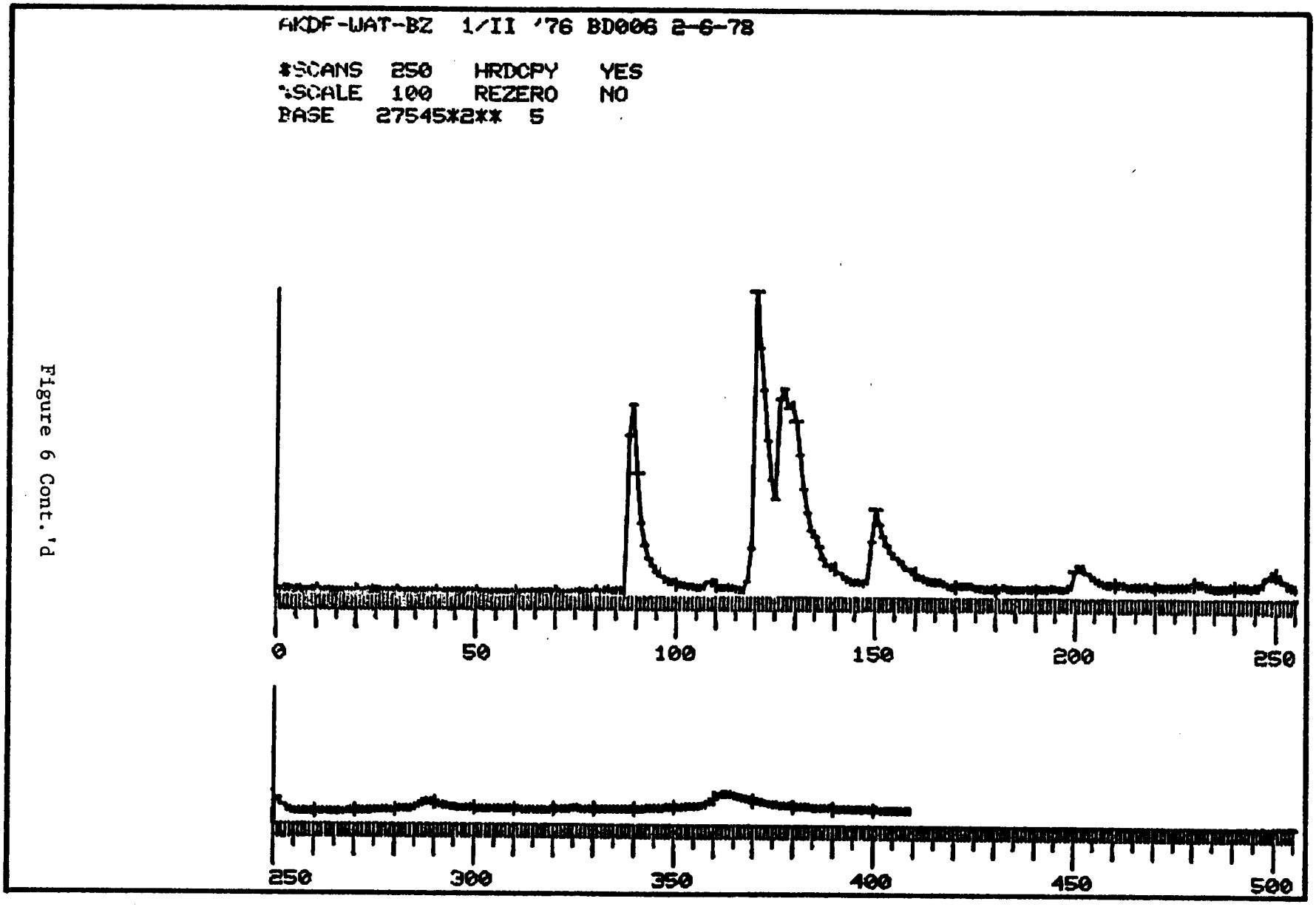


Figure 6 Cont.'d

```
SIGNIFPK
GC ID BP    125    DATE    2/ 3/78
AQRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      8

KIG-WAT-HEX 1/II PART  2-3-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
          INTN    OCCUR   IONS *2** 5

     41    1000       21    9617
     43    1000        1   20287
     45    1000      182   14480
     57    1000       40   23401
     56     902       21    5734
```

Figure 7. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AKDG, Collected During the April Monthly Sampling from Station I/II.

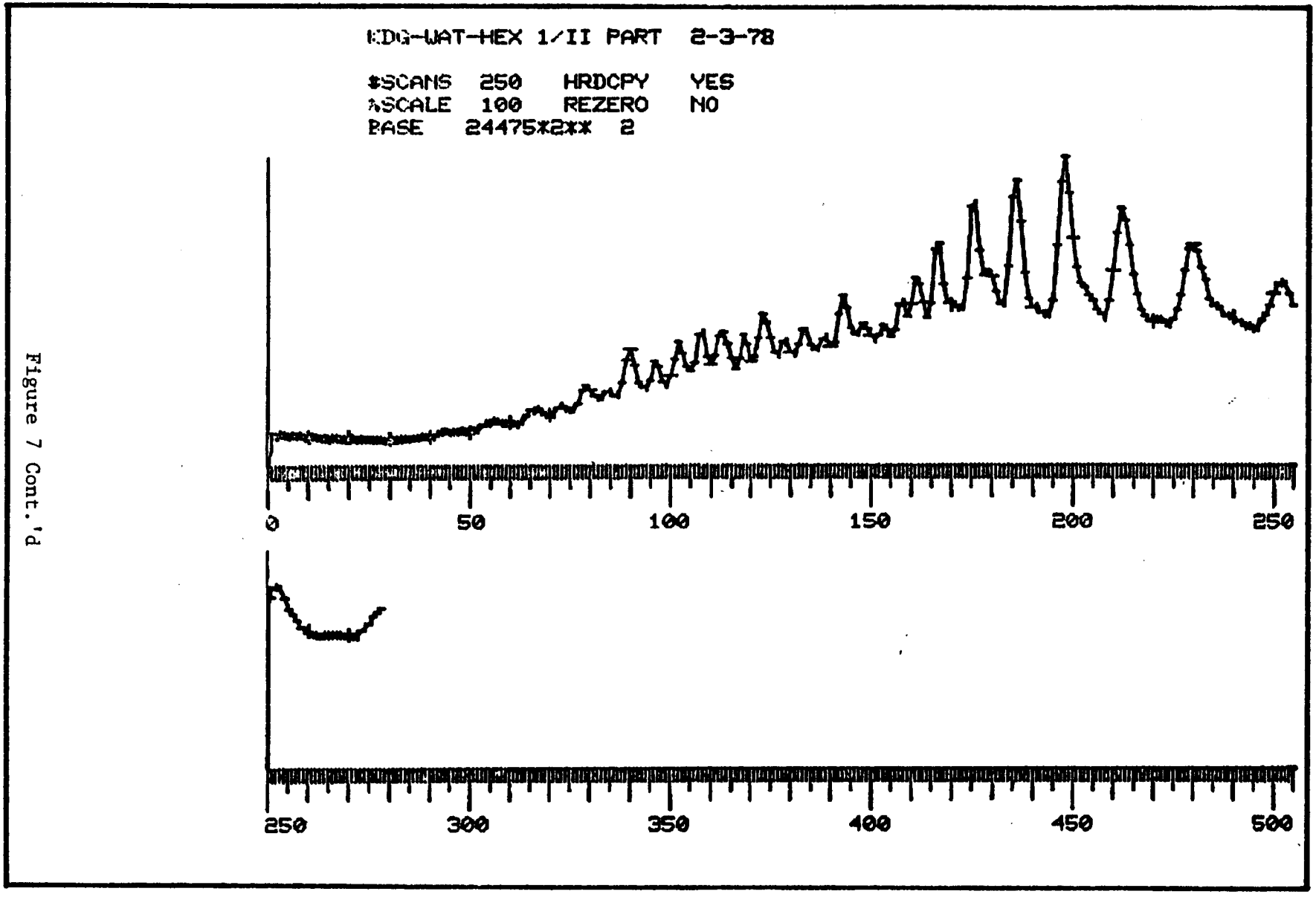


Figure 7 Cont.'d

```
SIGNFPK
SP ID BP    127    DATE    2/ 3/78
RSPNTE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      8

AKDG-WAT-BZ 1/II PART. 2-3-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 5

     43    1000       1   18275
     45    1000     163   26527
     55    1000     137    8784
     57    1000      13   12807
     74    1000      99    7680
    129    1000     182    1709
    149    1000     217    2837
    163    1000     130     732
     41     929      78    9729
     87     885     204    9258
     56     866      78    5158
     69     858     169    6408
```

Figure 8. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AKDG, Collected During the April Monthly Sampling from Station 1/II.

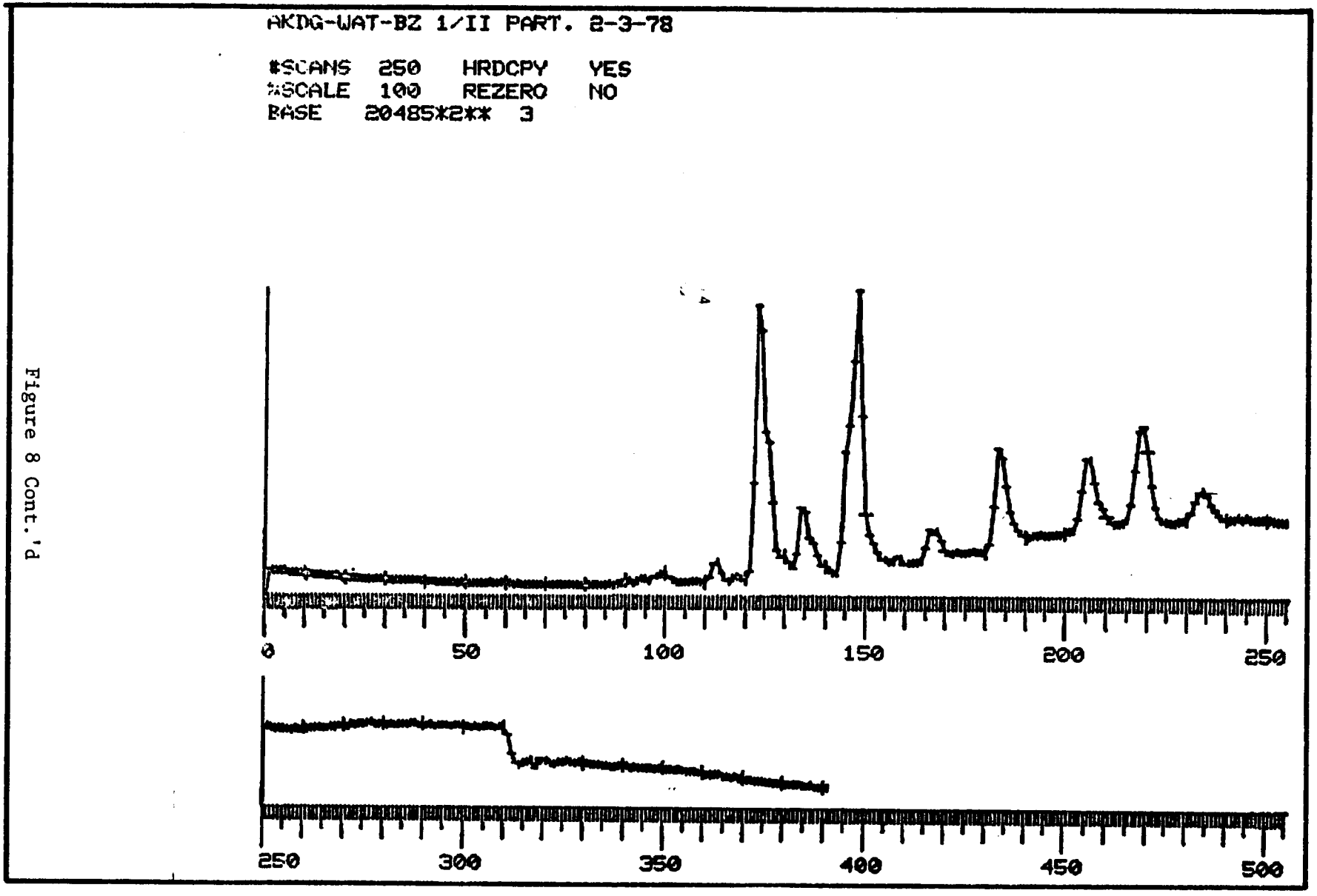


Figure 8 Cont.'d

```
SIGNFPK
GC ID BP    115    DATE    9/28/77
APRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      4

PLM WATER PART. 1/II SPRING,ALCV,BENZENE

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 5

     41    1000       53   15113
     43    1000        5   19153
     45    1000      103   11906
     56    1000       12   11951
     57    1000        1   16762
    149    1000      114    6515
     74     871       75     789
```

Figure 9. Total Ion Chromatogram and Significant Peak Indices for Water Sample, ALCV, Collected During the Spring Seasonal Sampling from Station 1/II.

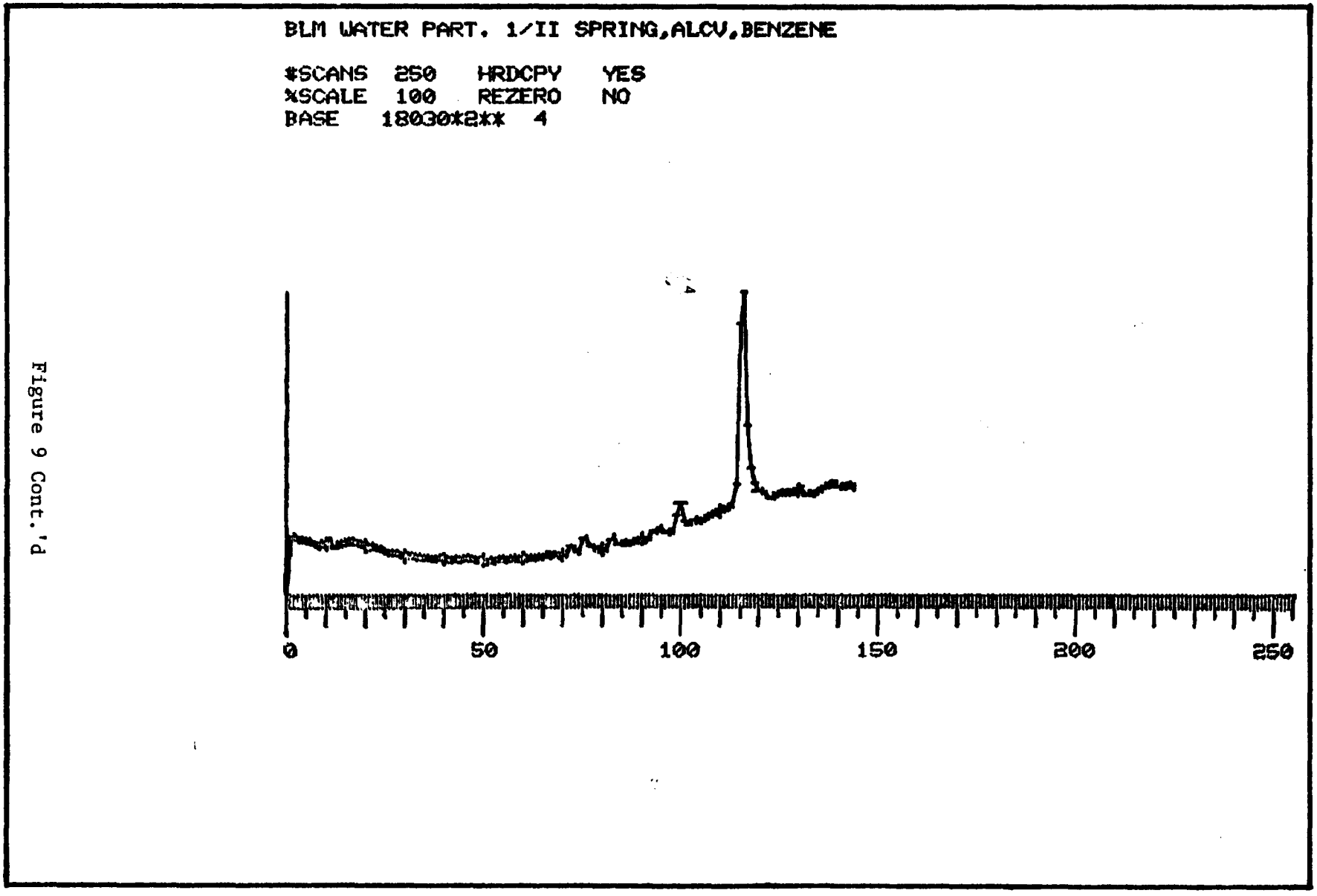


Figure 9 Cont.'d

```
 [illegible]FPK
 [illegible] ID BP    122    DATE    9/28/77
 [illegible]HTE         2    SCTIME       4    RESPWR    500
 HI[illegible]SS      500    THRESH       4

FLM WATER DIS SPRING 2/II,ALEM, BENZENE

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES
```

MASS	MAX INTN	FIRST OCCUR	SUM IONS x2xx 7
43	1000	20	22212
45	1000	122	12208
61	1000	105	8367
63	1000	80	11887
77	1000	77	14880
78	1000	1	14367
85	1000	23	14751
97	1000	46	12380
99	1000	89	11599
109	1000	91	13023
111	1000	65	15632
112	1000	81	8258
115	1000	30	5042
123	1000	54	13601
125	1000	45	10320
143	1000	114	6118
145	1000	98	8330
149	1000	128	8816
151	1000	67	6705
153	1000	71	3641
157	1000	94	7482
159	1000	69	8852
162	1000	86	2375
183	1000	62	9389
185	1000	87	9250
139	988	131	2824
147	965	78	9743
83	946	103	13709
41	942	56	11443
146	935	81	7039
219	933	73	8448
75	931	92	15092
89	928	46	13352
91	928	56	7754
117	914	35	4322
51	897	92	13414
87	883	100	9889
96	882	111	6656
55	867	58	11713

Figure 10. Total Ion Chromatogram and Significant Peak Indices for Water Sample, ALEM, Collected During the Spring Seasonal Sampling from Station 2/II.

BLM WATER DIS SPRING 2/II,ALEM, BENZENE

*SCANS 250 HRDCPY YES
%SCALE 100 REZERO NO
BASE 26065*2** 6

Figure 10 Cont.'d

SIGNFPK
GC ID BJ 6 DATE 9/26/77
AQRATE 2 SCTIME 4 RESPWR 500
HIMASS 500 THRESH 4

BLM WATER DISS SPRING 3/III ALLM,HEXANE

IGNORE 0, 0, 0, 0
MILOUT 850 HRDCPY YES

MASS	MAX INTN	FIRST OCCUR	SUM IONS XZXX 6
43	1000	14	2277
45	1000	131	1152
50	1000	99	7423
57	1000	1	1952
74	1000	85	7015
75	1000	83	10946
111	1000	84	10687
123	1000	104	1948
146	1000	13	18816
148	1000	27	12643
180	1000	38	9457
182	1000	36	9159
41	944	4	3333
115	927	84	1604
151	922	84	2009
51	867	103	4958

Figure 11. Total Ion Chromatogram and Significant Peak Indices for Water Sample, ALLM, Collected During the Spring Seasonal Sampling from Station 3/III.

DRAW GC
GC ID BJ 6 DATE 9/26/77
AQRATE 2 SCTIME 4 RESPWR 500
HIMASS 500 THRESH 4

BLM WATER DISS SPRING 3/III ALLM,HEXANE

#SCANS 250 HRDCPY YES
%SCALE 100 REZERO NO
BASE 19488*2** 5

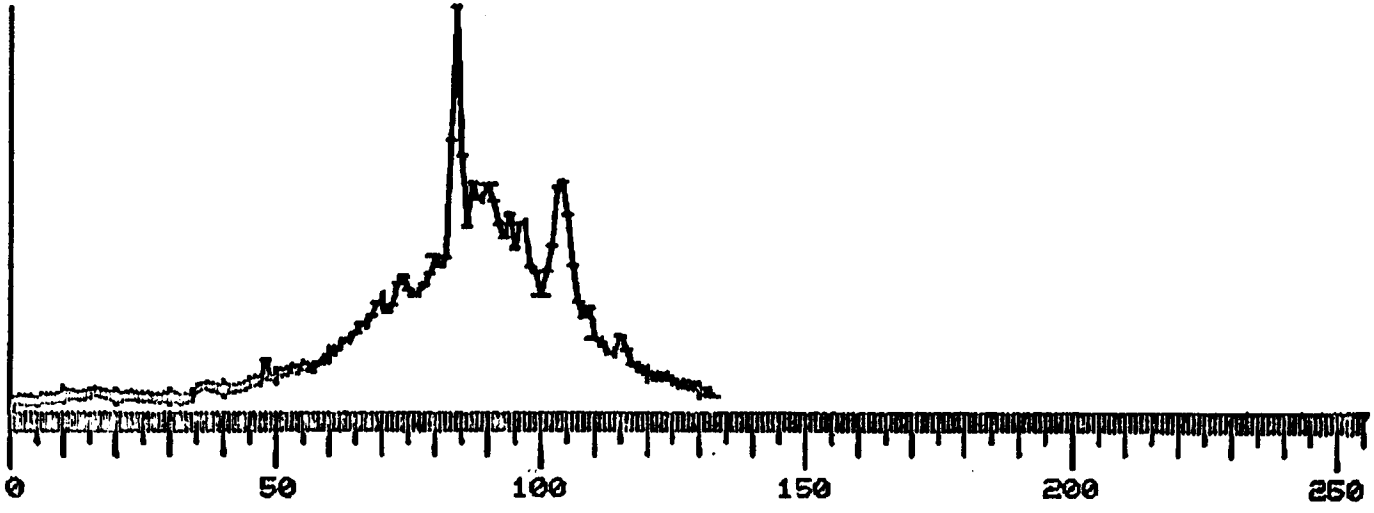


Figure 11 Cont.'d

```
[illegible]FPK
[illegible] ID BF    116    DATE    9/28/77
[illegible]PHTE        2    SCTIME      4    RESPWR    500
[illegible]MASS      500    THRESH      4

FLM WATER PART. 1/IV SPRING,ALNK,BENZENE

IGNORE       0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR    IONS *2** 4

     43    1000      79   19816
     45    1000     108   26104
    149    1000     109   22272
     57     989      87   16321
     41     917      80   15828
```

Figure 12. Total Ion Chromatogram and Significant Peak Indices for Water Sample, ALNK, Collected During the Spring Seasonal Sampling from Station 1/IV.

```
DRAW GC
GC ID BP    116    DATE    9/28/77
AQRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      4

BLM WATER PART. 1/IV SPRING,ALNK,BENZENE

#SCANS  250    HRDCPY    YES
%SCALE  100    REZERO    NO
BASE    17911*2**  5
```

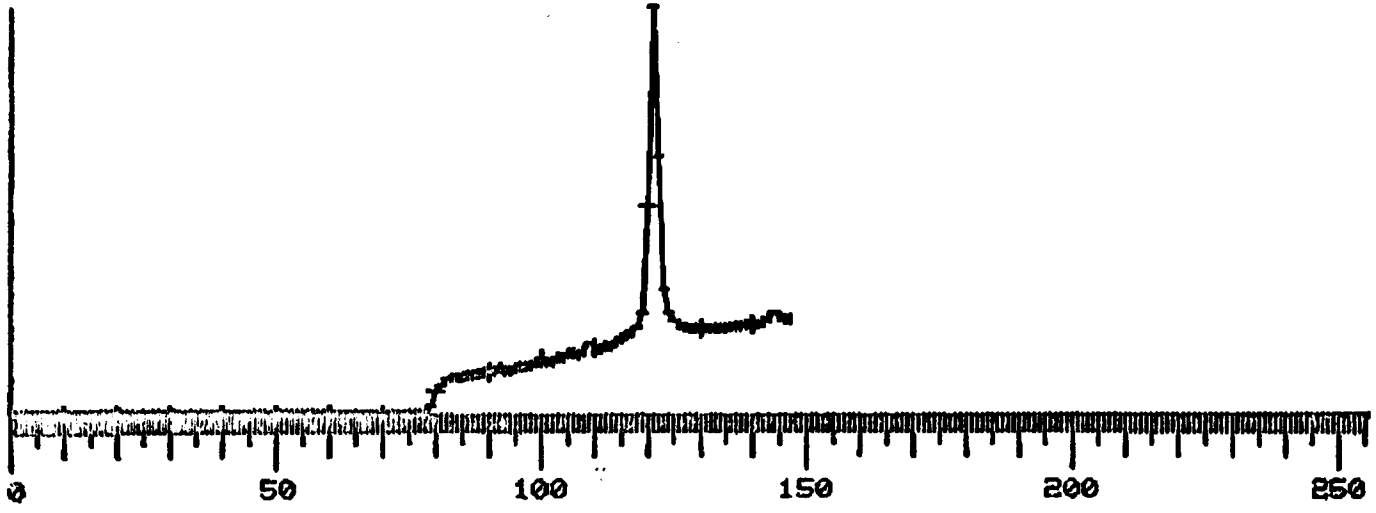


Figure 12 Cont.'d

GC ID BP 121 DATE 9/28/77
MSRATE 2 SCTIME 4 RESPWR 500
HIMASS 500 THRESH 4

ELM WATER DISS 3/I SPRING,ANZZ, BENZENE

IGNORE 0, 0, 0, 0
MILOUT 850 HRDCPY YES

MASS	MAX INTN	FIRST OCCUR	SUM IONS *2** 6
45	1000	120	16812
63	1000	54	21671
75	1000	68	26999
77	1000	58	26095
78	1000	1	22892
89	1000	9	25671
97	1000	34	19437
109	1000	66	23375
111	1000	80	24436
123	1000	52	23849
145	1000	75	8026
146	1000	84	18275
147	1000	73	10187
149	1000	115	18861
151	1000	14	19212
185	1000	53	17563
187	1000	35	16245
125	995	27	19517
159	990	62	12303
219	977	105	5253
157	964	62	8233
99	961	77	16840
148	914	104	13075
183	904	105	7874
65	862	54	14444
181	859	105	2307
110	858	21	16056

Figure 13. Total Ion Chromatogram and Significant Peak Indices for Water Sample, ANZZ, Collected During the Spring Seasonal Sampling from Station 3/I.

```
DRAW GC
GC ID BP    121    DATE    9/28/77
AQRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      4

BLM WATER DISS 3/I SPRING,ANZZ, BENZENE

#SCANS   250    HRDCPY    YES
%SCALE   100    REZERO    NO
BASE    17803*2** 6
```

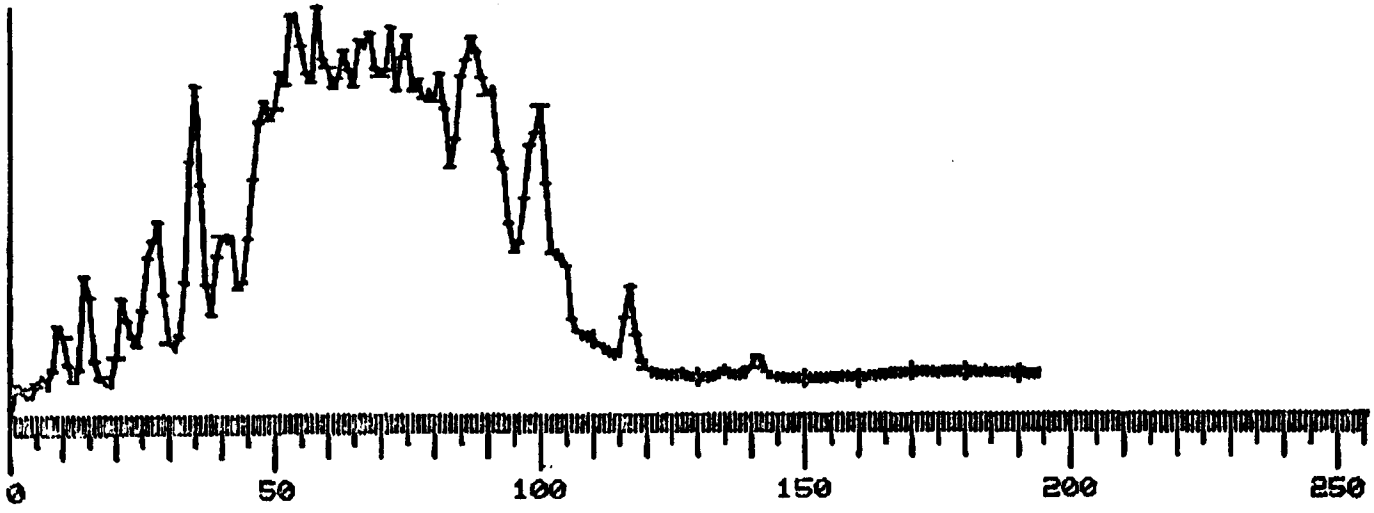


Figure 13 Cont.'d

```
 IGNFPK
 ID BP    117    DATE    9/28/77
 ORATE      2    SCTIME      4    RESPWR    500
 HIMASS   500    THRESH      4

BLM WATER PART. SPRING, 3,/III,AOAD, HEXANE

IGNORE       0,     0,     0,     0
MILOUT   850  HRDCPY   YES

  MASS    MAX    FIRST    SUM
         INTN    OCCUR   IONS *2** 5

    43    1000       3   25349
    45    1000     114   20922
    57    1000       1   23312
   149    1000     108    6036
    56     912       4   10999
    41     866       6   16125
```

Figure 14. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AOAD, Collected During the Spring Seasonal Sampling from Station 3/III.

DP/4U GC
VC ID BP 117 DATE 9/28/77
[illegible]RATE 2 SCTIME 4 RESPWR 500
HIMASS 500 THRESH 4

BLN WATER PART. SPRING, 3,/III,AOAD, HEXANE

*SCANS 250 HRDCPY YES
%SCALE 100 REZERO NO
BASE 27114*2** 3

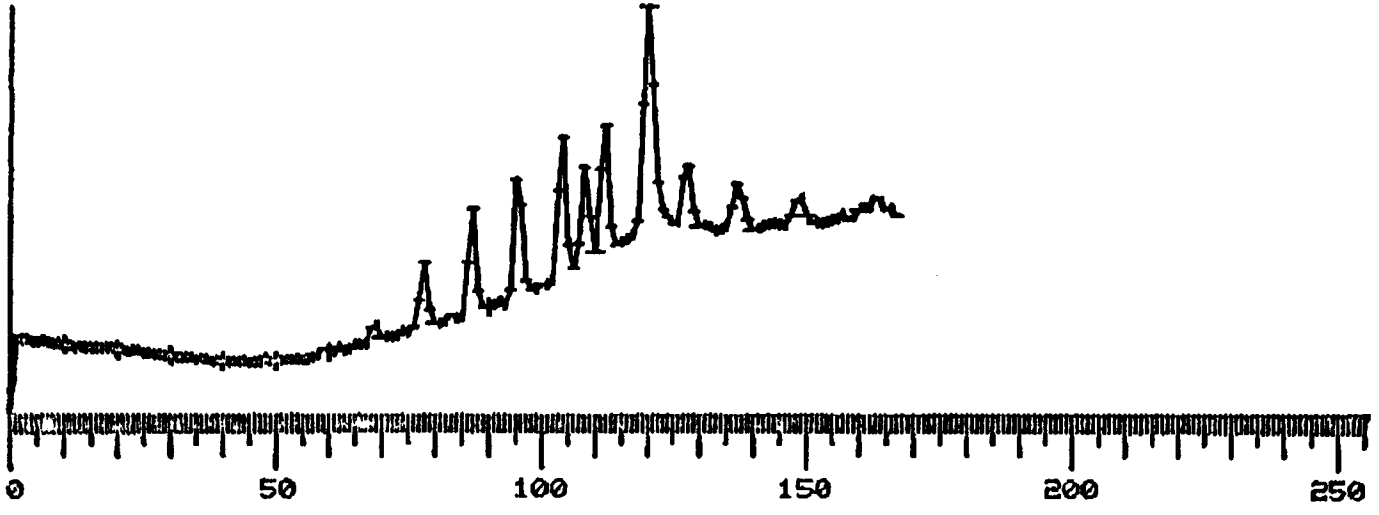


Figure 14 Cont.'d

```
SIGNFPK
GC ID BD        2    DATE    3/ 6/78
AORATE          3    SCTIME      4    RESPWR    500
HIMASS        500    THRESH      8

AOEK-WAT-BZ

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

  MASS    MAX    FIRST    SUM
          INTN   OCCUR   IONS *2** 5

    43    1000       1   10941
    45    1000     178   10871
    57    1000     141    8914
    73    1000      21   28434
   149    1000     123    4568
   129    1000     167    1410
   355     939      46   12620
    56     892      37    5483
    41     858       7    7063
```

Figure 15. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AOEK, Collected During the July Monthly Sampling from Station 3/II.

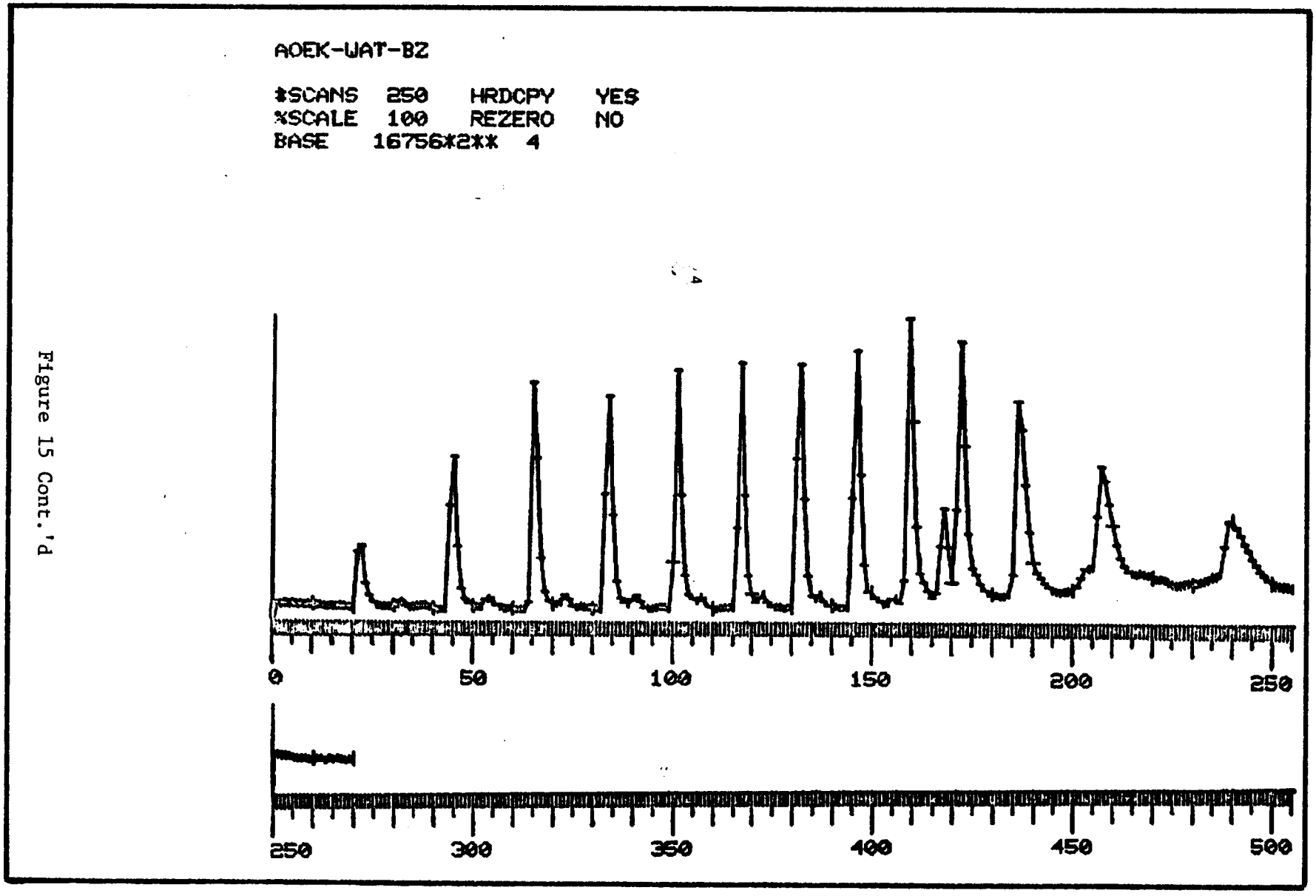


Figure 15 Cont.'d

```
*ICNFPK
GC ID BD       4    DATE    3/ 6/78
ANPHTE         2    SCTIME      4    RESPWR    500
HI:MSS       500    THRESH      8

APHM-WAT-BZ  3/II 76   8D004 2-6-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY    YES

  MASS    MAX     FIRST    SUM
          INTN    OCCUR    IONS *2** 5

    41    1000       16   12916
    43    1000        1   15470
    45    1000      177    9981
    57    1000        2   20589
   149    1000      138   31208
    56     997       43    9333
    73     854      132    3584
```

Figure 16. Total Ion Chromatogram and Significant Peak Indices for Water Sample, APHM, Collected During the August Monthly Sampling from Station 3/II.

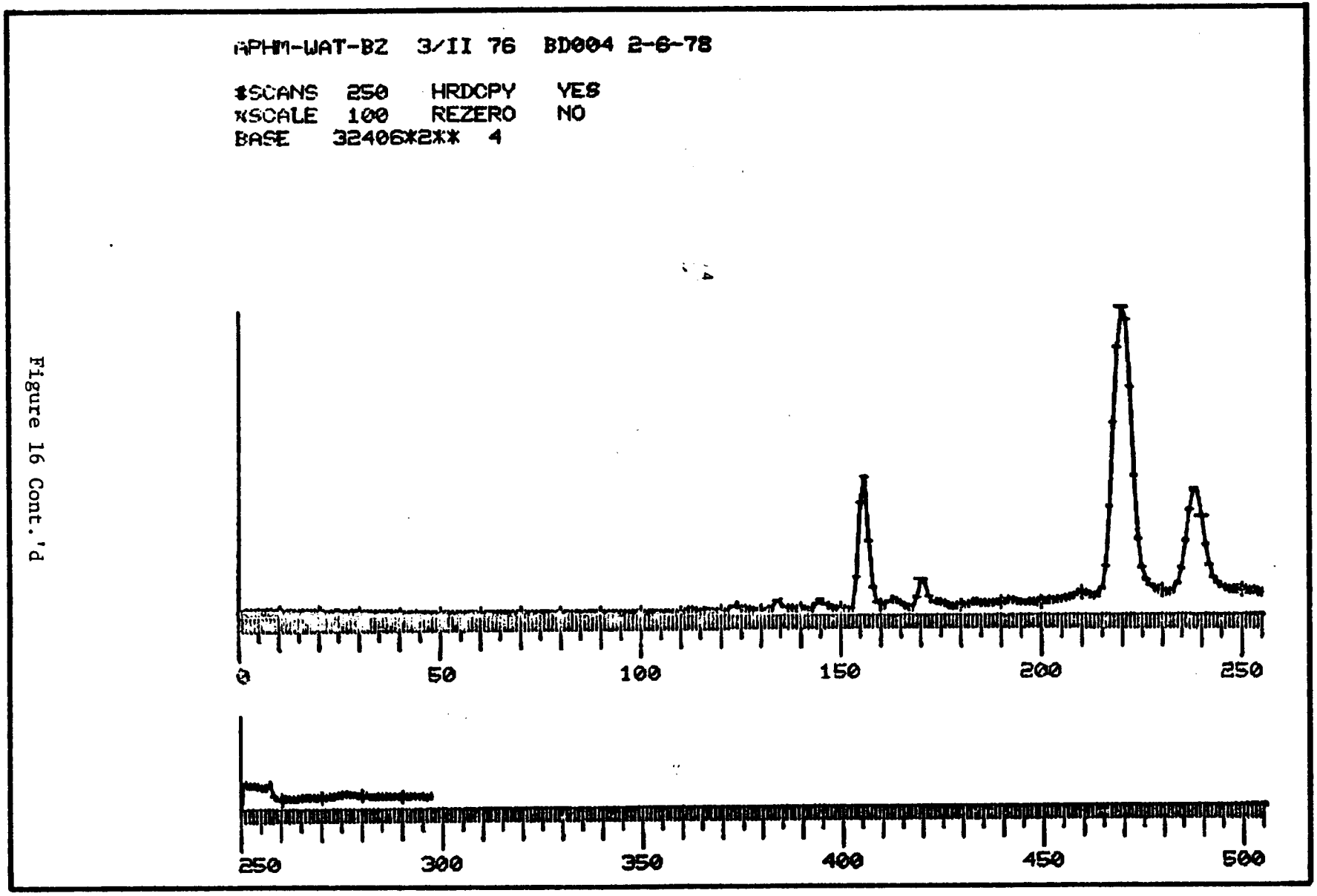
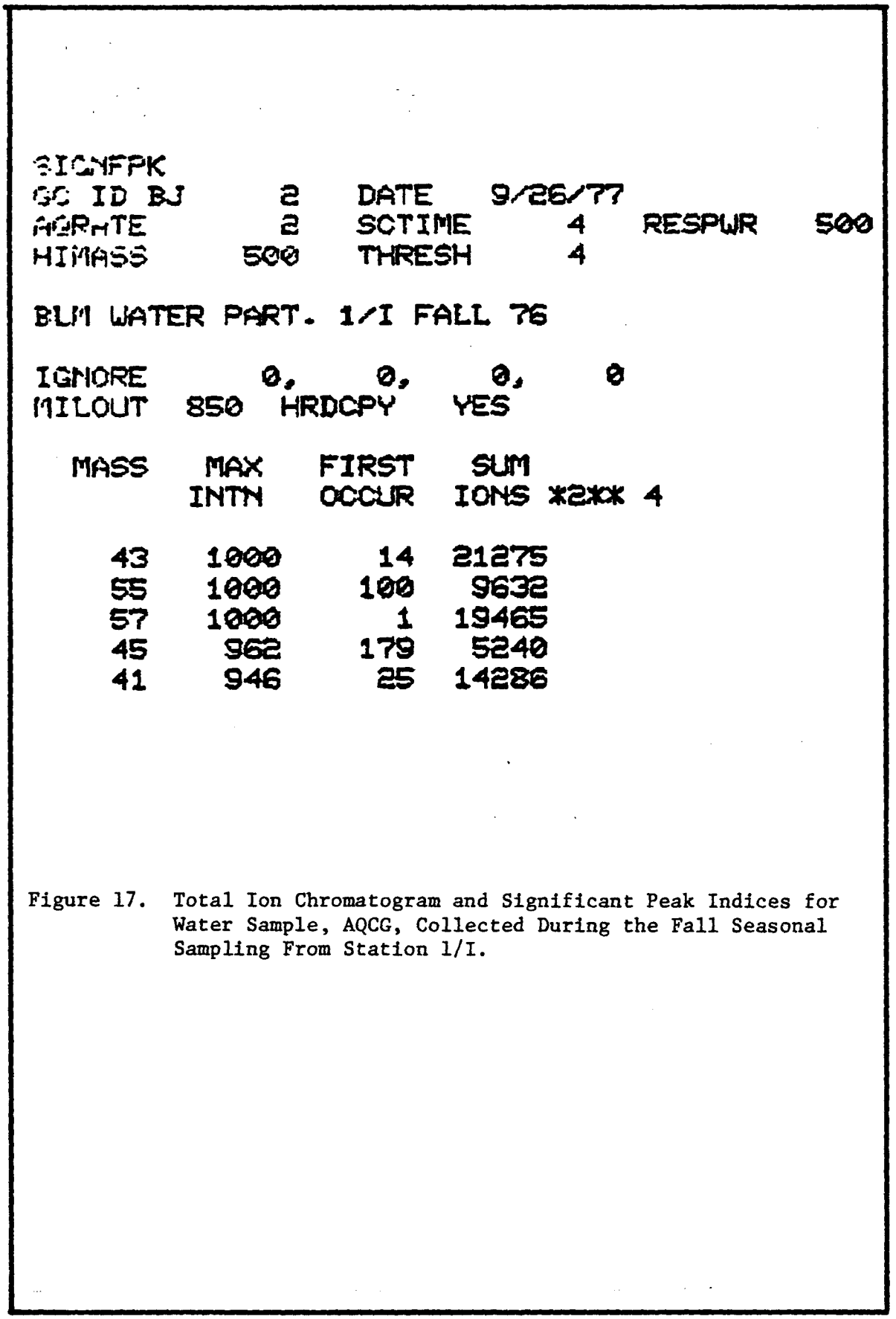


Figure 16 Cont.'d



```
SIGNFPK
GC ID BJ      2    DATE   9/26/77
AGRATE        2    SCTIME     4    RESPWR    500
HIMASS      500    THRESH     4

BUM WATER PART. 1/I FALL 76

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

  MASS    MAX    FIRST    SUM
         INTN    OCCUR   IONS *2** 4

    43    1000      14   21275
    55    1000     100    9632
    57    1000       1   19465
    45     962     179    5240
    41     946      25   14286
```

Figure 17. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AQCG, Collected During the Fall Seasonal Sampling From Station 1/I.

```
DRAW GC
GC ID BJ      2    DATE   9/26/77
ACQRATE       2    SCTIME     4    RESPWR   500
HITMASS     500    THRESH     4

ELM WATER PART. 1/I FALL 76

#SCANS  250   HRDCPY   YES
%SCALE  100   REZERO   NO
BASE   18866*2** 1
```

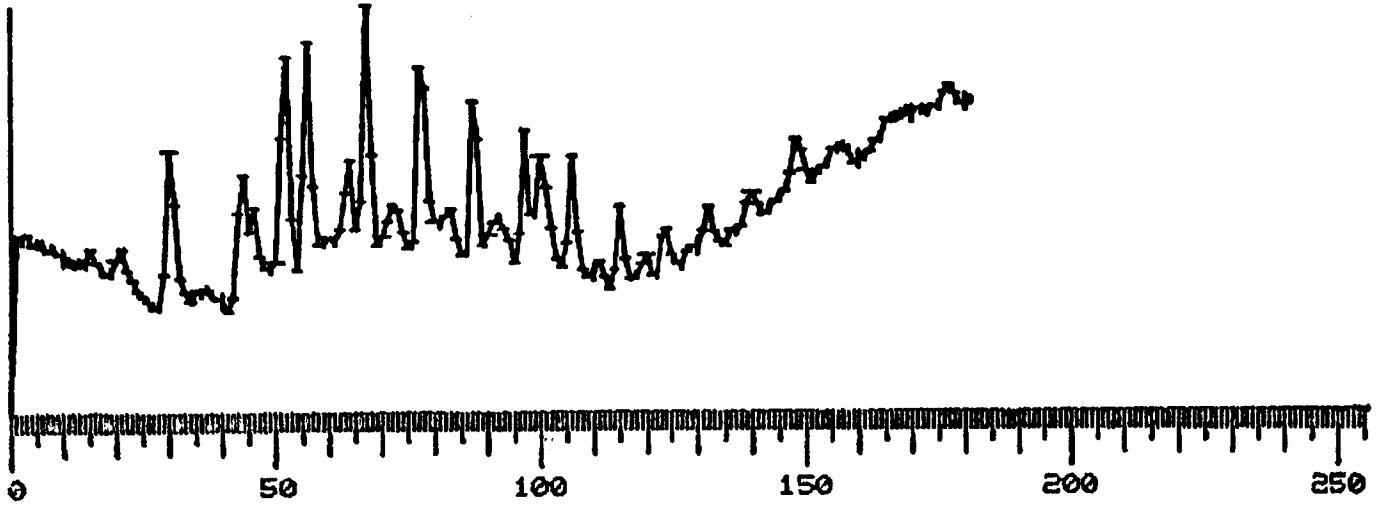
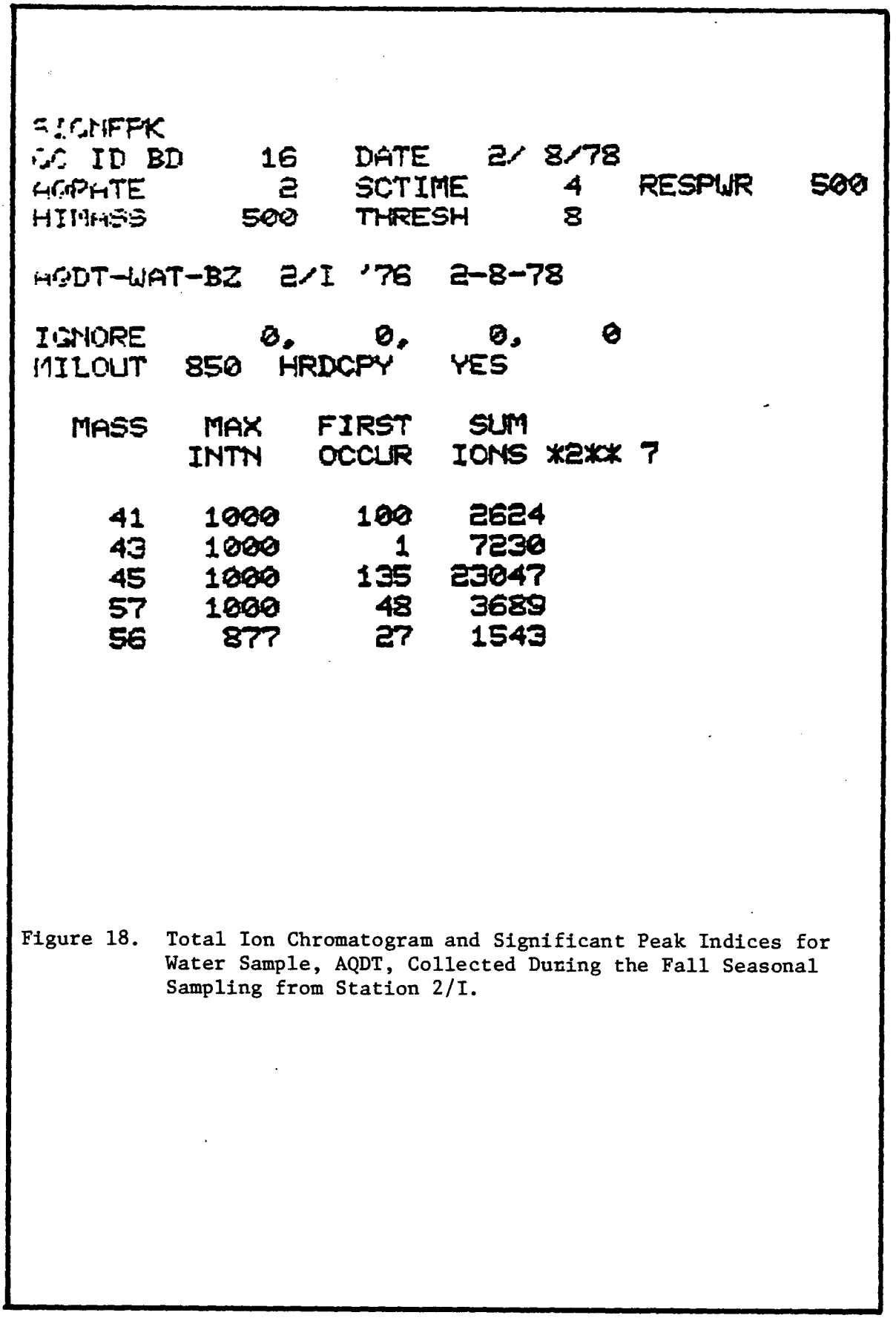


Figure 17 Cont.'d



```
SIGNFPK
GC ID BD     16    DATE    2/ 8/78
AQPATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      8

AQDT-WAT-BZ  2/I '76  2-8-78

IGNORE        0,     0,     0,     0
MILOUT  850  HRDCPY   YES

  MASS    MAX    FIRST    SUM
         INTN    OCCUR   IONS *2** 7

    41    1000     100    2624
    43    1000       1    7230
    45    1000     135   23047
    57    1000      48    3689
    56     877      27    1543
```

Figure 18. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AQDT, Collected During the Fall Seasonal Sampling from Station 2/I.

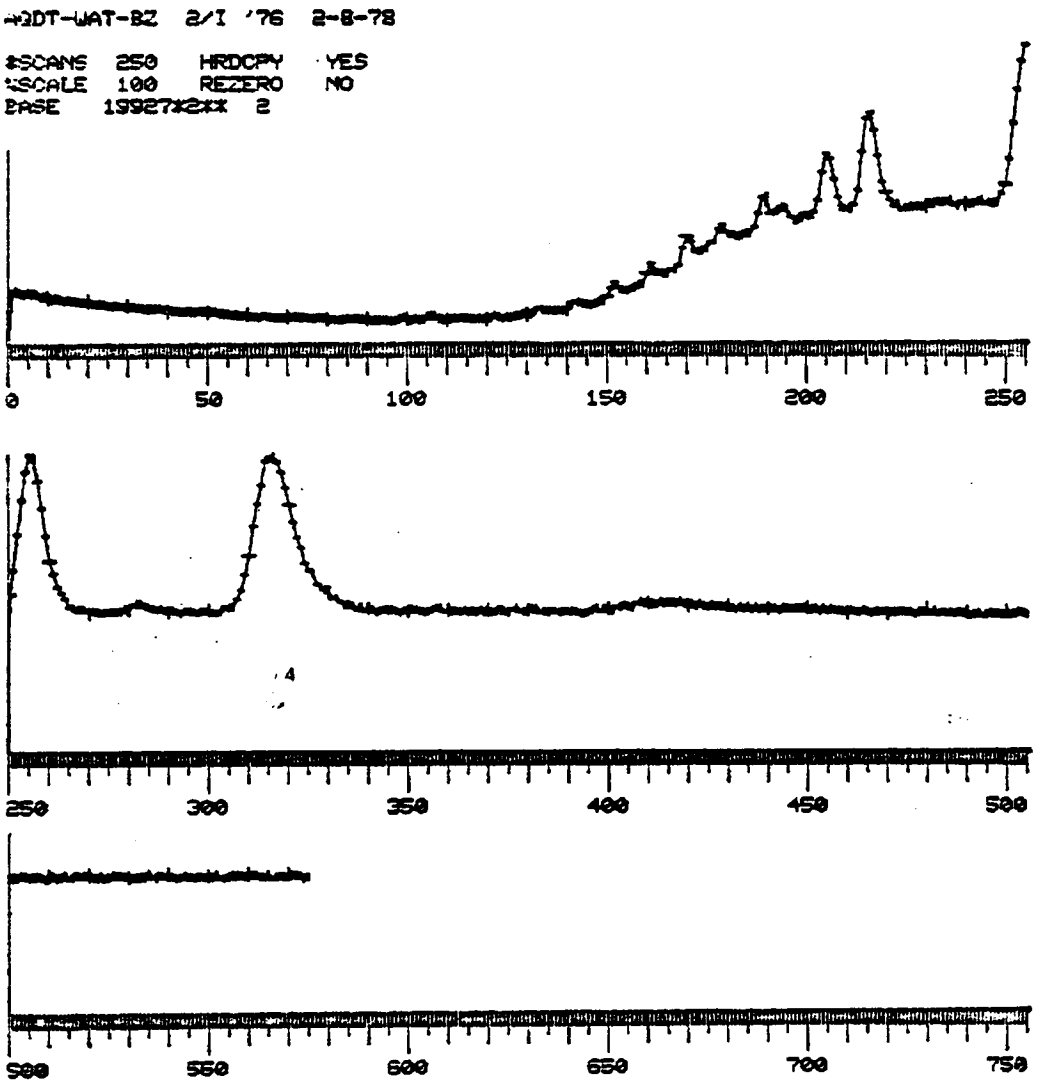


Figure 18 Cont.'d

```
SIGNIFPK
GC ID BD      13    DATE    2/ 7/78
ASPHTE         2    SCTIME      4    RESPWR    500
HIMASS       500    THRESH      8

AQLP-WAT-HX  1/III '76 2-7-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
          INTN    OCCUR   IONS *2** 6

     43    1000     122    4210
     45    1000     123   18362
     57    1000     112    2216
     78    1000       1    2376
```

Figure 19. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AQLP, Collected During the Fall Seasonal Sampling from Station 1/III.

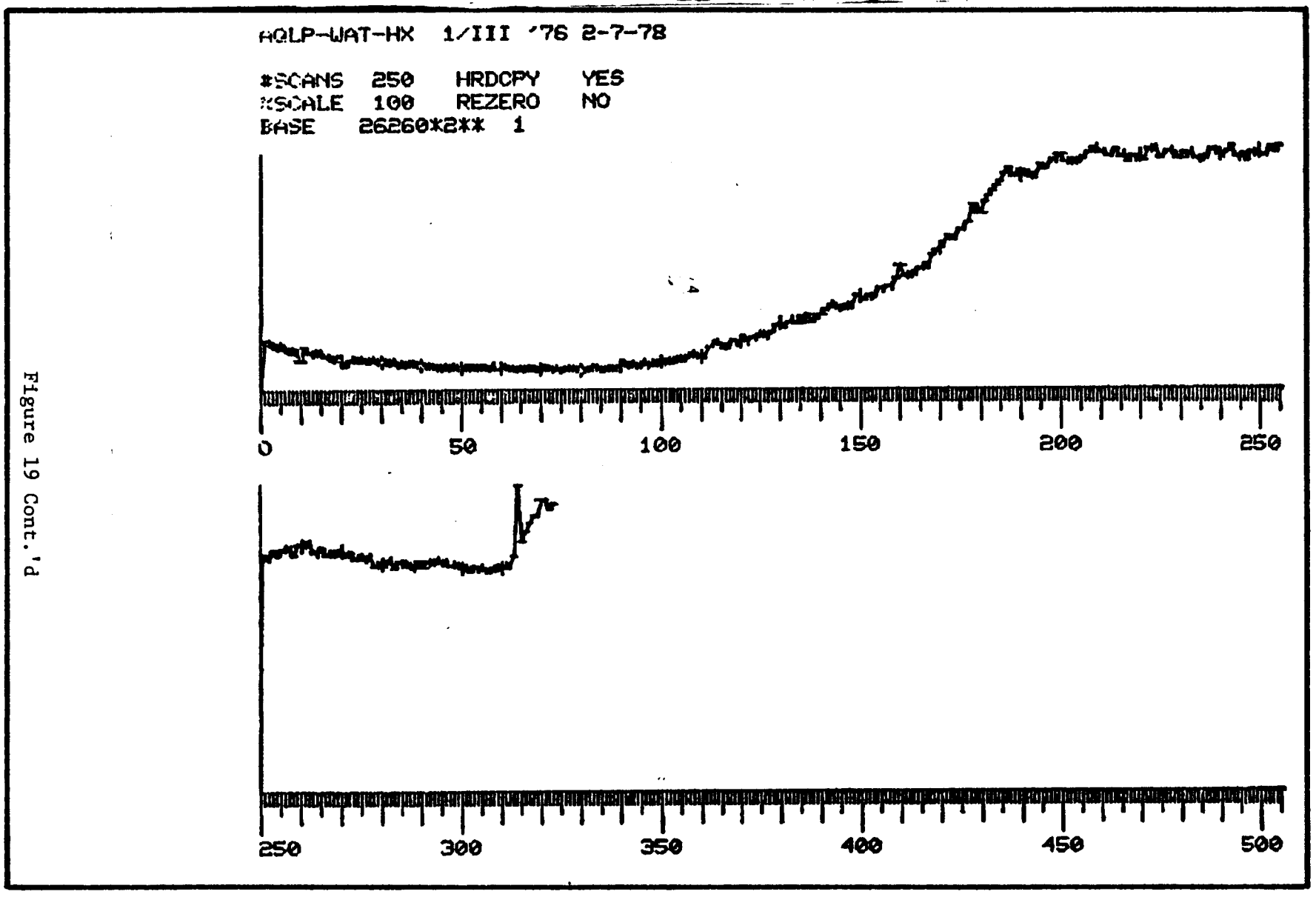


Figure 19 Cont.'d

```
SIGNFPK
GC ID BD      15    DATE    2/ 7/78
AQRATE         2    SCTIME      4    RESPWR    500
HIMASS       500    THRESH      8

AUYG-WAT-BZ  1/IIC NOV '76  2-7-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 5

     41    1000      23    6662
     43    1000       1   11911
     45    1000     148   22763
     55    1000     155    3529
     57    1000      13    7582
     74    1000     106    3892
     56     960      32    4744
```

Figure 20. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AUYG, Collected During the November Monthly Sampling from Station 1/II.

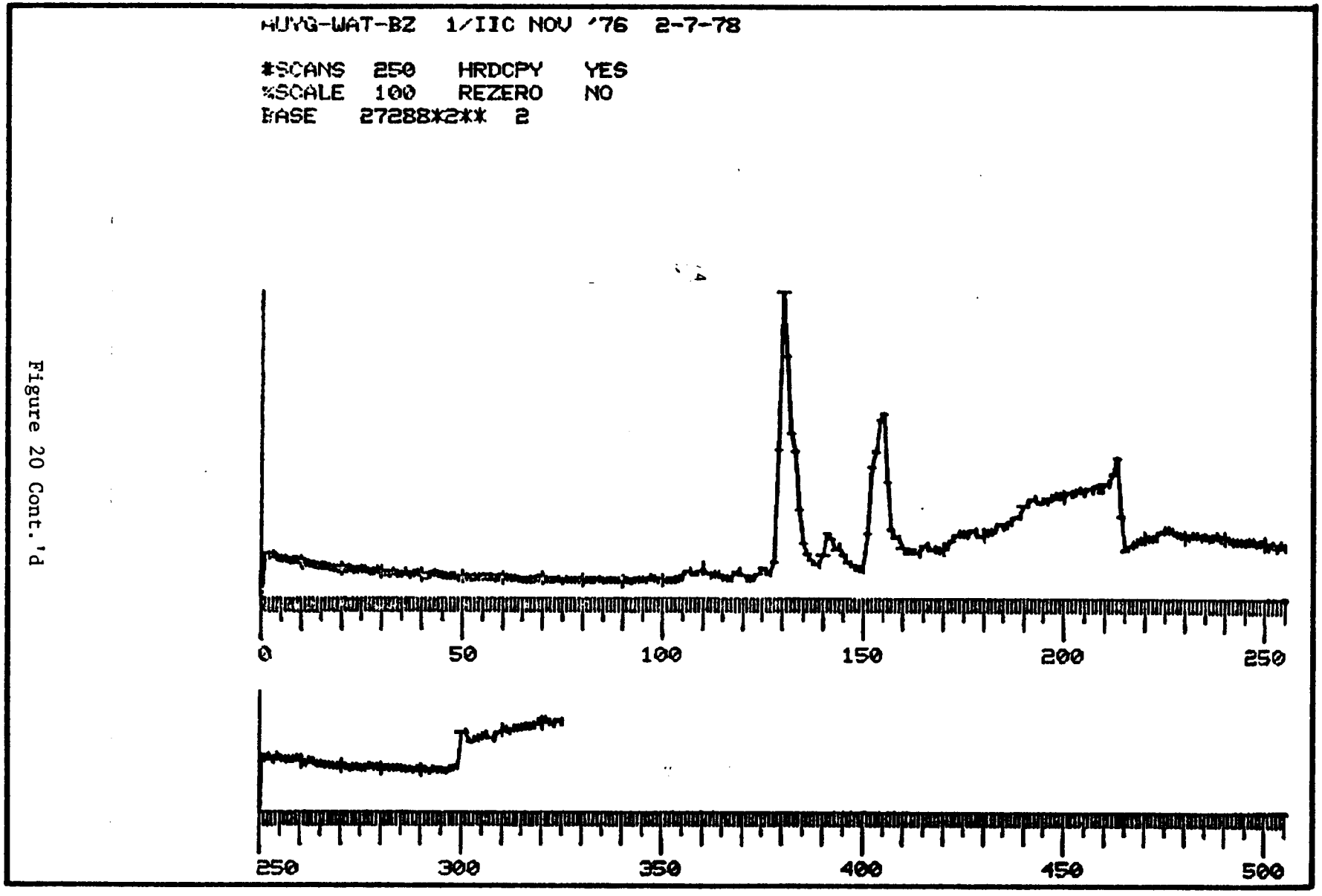


Figure 20 Cont.'d

```
SIGNFPK
GC ID BP    119    DATE    9/28/77
AQRATE        2    SCTIME      4    RESPWR    500
HIMASS      500    THRESH      4

BLM WATER PART DECEMBER 1/II, AVRY, BENZENE

IGNORE       0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 5

     69    1000        1   32733
    149    1000       92     635
```

Figure 21. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AVRY, Collected During the December Monthly Sampling from Station 1/II.

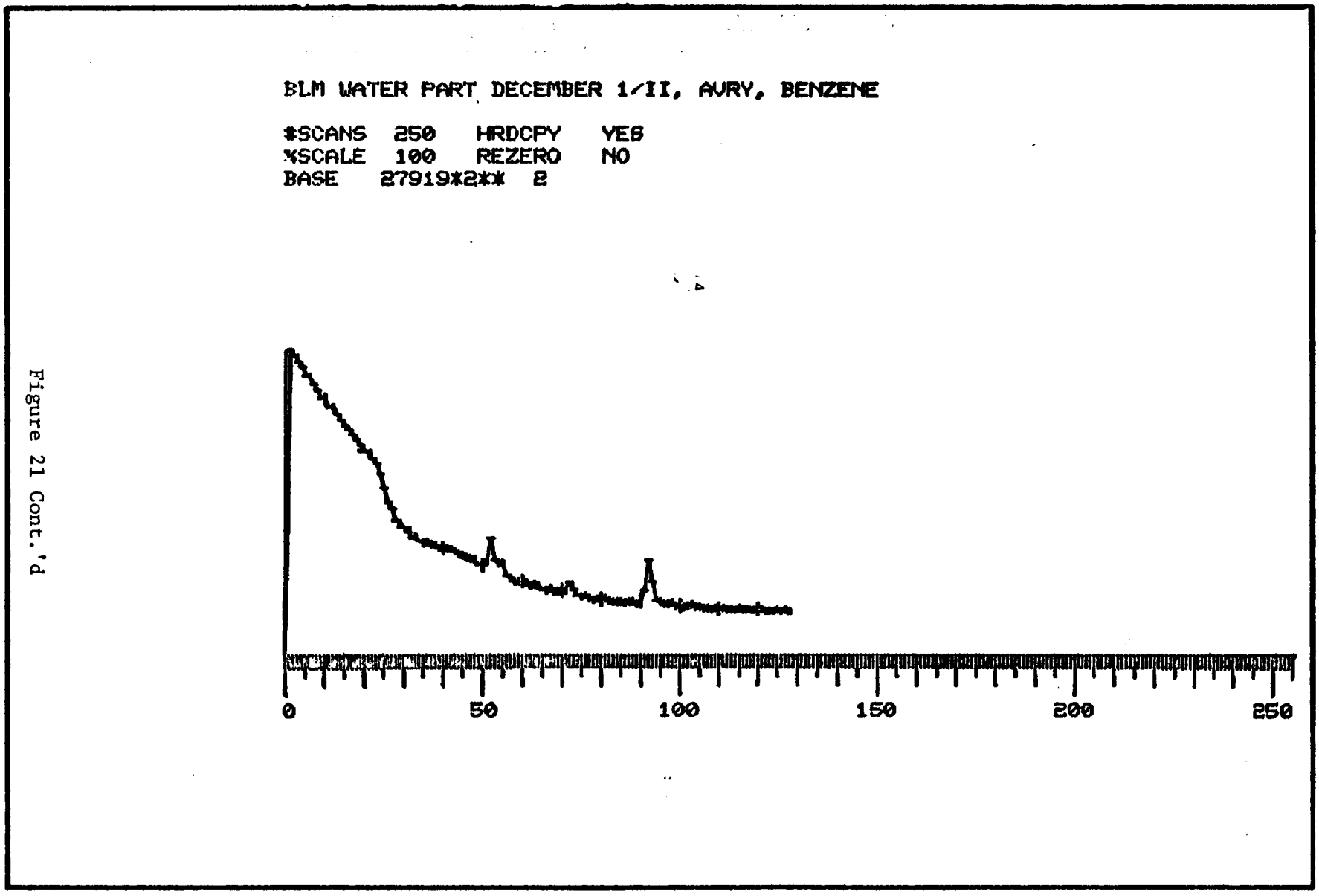


Figure 21 Cont.'d

```
SIGNIFPK
MC ID BD      10    DATE    2/ 7/78
NORATE         2    SCTIME      4    RESPWR    500
HIMASS       500    THRESH      8

AWWA-WAT-HX  3/II DEC '76 2-7-78

IGNORE        0,     0,     0,     0
MILOUT   850  HRDCPY   YES

   MASS    MAX    FIRST    SUM
           INTN   OCCUR   IONS *2** 7

     43    1000       1   13342
     45    1000     230    4585
     57    1000       3   17464
     69    1000     180   10398
    149    1000     142    3350
     41     912       4    7738
```

Figure 22. Total Ion Chromatogram and Significant Peak Indices for Water Sample, AVWA, Collected During the December Monthly Sampling from Station 3/II.

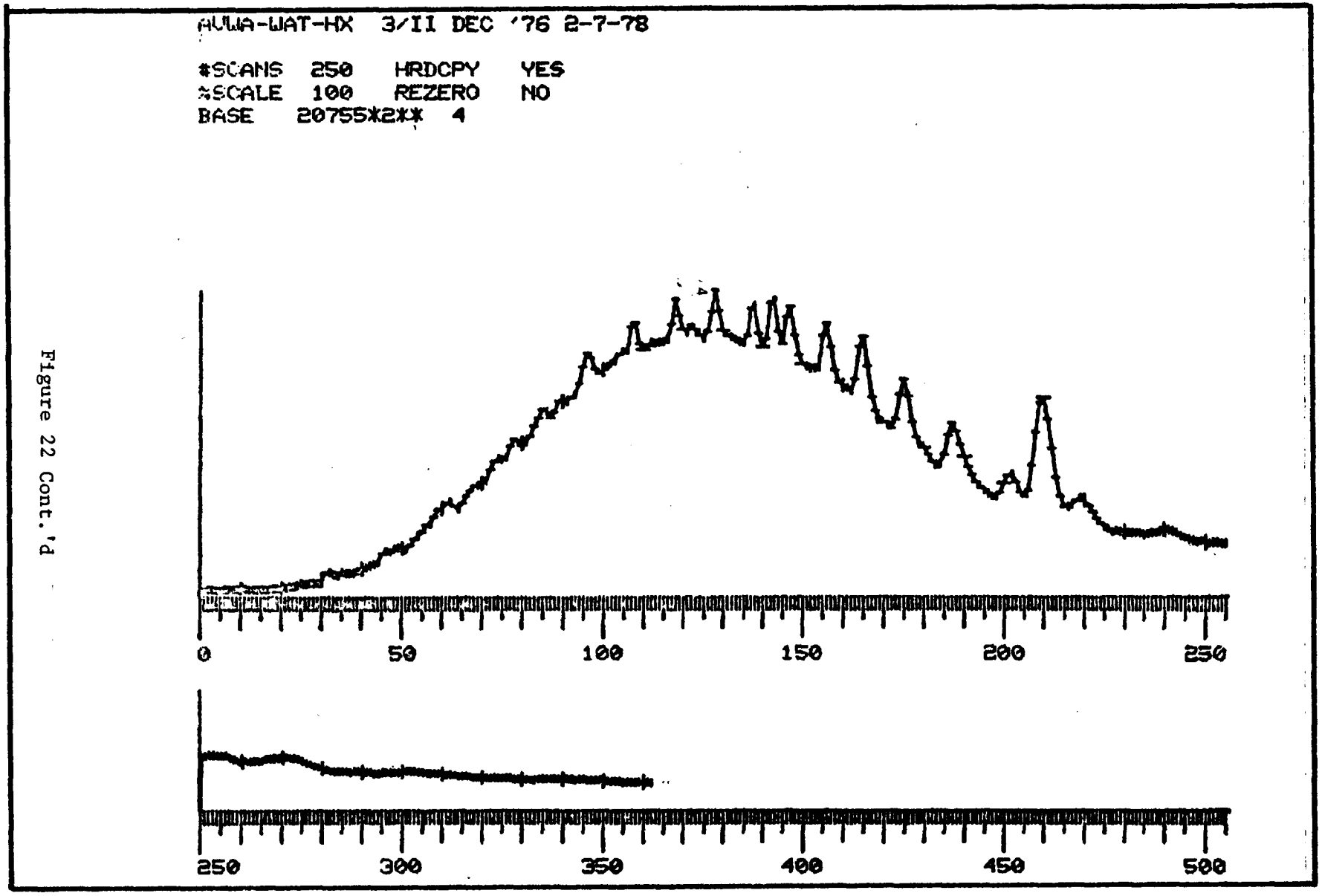


Figure 22 Cont.'d

by reactions resulting from the use of chloroform as the extraction solvent.

Table 1 summarizes the GC/MS analysis of the fractions analyzed.

TABLE 1

IDENTIFICATION OF COMPOUNDS IN WATER EXTRACTS USING GC/MS

Sample Code	Type	Fraction	Location	Period	Compound Characterized
AGGK	PAR	HX	2/IV	Winter	nC18 - nC26, 2 phthalates
AGIB	DIS	BZ	3/IV	Winter	All major peaks appear chlorinated
AGUW	DIS	BZ	3/II	Winter	2 phthalates
AGYQ	DIS	BZ	2/III	Winter	nC23 - nC29, 1 phthalate
AKDF	DIS	HX	1/II	April	nC20 - nC30, 4 phthalates
AKDF	DIS	BZ	1/II	April	7 chlorinated compounds, 1 phthalate
AKDG	PAR	HX	1/II	April	nC22 - nC36
AKDG	PAR	BZ	1/II	April	6 fatty acid methyl esters (16,17 18,20,24,26) phthalate, 3 aliphatics
ALCV	PAR	BZ	1/II	Spring	1 phthalate
ALEM	DIS	BZ	2/II	Spring	all major peaks appear chlorinated
ALLM	DIS	HX	3/III	Spring	Insufficient signal/noise ratio
ALNK	PAR	BZ	1/IV	Spring	1 phthalate
ANZZ	DIS	BZ	3/I	Spring	Chlorinated hydrocarbons, 1 phthalate
AOAD	PAR	HX	3/III	Spring	nC22 - nC30, 2 phthalates
AOEK	DIS	BZ	3/II	July	Silicon compounds
APHM	DIS	BZ	3/II	August	4 phthalates
AQCG	PAR	HX	1/I	Fall	nC15 - nC28, pristane, phytane, 1 phthalate
AQDT	DIS	BZ	2/I	Fall	1 phthalate, 3 long chain wax esters
AQLP	DIS	HX	1/III	Fall	Insufficient signal/noise ratio
AUYG	PAR	BZ	1/II	November	3 fatty acid methyl esters (16, 17, 18)
AVRY	PAR	BZ	1/II	December	Fatty acid methyl ester (16)
AVWA	DIX	HX	3/II	December	nC23 - nC33, 2 phthalates, squalene (trace amount)

CHAPTER SEVENTEEN

HEAVY MOLECULAR WEIGHT HYDROCARBONS IN MACROEPIFAUNA AND MACRONEKTON

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Grace Neff
Mrs. Yang Hrung
Darlene Garey
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ABSTRACT

A total of 278 samples of macroepifauna and macronekton from the South Texas Outer Continental Shelf were analyzed for heavy hydrocarbons by the techniques outlined in the BLM Work Statement, Draft VII. These analyses included samples of muscle, liver and gills. Selected samples were also analyzed by GC-MS techniques as required. Most muscle samples had very low hydrocarbon levels (generally less than 2 ppm), with C_{15} and C_{17} n-alkanes predominating. The hydrocarbons detected were mainly of biogenic origin, as indicated by the dominance of the C_{15} and C_{17} n-alkanes, pristane and by the absence of aromatic hydrocarbons (squalene was the only compound detected in the aromatic fraction). The absence of correlations in the pristane/phytane/C_{17}, phytane/C_{18} and CPI_{14-20} ratios also implied the absence of significant levels of petroleum in the study area. Thus, the data obtained provide present baseline hydrocarbon concentrations and distributions against which future monitoring data can be compared.

INTRODUCTION

The purpose of this project was to analyze macroepifauna and macronekton samples from the South Texas Outer Continental Shelf (STOCS) for heavy molecular weight hydrocarbons. The results of these analyses provide baseline and monitoring data for the STOCS as outlined in the BLM contract.

Although petroleum hydrocarbons are known to be taken up relatively rapidly by marine organisms (Anderson *et al.*, 1974) and have been detected at parts per million levels in animals from highly polluted areas (Farrington and Quinn, 1973; Scarrett and Zitko, 1972), little is known about detecting trace or parts per billion levels of petroleum hydrocarbons. One obstacle to low level detection of petroleum hydrocarbons is interference from biogenic hydrocarbons. This interference may be minimized by obtaining profiles of biogenic hydrocarbons from different species. Such biogenic profiles can then be subtracted from profiles obtained from organisms later monitored to better detect trace levels of petroleum hydrocarbons. Thus, our major effort has been directed to defining biogenic profiles and determining which organisms are most consistent and most suitable for monitoring purposes.

Samples for these studies consisted of macroepifauna obtained from the 12 primary STOCS stations. The macronekton were taken from the Topographic Features Southern Bank and Hospital Rock stations. The macroepifauna consisted of representatives from three classes of organisms, namely molluscs, crustaceans and fish. The macronekton were mainly fish of the snapper family. The laboratory analyses were based on methods outlined in Attachment A of BLM Contract AA550-CT6-17. Our methods are detailed in the following section. Interpretation of data was based on our previous experience (Giam *et al.*, 1976; Farrington *et al.*, 1972; Farrington *et al.*, 1976), on the report of Clark (1974) and on gas chromatography-mass spectral data.

METHODS AND MATERIALS

Sampling

All samples were collected by personnel of UTMSI/PAML. Epifaunal and demersal fishes samples were collected with a 35-ft (10.7-m) Texas box otter trawl and macronekton samples were caught by hook and line. Every reasonable precaution was taken to avoid contamination during sampling. Samples were placed in cleaned glass jars fitted with teflon or aluminum foil. When potentially contaminating sediment or other foreign material was adhering to the organisms they were rinsed with seawater. All samples were immediately frozen and so kept until analysis.

The sampling scheme and frequency as presented below were routinely followed, whenever possible. Epifaunal samples for hydrocarbon analyses were collected three times (seasonally) at each of the 12 primary STOCS stations. These samples consisted of five individuals each of three species. In addition, at one station during one season only, a sample was collected to allow the analysis of 12 individuals of the same species as well as one pooled sample comprised of material from the 12 individuals. Macronekton were collected at Southern Bank and Hospital Rock seasonally and monthly; samples were to represent five individuals of each of two species per bank station.

Laboratory Analysis

Materials[1]

Solvents were Mallinckrodt Nanograde^{R} and were used as received or redistilled when required. Silica gel (Woelm, 70-230 mesh) and Aluminum Oxide Woelm Neutral (Activity Grade 1) were activated at 200°C for at least 24 hr before use. Hydrocarbon standards were obtained from Analabs and Poly-

[1]Trade names of reagents, solvents and equipment, and the suppliers are included to facilitate recognition by interested readers of what we use; there is no implication that these are solely recommended.

science Co.

Instrumentation

A Hewlett-Packard 5830 gas chromatograph (GC) equipped with dual flame ionization detectors and a programmable integrator was used. It was equipped with 6' x 1/8" stainless steel columns of 5 percent FFAP on Gas Chrom Q 100/120. The injector was at 280°C and the detector at 350°C. The column oven was temperature-programmed from 100 to 250°C at 5°/min.

Procedure

(a) Background Reduction.

Prior to actual sample analysis, procedure blanks and recovery studies were performed. All solvents to be used in the procedure were concentrated to the extent required by the procedure and analyzed by gas chromatography. Any solvent exhibiting any impurities in the hydrocarbon region of the spectrum was rejected or redistilled in an all-glass system. Solid reagents were purified by heating in a 325°C oven for at least 24 hr; concentrates of solvent rinses of these materials were inspected by gas chromatography as described for solvents. Glassware and equipment were washed with Micro cleaning solution (International Products Corp.) and distilled water, rinsed with acetone, methanol and hexane and heated overnight at 325°C. After heating, they were rinsed with two portions of benzene and two of hexane. The final hexane rinse was concentrated and checked by gas chromatography. If any impurities were present, rinsing was repeated as needed to obtain an acceptable blank. Glassware checks accompanied each sample run and procedure blanks were performed at frequent intervals. Routine procedure blanks of less than 0.001 ppm were obtained.

(b) Extraction of Macrofauna.

Approximately 100 g of tissue were used for all analyses. When possible, a minimum of five organisms or portions thereof was used per analysis to minimize the natural variability of hydrocarbon content in conspecifics. Each sample was cut into smaller pieces and the wet weight determined. An aliquot of the sample was then placed in a tared beaker and dried at 60°C until a constant weight was obtained. In this manner, the wet and dry weights of the sample were obtained. The remainder of the sample was saponified.

(c) Saponification.

Saponification was conducted by refluxing the sample with 0.05 g KOH/g tissue in approximately 50 ml methanol. Saponification was continued until the tissues were digested. After the completion of digestion, an equal volume of purified water was added to the mixture. The mixture was then refluxed overnight. Upon completion of hydrolysis, the mixture was diluted with an equal volume of a saturated NaCl solution. The mixture was then extracted three times with n-pentane. The volume of n-pentane used for each extraction was equivalent to the volume of methanol initially used in the saponification. The n-pentane fractions were then combined and washed with an equal volume of water. The solvent was removed from the pentane extract, with a Kuderna-Danish evaporator, (for weight determination) prior to column chromatographic separation.

(d) Column Chromatography.

A weight ratio of about 100 parts alumina to one (1) part lipid sample and 200 parts silica gel to one (1) part lipid sample was used. The column had a length to inside diameter ratio of approximately 20:1. Both the silica gel and the neutral alumina were Activity I. The column was packed in hexane and rinsed with one column volume of n-pentane. At

no time was the column allowed to run dry. The extract taken up in a small volume of n-pentane was then applied to the column and the aliphatic fraction eluted with two column volumes of n-pentane. This was followed by elution of aromatics with two column volumes of benzene. The eluates from the two fractions were then taken to near dryness. They were then transferred to screw cap vials with teflon lined caps, and the remainder of the solvent was removed with a stream of purified nitrogen. Following column chromatography, all eluates were analyzed by gas chromatography.

(e) Gas Chromatographic Separations.

Each eluted fraction obtained from the column chromatographic separation was quantitatively dissolved in a small volume of carbon disulfide for injection into the GC. A stainless steel column (1/8" x 6') packed with 5 percent FFAP on Gas Chrom Q (100-120 mesh) was used for the analysis. The column resolved n-C_{17} from pristane and n-C_{18} from phytane with a resolution (R) of approximately unity, where

$$R = 2d/w_1 + w_2 \text{ and,}$$

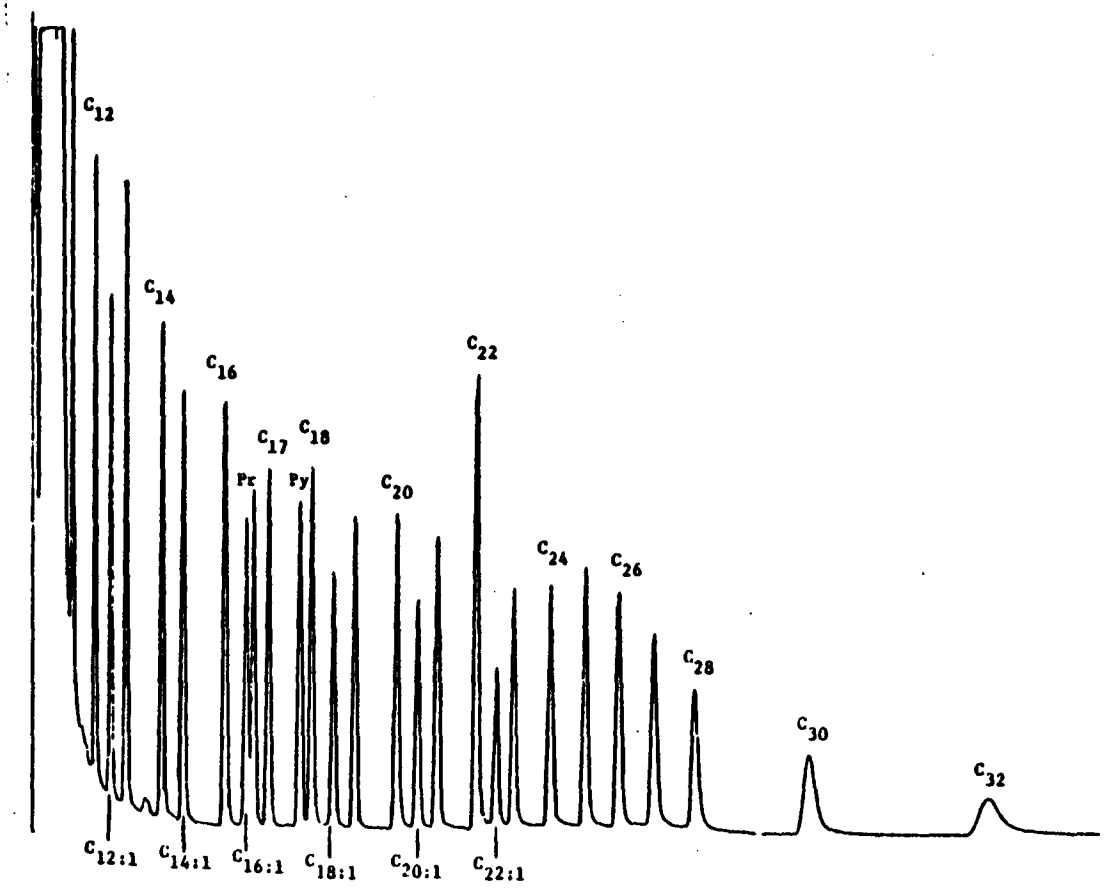
w is the width of each peak at the base on one phase for both pairs of components, and

d is the distance between apices.

The column was also capable of resolution of hydrocarbons from n-C_{14} through n-C_{36} (Figure 1). To assist identification, the following compounds were used as standards to match the retention times of peaks in the gas chromatogram: aliphatic hydrocarbons C_{15} - C_{32}; trimethylbenzene; 1,2,3,5-tetramethylbenzene; 1,2,3,4-tetramethylbenzene; naphthalene; 2-methylnaphthalene; 1-methylnaphthalene; 1, 5-dimethylnaphthalene; 2,3-dimethylnaphthalene; 4-phenyltoluene; 3,3'-dimethylbiphenyl; 4,4'-di-

FIGURE 1

GAS CHROMATOGRAM OF ALIPHATIC STANDARDS ON A 10' x 1/8" 5% FFAP COLUMN. GLC CONDITIONS: CARRIER GAS, N_2 at 20 cc/min, COLUMN TEMPERATURE 100-240°C at 5°/min.



methylbiphenyl; fluorene; 1-methylfluorene; phenanthrene; anthracene; 9-methylanthracene; fluoranthene; and chrysene (Figure 2).

(f) Gas Chromatography-Mass Spectrometry (GC-MS).

Aliquots of extracts from 12 percent of the GC samples were analyzed by GC-MS. The runs were made by Dr. R. Spraggins (of the Center for Trace Characterization) in cooperation with Dr. H. S. Chan. Since the concentrations of components were very low (often near the limit of detection of GC-MS), only major components found in gas chromatograms were identified.

Equipment

The analyses were run on a Hewlett-Packard 5980A dodecapole mass spectrometer interfaced to a 5710A gas chromatograph. This GC-MS system was supported with a 5933A Data System, a Tektronix 4012 CRT terminal, a Tektronix 4631 Hard Copy Unit, and a 15,000 spectra reference library stored on a single disc (Aldermaston).

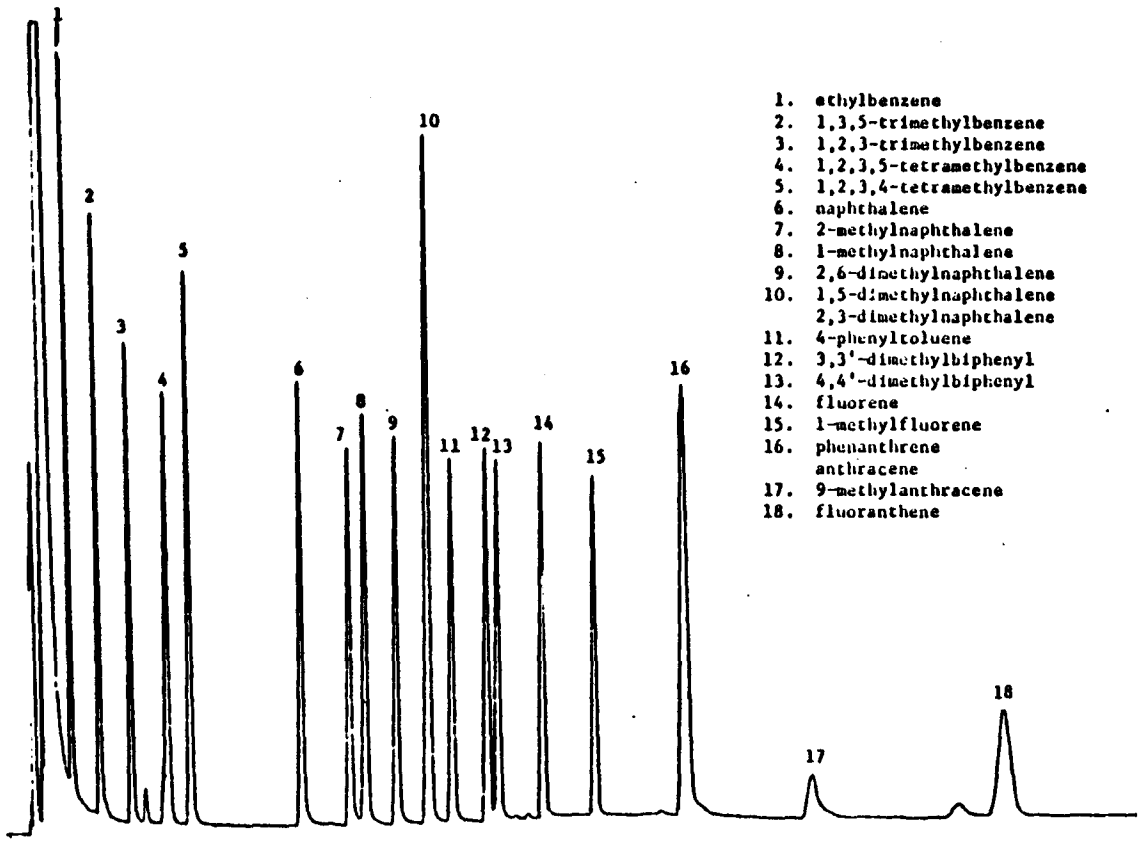
Gas Chromatography Conditions

The samples for GC-MS analysis were analyzed in four groups (quarterly). The first group of 10 samples was analyzed using a 6', 3 percent apiezon GC column for separation, with temperature programming from 150-270°C at a rate of 16°C/min and a helium flow rate of 60 ml/min.

The second group, containing 21 samples and all subsequent samples, were analyzed on 30-m glass capillary GC columns (G 50, #1176B and #1176 F) coated with OV-101 (J & W Scientific, Inc.). These columns provide baseline separation of n-heptadecane and n-octadecane from pristane and phytane, respectively. Hewlett-Packard's variable injection splitter was incorporated into the GC-MS system for capillary column analyses. A

FIGURE 2

GAS CHROMATOGRAM OF AROMATIC STANDARDS ON A
10' x 1/8" 5% FFAP COLUMN. (SAME GLC CONDITIONS AS IN FIG. 2)



1. ethylbenzene
2. 1,3,5-trimethylbenzene
3. 1,2,3-trimethylbenzene
4. 1,2,3,5-tetramethylbenzene
5. 1,2,3,4-tetramethylbenzene
6. naphthalene
7. 2-methylnaphthalene
8. 1-methylnaphthalene
9. 2,6-dimethylnaphthalene
10. 1,5-dimethylnaphthalene
    2,3-dimethylnaphthalene
11. 4-phenyltoluene
12. 3,3'-dimethylbiphenyl
13. 4,4'-dimethylbiphenyl
14. fluorene
15. 1-methylfluorene
16. phenanthrene
    anthracene
17. 9-methylanthracene
18. fluoranthene

split ratio (10:1) gave optimum peak shapes while still retaining good detection limits using column #1176 B. The capillary column was temperature-programmed from 150-250°C at a rate of 2°C/min. Column #1176 F gave optimum results using a 20:1 split ratio and a temperature program rate of 4°C/min.

Mass Spectrometer Conditions

The mass spectrometer was operated with an ion source temperature of 180°C and an analyzer temperature of 110°C. The maximum detector gain remained at 2 x 10^6 torr during sample groups 1 and 2. Maximum detector gain for sample group 3 was 1 x 10^7 torr.

The mass spectrometer was tuned on perfluorotributyl amine (PFTBA) prior to analysis. Optimum source potentials for each set of analyses were:

Source Potential Settings
May, 1976 (Sample Group 1)

Drawout len - 4
Repeller - 15
Ion focus len - 23
Electron energy - 70 ev
Emission - 20
Target - 20
X-ray - 1

Source Potential Settings
Sept., 1976 (Sample Group 2)

Drawout len - 6
Repeller - 16
Ion focus len - 18
Electron energy - 70 ev
Emission - 22
Target - 21
X-ray - 1

Source Potential Setting
December, 1976

Drawout len - 3
Repeller - 16
Ion focus len - 12
Electron energy - 70 ev
Emission - 20
Target - 15
X-ray - 1

Compounds Confirmed by GC-MS

The total ion chromatogram for each sample was permanently stored on auxillary discs. Major sample components which appeared in both GC and GC-MS were identified. Some minor sample components were not identified due to poor signal-to-background spectra. The electron-impact spectra of individual components were permanently stored on disc for comparison with library spectra or for other uses. Individual spectra from data files were compared: (1) by computer with spectra included in the Aldermaston Library on disc using the "search" routine; (2) with reference spectra run on our instrument; and (3) with the "Eight Peak Index of Mass Spectra" (Mass Spectrometry Data Center). Table 37, Appendix M, shows the compounds that were confirmed by GC-MS for each sample. Representative mass spectra of compounds confirmed in these samples is also included in Figures 3-12, Appendix M.

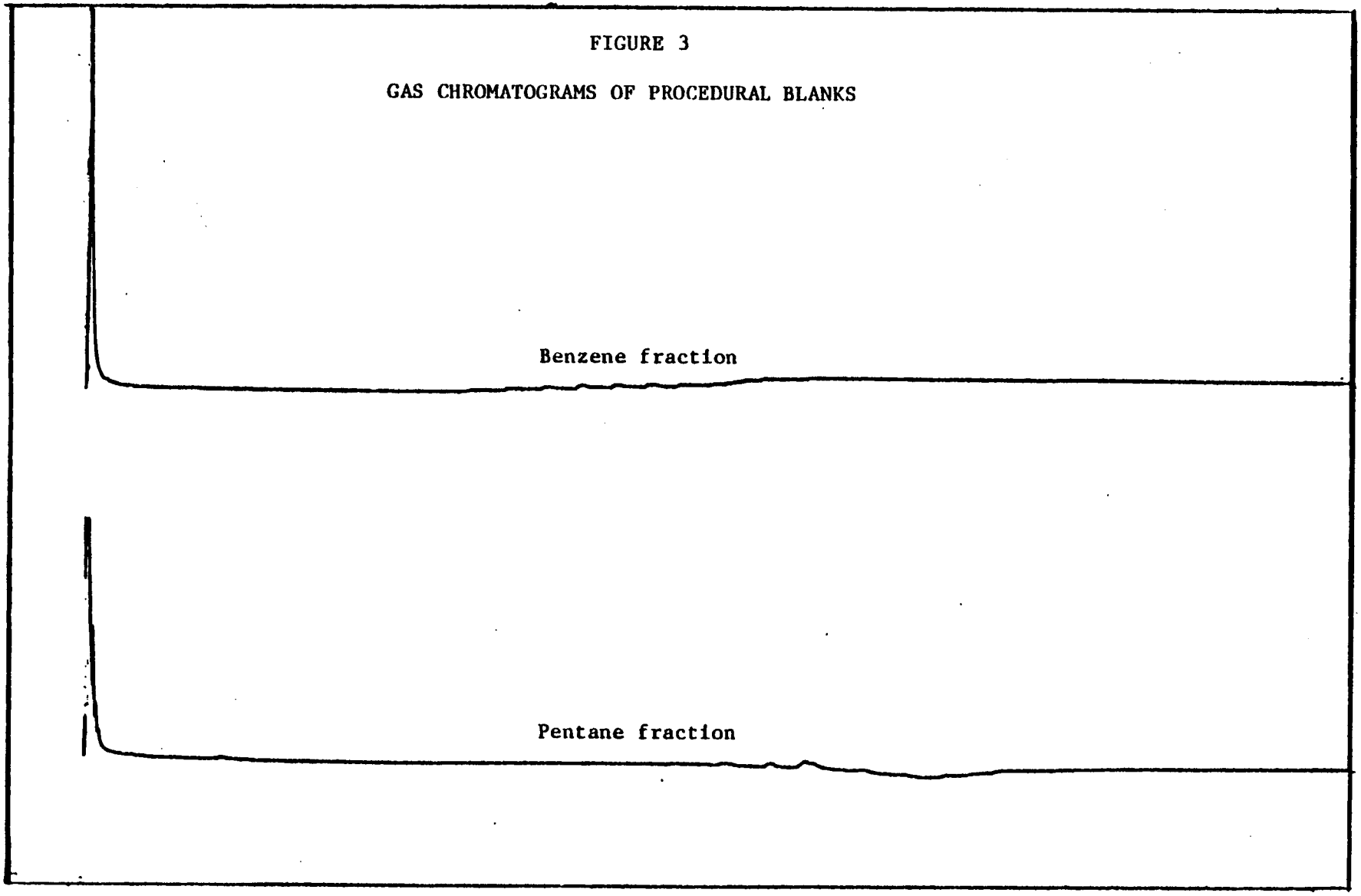
RESULTS

Analytical Procedures

Procedure blanks and recoveries of spikes were carried out prior to sample analyses. Excellent procedure blanks (Figure 3) were routinely obtained. For a detailed discussion on decontamination procedures, see Giam and Wong (1972) and Giam *et al.* (1975).

FIGURE 3

GAS CHROMATOGRAMS OF PROCEDURAL BLANKS



The analytical methods described in this report were essentially those required by BLM Contract No. AA550-CT6-17. The procedure, which included two evaporative concentration steps, gave very good recoveries (Table 1) for the higher molecular weight hydrocarbons, but yielded a lower recovery for lower molecular weight and more volative hydrocarbons. However, this lower recovery did not affect the characteristic (odd-even) distribution pattern of petroleum. Thus, if a biological sample was contaminated with petroleum, the analytical procedure would be capable of detecting the contamination. Moreover, the analyses of the replicate biological samples gave good reproducibilities (Table 2; Tables 10-12, Appendix M). The limits of detection were generally 0.005 ppm or better. Examples of typical gas chromatograms of procedure blanks and samples and the mass spectra of major components are shown in Figures 1-3; and Figures 1-12, Appendix M.

Analyses of Macroepifauna Samples

The results of the analyses of 148 organisms are detailed in Tables 1-12, Appendix M, as total concentration of alkanes, percent distribution of n-paraffins, the levels of pristane and phytane, and the ratios of pristane/phytane, pristane/C_{17} and phytane/C_{18} and the carbon preference index (CPI) ratios. A summary of the range of total concentrations of n-alkanes for the more dominant species of organisms are shown in Table 3, while Table 2 illustrates the variation found in replicate samples. In most cases, the C_{15} and C_{17} alkanes were the dominant n-alkanes. As shown in Table 4, they frequently constituted more than 70% of the total of n-alkanes. Pristane was present in almost all samples at relatively high levels, while phytane was present in approximately 15% of the samples at concentrations generally less than 0.1 ppm. The pristane/phytane ratios

TABLE 1

PROCEDURAL RECOVERY[1] OF ALIPHATICS AND AROMATICS

	PERCENT RECOVERY AVERAGE OF 14 DETERMINATIONS
C_{17}	75 ± 15
C_{18}	75 ± 10
C_{19}	93 ± 6
C_{20}	93 ± 3
C_{21}	97 ± 3
C_{22}	86 ± 5
C_{23}	96 ± 3
C_{24}	91 ± 5
C_{25}	89 ± 7
C_{26}	86 ± 9
1-methylnaphthalene	62 ± 19
2,6-dimethylnaphthalene	77 ± 14
1,5-dimethylnaphthalene	76 ± 16
4-phenyltoluene	88 ± 11
3,3'-dimethylbiphenyl	81 ± 10
4,4'-dimethylbiphenyl	88 ± 11
fluorene	93 ± 10
1-methylfluorene	76 ± 15
anthracene	66 ± 16

[1] Recovery of hydrocarbons (approximately 20 µg added) subjected to all steps in the analytical procedure in the absence of biota.

TABLE 2

CONCENTRATION OF n-ALKANES IN REPLICATE SAMPLES

Species	Station	Concentration, µg/g dry wt.			
Squid	1/I	0.07	0.07		
	1/II	0.00	0.06		
	3/II	0.24	0.22	0.04	
Longspine porgy	3/II	0.00	0.01	0.02	
Butterfish	2/I	17.62	17.17	16.00	
Wenchman	3/II	0.48	0.50	0.37	0.96
Blackeared bass	2/II	0.00	0.06	0.28	
	2/IV	0.55	0.69		
Brown shrimp	1/II	0.00	0.00	0.01	
	2/II	0.00	0.00	0.00	
	1/IV	0.00	0.02		
Shoal flounder	1/II	0.00	0.00	0.08	
	2/II	0.00	0.00	0.04	
Dwarf goat fish	1/III	1.02	2.17		
	2/III	2.38	2.60	2.48	
Rough Scad	1/II	5.25	6.32		
	1/III	3.26	3.21		
	1/IV	0.01	0.03		

TABLE 3

CONCENTRATION OF n-ALKANES (C_{14}-C_{32}) IN MUSCLE OF MAJOR SPECIES OF MACROEPIFAUNA FROM THE SOUTH TEXAS OCS, 1975-1976

Species	Concentration µg/g dry wt.		
	Range	Mean	Median
Squid (44)	0.00- 7.45	0.76 ± 1.37	0.22
Wenchman (32)	0.13-13.07	2.47 ± 3.58	1.17
Rough Scad (25)	0.01- 8.07	2.51 ± 2.51	1.22
Shoal flounder (19)	0.00- 1.82	0.76 ± 0.65	0.81
Longspine porgy (18)	0.00- 1.88	0.41 ± 0.49	0.22
Dwarf goatfish (18)	0.05- 9.73	2.54 ± 2.77	0.20
Blackeared bass (14)	0.00- 0.69	0.25 ± 0.23	0.24
	0.00-13.07		
Brown shrimp (36)	0.00- 0.32	0.07 ± 0.09	0.04

TABLE 4

PERCENTAGES OF C_{15} AND C_{17} IN TOTAL n-ALKANES OF MUSCLES OF SPECIES OF MACROEPIFAUNA FROM SOUTH TEXAS OCS (1975-1976)

Species	Percentages (C_{15} + C_{17})
Wenchman (27)	87 ± 13
Lizard fish (10)	86 ± 10
Atlantic bumper (7)	86 ± 6
Butterfish (6)	84 ± 3
Rough scad (17)	84 ± 13
Dwarf goatfish (16)	79 ± 19
Squid (32)	68 ± 26
Longspine porgy (17)	40 ± 31
Shoal flounder (15)	27 ± 35

ranged from 6 to 136. The pristane/C_{17} ratios were generally in the range of 1 to 10. The few phytane/C_{18} ratios that could be calculated were mainly in the range from 0.5 to 5. The majority of the CPI_{14-20} ratios were greater than two, with most values between 10 and 30. The CPI_{20-32} ratios were in the range of 0.2 to 46 with the majority of values from 0.5 to 2.

Analyses of Macronekton

The results of the analyses of 85 macronekton samples are presented in detail in Tables 13-36, Appendix M, as total concentration of alkanes, percent distribution of n-paraffins, the levels of pristane and phytane, and the ratios of pristane/phytane, pristane/C_{17}, phytane/C_{18} and the CPI ratios.

The range of total concentrations of n-alkanes in muscle, liver and gill of the two major species analyzed, vermilion and red snapper, are shown in Table 5. The C_{15} and C_{17} hydrocarbons were the dominant n-alkanes (Table 6). Pristane was present in almost all samples. Phytane was present in approximately 10 percent of the samples in concentrations from 0.004 to 0.165 ppm. The pristane/phytane ratios ranged from 0.5 to 92. The pristane/C_{17} ratios varied from 0.8 to 23, with most values around one. The phytane/C_{18} values ranged from 0.4 to 2.0. The CPI_{14-20} ratios ranged from 1.9 to 70, with most values between 10 and 30. The CPI_{20-32} ratios ranged from 0.4 to 8.5.

Analyses of Possible Shipboard Contaminants

Samples of oily bilge water, a ship's lubricant and a fuel oil were analyzed by the techniques similar to that used for biota. These substances yielded characteristic petroleum-like patterns of aliphatic hydrocarbon distribution with no odd-even predominance. Aromatics were

TABLE 5

CONCENTRATION OF n-ALKANES (C_{14}-C_{32}) IN MUSCLE, LIVER AND GILL IN MACRONEKTON FROM THE SOUTH TEXAS OCS, 1976

	Concentration µg/g, dry wt.		
Species	Range	Mean	Median
Vermilion snapper			
muscle (16)	0.01- 3.69	1.23 ± 1.28	0.69
liver (12)	0.00-29.93	5.89 ± 9.71	1.51
gill (13)	0.00-23.98	3.79 ± 7.02	0.31
Red snapper			
muscle (11)	0.01- 0.97	0.33 ± 0.36	0.17
liver (10)	0.63-13.33	5.48 ± 4.21	4.93
gill (10)	0.00-15.22	3.75 ± 5.00	1.44

TABLE 6

PERCENTAGES OF C_{15} AND C_{17} IN TOTAL n-ALKANES
OF MACRONEKTON FROM THE STOCS 1976

Species	Percentages (C_{15} + C_{17})
Red Snapper	
muscle (15)	95 ± 4
liver (15)	92 ± 8
gill (13)	90 ± 8
Vermilion Snapper	
muscle (19)	92 ± 10
liver (10)	91 ± 11
gill (11)	91 ± 11

found in the appropriate samples, *e.g.* the fuel oil. The results of these analyses are summarized in Table 37, Appendix M.

DISCUSSION

General Comments

The analytical methods were adequate for the main purpose of the project, namely to provide baseline data for hydrocarbons in biota and to evaluate if the hydrocarbons are of petroleum or biogenic origin. Because of the very low levels of hydrocarbons in biota (generally 2 ppm or lower), contamination during sampling and analysis must be scrupulously avoided. Good recoveries, blanks, and reproducibilities were obtained in spite of the low levels of hydrocarbons present. The mean concentrations of hydrocarbons in muscles of biota from South Texas OCS were in the low parts per million (μg/g dry weight) range, with brown shrimp having the lowest concentrations (Tables 3 and 5). For most species, C_{15} and C_{17} alkanes constituted the major component of the hydrocarbons (Tables 4 and 6). There were little or no phytane, aromatics or distribution patterns to indicate that the hydrocarbons were of petroleum origin.

Macroepifauna

Contractual obligations were fulfilled by the analysis of 124 samples of macrobenthic organisms, 16 more than required. (A summary of the analyses is found in Tables 1-12, Appendix M). The 24 replicate samples were also analyzed as required, but the 13 individual and pooled quality control samples were too small for individual analysis. (Attempts are being made by the Contractor to supply larger samples.) Another problem with contract compliance was that samples representing all three

species, a mollusc, crustacean and fish, were not received from every station; often, only fish were available. The diversity of fish samples and the lack of shrimp and squid from every station made statistical correlations between stations difficult. For example, some stations appeared to yield higher hydrocarbon levels or wider distributions of n-alkanes, but on close inspection of the data, this might have been due to the taxonomic characteristics of the species collected. For example, rough scad and butterfish contain numerous hydrocarbons while shrimp and squid have relatively few.

A number of trends were apparent from the data. The dominant hydrocarbons were pristane and the C_{15} and C_{17} n-alkanes. This was probably due to the diet of the organisms, as pristane is the major hydrocarbon in zooplankton (Blumer *et al.*, 1964) and the C_{15} and C_{17} n-alkanes are the dominant hydrocarbons in unpolluted algae (Clark and Blumer, 1967). The hydrocarbons present in more than 10 percent of the samples were confirmed by GC-MS; squalene was the only compound confirmed in the aromatic fraction. Phytane was detected in only 23 samples and tended to occur more frequently at some stations than others. None was detected at Stations 1/I, 3/I and 3/III; it was present in one sample from Stations 2/II, 3/II and 3/IV; in two samples from Stations 2/I and 1/IV; in three samples from Stations 1/II; and in four samples each from Stations 1/III, 2/III and 2/IV. As phytane is generally considered to be derived from petroleum rather than from biogenesis (Farrington *et al.*, 1972), these findings suggest that there could be sources of petroleum pollution at those stations. However, other indicators of petroleum such as aromatic hydrocarbons (Farrington *et al.*, 1972), were absent and the levels of phytane were very low implying the presence of very low levels of petro-

leum, if any. Also, the replicate samples studies shown in Table 2 indicated that higher hydrocarbon levels were present at some stations relative to others (*e.g.*, for rough scad, 1/II>1/III>1/IV), but the stations with the high levels were different from those containing more samples with phytane. If significant levels of petroleum were present in an area, its presence should be reflected in the hydrocarbon levels in the area's organisms as petroleum hydrocarbons have been found to be taken up rapidly from contaminated water (Anderson *et al.*, 1974) and to be retained for long periods from chronic exposure (DiSalvo *et al.*, 1975). Thus, if petroleum is present in the South Texas OCS study area, it is probably at very low levels.

There was also an absence of correlation between and within stations of the pristane/phytane, pristane/C_{17} and phytane/C_{18} ratios. These parameters are often used to identify sources of oil pollution and would be expected to be similar in organisms exposed to a single petroleum source, although there is some indication that biogenic hydrocarbons can affect the ratios (Farrington and Medeiros, 1975). In any case, the lack of correlation of the ratios further implies the absence of significant petroleum sources in the Texas OCS.

Another index of the presence of petroleum are odd-even ratios. In this study, carbon preference indices (CPI) were used as a measure of odd-carbon dominance and were calculated as follows:

$$CPI_{20-32} = 1/2 \left\{ \frac{\sum_{n=21}^{n=31} \text{HC odd}}{\sum_{n=22}^{n=32} \text{HC even}} + \frac{\sum_{n=21}^{n=31} \text{HC odd}}{\sum_{n=20}^{n=30} \text{HC even}} \right\}$$

$$CPI_{14-20} = 1/2 \left\{ \frac{\sum_{n=15}^{n=19} \text{HC odd}}{\sum_{n=16}^{n=20} \text{HC even}} + \frac{\sum_{n=15}^{n=19} \text{HC odd}}{\sum_{n=14}^{n=18} \text{HC even}} \right\}$$

The CPI_{20-32} is generally of the same order of magnitude for petroleum (mean 1.2) and for biological organisms (mean 1.0-1.5), but the CPI_{14-20} more accurately reflects the odd-carbon dominance of biological samples that is absent in petroleum. The CPI_{14-20} is almost always >2 for organisms, and averaged <1.0 for petroleum (Clark, 1974). In this study, most of the CPI_{14-20} values were between 10 and 30. The few values that were close to one were for samples with a small number of hydrocarbons whose distribution patterns did not resemble petroleum. Thus, this parameter also supports the other findings in indicating very low levels of petroleum in the South Texas OCS study area.

Macronekton

Only 85 of the contracted 108 macronekton samples were received and all were analyzed. Specifically, the August and November monthly samples had six rather than the required 12 samples, the Fall sampling was one organism, or three samples less than the specified amount, and one monthly suite of samples (12) was not received. Extra samples were received and analyzed in March, April and July. During the winter analyses, muscle but not liver and gill were analyzed because Work Statement Draft V did not require analyses of liver and gill. Samples received following receipt of Draft VI were analyzed for muscle, liver and gill. Most of the samples were red and vermilion snapper; a good profile of snappers in two banks was available.

The range of total hydrocarbon concentrations was from 0.003 ppm for gill to 290.7 ppm for liver. The dominant hydrocarbons were the C_{15} and C_{17} n-alkanes and pristane, as found for macroepifauna. Phytane was found in about 10 percent of the samples and was distributed equally between the two banks. The phytane appeared to be associated with higher hydrocarbon levels in the samples and CPI_{14-20} ratios were at the lower end of the average range (10-30), but this was not true in all cases. With the absence of aromatic compounds in the samples and a relatively small number of phytane-containing samples, the presence of petroleum in these samples was not conclusive.

The ranges of the pristane/phytane, pristane/C_{17} and phytane/C_{18} ratios were smaller than those found for macroepifauna, probably due to the smaller number of species sampled. These ratios were not sufficiently consistent at either bank, however, to suggest a source of petroleum hydrocarbons. The CPI_{14-20} values were also strongly suggestive of biogenic sources of the hydrocarbons detected.

CONCLUSIONS

As in the 1974-1975 study, heavy hydrocarbons of anthropogenic origins were not indicated in this study. The large number of different species, but few replicates of the same species studied, have made data correlations and interpretation statistically difficult. However, the study provides a wide data base which will serve as a baseline against which future data can be evaluated. It is hoped that fewer species but more replicates will be utilized in future studies to allow more detailed intercomparisons between sampling sites and intraspecific variability.

LITERATURE CITED

Anderson, J. W. J. M. Neff, B. A. Cox. H. E. Tatem, and G. M Hightower. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27:75-88.

Blumer, M., M. M. Mullin, and D. W. Thomas. 1964. Pristane in the marine environment. Helgolaender Wiss. Meeresunters. 10:187.

Clark, R. C., Jr. 1974. Methods for establishing levels of petroleum contamination in organisms and sediment as related to marine pollution monitoring. NBS Spec. Publ. 409. Marine pollution monitoring (petroleum). Proceedings of a symposium and workshop. Gaithersburg, Md. pp. 189-194.

_____, and M. Blumer. 1967. Distribution of paraffins in marine organisms and sediment. Limnol. Oceanog. 12:79.

DiSalvo, L. H., H. E. Guard, and L. Hunter. 1975. Tissue hydrocarbon burden of mussels as a potential monitor of environmental hydrocarbon insult. Environ. Sci. Technol. 9:247-251.

Farrington, J. W., C. S. Giam, G. R. Harvey, P. L. Parker, and J. Teal. 1972. Analytical techniques for selected organic compounds. Marine pollution monitoring: strategies for a national program workshop sponsored by NOAA at Santa Catalina Marine Biological Laboratory. October 25-28. pp. 152-176.

_____, and G. C. Medeiros. 1975. Evaluation of some methods of analysis for petroleum hydrocarbons in marine organisms. Proceedings of the 1975 conference on prevention and control of Oil pollution, San Francisco, Ca. pp. 115-121.

_____, and J. G. Quinn. 1973. Petroleum hydrocarbons in Narragansett Bay. I. Survey of hydrocarbons in sediments and clams. Estuar. Coastal Mar. Sci. 1:71-79.

_____, J. M. Teal, and P. L. Parker. 1976. Petroleum hydrocarbons. *in* E. D. Goldberg, ed. Strategies for marine pollution monitoring. Wiley-Interscience Publ. New York.

Giam, C. S., and M. K. Wong. 1972. Problems of background contamination in the analysis of open-ocean biota for chlorinated hydrocarbons. J. Chromatog. 72:283-292.

_____, H. S. Chan, and G. S. Neff. 1975. A sensitive method for the determination of phthalate ester plasticizers in open-ocean biota samples. Anal. Chem. 46:2225-2228.

_____. 1976. Distribution of n-paraffins in selected marine benthic organisms. Bull. Environ. Contam. Toxicol. 16:37-43.

Scarratt, D. I., and V. Zitko. 1972. Bunker C oil in sediments and benthic animals from shallow depths in Chedabusto Bay, N. S. J. Fish. Res. Bd. Can. 29:1347-1350.

CHAPTER EIGHTEEN

TRACE METALS IN EPIFAUNA, ZOOPLANKTON AND MACRONEKTON

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ABSTRACT

This report covers second year data from an ongoing study to determine baseline concentrations of trace metals in marine organisms from the STOCS area. This data will be used to assess the potential impact of future oil and gas exploration/production on biota in the area. Concentrations of 10 metals (Al, Ca, Cd, Cu, Cr, Fe, Ni, Pb, V, Zn) were determined in 312 samples including: zooplankton (62); muscle tissue from fish (140), shrimp (19) and squid (14); fish gill (31) and liver tissue (29); and whole oysters (15). Most samples came from 14 stations sampled at least 3 times during 1976. Concentrations were determined by flame atomic absorption spectroscopy (Cu, Fe, Zn), flameless AAS (Cd, Cr, Ni, Pb) and neutron activation analysis (Al, Ca, V).

No indication of substantial heavy metal pollution was observed. Levels of Cd, Cu and Pb appeared to be higher in the north. Sample groups in order of decreasing total trace metals content (except Al, Ca) were zooplankton, liver, oyster, gill, shrimp, squid and fish flesh.

No significant changes in annual mean trace metals concentrations were found for any sample group between 1975 and 1976. The number of metals in these comparisons was limited, however, because of the systematic overestimation in 1975 of Cd, Cr, Ni and Pb in many types of samples.

There was considerable variability in the trace metals data. Sample groups in order of decreasing variability were zooplankton, liver, fish flesh, gill, shrimp, squid and oyster. Variability within species was only moderately less than that in groups. With this level of variability, only differences of $\geq$ 100 percent could be resolved statistically.

Replication of samples for individual species was poor. Samples from 29 species were analyzed, but only 11 species had $\geq$ 9 samples for analysis. Statistically valid interspecific comparisons could only be made between the annual mean trace metals concentrations of these 11 species. Smaller scale geographical and seasonal comparisons could only be made within the larger groupings of similar species. Similar interspecific comparisons were infeasible. An improvement in the replication of samples by decreasing the number of species analyzed is essential for future work.

Incorporation of aluminosilicate detritus was significant only for zooplankton samples. This incorporation was lowest in the spring and decreased offshore in all seasons. Levels of Cu, Ni and Pb decreased in the south. Lead concentrations decreased offshore, and Cd levels were lower inshore.

Fish, shrimp and squid muscle tissue had generally low, uniform trace metals levels with few apparent geographical, seasonal or interspecific differences.

Gill and liver tissue from vermilion snapper had generally higher concentrations of trace metals than similar tissues from red snapper. Cd levels were higher in livers than gills for both species. Cadmium levels were higher in samples from Hospital Rock than from Southern Bank.

Oysters had generally higher levels of all trace metals. Levels of Cd, Cu, Pb and Zn were significantly higher in samples from the East Flower Garden than those from four other bank stations.

INTRODUCTION

The introduction of trace metals into the marine environment as a result of man's activities has been well demonstrated (Merlini, 1971). Metal pollution has been observed in sediments from Corpus Christi Bay (Holmes *et al.*, 1974) and the Houston Ship Channel - Galveston Bay area (Hann and Slowey, 1972). There are no reports of significant contamination in sediments from other areas on the continental shelf of the northwest Gulf of Mexico (Trefry and Presley, 1976). Offshore oil and gas exploration is increasing. The South Texas Outer Continental Shelf (STOCS) has been designated by the Department of the Interior as a potential area for such activities. The Bureau of Land Management (BLM) under the Department of the Interior is tasked with the proper development and sound management of these offshore mineral resources.

As part of BLM'S National OCS Environmental Studies Program, the purpose of the STOCS Monitoring Study is to provide a basis for predicting the impact of oil and gas exploration and development on this marine environment. Many metals are associated with drilling activities including Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Nickel (Ni), Vanadium (V) and Zinc (Zn), (Anon., 1975). The purpose of this project is to establish baseline data on trace metals concentrations in STOCS organisms before extensive oil and gas exploration begins. This baseline data can then be used to assess the impact of future petroleum drilling/production activities in terms of changes in organismal trace metals levels. To assess the geographical variability of trace metals in STOCS biota, a variety of organisms were collected from three stations on each of four transects across the shelf and from several topographic highs (fishing banks). All stations except a few bank

stations were sampled at least three times to determine any seasonal effects on trace metals levels.

This report covers the second (1976) year of the STOCS Monitoring Study Trace Metals Project. A total of 312 biological samples were analyzed for 10 metals (Al, Ca, Cd, Cu, Cr, Fe, Ni, Pb, V, Zn). The types of samples analyzed were zooplankton (62), fish muscle (140), fish livers (29), fish gills (31), shrimp muscle (19), squid muscle (14), whole oysters (15) and miscellaneous crustaceans (2). In 1975, 348 samples were analyzed for most of the same metals. The 1975 sample types were similar to 1976 except for 35 neuston samples.

The purpose of this report is to characterize the 1976 trace metals data in terms of average concentrations in the various types of organisms and compare these levels with similar data from 1975. Also the geographical and seasonal trends in 1976 organismal trace metals concentrations are discussed for each sample type, and, where possible, for individual species. Finally, an attempt is made to assess the nature and magnitude of possible future changes in STOCS organismal trace metals levels that could be detected using the current baseline data set. Also, ways in which the baseline data set can be improved by future work to resolve smaller scale changes in organismal trace metals levels are discussed.

METHODS AND MATERIALS

All samples were collected by personnel of the University of Texas, Port Aransas Marine Laboratory. Epifaunal and demersal fish samples were collected with a 35 ft (10.7 m) Texas box otter trawl. Zooplankton samples were collected with a metal-free, 1-m, 220 μm mesh net. Macronekton samples were caught by hook and line. Every reasonable precau-

tion was taken to avoid contamination during sampling. Epifaunal, demersal fish and macronekton samples were placed in polyethylene bags. When potentially contaminating sediment or other foreign material was adhering to the exterior surfaces of the organisms collected, they were rinsed prior to being put into the polyethylene bags. Zooplankton samples were transferred from the cod-end of the net to plastic snap-cap vials. To avoid any release of metals from the organisms caused by microbial activity, all samples were immediately frozen on board ship and remained frozen during transportation and storage until prepared for analysis.

Epifaunal and demersal fish samples were collected at Stations 1-3, all transects, during the three seasonal sampling periods. Five individuals of the three most abundant species were collected at each station. The species analyzed in each of the major sample groups are listed in the Results section. In addition, one sample consisting of twelve individuals of one species was collected. A total of 157 epifaunal samples was collected, including 12 sets of replicates and 24 quality control samples.

Macronekton samples were collected from Southern Bank and Hospital Rock during the three seasonal and six monthly sampling periods. Optimally, five individuals of two species (*Rhomboplites aurorubens* and *Lutjanus campechanus*) were collected from each bank during each sampling period. A total of 79 macronekton samples was collected. Four quality control subsamples were taken from the pooled analytical sample.

Zooplankton samples were collected from Stations 1-3, all transects, during the three seasonal sampling periods. A total of 72 zooplankton samples was collected, including 12 sets of replicate and 12 quality control samples.

Additional macronekton samples and samples of the spiny oyster (*Spondylus americanus*) were collected during the Topographic Features Study.

Sample Preparation

Non-contaminating procedures used to prepare each of the major sample types (*i.e.* zooplankton, shrimp and other Crustacea, squid, oysters, fish flesh and liver and gills) are detailed below.

No sorting was made of the diverse and variable group of zooplankton organisms collected. Each sample was thawed and poured into an acid-cleaned 140 mm diameter plastic petri dish. Filtered seawater was added as necessary to cover the entire sample. The sample was carefully inspected using a dissecting microscope. Any foreign material was removed and the relative proportion of major types of zooplankton (*e.g.* copepods, ostracods, crustacean larvae, chaetognaths, etc.) in the sample was estimated. The sample was poured onto a 220 μm NITEX screen and rinsed sparingly with deionized water. The deionized water used for all work in this study was prepared by passing distilled water through an ultrapure, mixed-bed demineralizer column (BARNSTEAD D0809). Excess water was removed by placing the screen over a series of paper towels and gently squeezing the sample with a teflon spatula, and weighed immediately to determine wet weight. The vial was covered with parafilm and placed in a freezer until it could be freeze-dried.

Fish and macroinvertebrate samples were thawed just prior to being prepared for freeze drying. They were rinsed with deionized water as necessary to remove any mud or other foreign material adhering to the exterior surfaces of the organisms. All dissections were done in a clean room on acrylic plastic cutting boards using stainless steel

scalpels, scissors and nylon or teflon tweezers as required. At no point during the dissection were the preparer's fingers allowed to touch the tissue to be analyzed. All dissecting equipment was thoroughly rinsed with 1 N nitric acid (HNO_3) and deionized water between each sample. At the end of each sample preparation, all equipment was thoroughly cleaned in a Na_2CO_3 solution and rinsed with 1 N HNO_3 and deionized water. The equipment was stored in polyethylene bags until the next use. The acrylic boards were soaked in 0.5 N HNO_3 between each use.

Muscle tissue from all fish, shrimp and squid and liver and gill tissue from selected fish were prepared for analysis. A maximum amount of the appropriate tissue from each individual was prepared for freeze drying as described below. This action was taken to insure that extra freeze-dried material would be available for repeat analyses when necessary and to avoid having material from the same sample stored in two different ways for long periods. Except for samples prepared as whole organisms (*i.e.* crab, oyster and zooplankton), an equivalent wet weight tissue aliquot was taken from up to 10 individuals in the sample (if available) and pooled in a tared plastic, snap-cap vial to give a total wet weight if possible of 6-12 g. After dehydration this pooled sample yielded a dry weight of 1-3 g, all of which was analyzed for trace metals. Pooled samples were prepared in this manner to insure that the trace metals concentrations in the pooled sample represented a true average of the concentrations existing in each of the individual organisms included in the sample, and also to avoid having to homogenize a large, pooled sample with a ball mill or mortar and pestle and risk contamination. If sufficient tissue remained, reserve pooled samples identical to the first were prepared in separate vials. If there was

insufficient tissue remaining to prepare a second replicate pooled sample, or if there was still tissue left over after the preparation of the additional replicates, the remaining tissue from each individual was placed in separate vials for possible future use.

The abdominal musculature was removed from whole shrimp for analysis. The total length (from rostrum to tail) and sex (whenever possible) were determined for each individual prior to dissection. The head and thorax were cut off and discarded. The abdominal musculature was removed by making a mid-ventral incision with scissors and peeling off the exoskeleton. The mid-ventral artery was removed from the surface of the muscle and the digestive tract and dorsal artery excised by making a mid-dorsal incision. This procedure was done to reduce the variability in sample trace metal concentration since vascular and digestive tissue could have significantly different trace metals content than the muscle tissue. The muscle tissue was rinsed sparingly with deionized water to remove any remnants of the arteries or digestive tract and was then handled as described below. The single stomatopod sample was prepared similarly, except the digestive gland which is closely associated with the abdominal musculature was also removed. Whole crabs, due to their small size, were freeze-dried. The legs and dorsal carapace were removed before drying to decrease the concentration of calcium and other interferants from the exoskeleton in the final sample matrix.

For squid, the mantle length (from the dorsal anterior margin to the tip of the tail) and the sex (whenever possible) of each was determined. The mantle was slit from the funnel to the tail and laid open. The pen, viscera, exterior skin and tail fins were removed. The remain-

ing mantle muscle tissue was rinsed sparingly as required with deionized water to remove any remnants of viscera, etc. and treated as described below.

The shell length and width of each oyster were measured. The whole organism was then removed and placed in an acid-cleaned 140 mm plastic petri dish. Mucus, particulates and other foreign material were washed off with filtered seawater. Portions of the gut containing ingested material were also removed. The whole oyster was then placed in a tared, snap-cap vial and handled as described below.

The standard length and sex (whenever possible) of each individual fish was determined. In dissecting out the lateral trunk musculature, a concerted effort was made to avoid contamination of the muscle sample which could occur if the sample came into contact with the exterior surface of the skin. On each side of the fish, a dorso-ventral incision was made along the anterior margin of the lateral trunk musculature. This incision was continued posteriorly just lateral to the mid-dorsal and mid-ventral planes. The skin was flayed off and discarded. The muscle was cut away from the axial skeleton, and, when sufficient tissue was available, the margins, where possible contact with the exterior skin could occur, were trimmed off and discarded. If there was insufficient tissue available, these margins were rinsed sparingly with deionized water. For macronekton samples, gill and liver tissue were also sampled. The gills (including gill rakers) were removed by cutting the dorsal and ventral attachments and were rinsed sparingly with deionized water to remove any foreign material. As the last step, the body cavity was opened and the liver excised. All tissues once removed were handled as described below.

To check possible sources of contamination during sample preparation, the following experiment was conducted. Eight replicate aliquots of flesh from a single red snapper (*Lutjanus campechanus*) were prepared as described above. Two replicates were frozen immediately. Four aliquots were refrigerated for 18 hours and then frozen. During refrigeration one replicate received no further treatment. The blade end of a scalpel which had been used in numerous previous dissections was put directly into the second replicate. The blade end of a similar scalpel which had never been used was put into the third replicate. The cutting blades of a pair of dissecting scissors were put into the fourth aliquot. The remaining two aliquots were placed directly onto an acrylic cutting board or a WHATMAN Number 1 filter paper for 20 minutes at room temperature and then frozen. All samples were then handled as described below. The only significant contamination observed was a $\geq$ 30-fold increase in the Cr concentration of the aliquot exposed to the scissors. The use of dissecting scissors in sample preparation throughout the study was minimized. However, the time of contact between tissue and dissecting instruments during this experiment was much longer than would ever occur during actual sample preparation. This experiment suggests that the preparation techniques were not a source of sample contamination for the eight metals measured.

At the end of each sample dissection, the tissue sample(s) was placed immediately in a tared snap-cap vial and weighed immediately to determine wet weight. The samples were covered with Parafilm and placed in a freezer. When sufficient number of samples had accumulated, all samples were freeze-dried for 24 to 96 hours to a constant weight. After removal from the freeze dryer, the samples were reweighed to determine dry weight and the percentage of moisture lost by each sample was cal-

culated. Samples were then stored in a desiccator until analyzed.

Digestion (Wet Oxidation) of Samples

Freeze-dried samples were prepared for atomic absorption spectrophotometric (AAS) analysis using a nitric (HNO_3): perchloric ($HCLO_4$) acid digestion procedure as described in Method 3 of Attachment B to the 1976 BLM STOCS contract (AA550-CT6-17). Unacceptably high procedural blanks for Cd, Cr and Pb were observed in preliminary sample digestions using this method.

The primary source of contamination was perchloric acid ($HCLO_4$, double redistilled) and to a much lesser extent nitric acid (HNO_3, double redistilled) since up to 25 ml HNO_3 were being used per sample digested. To minimize this blank problem, a new lot of $HCLO_4$ containing considerably lower concentrations of Cd, Cr and Pb was obtained and the amount of HNO_3 and $HCLO_4$ used to digest each sample was significantly reduced. This reduction was realized by changing to an essentially closed refluxing system. A 1-3 g dry-weight sample was placed in a spoutless, electrolytic style pyrex beaker and 4-5 ml of 70 percent HNO_3 per gram of sample and 1 ml total of $HCLO_4$ were added. The beaker was covered with a 75 mm, non-ribbed pyrex watchglass and allowed to sit overnight at room temperature. The mixture was then refluxed at low heat on a hotplate for 6-24 hours. A bent glass rod was placed between the beaker lip and the watchglass and the heat increased to permit HNO_3 evaporation. At the first sign of white $HCLO_4$ fumes (*i.e.* when most of the HNO_3 was gone), the glass rod was removed allowing the watchglass to again rest flush on top of the beaker. The sample was allowed to reflux until cleared completely. If the sample did not clear, an additional 1 ml HNO_3 and 0.5 ml $HCLO_4$ were added and the refluxing continued

until clearing occurred. This step was repeated once if necessary. Finally, the watchglass was removed and the mixture was allowed to evaporate to near dryness.

Each digested sample was transferred to a tared 30 ml Oak Ridge-type, screw-top polypropylene centrifuge tube by washing the beaker several times with 0.1 N HNO_3 (BAKER ULTREX grade) and pouring the resultant solutions into the centrifuge tube. Each sample was brought to approximately 25 ml, thereby diluting the original dry weight sample 10-20 times. The volume of each sample was determined by reweighing the filled sample tube and making a small correction (*e.g.* 1.01 - 1.04, pH $\sim$ 0.5 - 1) for the specific gravity of the sample solution which was determined for each digestion. Insoluble residue which occurred in significant amounts in several zooplankton and fish gill samples was allowed to gravity settle in the tubes. Further dilutions from the original solution were made on a weight/weight basis in 5 dram snap-cap vials using 0.1 N HNO_3.

All digestion glassware was soaked immediately after use in a solution of "Micro" detergent and distilled water in covered polyethylene pans for up to several days. The glassware was then rinsed thoroughly with deionized water and soaked in 3 N reagent grade HNO_3 in covered polyethylene or polypropylene pans until the next use. The centrifuge tubes were prepared for use by cleaning in a "Micro" solution. They were then filled with 5 N reagent grade HNO_3, heated for several days at 50°C and stored in room temperature until used. Prior to use, the tubes were emptied, rinsed thoroughly with deionized water and tared. The 5 dram snap-cap vials used for further dilutions were filled with 1 N reagent grade HNO_3 and allowed to sit at room temperature for several days. Prior to use they were emptied, rinsed with deionized water

and tared.

About fifty samples and blanks were digested at any one time using the above procedure. Three to five procedural blanks were included in each digestion to determine the amount of each metal contributed to the samples by the digestion glassware and reagents. These blanks received the same reagents and treatment as the tissue samples. An aliquot of the 0.1 N HNO_3 used to transfer and dilute the sample was placed in a centrifuge tube and analyzed with each digestion as a diluent/tube blank. Reagent blanks were analyzed for all bottles of acid prior to their use in sample digestion. These blanks were prepared by taking ≥ 10 ml of acid, evaporating it to near dryness in digestion glassware and transferring the residue to a centrifuge tube in the same manner described above. For each series of dilutions made using 5 dram vials, one or more vial blanks were prepared and analyzed.

To determine if any of the metals of interest was being lost from samples during digestion, spike recovery experiments were conducted during four different digestions. Three experiments used aliquots of fish flesh and one used shrimp flesh. In each experiment two replicate aliquots of tissue were placed in separate beakers and digested as described above. One aliquot was spiked during initial heating with the following amounts of metals: Cd (.025 µg), Cr (0.25 µg), Cu (50 µg), Fe (50 µg), Ni (2 µg), Pb (0.5 µg) and Zn (50 µg). Replicate aliquots of the spike were placed in two separate tared centrifuge tubes and brought to 20 ml with 0.1 N HNO_3 diluent. These two samples were analyzed to determine the actual amount of each metal in the spike. The unspiked tissue sample was analyzed to determine the amount of each metal in the sample itself. The total amount of each metal expected in the spiked sample was calculated using these two values. Percent recovery was determined by com-

paring the expected amount of each metal with the actual amount measured in the spiked sample. The average percent recovery was as follows: Cd 93%, Cr 94%, Cr 94%, Cu 107%, Fe 93%, Ni 95%, Pb 103% and Zn 107%. Considering the low levels of metals used in the spikes and the precision of the analyses, these results were quite acceptable. They indicate that there was no significant loss of any of the metals studied during the digestion procedure.

Atomic Absorption Spectroscopy (AAS) Procedures

Eight elements (Cd, Cr, Cu, Fe, Ni, Pb, V and Zn) were analyzed in biological samples from the 1976 STOCS study. Cadmium, Cr, Ni and Pb, which occurred at low levels, were measured using flameless AAS. These analyses were made using a PERKIN-ELMER Model 306 atomic absorption spectrophotometer equipped with an HGA-2100 graphite furnace atomizer. A summary of the instrumental operating conditions and the average procedural blanks for all eight digestions are given in Table 1. External and internal furnace purge gas flow rates were verified at specified levels of 0.9 and 0.3 l per minute repectively at 40 psi delivery pressure. Injection volume was 25 µl. The furnace temperature gauge was calibrated using a clamp-on (inductive) ammeter and an optical pyrometer. Dry, char and atomization temperatures and times were optimized for each metal using selected representative samples according to the manufacturer's recommendations (Anon., 1974). Non-resonance lines used for this optimization to estimate the magnitude of broad band molecular absorption for various sample types were 226.5 (Cd), 231.6 (Ni), 282.0 (Pb) and 352.0 (Cr) nm. Corrections for non-specific or broad band molecular absorption were made by a deuterium arc background corrector. For Cd and Pb, sample dilutions $\geq$ 1/50 were used for quantitation, and for Cr and Ni,

TABLE 1

SUMMARY OF OPERATING CONDITIONS FOR FLAMELESS ATOMIC ABSORPTION ANALYSIS

Element	Wavelength (nm)	Source[1]	Temperature (°C) Time (seconds) dry	char	atomize	Minimum Detectable Concentration[3] (ppb)	Sensitivity[2] (pg)	Average Procedural Blank (ng)
Cd	228.8	EDL (5) HCL (8)	85° 60 sec	300° 60 sec	1800° 8 sec	0.025	9	6
Cr	357.9	HCL (10)	85° 60 sec	800° 30 sec	2600° 8 sec	1	25	<27
Ni	232.0	HCL (20)	85° 60 sec	1200° 30 sec	2500° 8 sec	4	100	<107
Pb	283.3	EDL (9)	85° 60 sec	500° 60 sec	2000° 8 sec	0.3	25	39

[1] Electrodeless discharge lamp (EDL). Numbers in parentheses are source energy in watts. Hollow Cathode Lamp (HCL). Numbers in parentheses are source current in milliamps.

[2] Average amount of metal injected giving a signal of .0044 absorbance units.

[3] At 10x scale expansion and approximately 1 chart unit; except Ni at 3x and 2 chart units.

dilutions of $\leq$ 1/50. Chemical interference was evaluated and corrected as necessary by frequent use of the standard additions technique and check dilutions. Mixed standard metal solutions were prepared in dilute HNO_3 (BAKER ULTREX grade) by diluting concentrated commercial atomic absorption standards. Samples were quantitated by peak height comparison with bracketing standards injected before and after the sample. Consideration was given to temporal variations in instrumental sensitivity, non-linearity between bracketing standards and gross differences in peak shape.

Copper, Fe and Zn were analyzed by flame AAS using a JARRELL-ASH Model 810 atomic absorption spectrophotometer. Analyses were carried out following the manufacturers recommended procedure (Anon., 1971; 1972). A summary of the operating parameters for these analyses is given in Table 2. Non-specific absorption was monitored by measuring simultaneously the absorbance of a non-resonance line and the analytical line of the element of interest. A fairly lean air-acetylene flame with flow rates of circa 7 and 2.5 l per minute, respectively, were used for all three elements. Aspiration rate was generally 5 to 6 ml per minute. Chemical interference was checked by use of the standard additions technique. Mixed standards used were prepared as described above.

The accuracy and precision of AAS analysis was evaluated by analyzing two NBS standard biological reference materials (*i.e.* #1571 orchard leaves and #1577 bovine liver) with each digestion. The results of these analyses as compared to NBS values are given in Table 3. These results indicate the AAS techniques used were acceptable. The only significant deviation occurred with Fe in orchard leaves. We were consistently below this NBS value using a variety of different batches of

TABLE 2

SUMMARY OF OPERATING CONDITIONS FOR FLAME ATOMIZATION ATOMIC ABSORPTION ANALYSIS

Element	Analytical Wavelength (nm)	Non-resonance Wavelength (nm)	Sensitivity[1] (ppm)	Average Procedural Blank (ng)
Cu	324.7	322.9	0.05	< 75
Fe	248.3	247.3	0.07	<100
Zn	213.9	220.2	0.02	< 75

[1] Average concentration giving a signal of .0044 absorbance units. Minimum detectable concentration was generally about one half of the sensitivity.

TABLE 3

ACCURACY AND PRECISION OF ATOMIC ABSORPTION ANALYSIS

Standard Reference Material	Concentration (ppm dry weight ± 1 standard deviation)						
	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Bovine liver (NBS No. 1577)							
This study (8)	0.31 ± .03	0.08 ± .01	198 ± 22	257 ± 68	0.09 ± .03	0.39 ± .09	130 ± 13
NBS values	0.27 ± .04	<0.2[2]	193 ± 10	270 ± 20	<0.2[2]	0.34 ± .08	130 ± 10
Precision[1]							
This study	10	13	11	26	33	23	10
NBS values	15	NA	5	7	NA	24	8
Orchard Leaves (NBS No. 1571)							
This study (8)	0.11 ± .02	2.2 ± 0.4	12 ± 1	220 ± 40	1.1 ± 0.1	43 ± 3	24 ± 6
NBS values	0.11 ± .02	2.6 ± 0.2	12 ± 1	300 ± 20	1.3 ± 0.2	45 ± 3	25 ± 3
Precision[1]							
This study	18	18	8	18	9	7	25
NBS values	18	8	8	7	15	7	12

[1] Precision expressed as percent coefficient of variation *i.e.* std. dev./mean x 100.

[2] Not certified values.

AA standards. We feel that this plant material may be resistant to complete disillusion by HNO_3:$HCLO_4$ and are trying other digestion procedures.

Analysis of Vanadium in Organisms

The sensitivity for V determination by AAS analysis is very low with a minimum detectable quantity of $\geq$ 100 ng. Instrumental neutron activation analysis (INAA) was prescribed by BLM in Attachment B to the 1976 STOCS Study Contract in an effort to improve the sensitivity of V analysis. The primary difficulty that must be overcome when using INAA for V in marine organisms is interference from Na-24 and Cl-38 background levels produced during irradiation. BLM prescribed the use of sulfuric acid (H_2SO_4) and hydrated antimony pentoxide (HAP) as pre-irradiation chemistry reagents for the removal of Cl and Na, respectively, from acid digests of organism samples.

These pre-irradiation chemical separations required considerable effort to implement in the laboratory. A method had to be developed for the synthesis of HAP ($Sb_2O_5 \cdot 4H_2O$) since the sole commercial source of HAP is in Italy, delivery is slow and the product variable in Na affinity. Also large amounts of Cl, which are very difficult to remove, are introduced into the samples by using $HCLO_4$ as the oxidizing agent. To keep Cl below the interference level in the samples, it was necessary to do separate, duplicate digestions of each sample for V analysis using hydrogen peroxide (H_2O_2) as an oxidizing agent (*i.e.* Method 2, Attachment B, 1976 contract). Chlorine concentrations after this digestion procedure were at acceptable levels and no further treatment was required. The HAP procedure was modified from Girardi and Sabbioni (1968). A batch method was used to remove Na from the sample digests. The HNO_3:H_2O_2 digest of a 0.5 to 1 g dry-weight sample was added to a 50 ml screw-top,

polyethylene centrifuge tube containing from 0.1 to 0.5 g of HAP. Enough 70 percent HNO_3 was added to provide a final acid concentration of about 10 N. After shaking for 5 minutes, the samples were centrifuged and the supernatant poured into a 50 ml teflon beaker. This step was repeated using 10 ml of 70 percent HNO_3. The teflon beaker contents were then evaporated to a volume which could conveniently be poured into a 1.5 ml irradiation polyvial used by the Texas A & M University Nuclear Science Center. The vial was heat-sealed to prevent sample loss during analysis.

Each sample was irradiated separately for 2 minutes by a 1 MW TRIGA REACTOR. This process was facilitated by a pneumatic transport system which can rapidly transfer samples in and out of the reactor core. The sample was first placed in a secondary poly vial, together with an aluminum flux monitor, before being transported to a core for irradiation. Standards prepared from commercial AAS standards or pure metals were used.

After return of the sample and a 1-minute delay, the aluminum flux monitor was counted by a multi-channel pulse height analyzer. After an appropriate delay period (usually 3-5 minutes, so that the dead time was < 30 percent), the irradiated sample was placed on a ORTEC GE (Li) detector and counted using a separate GEOS Quanta 4096 channel pulse height analyzer. After a 5-minute counting period, the spectrum was stored on magnetic tape.

Data reduction was done using the program HEVESY (Schlueter, 1972). This program calculates peak intensities and converts them to concentration by comparison with standards. Corrections were made for varying delay times, dead times and neutron fluxes.

In spite of achieving acceptable levels of Na and Cl in the sample digests, the average minimum detectable quantity (MDQ) for V by INAA under the conditions used in this study was about 60 ng. Many samples were below the limit of detection. This relatively poor sensitivity was apparently due to interference from the considerable remaining sample matrix. This problem was exacerbated by the fact that the concentration in many samples was low (*i.e.*, 0.3 ppm), and relatively large samples were required to get a sufficient amount of V for analysis. Another problem on a small percentage of samples was Sb carryover to the treated samples. Although Sb lends no direct interference to INAA determination of V, its neutron cross-section is large enough to render the sample sufficiently "hot" after even 2 minutes irradiation to increase the dead time to a prohibitive level. Sometimes this situation can be compensated for by altering the counting geometry. In either case, the sensitivity for detecting V-52 is severely reduced and most often no usable data derived.

One characteristic of INAA is its capability for analyzing several elements from a single irradiation. Concurrent with V analysis, the concentrations of Al, Ca and Cu were determined. However, the analytical conditions could not be optimized for all four elements during a single irradiation. The sensitivity for Al was good and the Al concentration data satisfactory. However, the sensitivity for Ca was marginal and many of the samples were below the detection limit which was quite variable and often very high. The sensitivity for Cu was very poor and almost all the samples were below the elevated detection limit. These less-than values were consistent with Cu concentrations for the same samples determined by AAS analysis, but are of no practical use and are not reported in the raw data tabulation in Table 1, Appendix N.

RESULTS AND DISCUSSION

Table 1, Appendix N, gives the complete trace metals project data set and includes the concentrations of 10 elements in 312 samples. Aluminum, Ca and V data are missing from several samples for one of two reasons. First, Sb carryover in some samples prepared for INAA resulted in excessive background activity as discussed under Methods. For a few samples, no additional sample material was available for the separate HNO_3:H_2O_2 required for INAA sample preparation.

Table 4 summarizes the 312 samples analyzed according to the major sample groups specified in the 1976 STOCS contract (AA550-CT6-17): Zooplankton (ZPL), Macroepifauna and demersal fish (EPI) and macronekton (MNK). These three groups are further divided into seven groups of similar types of organisms or tissues: zooplankton, shrimp, squid, oyster, fish muscle, fish livers and fish gills. These seven groups included twenty-nine identified species of marine organisms and zooplankton. This table emphasizes that there were only a few individual samples for a single species and no species provided significant coverage of the STOCS study area in either space or time. This situation resulted from both the difficulty of consistently sampling the same species at selected locations over a year and the contract requirement to analyze the three dominant EPI species at each transect station each season regardless of species. For MNK, the situation was better because there were essentially only two species at two stations. However, these MNK samples were spread over eight separate sampling periods during the year. Thus, temporal and spatial intraspecific comparisons within the STOCS study area, based on reasonable numbers of samples, were generally not justified with this data set. To make such comparisons, individual species

TABLE 4

ANALYSES BY SAMPLE TYPE, SPECIES AND COLLECTION DATA

Sample type and species	Species collection data: Number of Seasons Sampled	Number of Transect Stations Sampled	Number of Bank Stations Sampled	Number of Samples Analyzed
Zooplankton (ZPL)	3	12	-	62
Macroepifauna and demersal fish (EPI)				
A. Shrimp (flesh)				
1. *Penaeus aztecus*	3	7	-	9
2. *Penaeus setiferus*	2	2	-	5
3. *Solenocera vioscai*	2	1	-	4
4. *Parpandalus sp.*	1	1	-	1
B. Squid (flesh)				
1. *Loligo pealei*	3	7	-	12
2. *Loliguncula brevis*	2	2	-	2
C. Demersal fish (flesh)				
1. *Chloroscombrus chrysurus*	2	2	-	5
2. *Cynoscion arenarius*	2	2	-	4
3. *Gonioplectrus hispanus*	1	1	-	1
4. *Larimus fasciatus*	1	1	-	1
5. *Leiostomus xanthurus*	1	1	-	3
6. *Lutjanus campechanus*	1	1	-	1
7. *Micropogon undulatus*	1	2	-	2
8. *Peprilus burti*	2	2	-	4
9. *Polydactylus octonemus*	2	2	-	2
10. *Prionotus paralatus*	2	2	-	2

TABLE 4 CONT.'D

Sample type and species	Species collection data: Number of Seasons Sampled	Number of Transect Stations Sampled	Number of Bank Stations Sampled	Number of Samples Analyzed
C. Demersal fish (cont.)				
11. *Pristipomoides aquilonaris*	3	5	-	28
12. *Serranus atrobranchus*	2	7	-	11
13. *Stenotomus caprinus*	3	7	-	9
14. *Syacium gunteri*	1	4	-	8
15. *Synodus foetens*	3	7	-	10
16. *Trachurus lathami*	3	7	-	12
17. *Trichopsetta ventralis*	1	1	-	1
18. *Upeneus parvus*	3	4	-	5
D. Miscellaneous Invertebrates				
1. *Callinectes similis* (crab, whole)	1	1	-	1
2. *Squilla empusa* (stomatopod, flesh)	1	1	-	1
3. *Spondylus americanus* (oyster, whole)	1	-	5	15
Macronekton				
A. Flesh				
1. *Holocentrus rufus*	1	-	1	1
2. *Lutjanus campechanus*	8	-	6	16
3. *Rhomboplites aurorubens*	9	-	6	14
B. Liver				
1. *Holocentrus rufus*	1	-	1	1
2. *Lutjanus campechanus*	8	-	6	16
3. *Rhomboplites aurorubens*	8	-	6	12

TABLE 4 CONT.'D

Sample type and species	Species collection data: Number of Seasons Sampled	Number of Transect Stations Sampled	Number of Bank Stations Sampled	Number of Samples Analyzed
C. Gills				
1. *Holocentrus rufus*	1	-	1	1
2. *Lutjanus campechanus*	8	-	6	16
3. *Rhomboplites aurorubens*	9	-	6	14

must be organized into larger, meaningful groups.

The groups selected for discussion in this report were zooplankton, fish muscle (= flesh), fish liver, fish gills, shrimp flesh, squid flesh and *Spondylus americanus* (spiny oyster, whole organism). The zooplankton were a diverse group resulting from a specific sampling technique, and no sorting was done on the resultant samples. The fish-flesh group included samples of MNK from six bank stations and EPI from 12 transect stations, and, thus, was the group which had the largest geographical distribution.

Table 5 compares the annual mean concentrations of 10 elements in the above seven groups for 1976 with similar data from 1975 analyses. These annual averages, although derived by grouping several similar species, provide a concise and general way of comparing differences in trace metal concentrations among different types of organisms and between the same kind of organisms from two consecutive years. These averages for both years were calculated using all less-than values at the indicated limit of detection to avoid excluding too much data from consideration. In 1975, there were undetectable values only for V and this was predominantly in EPI and MNK flesh samples. There were few undetectable values for the other metals due to the systematic overestimation of the concentrations of several metals in many sample types for reasons discussed later (cf. 18-33). In 1976, there were more undetectable values as a result of the more sensitive flameless AAS analysis used. Fish flesh had the most frequent undetectable values for Cr, Ni and V and less frequently for Cd and Pb. Undetectable values for the same metals also occurred infrequently in shrimp and squid samples. Because less-than values were used in these calculations, the true means for the above elements and sample groups were lower than indicated values. However, since the 1976 detection limits for Cd, Cr, Ni and Pb were quite low (*i.e.* < 0.01 to 0.1 ppm dry weight),

TABLE 5

COMPARISON OF ANNUAL AVERAGES OF TRACE METALS IN MAJOR SAMPLE TYPES FROM 1975 AND 1976

Sample Type	Number of Species	Number of Samples	Year Collected	Concentration (ppm dry weight ± 1 standard deviation)									
				Cd	Cr	Cu	Fe	Ni	Pb	V	Zn	Al	Ca
Zooplankton	-	62	1976	2.9±1.4	4.3±4.2	20±22	2100±3000	6.6± 6.2	20±45	13±22	120±170	3100±5600	34000±22000
(ZPL)	-	70	1975	3.5±1.5	4.4±2.6	13± 7	-	7.1± 3.4	11± 9	17±15	113± 38	-	-
Fish Muscle	20	140	1976	0.02±0.02	0.04±0.03	1.1±0.5	6±4	0.09±0.06	0.05±0.05	0.2±0.3	12± 7	26±13	2100± 5500
(EPI, MNK/TOPO)	16	118	1975	0.11±0.07	1.8±1.3	1.2±0.6	-	1.0 ±1.1	0.97±0.52	1.8±1.4	16± 6	-	-
Fish Gills	3	31	1976	0.82±1.20	0.12±0.07	2.9±2.5	140±70	0.48±0.14	1.4±1.6	2±2	82± 48	110±140	75000±38000
(MNK/TOPO)	1	6	1975	0.67±0.25	3.8±0.2	1.0±0.4	116±10	4.6 ±0.4	7.9±1.8	-	64± 7	-	-
Fish Livers	3	29	1976	7.2±8.5	0.07±0.05	25±23	850±640	0.24±0.22	0.38±0.36	3±6	320±350	79±120	62000±69000
(MNK/TOPO)	1	6	1975	4.5±1.4	2.2±0.2	12± 2	563±176	0.87±0.10	2.8±2.4	-	160± 67	-	-
Shrimp	4	19	1976	0.09±0.08	0.04±0.02	24±8.6	4±3	0.21±0.14	0.08±0.06	0.5±0.9	63± 18	39±40	1800±1700
(EPI)	5	29	1975	0.15±0.07	1.8±0.8	25± 4	-	0.93±0.66	0.86±0.50	2.2±0.8	51± 9	-	-
Squid	2	14	1976	0.22±0.20	0.03±0.02	17±17	5±5	0.16±0.12	0.13±0.10	0.2±0.2	47± 8	31±14	490±250
(EPI)	1	24	1975	0.39±0.54	2.7±2.5	10± 4	-	5.4±10	1.0±0.58	2.6±0.8	44± 7	-	-
C. americanus (oyster, TOPO)	1	15	1976	28±18	2.6±1.7	26±35	140±92	41±17	3.3±2.9	9±7	170± 99	500±1500	3300±2100

overestimation of these means should not be excessive and should not
ect the relationships in these general comparisons.

Two approaches were used to characterize systematically the relative
els of trace metals among the groups in Table 5. The first approach
to rank the groups numerically from one to seven for each of eight
als (except Al, Ca). The average ranking for each group was then
culated (*i.e.* sum of ranks for each group/eight). *Spondylus* has gen-
lly the highest trace metals concentrations with zooplankton having a
ghtly lower level. The order of the remaining groups was liver, gill,
rimp, squid and fish-flesh. Liver and gill tissues showed a complimen-
ry distribution of several metals. Livers had higher concentrations
the metabolically important metals, Cu, Fe and Zn as well as Cd.
lls had higher levels of Al, Cr, Ni and Pb which do not form organic
mplexes as readily as do the metabolically active metals. Aluminum,
and Ni may tend to be incorporated in the non-living portions of tissues
ch as bone and cartilage. The higher levels of Al, Cr and Ni in gills
ght result from the fact that gill samples included a larger proportion
these non-living, structural tissues than did liver samples.

The second approach was to order the seven groups according to the
m total of the eight metals in each one. The relative positions of
nly the first three groups changed using the second criteria. Zooplank-
on had the highest total metal content (2.3 mg/g) with livers second
1.2 mg) and oysters third (0.42 mg). Gills had a total of 0.23 µg with
he three remaining groups all having <0.1 mg. Zooplankton were first
olely because of the extremely high concentration of Fe. Comparing
he Al and Fe concentrations in all groups with the mean concentrations
f these metals in bottom sediments from the study area (Berryhill, 1977),
suggested how significant the incorporation of iron-bearing aluminosili-

cates is in each sample type. This comparison showed that such incorporation was only significant for zooplankton and was the most likely explanation for the high Fe concentration in this group. Livers were second on the basis of total metal content because of the higher concentrations of the important metabolites, Fe and Zn, in this metabolically active tissue. Oysters were third because this group had relatively high concentrations of all metals but none matched the high levels that occurred in zooplankton or livers. The remaining four groups were in the same relative order according to both criteria. They had generally lower concentrations of most metals with expected higher concentrations for only the metabolically active metals, Cu, Fe and Zn.

Variability in this data is one of the key factors in determining how small a change in the concentration of one of the trace metals could be detected during some future monitoring program. Relative variability in trace metals content among the seven groups (Table 5) was quantitatively characterized in two ways. First, the mean percent coefficient of variation (C.V.) for eight metals was calculated for each group (percent C.V. = std. dev./mean x 100). Zooplankton had the greatest variability in trace metals concentrations (percent C.V. = 128) with liver samples second (percent C.V. = 105). The remaining groups in order of decreasing variability were fish flesh, gills, shrimp, squid and oysters (percent C.V. = 85, 84, 77, 74, 74, respectively). The high variability in trace metal content among zooplankton samples was most likely a result of the variable species composition of the samples and the variable incorporation of aluminosilicate detritus by zooplankton over space and time. Several factors contributed to variability in the remaining groups. A small amount of the observed variability

resulted from lumping several different species in a single group. For example, similar calculations on single species yielded mean percent coefficients of variations which represented only moderate reductions from the group averages (*P. aztecus*, 56; *L. pealei*, 78; and *P. aquilonaris*, 64). Some variability may have been an artifact of including less-than values when calculating the averages and variances for many sample groups. Some variability could also arise from differences in sample preparation techniques such as taking tissue from different body areas of various individuals for a pooled sample or from individuals of very different size, etc. The contribution from this factor should be small because an effort was made during preparation to minimize these differences. Much of the variability may be simply that naturally found in similar individuals of the same species from any one place and time. This suggestion was supported by data from the separate analyses of 12 individual *P. aquilonaris* from the same trawl. The percent coefficients of variation for Cu, Fe and Zn from these samples were 20, 42 and 58, respectively (mean percent C.V. = 40).

The second way of examining the relative variability in trace metals content (Table 5) was to calculate for each metal (except Al, Ca) the average of the individual percent coefficients of variation from each of the seven sample groups. Nickel had the lowest variability (percent C.V.= 66). The remaining elements in order of increasing variability were Zn, Cr, Fe, Cu, Cd, Pb and V (percent C.V. = 67, 71, 81, 86, 97, 112, 137, respectively). Zinc had the lowest variability among the three metabolically important elements, Cu, Fe and Zn. This fact suggests that Zn metabolism in the organisms sampled may be more independent of environmental and organismal factors than Cu and Fe metabolism. Copper and Fe are directly involved in respiration, a physiological process sensitive

to environmental change. Cadmium and Pb both had higher variability. Their distribution in the marine environment is significantly affected by man's activity. Consequently, with industrial point sources along the Texas coast and changeable transport processes, the distribution of Cd and Pb and their resultant incorporation into organisms would be expected to be quite variable within the STOCS study area.

The amount of variability in this data set would permit the detection of only large-scale concentration changes (on the order of several-fold). Variability in trace metal data from this study must be reduced, if possible. Some reduction may be possible by further analysis of the data involving factors related to sample composition. However, the most direct approach would be to maintain the same analyses effort, but concentrate on fewer species and analyze a significant number of each species. With the current sampling program, this approach would result in some missing values from the station - season data matrix. However, such missing data would probably have a small effect on our ability to make projections about any part of the study area. The important gain would be the probable decrease in data variability and the resultant increase in our capability to detect smaller-scale changes in organismal trace metal content.

Three factors must be considered when comparing mean trace metals concentrations in the same sample groups for 1975 and 1976 (Table 5). First, Fe data was obtained in 1975 from only a limited number of zooplankton, shrimp and squid samples. This 1975 Fe data cannot be realistically compared with 1976 Fe values for these groups. Second, between different years of this study, an effort was made in 1976 to analyze the same species that were analyzed in 1975. Consequently, the species compo-

sition of the seven sample groups was quite similar for both 1975 and 1976, although the proportion of any species within a given sample group varied between years. In general, over 90% of the samples in each group were from the same species in both years. Liver and gill tissues from only *Rhomboplites aurorubens* were analyzed in 1975 as compared with similar tissues from this same species plus *Lutjanus campechanus* in 1976. No *Spondylus* or molluscs of any kind were analyzed in 1975.

The third factor was the change in 1976 to the more sensitive flameless atomic absorption spectrophotometry (AAS) analysis for Cd, Cr, Ni and Pb because low levels of these metals were encountered in the organisms sampled. Analysis of seven elements (except Al, Ca, V) in 1975 was by flame atomization AAS. The concentrations of trace metals in zooplankton samples from 1975 and 1976 were very similar (Table 5). This fact demonstrates that the different AAS techniques used in 1975 and 1976 gave comparable results when the metals analyzed were at concentrations above the detection limits for flame AAS. For other sample groups, the 1976 mean values of Cr, Ni and Pb were significantly lower than those from 1975. It is clear now that this difference is an artifact due to errors in the 1975 data. Reanalyses of 1975 samples in 1976 (Table 6) shows that the concentrations of Cr, Ni and Pb in the involved sample types are below the detection limits of flame AAS. Determination of the concentrations of Cr, Ni and Pb in these samples by flame AAS resulted in a systematic overestimation of the true concentrations. The situation was similar for Cd, but since the sensitivity of Cd analyses by flame AAS is much better than for Cr, Ni and Pb, the overestimation was of a smaller magnitude.

For 1975 gill and liver samples, the Cd concentration was high enough to permit accurate analysis by flame AAS.

This conclusion concerning Cd, Cr, Ni and Pb levels in 1975 and 1976 samples was supported by two additional checks which were made in 1976. As discussed in the Methods, several spike recovery experiments showed that there was no significant loss of any metals studied using the 1976 procedures for sample digestion. Second, homogenized freeze-dried samples which had been collected, prepared and analyzed in 1975 were reanalyzed this year. Table 6 summarizes results from the reanalyses of fourteen 1975 samples in 1976 and compares this data with results from the 1975 analyses of the same samples. The agreement between 1975 and 1976 analyses of the zooplankton sample was good for all metals. Zinc and Cu concentrations in the remaining species also agreed well. The concentrations of Cd, Cr, Ni and Pb in these species were all much lower in the replicate aliquots reanalyzed in 1976. As discussed above, the 1975 data are artifacts from trying to determine the concentrations of these metals by flame AAS in samples where the actual concentrations were below the detection limits for this method. However, these differences due to changes in analytical techniques cannot account for all of the differences for the same metals and species observed between samples collected, prepared and analyzed in 1975 (Table 6) or 1976 (Tables 7 and 9). The remaining difference could be due to a change in metals levels in STOCS biota between 1975 and 1976 or a result of some contamination of 1975 samples during preparation. Our current data is inadequate to distinguish between these two possibilities.

Considering the above three factors, comparisons between 1975 and 1976 trace metals concentrations in each sample group (Table 5) can be discussed. The mean concentrations for both years were generally quite

TABLE 6

COMPARISON OF AVERAGE TRACE METALS CONCENTRATIONS IN
SAMPLES COLLECTED AND ANALYZED IN 1975 and REANALYZED IN 1976

Sample Description	Number of Samples Reanalyzed	Year Analyzed	Concentration (ppm dry weight ± 1 standard deviation)					
			Cd	Cr	Cu	Ni	Pb	Zn
Zooplankton	1	1976	2.70	4.7	13	6.7	3.7	140
		1975	2.86	4.1	8.6	11.	3.5	130
Penaeus aztecus	4	1976	0.06±0.02	0.89±0.90	26±1.3	0.65±0.54	0.12±0.04	50±2.4
		1975	0.15±0.05	2.25±0.9	26±2.1	1.6±1.1	0.85±0.33	47±3.3
Loligo pealei	4	1976	0.10±0.07	1.0±1.0	12±6.4	0.71±0.70	0.25±0.19	39±5.5
		1975	0.17±0.04	3.2±1.1	11±4.2	2.0±0.8	1.2±0.6	42±8.1
Lutjanus campechanus	1	1976	<0.01	0.04	0.8	<0.05	<0.05	12
		1975	0.11	1.4	0.9	1.1	1.1	11
Prionotus paralatus	1	1976	0.02	0.38	0.9	0.25	0.47	15
		1975	0.11	2.6	0.8	0.8	1.5	16
Pristipomoides aquilonaris	2	1976	0.09±0.06	0.15±0.18	1.7±1.0	0.05±0.01	0.16±0.17	22±13
		1975	0.08±0.03	1.6±1.1	1.1±0.2	0.5±0.5	0.6±0.4	18± 8
Rhomboplites aurorubens	1	1976	0.03	0.22	1.1	0.11	0.17	11
		1975	0.13	1.7	1.2	1.5	2.0	11

similar for every sample group. No significant differences were found between the mean concentrations of seven trace metals (Table 5) in zooplankton from 1975 and 1976. For fish flesh, shrimp and squid, only Cu and Zn data from both years could be compared. The levels of these two trace metals were similar in 1975 and 1976 within all three groups. Similar 1975/1976 comparisons of annual mean concentrations of trace metals were made involving individual species of fish (*P. aquilonaris, S. atrobranchus, S. caprinus, T. lathami*), shrimp (*P. aztecus*) and squid (*L. pealei*) with sufficient samples analyzed in both 1975 and 1976. The results were the same as for the sample groups' comparisons within each species, *i.e.* Cu, Zn levels from both years were similar. This observation showed that Cu and Zn concentrations among individual species of fish, shrimp or squid were generally uniform. Thus, comparisons within a given grouping of similar species gives similar results as comparisons within each individual species making up a significant portion of that group. Vanadium was undetectable in many fish flesh, shrimp and squid samples from both years. The differences in mean V levels between the two years (Table 5) in these three groups was simply a reflection of increased sensitivity of the V analysis techniques used in 1976 (see Methods). Gill and liver tissues from only *Rhomboplites* were analyzed in 1975. Cadmium, Cu, Fe and Zn data in 1975 can be realistically compared to 1976 data for reasons discussed above. The 1975 levels of the four metals in both tissues were lower than similar data from 1976 *Rhomboplites* samples. One possible reason was that five of the six 1975 gill samples came from bank stations not sampled in 1976.

The comparisons of 1975 and 1976 data discussed above, allow the detection of changes in trace metals levels which occur over a large

portion of the STOCS study area during an entire year. However, to detect changes on smaller geographical and temporal scales requires comparisons of trace metals data collected at several different times from stations located throughout the study area. Such geographical and seasonal comparisons of trace metals data for each sample group are discussed in separate sections below. Similar comparisons, as well as annual mean trace metals levels, are given for individual species with sufficient numbers of samples analyzed in 1976. Such intraspecific comparisons were, however, often based on only two-to-five samples per season or geographic area (*e.g.* all samples from Transect I, all samples from all inshore stations 1/I, 1/II, 1/III, 1/IV, etc.). Comparisons among individual stations (*e.g.* 1/I vs 2/I, etc.) were infeasible because replication of samples at individual stations for any species was too infrequent.

Zooplankton

The levels observed in zooplankton (Table 5) were generally similar to those of previous studies (Sims, 1975; Martin and Knauer, 1973). Several 1976 zooplankton samples showed extremely high Pb concentrations suggestive of contamination. These samples also showed relatively high Fe concentrations, probably due to incorporation of aluminosilicate detritus. However, the concentration of Pb in such detritus (Berryhill, 1977) was too low to account for the high Pb levels observed. Two samples of shipboard contaminants (paint chips and lube oil) were analyzed in 1976 (Table 2, Appendix N). The observed Pb contamination did not appear to involve inclusion of either of these contaminants in the samples. Only Cr in lube oil was at a sufficiently high concentration to contaminate samples. Iron, Pb and Zn concentrations in paint

chips were high enough to contaminate zooplankton samples, but the contaminated zooplankton samples showed no elevated Zn levels.

The mean trace metals concentrations were generally similar among the three seasons sampled. However, incorporation of suspended matter was distinctly lower in the spring samples as shown by the lower Al, Fe and V concentrations.

Several geographic trends were apparent in the 1976 zooplankton data. The incorporation of aluminosilicate detritus appeared to be maximum in the samples from Transect III. The amount of incorporation was also maximum at inshore stations and decreased with increasing distance from shore. This trend was reflected by decreasing concentrations of Al, Cr, Fe and V offshore since these metals occurred in significant concentrations in aluminosilicate detritus. This trend also correlated well with the decreasing amount of aluminosilicate-rich suspended matter offshore (Berryhill, 1977).

Copper, Ni and Pb concentrations generally decreased from north to south. A decrease in Pb concentrations away from shore was also apparent. This trend was probably a result of offshore samples being farther away from coastal sources of Pb input to the STOCS area. Cadmium showed the reverse trend from Pb. The Cd increase offshore, also observed in 1975, correlated with the decrease in zooplankton biomass observed offshore (see Dr. Park's zooplankton report). This trend suggested a dilution phenomenon where, as the zooplankton biomass increased, the amount of Cd accumulated per unit biomass decreased. The opposite trends for Pb and Cd suggest that more Cd than Pb was transported offshore. Another possibility would be an additional source of Cd offshore such as gas seep.

Fish Muscle

The mean trace metals concentrations observed in 1976 (Table 5) were

similar to those reported in earlier studies, except that Cr and Ni were generally lower (Goldberg, 1972). The trace metals in fish muscle data were very uniform with no major seasonal or geographic trends. Even *Rhomboplites* and *Lutjanus* collected at six bank stations had flesh trace metals levels similar to fish collected from the 12 transect stations. Adequate annual baseline trace metals data were obtained for seven species of fish which had a sufficient number of samples distributed among several stations and two or more seasons analyzed in 1976 (Table 7). The only significant difference in metals levels among these species was that *T. lathami* had significantly higher levels of Fe and Zn than several of the other species. Intraspecific comparisons of trace metals data by seasons and sampling stations were made. Again, no significant trends were observed. For several species, Fe levels in winter samples were higher than in samples from the other two seasons. No differences between any of the bank stations or seasons for either of the two major macronekton species (*L. campechanus, R. aurorubens*) were observed.

The low and uniform levels of trace metals in fish flesh provide a potentially good opportunity for detecting any future increases of metals in the STOCS study area due to man's activities. However, many fish flesh samples in 1976 were below the limits of detection even by flameless AAS. These less-than values will permit the detection of any future increases in trace metals levels, but not with the resolution provided by knowing the actual concentrations in the samples. Such actual concentration data, unless quite variable, would allow the early detection of small increases. To measure these low levels would require additional separation and concentration techniques with greater potential for sample contamination. These procedures would significantly increase sample prep-

TABLE 7

AVERAGE CONCENTRATIONS OF TRACE METALS IN FISH MUSCLE IN 1976

Species	Number of Samples	Concentration (ppm dry weight ± 1 standard deviation)									
		Cd	Cr	Cu	Fe	Ni	Pb	V	Zn	Al	Ca
Lutjanus campechanus	17	0.03±0.00	0.03±0.01	0.8±0.3	5.4±2.8	0.06±0.02	0.03±0.01	0.2±0.1	12±10	31±25	4300± 7000
Pristipomoides aquilonaris	28	0.02±0.02	0.04±0.04	1.3±0.5	3.8±1.8	0.08±0.03	0.04±0.02	0.2±0.1	8.2±4.8	28±10	1600± 3000
Rhomboplites aurorubens	14	0.01±0.01	0.03±0.03	1 ±0.2	6.9±2.7	0.05±0.02	0.03±0.02	0.2±0.1	11±6.3	21± 7	6600±13000
Serranus atrobranchus	11	0.02±0.01	0.03±0.01	0.8±0.3	2.9±1.5	0.08±0.02	0.04±0.04	0.2±0.1	8.5±3.8	28± 7	1000± 300
Stenotomus caprinus	9	0.02±0.01	0.03±0.01	0.9±0.2	4.6±1.9	0.10±0.03	0.05±0.04	0.2±0.1	11±3.7	28±13	820± 490
Synodus foetens	10	0.01±0.01	0.03±0.01	0.9±0.3	3.6±2.0	0.06±0.01	0.10±0.13	0.2±0.1	12±4.1	23± 6	920± 410
Trachurus lathami	12	0.05±0.03	0.04±0.05	2.1±0.7	13±6.1	0.13±0.10	0.07±0.04	0.1±0.1	21±4.1	20± 8	710± 240

aration time.

Fish Gill and Liver Tissue

Adequate baseline trace metals data were obtained for liver and gill tissues from two species of fish, but only for two bank stations where each species was sampled several times (Table 8). Copper and Fe concentrations were higher in livers than gills, as would be expected for this metabolically active tissue. Cadmium was significantly higher in liver tissue from both species than in the gills.

Trace metals levels in both tissues of *Rhomboplites* were generally not significantly different from levels in the same tissues from *Lutjanus*. At Hospital Rock, *Rhomboplites* gill and liver tissue had significantly greater Cd concentrations than did *Lutjanus* gills and livers. However, in almost all cases (31/36 comparisons) the metals levels in *Rhomboplites* gill and liver tissue at each station were higher than those in similar tissues from *Lutjanus* from the same station. Both species were collected at the same time from the same station. One possible reason for this consistent difference is differential exposure of the two species to metals caused by differences in habits or diets. Within each species, the concentrations of all metals studied in each tissue were similar for both Hospital Rock and Southern Bank.

No seasonal trends in trace metals concentrations in gill and liver tissue from either species were observed. The data were quite variable making small trends difficult to see.

Shrimp

Trace metals levels in shrimp (Table 5) were generally similar with those of other studies (Sims and Presley, 1976). The 1976 shrimp trace metals data were quite uniform. No seasonal or geographic trends were

TABLE 8

AVERAGE CONCENTRATIONS OF TRACE METALS IN FISH GILL AND LIVER TISSUE IN 1976

Species	Bank[1] Station	Number of Samples	Tissue[2]	Concentration (ppm dry weight ± 1 standard deviation)									
				Cd	Cr	Cu	Fe	Ni	Pb	V	Zn	Al	Ca
Lutjanus campechanus	HR	6	G	0.16±0.14	0.10±0.06	1.8±0.8	100± 44	0.50±0.18	1.0 ±1.7	0.5±0.2	72± 23	93±100	91000± 38000
	SB	6	G	0.49±0.43	0.10±0.07	1.5±0.6	110± 32	0.44±0.20	0.54±0.29	0.6±0.2	74± 32	110± 98	110000± 19000
Rhomboplites aurorubens	HR	3	G	0.62±0.10	0.21±0.10	2.7±1.0	240± 110	0.60±0.13	3.3 ±3.9	6	160±140	220	12700
	SB	7	G	2.14±1.82	0.12±0.07	4.3±3.9	160± 76	0.42±0.10	1.6 ±1.4	2±1	75± 8	76± 60	66000± 43000
Lutjanus campechanus	HR	6	L	1.5 ±0.6	0.05±0.01	22±23	680± 300	0.14±0.08	0.24±0.08	0.9±0.7	130± 54	120±130	25000± 28000
	SB	6	L	1.6 ±0.5	0.06±0.02	13± 6	350± 150	0.14±0.07	0.21±0.14	0.4±0.1	113± 30	32± 5	58000± 50000
Rhomboplites aurorubens	HB	3	L	15 ±4	0.13±0.08	14± 4	1900±1100	0.67±0.40	0.76±0.47	4±1	490±350	63± 24	130000±180000
	SB	5	L	8.8 ±6.4	0.08±0.03	34± 46	1100± 640	0.20±0.06	0.36±0.22	1±0.5	340±390	30± 19	50000± 69000

[1] HR = Hospital Rock; SB = Southern Bank

[2] G = gill tissue; L = liver tissue.

apparent. No significant interspecific differences were found. Adequate annual baseline data were obtained for *Penaeus aztecus* (Table 9). Too few samples were collected to permit any intraspecific comparisons on the basis of season or area sampled. A single stomatopod crustacean (*Squilla empusa*) sample was analyzed in 1976. This species is sympatric in many areas with the shrimp species analyzed. *Squilla* had distinctly higher concentrations of Cd, Ca, Fe and Zn than shrimp. Although only one sample was analyzed in 1976, this relationship has been observed consistently with shrimp and stomatopod samples from the STOCS area analyzed in this lab.

Squid

Squid trace metals levels were also quite uniform in the STOCS area. No seasonal trends were observed. Both species of squid analyzed had similar concentrations of trace metals. Sufficient annual average baseline data were obtained for *Loligo pealei* (Table 9). The only geographic trend noted was the decrease in Cd and Cu levels from north to south. Copper in zooplankton showed a similar relationship. There was also some indication that Cd concentrations decreased offshore.

Spondylus americanus (oyster)

Spondylus samples were only analyzed in 1976. All samples were collected over a 2 to 3 week period. Compared to sediment trace metals levels (Berryhill, 1977), Cd, Cu, Ni and Zn were enriched in *Spondylus*. The enrichment in Ni was especially noteworthy since Ni was generally very low in the other organisms analyzed. The concentrations of Al and Fe were generally low, showing that incorporation of aluminosilicate detritus was not a significant source of trace metals. The levels

TABLE 9

AVERAGE CONCENTRATIONS OF TRACE METALS IN PENAEUS AZTECUS AND LOLIGO PEALEI MUSCLE IN 1976

Species	Number of Samples Analyzed	Concentration (ppm dry weight ± 1 standard deviation)									
		Cd	Cr	Cu	Fe	Ni	Pb	V	Zn	Al	Ca
P. aztecus (shrimp)	9	0.08±0.04	0.04±0.02	30± 5	3.7±3.4	0.17±0.11	0.07±0.06	0.3±0.2	58±6	27± 7	960±650
L. pealei (squid)	12	0.18±0.20	0.03±0.02	13±13	4.6±4.5	0.16±0.13	0.13±0.10	0.2±0.2	47±9	30±15	450±250

observed were similar to concentrations reported in other studies (Sims and Presley, 1976), although the reported trace metals concentrations in oysters were quite variable.

The concentrations of Cr, Ni and V were similar among the five bank stations sampled. The levels of Cd, Cu, Fe, Pb and Zn were quite variable among these stations. *Spondylus* from East Flower Garden had the highest levels of Cd, Cu, Pb and Zn. This mean Cd concentration was significantly greater than the levels at Southern Bank and Stetson Bank. The Cu concentration was significantly greater than those at all other stations. The Pb and Zn levels were probably not significantly different from any of the other stations. Oysters from Hospital Rock had the highest concentration of Fe which was significantly different from the lower levels at East Flower Garden and 28 Fathom Bank. The variability in trace metals levels among *Spondylus* from a single bank station was generally quite low for most metals.

CONCLUSIONS

The primary purpose of this study is to establish current baseline concentrations of trace metals in marine organisms within the STOCS study area. This baseline data can then be used to determine if future oil and gas exploration in the area contribute significant amounts of trace metals to the biota. Adequate baseline data have been obtained for several species of organisms in the form of mean concentrations of eight metals (except Al, Ca) in all 1976 samples of each species (Tables 5, 7, 8 and 9). There was considerable variability in the trace metals data; however, it is probably an accurate reflection of the natural variability within each species since an effort was made to control potential sources of additional variability (contamination, metal loss, erratic dissection

procedures, etc.) during sample preparation. Large (≥ 100 percent) changes in organismal trace metals levels which occur over significant portions of the STOCS study area could be detected with this data. Similar annual averages for groups of related organisms (Table 5) had generally more variability than the species averages and could detect only larger changes.

To fully meet the needs of the BLM OCS program, geographical and seasonal trace metals baseline data are needed. Such data permit the detection of smaller scale changes (*i.e.* station - station differences and seasonal changes at one station) such as might result from oil and gas exploration/production confined to only a few petroleum lease blocks. Adequate geographical and seasonal trace metals baseline data for individual species have been slow to accumulate due to difficulties in consistently sampling the same species throughout the year. With the intraspecific variability in trace metals concentrations observed in 1976, at least 5 to 10 replicates would be needed per station to detect a 75-100 percent difference between mean concentrations of a metal in the same species at two stations. Far too few samples of any one species were collected in 1976 to permit this magnitude of replication at any station or season. To increase the number of replicates being compared, pooling of similar species into larger groups (Table 5) was done. However, little was gained since the variability of trace metals levels in these groups was generally higher than for individual species, and more replication was required to achieve the same percent level of resolution. The only valid solution is to analyze many more samples of fewer species. This approach increases the number of replicate analyses per species, thereby providing a better opportunity for intraspecific geographical and seasonal

comparisons between individual sampling stations. It also minimizes the waste of effort involved in analyzing just two-to-three samples of several species. Such data can only be used as part of pooled group comparisons.

The choice of which species to emphasize in collection and analyses is important. Species selected should be widely distributed within the STOCS study area and available during all seasons. Consideration must be given to species analyzed in previous years so that good continuity among yearly trace metals data sets will be maintained. With these factors in mind, the following species should be emphasized in future work: *L. campechanus*, *R. aurorubens*, *P. aquilonaris*, *S. atrobranchus*, *T. lathami*, *P. aztecus* and *L. pealei*.

LITERATURE CITED

Anonymous. 1971. Instruction manual for Jarrell-Ash Model 810 atomic absorption spectrophotometer (Engineering Pub. #82-810). Jarrell-Ash Division, Fisher Scientific Company, Waltham, Massachusetts. 54pp.

_____. 1972. Atomic absorption analytical methods. Jarrell-Ash Division, Fisher Scientific Company, Waltham, Massachusetts.

_____. 1974. Analytical methods for atomic absorption spectroscopy using the HGS-2100 graphite furnace (#990-9972). Perkin-Elmer Corporation, Norwalk, Connecticut. 43pp.

_____. 1975. Summary recommendations of the trace metal and hydrocarbon seminars. Bureau of Land Management, Washington, D. C.

Berryhill, H.L. 1977. Environmental studies, South Texas Outer Continental Shelf, 1975: An atlas and integrated report. Bureau of Land Management, Washington, D. C. 303pp.

Girardi, F. and E. Sabbioni. 1968. Selective removal of radiosodium from neutron-activated materials by retention on hydrated antimony pentoxide. J. Radio. and Chem. 1:169-178.

Goldberg, E. 1972. Baseline studies of pollutants in the marine environment. Brookhaven National Laboratory, 24-26 May 1972. NSF/IDOE, Washington, D. C. 799pp.

Hann, R. W., Jr. and J. F. Slowey. 1972. Sediment analysis-Galveston Bay: Env. Eng. Div., Texas A & M University, Tech. Rept. 24. 57pp.

Holmes, C. W., E. A. Slade and C. J. McLerran. 1974. Migration and redistribution of zinc and cadmium in marine estuarine systems. Environ. Sci. Technol. 8:255-259.

Martin, J. H. and G. A. Knauer. 1973. The elemental composition of plankton. Geochimica et Cosmochimica Acta 37:1639-1653.

Merlini, M. 1971. Heavy metal contamination. *In* Impingement of man on the oceans. D. W. Hood, ed. John Wiley and Sons.

Schlueter, D. J. 1972. HEVESY, a general activation analysis computer program. Report TEES. 9002-1972-1. Texas A&M University, College Station, Texas. 41pp.

Sims, R. R., Jr. 1975. Selected chemistry of primary producers, primary consumers and suspended matter from Corpus Christi Bay and northwest Gulf of Mexico. Masters Thesis. Texas A&M University, College Station, Texas. 65pp.

_____, and B. J. Presley. 1976. Heavy metal concentrations in organisms from an actively dredged Texas bay. Bull. Environ. Contam. and Toxicol. 16(5):520-527.

Trefry, J. H. and B. J. Presley. 1976. Heavy metals in sediments from San Antonio Bay and the northwest Gulf of Mexico. Env. Geol. 1:283-294.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.