

USE OF ORDINATION AND CLASSIFICATION  
PROCEDURES TO EVALUATE PHYTOPLANKTON  
COMMUNITIES DURING SUPERFLUX II

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SUMMARY

Cluster analysis and an ordination procedure were performed on two data matrices to investigate real and environmental spatial relationships. Multiple regression analysis was used to relate the measured environmental variables to the phytoplankton community changes. Qualitative type phytoplankton data proved to be less structured in both of these spaces, relative to the biomass data. The salinity gradients of the northern transects covaried significantly with the phytoplankton association changes. In the southern transects the light variable was most important in explaining the variance in the ordination axes. These data suggest the close relationships between phytoplankton community changes and the physical hydrology of the area.

INTRODUCTION

The purposes of this study were: 1) to investigate phytoplankton community structure within the three-dimensional spatial confines of the Chesapeake Bay plume and 2) to examine the changes of community structure in a multidimensional environmental space. To realize the first objective, cluster analysis was used. A similar approach was followed in the study of plankton associations in the North Sea (ref. 1, 2), and to associate phytoplankton assemblages with major water masses in the West Indian Ocean (ref. 3). More recently cluster analysis has been applied in the impact assessment field and community structure studies (ref. 4, 5, 6, 7).

An ordination procedure was performed for the second objective. Polar ordination was used to place collection sites into a theoretically continuous environmental space (ref. 8). Eigenvector ordination techniques have also been used to investigate phytoplankton associations without any real efficiency (ref. 2, 9). Similar techniques were followed with more success to ordinate species samples from transient beach ponds (ref. 10). Polar ordination was selected for this study because of its relative simplicity (ref. 11) and the general failure of the other techniques previously applied in plankton research (ref. 12). The merits of this procedure with respect to

other ordination techniques have been previously discussed (ref. 13, 14, 15). A major assumption made by environmental ordination techniques is that species distributions in space and time are a result of specific responses to environmental variables. The assessment of such a multidimensional space could provide insight into the controlling factors of phytoplankton interrelationships.

The use of two data matrices in the following analysis allows the investigation of two fundamentally different questions. From the qualitative presence-absence matrix, species presence without reference to quantity is investigated. Are the species lists at the observed stations different within the sampling regime? Are there pronounced different qualitative regions within the study area relative to phytoplankton populations? The other matrix, the cell volume matrix, assesses the quantities of the phytoplankton species at the stations.

## MATERIALS AND METHODS

### Sampling Program

The phytoplankton samples for this study were collected during the June 1980 Chesapeake Bay plume studies aboard the NOAA vessels Kelez and Delaware II. The study area has a complex circulatory system represented by a southward flowing, low salinity mass of water originating from the Chesapeake Bay which generally holds to the Virginia and North Carolina coasts (ref. 16, 17). Such circulatory systems may be responsible for major phytoplankton dispersions (ref. 18), with areas of contrasting community structure.

The station numbers used in this study represent the 24 standard stations (see Marshall, Figure 2, paper no. 32 of this compilation) with each depth being assigned a station number. A total of 101 such station depth events occurred during the cruises. The samples were collected over a five day period. The study area was located between 37.00.6' and 35.50.2' N latitude and 76.02.9' and 75.17.1' W longitude. Parameters measured during the cruises were secchi depth, salinity, water temperature, dissolved oxygen, total suspended matter, nitrites, nitrates, ammonia, silicon, phosphates, and light. Appreciation is expressed to Dr. George Wong of Old Dominion University for supplying the station concentrations of nitrites, nitrates, phosphates, ammonia, and silicates; to Dr. Paul Zubkoff of the Virginia Institute of Marine Science for the daily isolation curves; and to Dr. James Thomas and Craig Robertson of NOAA for the salinity, dissolved oxygen, and temperature data. Special appreciation is given to Dr. James Matta of Old Dominion University in reference to the application of the multivariate techniques in this study. These data were selected for this study because of their historic relationships to phytoplankton dynamics.

The samples were collected with 20-liter Niskin sampling bottles. Different depths were selected at each station in relation to the thermostructure of the water column as assessed by using an expendable bathythermographic probe.

## Phytoplankton Analysis

For phytoplankton analysis a measured subsample (500 ml) of seawater was withdrawn from the Niskin sampler at each station depth and transferred directly into a polypropylene bottle which contained 20 ml of buffered formalin. Upon returning to the laboratory, the bottles were allowed at least 72 hours for the sedimentation of cells. A siphoning procedure followed that resulted in a 20-ml concentrate for each sample. For quantifying and identifying the cells either aliquots or whole concentrates were placed into settling chambers and allowed to re-settle; they were then examined and counted using a Zeiss inverted plankton microscope. Random fields of the chamber were selected and counts were made to give 85% confidence intervals on the mean concentration (ref. 19). A total of 168 species were identified from the 101 station depths.

To compute cell volumes, the identified species were assigned geometric shapes according to Kovala and Larrance (ref. 20). This scheme allowed for 18 phytoplankton shapes to choose from to approximate the shape of each species, with up to 10 dimensions applicable for the more complex forms. Average cell dimensions were determined from the literature with spot measurements also made for major species in the collections. A FORTRAN program was written to compute these volumes using the cell dimensions and appropriate formulae from Kovala and Larrance (ref. 20). Cell volumes per liter were computed for each station by multiplying the species volume by the number of cells per liter. This data base formed the volumetric matrix. This matrix was reduced to  $64 \times 101$  (species  $\times$  station-depths) by arbitrarily setting a cut-off criterion of 1%. Volumetric percentages for each species-station possibility were calculated and if a species did not account for at least 1% of the volume at any station it was removed from the matrix.

The qualitative matrix consisted of ones and zeroes. Wherever a species was present within the 168 by 101 matrix a value of 1 represented presence, zero for absence. This matrix was reduced to a  $72 \times 101$  dimension by setting the cut-off criterion to 5%.

### Other Variables

The light variable at each station was calculated using Riley's (ref. 21) equation:

$$\langle I \rangle = \frac{I_0}{kZ} (1 - e^{-kZ})$$

where  $\langle I \rangle$  is the amount of light received by the phytoplankton in a well-mixed water column of depth  $Z$  and extinction coefficient  $k$ .  $I_0$  is the surface radiation.

The extinction coefficient,  $k$ , was determined using the equation of Poole and Atkins (ref. 22):

$$k = \frac{1.41}{Z_{sd}}$$

where  $Z_{sd}$  is the depth of disappearance of the secchi disc (m.). Stations performed during darkness were assigned values of 0 at each depth.

A tide-related variable was also calculated. From standard tide tables (ref. 23) the tidal height at collection time for each station was determined. Values for the tide-related variable (TRV) were also calculated for offshore stations using the station time and the calculated tidal height (ft) for the closest subordinate standard tidal station. The variable was calculated as follows:

$$TRV = \left( \frac{TH}{2 DS \cdot DBM} \right)^{0.5}$$

where TRV is the tide-related variable, TH is tidal height at the closest subordinate station, DS is the distance from the collection station to the subordinate tidal station and DBM is the distance to the bay mouth (distances used were relative map units). This computation allows a simple approach for viewing the nonsynoptic nature of the sampling schedule as it relates to the tidal variable. The variable assigns smaller values for offshore stations. The variable is also inversely proportional to the distance from the bay mouth. Figure 1A shows the behavior of the variable if synoptic data were taken, Figure 1B is the variable calculated for the actual times of the standard stations.

## NUMERICAL METHODS

### Cluster Analysis Techniques

The purpose of the cluster analysis in this study was to segregate the 101 station depth events into a fewer number of station clusters. The intention of this technique is that stations within a defined cluster of stations are more closely related to each other than they are to stations of other clusters, relative to phytoplankton composition.

The computer program used was ORDANA (ref. 24). It has a sequential, agglomerative, heirarchical, non-overlapping algorithm.

For the qualitative data in this study the Jaccard coefficient was used. The Jaccard coefficient ( $D_{ij}$ ) was computed as follows:

$$D_{ij} = \frac{C_{ij}}{N_i + N_j - C_{ij}}$$

where  $C_{ij}$  is the number of conjoint presences within the two stations  $i$  and  $j$ .  $N_i$  and  $N_j$  are the numbers of species at the respective stations. The theoretical maximum value of 1.0 would indicate qualitatively perfect matching of species at the two stations.

For quantitative data the Czekanowsky similarity coefficient was used according to the following formula where  $S_{jk}$  = similarity between samples  $j$  and  $k$ ,  $X_{ij}$  = abundance of  $i$ -th species in the  $j$ -th sample, and  $n$  = the total number of species.

$$S_{jk} = \frac{\sum_{i=1}^n \text{MIN}(X_{ij}, X_{ik})}{\sum (X_{ij} + X_{ik}) - \sum \text{MIN}(X_{ij}, X_{ik})}$$

Again the theoretical maximum value for this coefficient was 1.0, indicating like species in similar quantities at each station.

The sorting strategy selected was group average, which is a space-conserving algorithm (ref. 11). This sorting strategy was chosen as it generally maximizes the correlation between the similarity values and the cluster analysis results.

All quantitative data (volume matrix) were transformed using  $X = (\ln X + 1)$  to reduce the scale problem inherent in the data and to rid the matrix of zeroes.

### Ordination Techniques

Polar ordination, developed by Bray and Curtis (ref. 8), is one of the simplest and most effective techniques available. Its major drawback is the required knowledge of endpoints along the ordination axis. To perform the ordination the following steps were taken:

1. Computation of a dissimilarity coefficient (determined by subtracting each similarity value from its theoretical maximum).

2. Selection of station endpoints which reflect the most dissimilar species populations. The endpoints were selected using the dissimilarity matrix. As hypothesized, the most dissimilar station-depth pair was between a bay mouth station (Standard station #801) and an offshore station (Standard station #816), where  $D=0.924$ . These two points are the anchors of the ordination axis with the distance between them,  $L$ .
3. The distances for all other stations were assessed from the dissimilarity matrix relative to the endpoints.
4. The positions of the other  $i$  samples,  $X_i$ , along the ordination axis were computed as follows:

$$X_i = \frac{L^2 - D_{1i}^2 - D_{2i}^2}{2L}$$

and the distance  $E_i$  of the sample from the axis is:

$$E_i = \left( D_{1i}^2 - X_i^2 \right)^{0.5}$$

The  $X_i$  values are an ordering of the species along a continuous axis. The  $E_i$  values are related to possible distortion of the axes. Second and subsequent axes may be calculated by selecting those two points which are closest to the median x-axis value for the next endpoints.

Multiple linear regression of environmental variables on these axes was performed to ascertain those variables which account for most of the variance in these axes. Violations in the assumptions of regression analysis were assessed by graphical interpretation of the residual plots. The light variable was transformed using the common log function.

## RESULTS

### Cluster Analysis

The results of the cluster analysis for the qualitative 72 X 101 (species x station-depths) matrix are schematically represented in dendrogram form in Figure 2. Two major clusters were observed to fuse at a similarity value of 0.317. Table 1 is a listing of the station-depth sites which are grouped under the major sub-groups in the dendrogram. Six clusters were observed (labeled A-F). These clusters are presented relative to their geographical locations in Figure 3. The two major clusters (B and C) accounted for 83.16% of the stations. The remaining 17 stations were grouped among 4 clusters which appeared to be randomly distributed among the stations. The depth stations at

each location generally grouped together indicating vertical homogeneity of plankton populations. The qualitative phytoplankton associations do not appear to be related to major water masses as might be expected from this region.

The results of the cluster analysis for the 64 X 101 cell volume matrix are presented in Table 2. In this matrix, phytoplankton biomass as measured by cell volumes is assessed. Two major clusters again result in 92 of the 101 station-depths being grouped to form a major dichotomy. These two major sub-groups fused at a similarity value of 0.493. A general large scale relationship between these sub-groups and their relation to a "plume" may be inferred (Figure 4). All standard stations closest to the coast clustered in one of these groups. Of the northernmost 21 standard stations only 2 standard stations (including their depths) seem to be outliers. Considering the possible patchy nature of phytoplankton populations these results appear to be representative of a plume or an onshore/offshore pattern. Three standard stations (801, 69 and 805) at the bay mouth clustered in such a way as to suggest that phytoplankton associations there are indicative of microscale changes within the water column. These results appear plausible considering the complexity of the currents in this general vicinity (ref. 16, 17). The southernmost transect seems to represent a reversal of the onshore versus offshore generality. It is noted that the results of the cluster analysis, as have been used here, are not hypothesis concluding. The procedure only allows a more objective approach at developing complex associations.

#### Polar Ordination

Polar ordination was performed on both of the matrices with varying results. As indicated by the results of the cluster analysis, the qualitative data were characterized by somewhat random distributions. Consequently, the ordination axes computed for these data were not significantly related to the environmental variables as assessed by multiple linear regression analysis. These results suggest the interactions by the species with the environmental variables measured are not sufficient to explain their qualitative distribution.

From the triangular dissimilarity matrix representing the 64 X 101 (species-volumes x station-depths) pairs a polar ordination was performed. Two ordination axes were computed. Regression analysis showed the first ordination axis was only weakly related to salinity which accounted for 23.3% of its variance (Table 3). A significant ( $\alpha = 0.05$ ) correlation between salinity and the first polar ordination axis existed. None of the remaining variables was significantly related to the dependent variable. There was no significant regression relationship between any of the environmental variables and the second polar ordination axis.

These results indicate a weak association between salinity and the change in species associations as assessed by the ordination technique. The general failure of environmental ordination procedures in cases involving many sites has been suggested by Boesch (ref. 25). As the number of sites increases, so does the inefficiency.

Ordinations of transect data were individually performed to decrease the site number and increase the efficiency of the ordination procedure. The station-depths from the six major transects (omitting standard stations #800 and 801) were ordinated (Figure 5A-F). The first polar ordination axes were observed to generally order the sites into an onshore versus offshore transition. The second polar ordination axes are not so easily generalized from the graphical presentation.

The results of the regression analyses of environmental variables on both the first and second axes are presented in Table 4. Of the measured variables, salinity was observed to account for most of the variance in the computed first polar ordination axes in two of the three northernmost transects and was significantly related to the second transect. Inorganic nutrients were observed to explain most of the variance in the second polar ordination axes for these northern transects. In the southern three transects the light and tide variables account for most of the variance in the ordination axes.

#### DISCUSSION

The existence of a biotic plume, as measured by phytoplankton volumes, is supported by the results of the classification and ordination analyses. While being a non-conservative property within the environment, phytoplankton biomass associations were observed to significantly covary with some conservative variables (salinity, silicates and phosphates).

The six major transects may be conveniently divided into two regions (northernmost three, southernmost three) which appear to have fundamentally different factors affecting their endemic phytoplankton populations. The basic environments of the populations within these two major regions appear to be different in lieu of the ordination results. The low salinity plume of water originating from the Chesapeake Bay which generally holds to the coast is a region of high division rates and standing crops (see Marshall, paper no. of this compilation). Within the southern three transects, of the measured variables, the light variable is most important in accounting for the variance in the population biomass shifts.

These results suggest relationships between the physical hydrology of the region and the phytoplankton communities. As indicated by the salinity data and previous summer studies (ref. 16) the water columns of the study area are generally stratified with a pronounced salinity gradient seaward. This gradient has an influence on the biomass associations as far south as the North Carolina border. Further south this effect is superseded by the summer stratification as indicated by the large proportion of variance in the ordination axes explained by the light variable.



The linear models used in this study were not expected to accurately describe the biological events. Walsh (ref. 26) has stated the problems of using linear models in biology most succinctly:

"Linear regression analysis is appropriate for preliminary insight into a complex system, but is an inadequate description of biological phenomenon. Linear relations...cannot be expected to fully describe or predict biological relationships which are basically non-linear and consist of thresholds, timelags, and saturation and inhibition effects."

#### NUMERICAL SUMMARY

1. The volumetric-biomass data proved more informative as related to environmental variables using regression analysis. These data more closely approximate the "plume" situation than the qualitative data.
2. The factors which influence phytoplankton populations within the region appeared to be complex.
3. A salinity gradient which was present within the northern transects covaried significantly with the phytoplankton biomass association changes.
4. Within the southern transects the normal summer stratification was related to phytoplankton populations with the light variable most important in explaining the variance.
5. The results suggest the importance of the physical hydrology in this system in influencing the phytoplankton associations.
6. Of secondary importance, the inorganic nutrients (silicates and phosphates) significantly covaried with the biomass changes within the northern transects.
7. The numerical methods, borrowed from the social scientists and the terrestrial phyto-sociologist, seemed to perform moderately well to extract information from large complex phytoplankton data matrices.

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Table 1. Order of depth-sites from dendrogram: qualitative data.

Station Sequences	
Group A S = .336	101, 100, 33, 24, 7, 5, 6, 23, 25, 15
Group B S = .339	98, 92, 97, 96, 95, 44, 43, 67, 51, 61, 40, 94, 49, 65, 59, 57, 58, 50, 42, 41, 30, 38, 66, 93, 91, 31, 87, 86, 85, 32, 52, 90, 89, 88, 39, 78, 77, 28, 76, 75, 60, 74, 63, 62, 73, 64, 26, 27, 29, 3, 2, 1
Group C S = .330	84, 83, 56, 72, 71, 70, 45, 55, 37, 48, 35, 36, 54, 53, 20, 19, 18, 47, 46, 34, 69, 68, 17, 16, 13, 12, 11, 4, 82, 81, 80, 79
Group D S = .367	22, 21, 14
Group E S = .259	10, 9, 8
Group F S = .209	99

Table 2. Order of depth-sites from dendrogram: quantitative data.

Station Sequences	
Group A S = .455	97, 90, 92, 88, 96, 95, 94, 51, 61, 40, 52, 44, 43, 93, 91, 67, 59, 57, 66, 58, 30, 50, 49, 31, 89, 88, 87, 85, 65, 32, 42, 41, 38, 39, 1, 2, 3, 78, 60, 77, 28, 29, 76, 75, 63, 62, 74, 73, 64, 26, 27, 24, 7, 6, 5, 23, 9, 8
Group B S = .482	84, 83, 72, 71, 48, 70, 55, 45, 69, 68, 56, 20, 19, 54, 53, 46, 36, 34, 18, 37, 35, 47, 17, 16, 13, 12, 11, 4, 33, 15, 25, 22, 21, 14
Group C S = .420	101, 100, 10
Group D S = .348	99, 98
Group E S = .310	82, 81, 80, 79

Table 3. Multiple regression results: polar ordination of 101 station-depths.

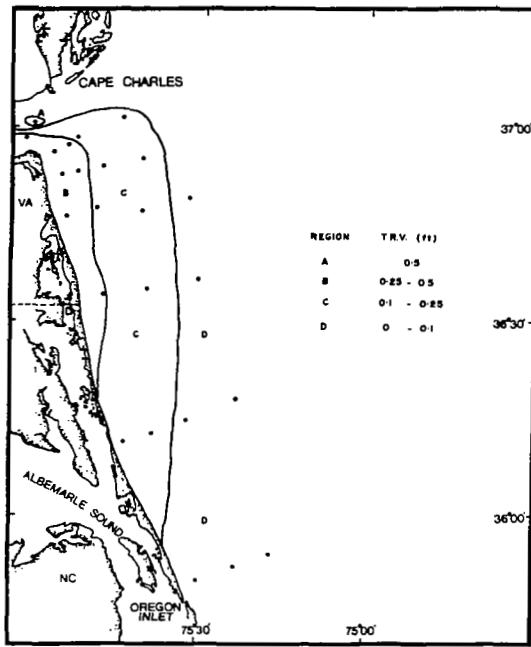
Dependent variable: polar ordination axis #1				
Independent Variables	F	R <sup>2</sup>	R <sup>2</sup> Change	Simple R
Salinity	29.13*	0.23283	0.23281	0.48253*
Temperature	20.52	0.30169	0.06885	-0.09978
Dissolved oxygen	15.17	0.32624	0.02456	0.09680
Total suspended matter	12.06	0.34151	0.01526	-0.23809*
Light	9.79	0.34731	0.00580	0.10983
Si	8.20	0.35110	0.00379	0.05055
Ammonia	7.04	0.35379	0.00269	0.07745
Nitrates	6.15	0.35615	0.00239	0.05675
Tide variable	5.44	0.35744	0.00125	-0.19218
Nitrites	4.85	0.35823	0.00079	-0.05649
Phosphates	4.38	0.35937	0.00114	-0.16556

\* = Significant,  $\alpha = 0.05$

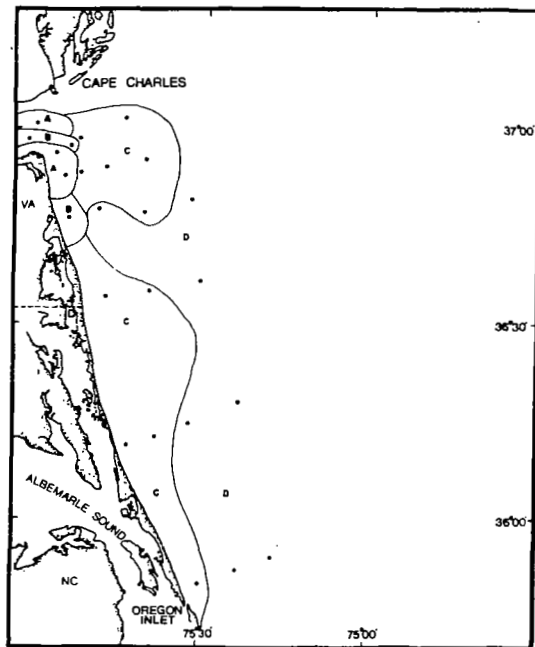
Table 4. Multiple regression results: polar ordination of transect data.

Transect	Dependent Variable	Independent Variable	R <sup>2</sup>	F
69-804	P.O. axis 1	Salinity	0.62609	18.418*
69-804	P.O. axis 2	Silicates	0.70027	25.699*
805-807	P.O. axis 1	Light	0.60840	20.203*
805-807	P.O. axis 1	Salinity	0.11008 (increase)	15.318*
805-807	P.O. axis 2	Silicates	0.46809	11.441*
808-811	P.O. axis 1	Salinity	0.43780	9.838*
808-811	P.O. axis 2	Phosphates	0.58076	18.008*
71-813	P.O. axis 1	Tide variable	0.73793	28.154*
71-813	P.O. axis 2	- - - - -	- - - - -	- - -
814-816	P.O. axis 1	Light	0.57611	24.514*
814-816	P.O. axis 2	- - - - -	- - - - -	- - -
73-818	P.O. axis 1	Light	0.60177	16.622*
73-818	P.O. axis 2	Tide variable	0.34562	5.809*

\* = Significant,  $\alpha = .05$



(a) Synoptic sampling.



(b) Superflux II sampling.

Figure 1.- Tide-related variable.



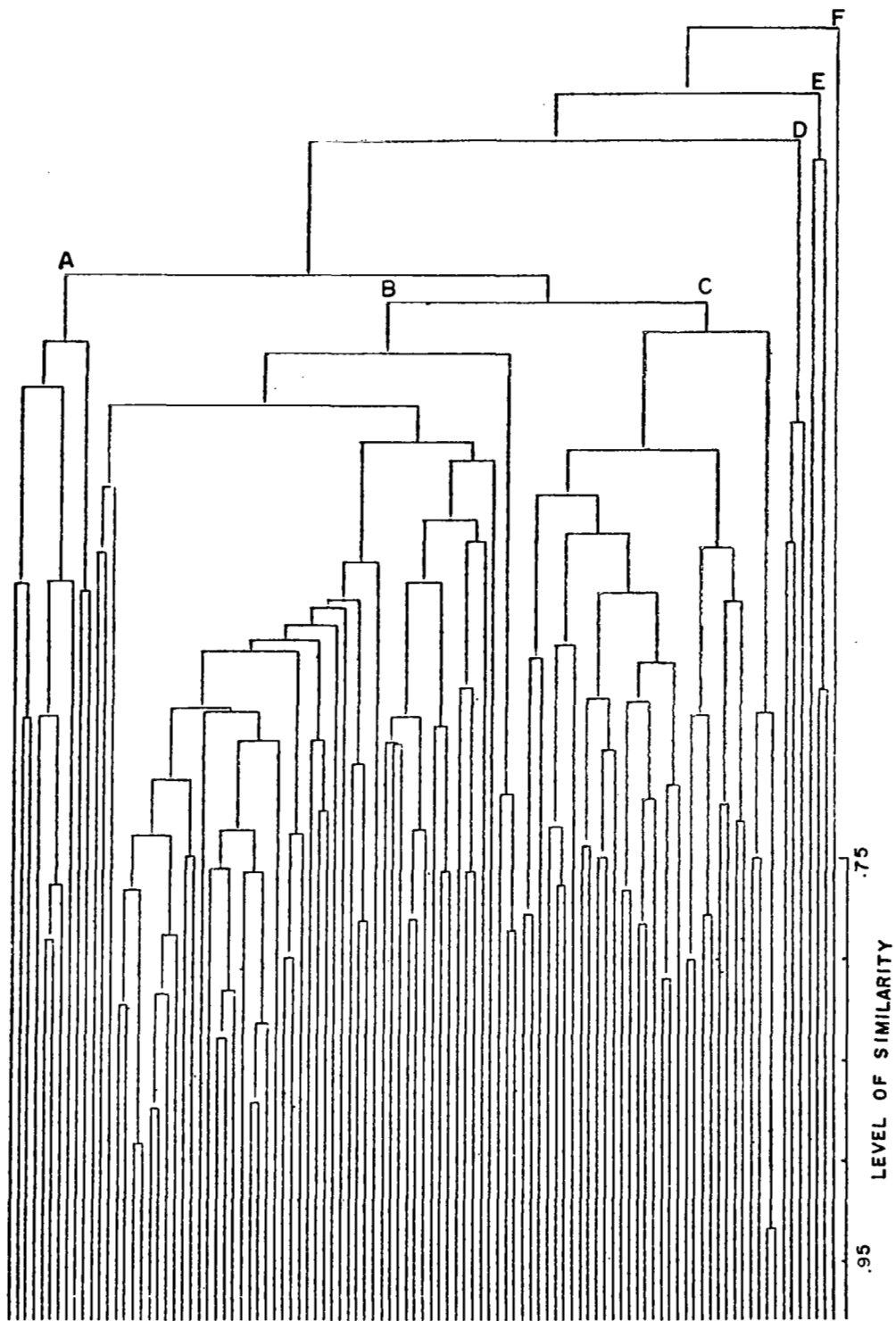


Figure 2.- Dendrogram sequence of stations.

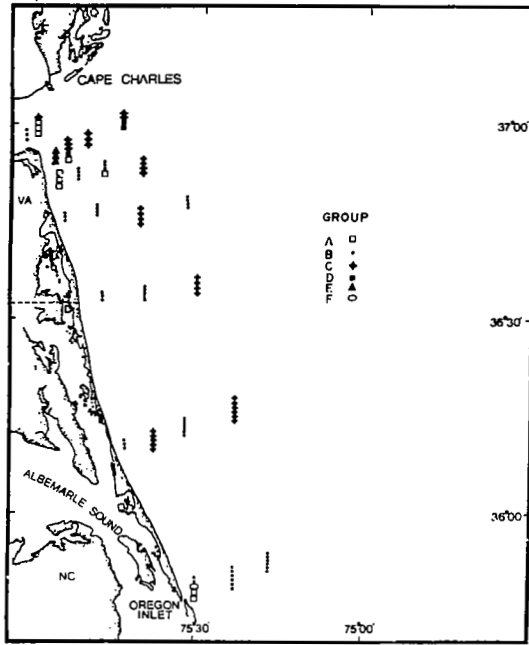


Figure 3.- Major qualitative clusters.

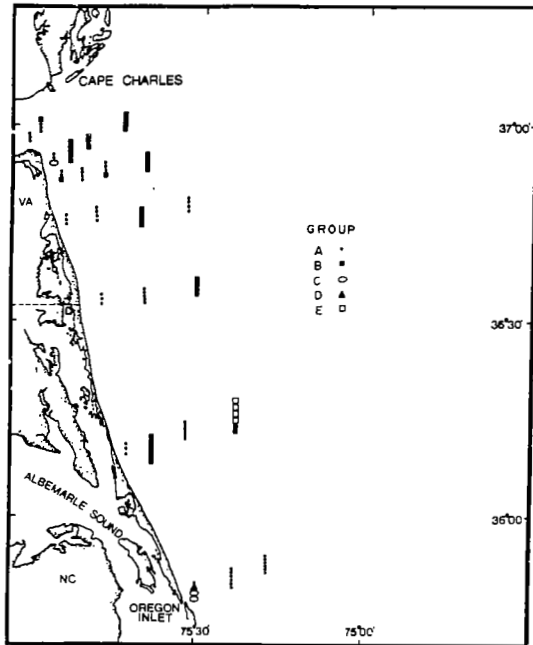
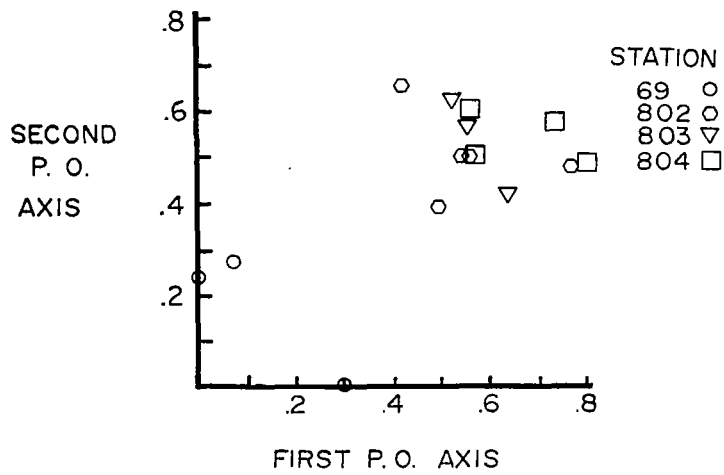
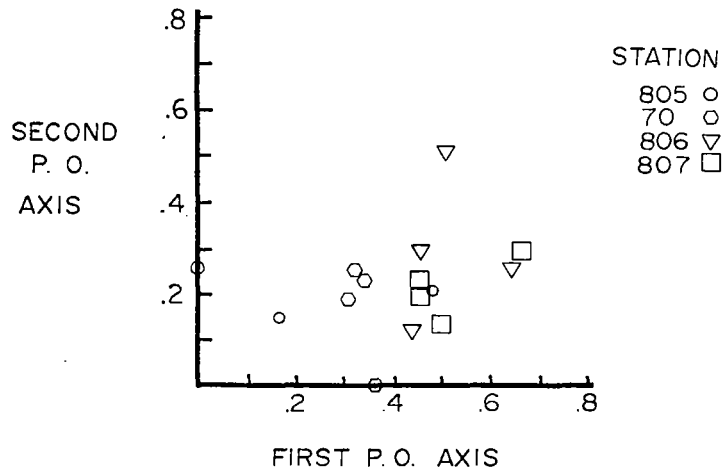


Figure 4.- Major qualitative clusters.

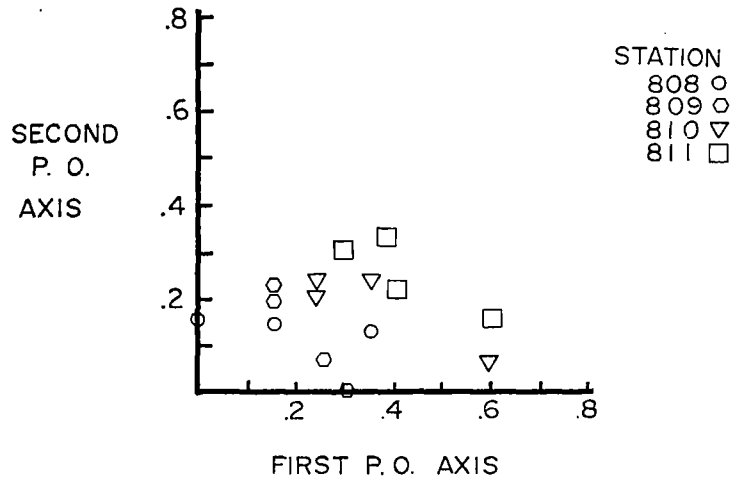


(a) Transect 69-804.

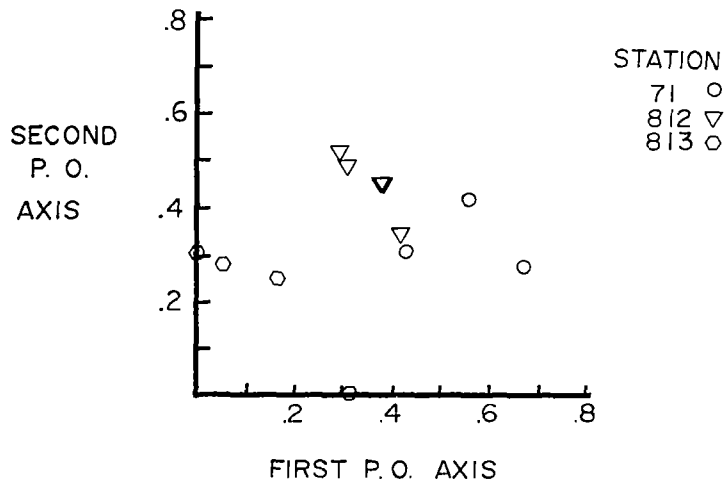


(b) Transect 805-807.

Figure 5.- Site ordination.

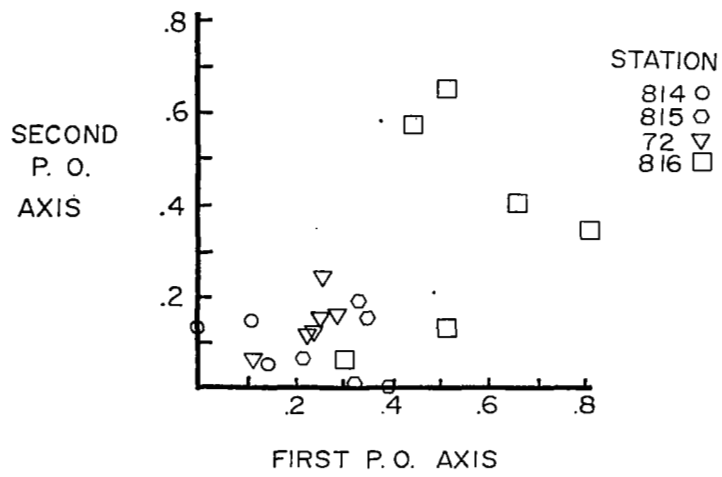


(c) Transect 808-811.

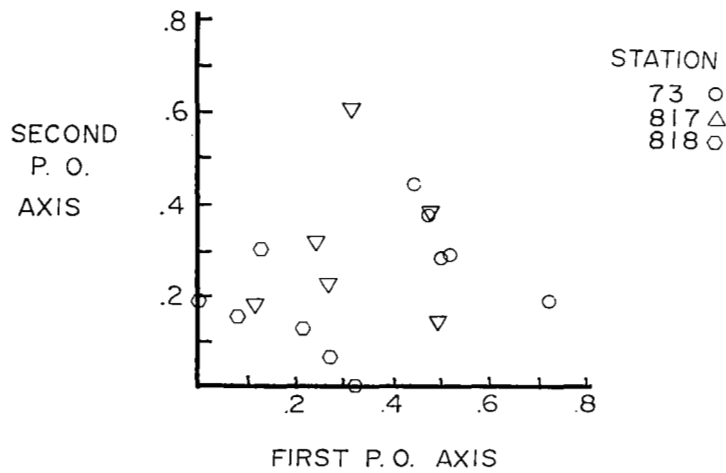


(d) Transect 71-813.

Figure 5.- Continued.



(e) Transect 814-816.



(f) Transect 73-818.

Figure 5.- Concluded.