Analysis of Baseline Studies of Zooplankton From Offshore Oil Lease Sites in the Eastern Gulf of Mexico

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Introduction

Variability in patchiness and composition of zooplankton populations present difficult problems in analyzing and obtaining a clear understanding of zooplankton community structure. Our first objective in this baseline study was to establish estimates of standing crop levels of selected zooplankton categories. These estimates which included numbers per cubic meter, biomass determinations, and diversity measurements, were used as a basis for the multivariate analysis and have been submitted as Monthly Report No. VIII. Supplements; Supplement III & IV. Since a number of factors in the water column, both biotic and abiotic, affect community structure either singly or in varying combinations, a multivariate analysis was used. {See Appendix II for a review of hydrocarbon effects of zooplankton). This multivariate approach allowed for: 1) correlation of the categories with available water column parameters; and 2) the examination of the variance-covariance structures to determine species/category associations. In addition, this study provides some recommendations for a longer term monitoring study.

Statistical Considerations

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The problems of analysis and interpretation of zooplankton data seem to require more than the "ordinary" statistical approaches. The results of earlier investigations (e.g. Cassie, 1963) suggest that zooplankters are not randomly distributed throughout the water column. Rather, they seem to show distributions that are highly correlated with the conditions of their immediate environment. Thus, an analysis and interpretation approach that would attempt to explain the observed variation in the standing crops of various types of zooplankters should incorporate as much information about the environment as is possible. This information must be relevant to the zooplankton sample obtained and therefore one should strive for as near to simultaneity of water column sample collection as is possible.

Another aspect of zooplankton analysis that rules out the more standard statistical approaches concerns the diversity of the samples. In many cases, of which this is one, the researcher is interested in most, if not all, components of the zooplankton community. Analysis by univariate statistical methods (i.e., considering each component species or group separately) assumes that each of the zooplankton categories behaves independently of all the other categories. This type of approach tends to ignore the importance of interaction, or covariance, among the members of the zooplankton community. Another related problem of the

univariate approach results from the confusing multitude of patterns possible from many univariate analyses of the same statistical model. With thirty or more zooplankton categories each to be used as a univariate dependent variable for a regression or analysis of variance model, there is a distinct possibility that no two categories will show the same results. In"addition, the time consumed in carefully interpreting the results of each univariate analysis often makes adequate analysis difficult within reasonable time constraints. All of this adds up to the result that a multivariate analysis approach supplies the best techniques for interpreting the zooplankton data. This is true from a theoretical statistics aspect, since we have multiple intercorrelated variables (the standing crop of zooplankton categories) from each observation, and from a biological standpoint as the multivariate approach is best suited to supply information for inference into the biological questions. "The ability to discern patterns of zooplankton abundances, identify species assemblages, and detect statistically significant differences in zooplankton communities, both in structure and abundances are afforded via multivariate analysis.

In a univariate analysis, one considers a statistical model with single dependent variate (e.g., density of calanoid copepods/cubic meter) and an independent set of variables that vary in their number and complexity. Since there is only one variable, each sample could be represented by its location on a line or single axis. In fact, most of the statistical hypothesis testing performed revolves about hypotheses concerning the location parameter (e.g., mean, mode, or median) of groups of samples. If one were to add another dependent variable to consider simultaneously with the first (e.g. density of

cyclopoid copepods") then each bivariate sample could be defined by its location in a two dimensional coordinate system. One axis would be the density of calanoid copepods and the other axis would be the density of cyclopoid copepods. If one adds variables, then one also adds axes to the coordinate system, until one has a multivariate system with a dependent vector of p-variates described by a p-dimensional hyper-space. Thus, at least part of multivariate analysis involves the testing of hypotheses concerning the location parameters, mean vectors, of various groups of samples, where each sample involves the measurement of more than one variable on each unit of observation (e. g., a parcel of Eastern Gulf water with the standing crop of various zooplankton categories being the dependent variables).

All samples have variability which can be apportioned to various sources: noise in measurement, environmentally-induced variations, and covariance with other dependent variables. This necessitates the consideration of the dispersal parameter of a sample (e.g. variance, range) when testing hypotheses about the location parameter. The . . . variability of the data and our ability to reduce this variability through experimental design and explaining it via covariance with other variables determines the precision with which we can place the location parameter of a group of samples. Thus, in much of our hypothesis testing we are asking "within the precision afforded by the data is it possible to distinguish between the location parameters of these groups of samples"? Other types of testing are concerned with specific hypotheses about the dispersion parameters themselves.

It is this dispersion or variability of the data that requires confidence intervals to be associated with the location parameter. Thus, in the univariate case, the location and dispersion parameters

help to define a line segment on the coordinate axis. In a bivariate system one obtains an ellipse with the intersect" on of its major and minor axes being the location parameter. In 'the p-dimensional, multivariate case the result is a hyper-ellipse. Therefore, we can mentally visualize the basis for multivariate hypothesis testing, much of which is, at least conceptually, a generalization from the univariate, single dimension case to the multivariate case. For example, there are multivariate t-tests and analyses of variance <MANOVA).

Considering the dependent variables as axes for a multidimensional coordinate system, we then utilize a multivariate analysis of variance with covariates (MANCOVA) as the tool for ascertaining the information contained in the raw data. The analysis of variance terms in the model are those factors that do not vary continously but have several discrete states, such as lease tract areas, stations within lease tract, etc. These are coded as dummy variables as explained by Searle (1971). The covariates are continuous variables that represent various aspects of the environmental condition at the time of the sample collection. The test for significance of the terms in the model involved a transformation of the multivariate test statistic Wilks' λ to an approximate F statistic. The transformation was developed by Rao (1952) and explained fully in Cooley and Lohnes (1971). The transformation is as follows:

$$(MS - 2B) / (PQ) (1 - \lambda^{1/S}) \lambda^{1/S} \sim F_{P,MS-2B,\alpha}$$

where,

M = (error degrees of freedom) - $.5(P-Q+1) \ln(\lambda)$

$S = (P^2 Q^2 - 4) / (P^2 + Q^2 - 5)$

$B = (PQ - 2) / 4$

" P= Number of dependent variables

Q = Rank of hypothesis matrix

The significance of one of the terms in the model has the same basic interpretation as in ANOVA. For covariates it indicates that a significant portion of the dependent variable's variance and co-variance, or multivariate dispersion parameter, is "explained" by variation in the covariate. For the AOV variable it shows a detection of a significant difference in the mean vectors, location parameter, of the discrete states of the factor under consideration.

Once significance is detected, the next problem encountered involves explaining the results in a biologically meaningful manner. For this we use the approach known as Canonical Discriminant Analysis (Cooley and Lohnes, 1971). Since the dependent variables show covariance (if this weren't so, we would use univariate statistics), there "is some redundancy contained in the original coordinate axes. (In the following discussion, the coordinate axes are equivalent to the original dependent variables). Thus, it is possible to make a rotation in axes with the following constraints: (1) the first axis will contain the greatest amount of between groups variation, (2) all succeeding axes are formed so that they are orthogonal (i.e., perpendicular or independent) to the preceding axes and explain the next greatest amount of between group variation and (3) all axes are linear combinations (e.g., linear axes formed by rotation of the original coordinate system) of the original variables. Thus, by concentrating the information content of the original coordinate system, we reduce the dimensionality of the problem with a minimal loss of information. The procedure is further constrained so that (4) the axes maximize the following determinental equation:

$$(\underline{W}^{-1}\,\underline{A} - \underline{\lambda}I)\,\underline{V} = 0$$

where,

$\underline{W}$ represents the within groups sum of squares matrix,

$\underline{A}$ represents the among groups sum of squares matrix, and

$\underline{\lambda}$ and $\underline{V}$ are the characteristic roots and vectors.

This procedure supplies u's with a technique for graphically displaying the differences between groups (e.g., between lease tracts, between stations, between surface, midwater and bottom, or any interactions). Additionally, the axes provide the key for interpreting and discovering what the differences represent. Since the axes are linear combinations, a score for a canonical discriminant function is defined as follows:

$$\text{CDF} = \sum_{i=1}^{p} \{\text{weighting for ith original variable as determined by the canonical discriminant function analysis)} * \text{(score for ith original variable)}.$$

The weighings are obtained from the. normalized characteristic vector of the hypothesis matrix." One may then calculate correlations between the original variables and the newly formed CDF variables (remember variables are equivalent to axes). The sign of the correlation and its magnitude signify the effect the variable has on the CDF score for a sample. For example, a large positive correlation indicates that the variable will have the effect of increasing the CDF score, a large negative correlation will indicate a reduced CDF score, and a correlation close to zero will show no effect on the CDF.

In interpreting the results, one should make use of the original variable correlations with the CDF axes to identify what the axes represent and the position of the points on the graph to see the

relationship of the groups to each other. In addition, the characteristic roots supply information concerning the portion of the total variation explained by the factor under consideration that is contained by a particular axis:

CDF axis proportion of variation explained = $\lambda i / \Sigma \lambda i$

The number of canonical discriminant axes possible is a function of two parameters. It is the minimum of either one less that the number of original variables (P - 1) or the degrees of freedom of the factor or covariate in question. Thus, if there are 30 variables and two groups, there is one CDF axis; or if there are two variables and 30 groups, there is one CDF axis possible.

Two-way interaction terms present an additional graphic approach to interpretation. If one plots the mean vector CDF scores for each discrete group outlined by a two-way interaction, polygons may be formed by connecting those points with common group values. Thus, for a station depth, interaction points for the three depths common to a single station would be joined or points of stations at a common depth could be joined. The size and orientation of the polygons thus joined supply information about the variability and community make-up of the various terms in the model.

The analysis of the control stations was handled somewhat differently than the other stations. The procedure used was to plot the control stations on the CDF coordinate system formed by the lease tract stations. This will show the relationships of the control stations with the various lease tracts without allowing the differences between the control stations and the lease tract stations to influence the forming of the axes. Thus, we will be able to assess the applicability of the control station selection.

It was found to be necessary to analyze separately the data from Lease Tracts i, II, and III and the data from Lease Tracts IV and V. This resulted primarily from significant differences in the assignments of counting and identification categories in the data sets of the two areas. Combination of the two data sets would have required an extreme reduction in the number of data categories used in analysis, since only comparable categories from each data set could have been used. This would have been most noticeable in the copepods, where the 11 and 16 data categories available under separate analysis of the two data sets would have been reduced to four with-no greater distinction than to Order. A great deal of information would have been lost by this. Also, samples from tracts IV and V were collected almost exclusively during the daylight hours, necessitating the exclusion of sunlight and sunlight-depth interaction terms from any model dealing with tract IV and V data. Again, considerable information would have been lost by this merger of data sets. Finally, in the case of each data set, a separate analysis was performed with total zooplankton density and species diversity, as measured by the Shannon-Weaver Species Diversity Index, in the place of individual zooplankter densities. Combining both data sets would have put the analyst in the position of comparing Shannon-Weaver indices that were not comparable in any sensible way, or of foregoing this analysis entirely.

Once the final data set from the five lease tract areas and four control stations had been assembled, statistical analysis was begun utilizing the Statistical Analysis System (SAS) Multivariate Analysis of Variance (MANOVA) routines on the University of Florida's Northeast Regional Data Center (NERDC) IBM 370/165 Computer.

It was first necessary to investigate the model which was to be used to study the zooplankton community structure in the five lease tract areas. For the purposes of this report, the following general model was selected:

$$\underline{Y}ijkl = \underline{\mu} + \underline{B}i + \underline{L}(i)j + \underline{D}k + \underline{BD}jk + \underline{H} + \underline{S} + \underline{SD} + [\underline{CH_4}] + [\underline{C_2H_4}] + [\underline{C_3H_8}] + E$$

Y = the vector of observed zooplankton responses.

μ = the vector of estimated general mean levels.

B = the vector of effects 'due to differences between lease tracts i = 1, 2, 3.

L = the vector of effects due to within-lease tract differences j = 1, 2, 3.

D = the vector of effects due to depth variation k = 1, 2, 3.

ED = the vector of effects due to interaction between depth effects and lease tracts effects.

H = the vector of effects associated with variations due to difference in time samples were collected.

S = the vector of effects associated with differences due to the presence or absence of sunlight.

SD = the vector of interactive effects involving differences in relative abundance of sunlight and depth of sample. --- '-"

$[CH_4]$ = the vector of effects due to the presence of methane in the water column.

$[C_2H_4]$ = the vector of effects "due to the presence of ethene in the water column.

$[C_3H_8]$ = the vector of effects associated with the presence of propane in the water column.

E = effects due to residual variance - covariance patterns.

In order to take a conservative approach to statistical inference and interpretation, we considered terms in the model to be statistically significant only if the probability that the observed test statistics would occur by chance was less than or equal to .005.

For analysis purposes, the 81 zooplankton categories used for identification and density counts of Lease Tract I, II, and 111 samples were condensed to 33 categories, and these 33 categories were transformed using the equation:

$$\text{density} = \log_{10}(\text{density} + 1)$$

so that their distribution more closely resembled the multivariate normal distribution. The condensing of categories was carried out according to the following scheme:

Reporting Category	Counting Category
1. *Globigerina*	*Globigerina*
2. Other Protozoa	*Pyrocystis* Tintinnids
3. Siphonophores	Siphonophores
4. Medusae	Hydromedusae Scyphozoan medusae
5. Polychaetes	Polychaetes
6. Gastropod veligers	Gastropod veligers
7. Pteropods	*Cavolina longirostris* *Clio* species *Creseis virgula* *Limacina inflata* *Limacina leseuri* Other Thecosomata *Desmopterus papilio* *Gymnosomata* species A
Reporting Category	Counting Category con't.
	Gymnosomata species B Other Gymnosomata
8. Bivalve larvae	Bivalve larvae
9. Cladocera	Cladocera
10. Ostracods	Ostracods
11. *Centropages furcatus*	*Centropages furcatus*

12. *Eucalanus* species	*Eucalanus elongatus* *Eucalanus* species, other
13. *Undinula vulgaris*	*Undinula vulgaris*, female
14. Other Calanoid copepods	*Candacia curta* *Euchaeta marine* *Mecynocera clausii* *Pontella* species *Rhincalanus cornutus* *Scoleothrix danae* *Temora* species Other Calanoids
15. Harpacticoid copepods	Harpacticoid copepods
16. *Corycaeus* species	*Corycaeus* species
17. *Oithona* species	*Oithona* species
18. *Oncaea* species	*Oncaea* species
19. Other Cyclopoid copepods	*Copilia mirabilis*, female *Copilia mirabilis*, male *Copilia quadrata*, female *Copilia quadrata*, male *Corissa* species *Farranula* species *Sappinrina* species *Vettoria* species Other cyclopoids
20. Copepodites	Calanoid copepodites Harpacticoid copepodites Cyclopoid copepodites
21. Copepod Nauplii	Copepod Nauplii
22. *Lucifer* species	*Lucifer faxoni* *Lucifer*, mysis-stage
23. Other shrimp-like forms	Other shrimp-like forms

Reporting Category	Counting Category con't.
24. Crab larvae	Crab zoea Crab megalops
25. Other crustacea	Barnacle larvae Stomatopod larvae Mysids Amphipods Euphausiids Phyllosoma larvae Anomurans Other crustaceans

26. Echinoderm larvae	Echinoderm larvae
27. Chaetognaths	*Sagitta enflata* *Sagitta hispida-helenae* complex *Sagitta tenuis-bipunctata* comple Other chaetognaths
28. *Oikopleura*	*Oikopleura*
29. *Fritillaria*	*Fritillaria*
30. Other Tunicates	Doliolida Salpida Other Thaliaceans Other Larvaceans
31. Fish eggs	Fish eggs
32. Fish larvae	Fish larvae
33. Other plankters	Heteropods Cephalopods Trochophore larvae Other plankters

B

D

B ,

Hydrocarbon Data

The data used here for CH_4, C_2H_4 and C_3H_8 levels in the MAFLA area leases were furnished by Dr. Sackett of Texas A & M University. The authors are indebted to Dr. Sackett for his prompt and clear delivery of data for inclusion in this effort to model statistically zooplankton community structure.

Other Environmental Data

Data from other participating investigators were not received in time to be included in this analysis. Inclusion of these data in any future analyses will add significantly to the detail of the model.

Model for Lease Tracts I, II, and 111
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Differences Between Lease Tracts

The significance of each term in the model and the percent variation accounted for by the CDFaxes for each term are presented in Table 3. Also included are the mean values of the canonical variables which are important for the analyses of each of the terms of the model. Figures 1 through 7 are basedupon these mean values.

Significant differences were found between lease tracts with respect to community structure as measured by the categories of the model. In Figure 1 the position of each lease tract in the CDF space is given based upon the CDF I and the CDF II axis. The analysis shows that CDF I accounts for 55.5% of the variation between lease tracts and that CDF II accounts for 44.50% of that variation.

Table 2 shows the composition of CDF I and CDF 11 and the correlation of each of the dependent variables with these functions'. Examination of these data reveal that CDF I has its largest positive correlation with other protozoans, other tunicates, Oncaea sp., gastropod veligers, Globigerina, and pteropods. There are no important negative correlations. CDF II has important positive correlations with ostracods, Centropages furcatus, bivalve larvae, shrimp and other tunicates.

It is seen in Figure 1 that Lease Tracts I and II have approximately the same coordinate in terms of CDF II, but very different coordinates on the CDF I axis; thus, it is CDF I which separates them. Conversely, CDF II serves to separate Lease Tract II from Lease Tract III.This means that Lease Tract 11 has some similarities with the lease tract to

Table 1 Summarized MANOVA Results for Lease Tract I, II, III Model

Source	Milks' λ	Significance	# of CDF axes Possible	%' Factor Variation Explained by each CDF Axis	CDF Mean Values		
					G r o	CDF I	CDF II
Lease Tract	.0407762	.0001	2	55.5	Tract I	-.381	.331
				44.5	Tract II	.371	.320
					Tract III	.369	-.218
Stations within Lease Tract	.0021804	.0001	6	40.70	Tract I Station 1	.358	-.426
				31.01	Tract I Station 2	.244	-.346
				11.80	Tract I Station 3	.574	-.200
				9.66	Tract II Station 1	-.173	.372
				3.51	Tract II Station 2	-.196	.547
				3.32	Tract II Station 3	-.468	-.162
					Tract 111 Station 1	-.227	.203
					Tract 111 Station 2	-.174	.313
					Tract 111 Station 3	-.148	.254
Depth	.085287	.0001	2	64.00	Surface	-.252	-.203
				36.00	Mid water	.207	.108
					Bottom	-.084	.317

Table 1 (Continued)

Source	Wilks' λ	Significance	# of CDF axes Possible	% Factor Variation Explained by each CDF Axis	CDF Mean Values		
					Group	CDF I	CDF II
Lease Tract by Depth	.01 15901	. 0001	4	41.33	Tract I. Surface.	.088	-.215
				26.28	Tract I. Mid water	.238	-.315
				21.09	Tract II Bottom,	.643	.026
				11.31	Tract II Surface	.096	.288
					Tract II Mid water	.410	-.120
					Tract II Bottom.,	.70.2<	-.269,
					Tract III Surface	. 367/	.165
					Tract III Mid water	.194.	.112
					Tract III Bottom	.069	.172
Depth by Sunlight	. 08581/	.0002:	4,	41.09	Surface Night	-.2,74.:	.39.8.
				31.86	Surface Dusk	-. 164	.3 25
				16.10	Stir-face. Day;	.062	. 246
				10.94':	Mid water Night	.184	..1 20

Table 1 (Continued)

Source	Wilks' λ	Significance	# of CDF axes Possible	% Factor Variation Explained by each CDF Axis	CDF Mean Values		
					Group	CDF I	CDF 11
Depth by Sunlight (Cont.)					Mid water Dusk	.098	.026
					Mid water Day	-.044	-.024
					Bottom Night	-.106	-.091
					Bottom Dusk	.064	.168
					Bottom Day	-.072	.071
Hour	.0021880	.3804		not significant			
Sunlight	.4758546	.7121		not significant			
CH_4	.5735710	.0857		not significant			
C_2H_4	.4848670	.0049	1	100%	---	---	---
C_3H_8	.3758282	.0001	1	100%	---	---	---

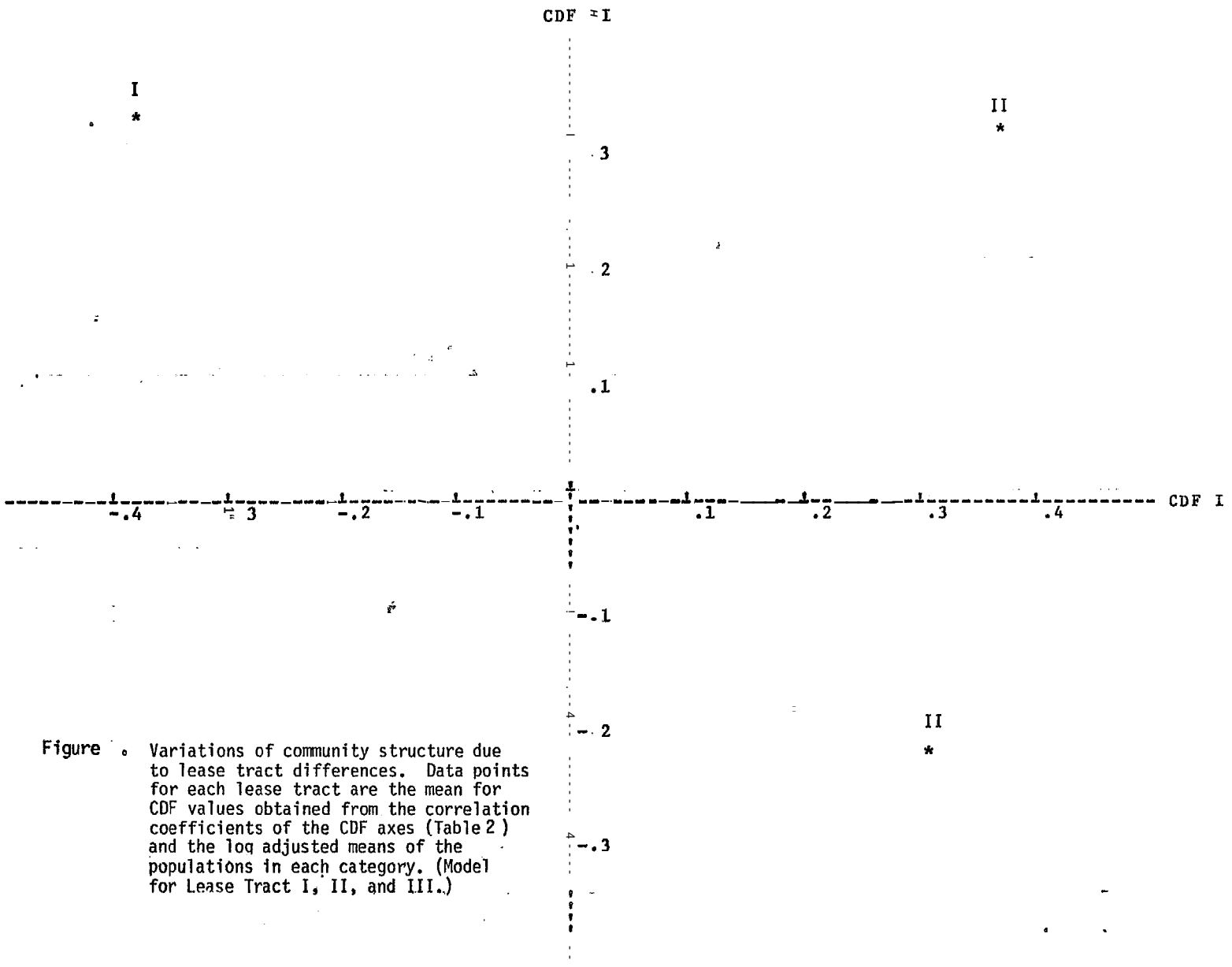


Figure . Variations of community structure due to lease tract differences. Data points for each lease tract are the mean for CDF values obtained from the correlation coefficients of the CDF axes (Table 2) and the log adjusted means of the populations in each category. (Model for Lease Tract I, II, and III.)

Table 2 Correlation coefficients between canonical variables and the dependent variables for between lease tracts (Model for lease tracts I, II, and III)

Categories	CDF I	CDF II
Globigerina	0.183	0.025
Other Protozoans	0.597	0.165
Siphonophores	0.065	-0.118
Medusae	0.010	-0.083
Polychaetes larvae	0.045	0.066
Gastropod veligers	0.204	0.090
Pteropods	0.181	0.002
Bivalve larvae	0.074	0.203
Cladocerans	0.077	0.135
Ostracods	0.053	0.393
Centropages furcatus	-0.066	0.266
Eucalanus sp.	0.123	0.069
Undinula vulgaris	-0.010	0.076
Other Calanoids	0.040	0.052
Harpacticoids	0.180	-0.071
Corycaeus sp.	0.088	0.020
Oithona sp.	0.164	-0.091
Oncaea sp.	0.232	-0.041
Other Cyclopoids	0.109	0.032
Copepod copepodites	0.032	0.110
Copepod nauplii	0.021	0.072
Lucifer faxoni	0.139	-0.034
Other shrimp-like forms	0.129	0.183
Crab larvae	-0.011	0.168
Other Crustaceans	0.047	0.046
Echinoderm larvae	0.000(4)	0.028
Chaetognaths	0.071	-0.015
Oikopleuridae	0.092	0.166
Fritillaridae	0.159	0.131
Other Tunicates	0.259	0.177
Fish eggs	0.085	-0.033
Fish larvae	0.060	0.011
Other zooplankters	0.059	0.010

the south and other similarities to the more northern lease tract. In other words, just as Lease Tract 11 occupies an intermediate position geographically, its position in the variation space is also intermediate.

When the differences between Lease Tracts I, II, and III are examined in light of the correlations presented in Table 2 the following conclusions can be drawn:

1. Lease Tract 11 has more other protozoans, other tunicates, Oncaea sp., gastropod veligers, Globigerina, and pteropods than Lease Tract I.

2. Lease Tract 111 has fewer ostracods, Centropages furcatus, bivalve larvae, shrimp, and other tunicates than Lease Tract II.

An important variation between Lease Tracts I and II is accounted for by the variation in other protozoans. The category "Other Protozoans" is largely made up of Pyrocystis. Thus, the separation of Lease Tracts II and III from Lease Tract I is largely due to a variation in the Pyrocystis catch.

Station Variations Within Lease Tracts

The model indicated that there were significant variations among stations within lease tracts. Figure 2 is a graphical presentation of the position of each station on the CDF space as defined by the first two CDF axes. CDF I accounts for 40.70% of the variation between stations and CDF II for 31.01%. The plane which is presented-therefore accounts for 71% of the variation between stations.

It is apparent from the examination of Figure 2, that Lease Tract III exhibited much less variation among its stations than did Lease Tracts I and II. The variation between stations in Lease Tract II is largest among CDF II. The largest variations in Lease Tract I are along CDF I.

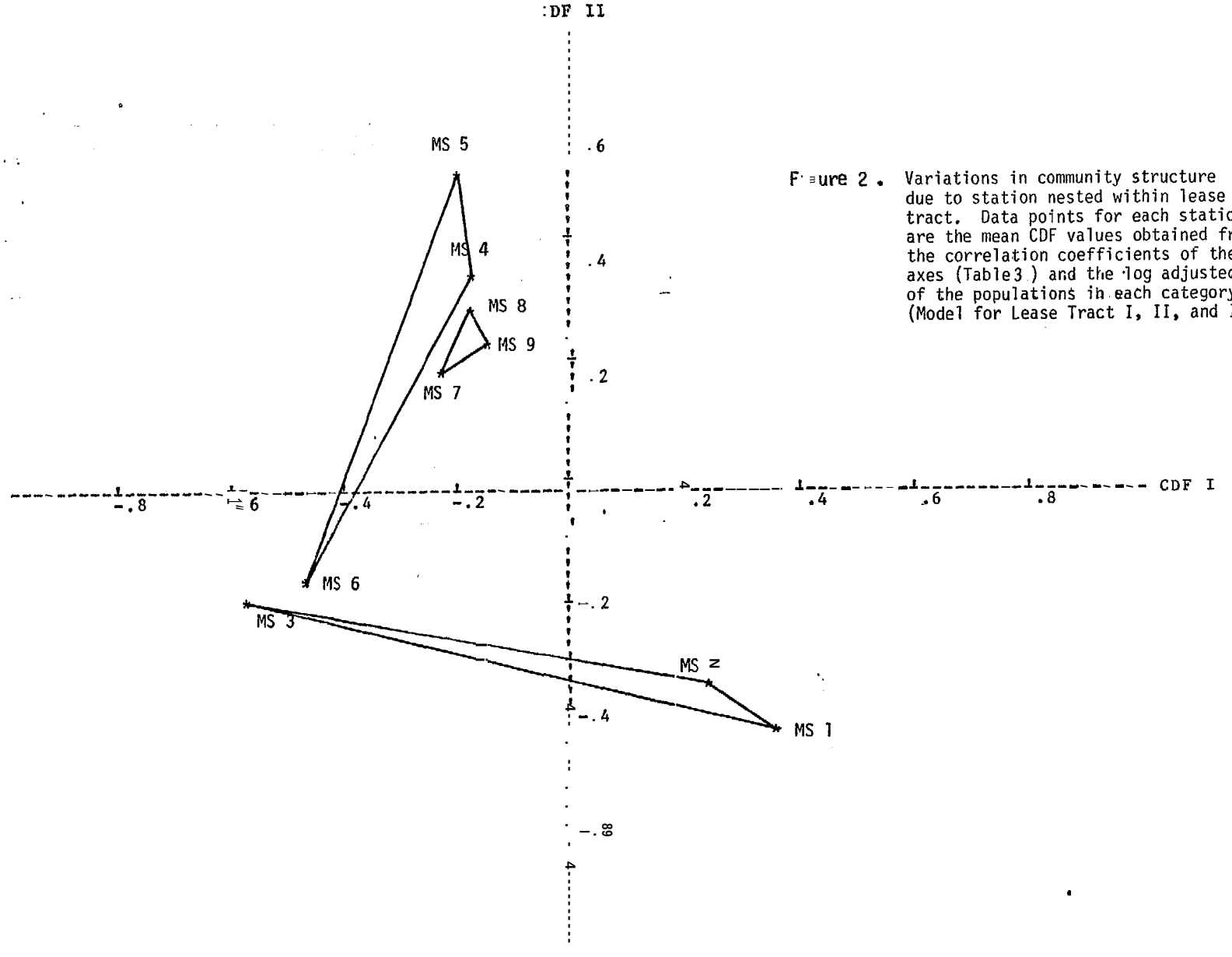


Figure 2. Variations in community structure due to station nested within lease tract. Data points for each station are the mean CDF values obtained from the correlation coefficients of the CDF axes (Table 3) and the log adjusted means of the populations in each category. (Model for Lease Tract I, II, and III)

Table 3 presents the correlations of the dependent variables with the CDF axes. CDF I has strong correlations with Oikopleura, Globigerina and Corycaeus also, an extremely strong correlation exists with other protozoans. As before, this category is strongly associated with the presence of Pyrocystis. CDF II has strong positive correlations with Oncaea, bivalve larvae, other protozoans, cladocera, pteropods, Corycaeus sp., and other cyclopoids. By comparison, there are no strong negative correlations.

The variations of the stations of Lease Tract I can now be associated with the zooplankton community. Station MS-3 varies from MS-1 and MS-2 most markedly along the CDF I axis. MS-3 has less Oikopleura, Globigerina and Corycaeus and more other protozoans than stations MS-1 and MS-2 of Lease Tract I. An examination of the means of "other protozoans" (Appendix XII reveals that MS-1 and MS-2 of Lease Tract I have far fewer other protozoans (Pyrocystis) than other stations. Now we can say with statistical confidence that the earlier separations of Lease Tract I from 11 and III due to a decrease in Pyrocystis is actually a reflection of the uniqueness of Stations MS-1 and MS-2 of Lease Tract I. It is really MS-1 and MS-2 which are different. Station MS-3 is much more like other lease tract stations in terms of its population of Pyrocystis. While Pyrocystis levels are higher in MS-3, the correlations with CDF I indicate that MS-3 has generally lower levels of Oikopleura, Globigerina and Corycaeus than do MS-1 and MS-2.

The variation of stations within Lease Tract II is largely along the CDF 11 axis. Particularly, MS-6 is separated from MS-5 by CDF II. CDF 11 is positively correlated with Oncaea sp., bivalve larvae, other protozoans, Cladocera, pteropods, Corycaeus and other cyclopoids. Thus,

Table 3 Correlation coefficients between canonical variables and the dependent variables for station nested within lease tract (Model for lease tracts 1, II, and 111)

Categories	CDF I	CDF II
Globigerina	0.215	0.208
Other Protozoans	-0.352	0.304
Siphonophores	0.012	0.134
Medusae	-0.005	0.053
Polychaetes larvae	0.138	0.157
Gastropod veligers	0.103	0.149
Pteropods	0.016	0.283
Bivalve larvae	0.166	0.347
Cladocerans	-0.009	0.293
Ostracods	0.016	-0.075
Centropages furcatus	-0.061	0.011
Eucalanus sp.	0.118	0.093
Undinula vulgaris	0.113	-0.112
Other Calanoids	0.118	0.216
Harpacticoids	0.068	0.025
Corycaeus sp.	0.177	0.278
Oithona sp.	0.132	0.116
Oncaea sp.	0.059	0.401
Other cyclopoids	0.081	0.272
Copepod copepodites	0.049	0.153
Copepod nauplii	0.141	0.064
Lucifer faxoni	0.000(3)	-0.059
Other shrimp-like forms	0.118	0.078
Crab larvae	0.160	-0.003
Other Crustaceans	0.058	0.055
Echinoderm larvae	0.171	-0.004
Chaetognaths	0.041	0.091
Oikopleuridae	0.300	0.212
Fritillaridae	0.167	0.103
Other Tunicates	-0.053	0.078
Fish eggs	0.110	0.040
Fish larvae	0.059	-0.088
Other zooplankters	0.049	0.037

Station 6 has fewer of these organisms than Stations 4 and 5.

Lease Tract III displayed the least variation among its stations.

As Figure 2 reveals, the stations are separated on the plane of CDF I and II into three rather distinct groups: 1) a group of five stations -- all of the Lease Tract III stations and MS-5 and MS-4 of Lease Tract II; 2) a group consisting of MS-3 (in Lease Tract I) and MS-6 (in Lease Tract II); and 3) a group consisting of MS-1 and MS-2 in Lease Area I. Note that the group of MS-3 and MS-6 is separated by CDF II from the largest group of MS-4, 5, 7, 8 and 9 and from the MS-1 and 2 group by CDF I. If the two smaller groups are characterized by separation from the largest group, the following conclusion can be reached: 1) the group of MS-3 and MS-6 is lower in Oncaea, other protozoans (Pyrocystis), bivalve larvae, and associated animals in CDF II; and 2) the group of MS-1 and MS-2 has even fewer other protozoans (Pyrocystis) and more Oikopleura.

These groups can possibly be related to water mass configurations occupying the eastern Gulf of Mexico at the sampling time. More hydrographic data are needed for inclusion in the model in order to investigate this possibility.

See the later section dealing with control stations for a discussion of the association of control and master stations.

Variations Due to Depth

Figure 3 shows the variation due to depth as determined by the CDF I and CDF II axes. CDF I accounts for 64.0% of the variation due to depth and CDF 11 accounts for 36.0%. The depth of tow was classified for the purposes of this analysis as surface, midwater, or bottom. These depth samples are marked" y separated on the CDF I and CDF II pane.

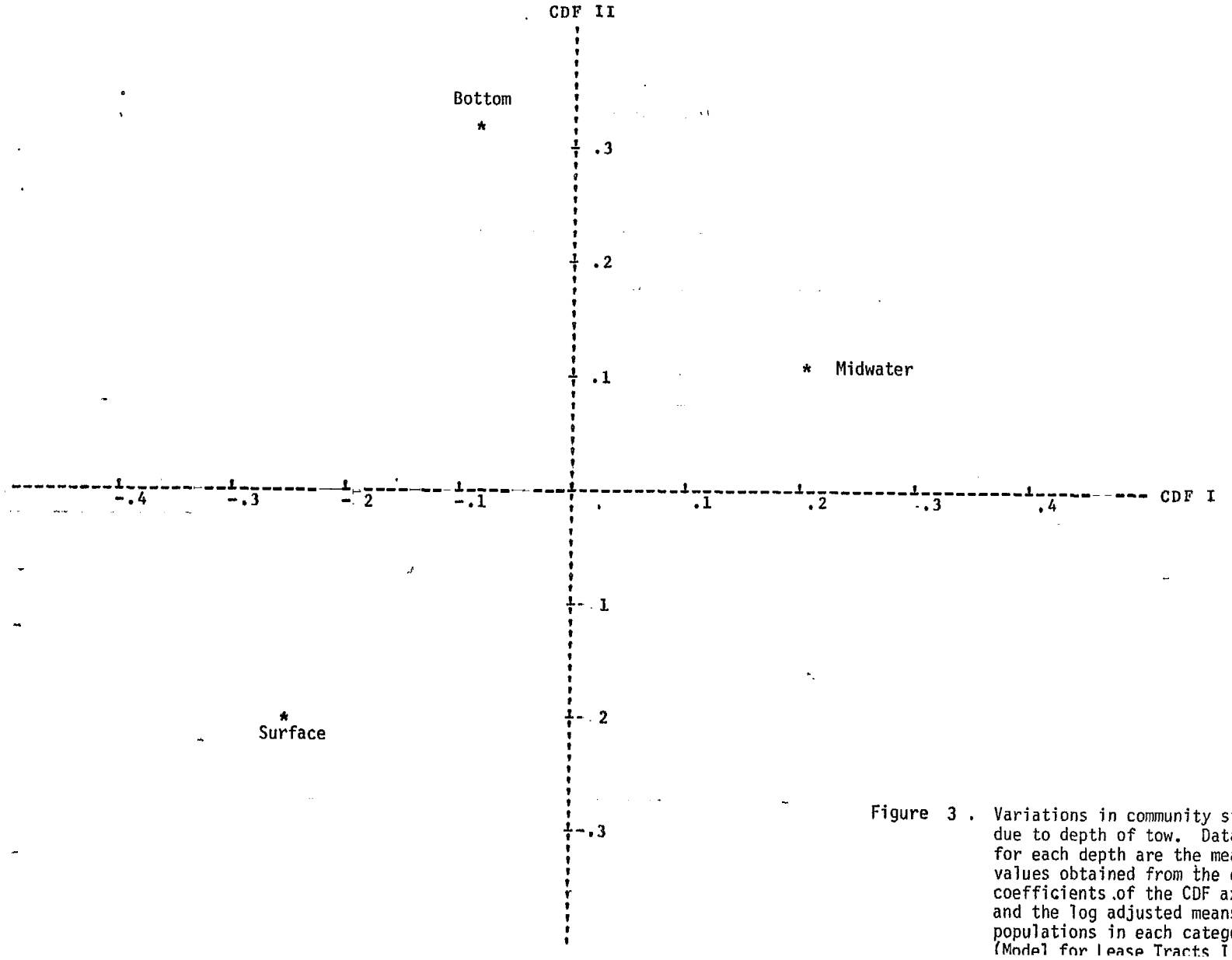


Figure 3 . Variations in community structure due to depth of tow. Data points for each depth are the mean CDF values obtained from the correlation coefficients of the CDF axes (Table 4) and the log adjusted means of the populations in each category. (Model for Lease Tracts I, II, and III).

Table 4 shows that CDF I is positively correlated with Oithona sp., and Fritillaria, and negatively correlated with levels of Corycaeus sp., bivalve larvae, cladocera, and copepodites. CDF II is positively correlated with Oithona sp., Oncaea sp., pteropods, and ostracods.

In Figure 3 movement from surface to midwater to bottom represents an increase along CDF II. This corresponds to an increase in Oithona sp., Oncaea sp., pteropods, and ostracods with increasing depth. This group's strong depth correlation indicates little vertical migration. Midwater samples are separated from surface and bottom samples along CDF I. When midwater is compared to bottom and surface tows, observations of the means (Submitted as Monthly Report No.VIII Supplements; Supplement III) plus the correlations in Table 4 reveal that this separation is a result of the relative scarcity of Coryaeus sp., bivalve larvae, and cladocera and the abundance of Fritillaria.

Lease Tract Depth Interaction

A significant lease tract depth interaction was found. In this term, the uniqueness of each depth of tow at each lease tract is investigated.

Recall that the CDF plane as represented by the CDF axis I and 11 is a region of variation in community structure of zooplankton as revealed by the separate categories of the model. CDF I accounts for 41.33% of the variation attributable to lease tract depth interactions and CDF 11 accounts for 26.28%. The plane of CDF I and II represented in Figures 4 and 5 allows mapping of almost 78% of the variation of this term of the model. The points for each lease tract depth combination are placed on the plane of CDF I and II according to coordinates produced by the model which are unique for each point. Points with

Table 4 Correlation coefficients between canonical variables and the dependent variables for between depths (Model for lease tracts I, II, and III

Categories	CDF I	CDF II
Globigerina	-0.069	0.068
Other Protozoans	0.109	0.151
Siphonophores	0.015	0.124
Medusae	0.122	0.143
Polychaetes larvae	-0.004	-0.118
Gastropod veligers	-0.182	0.033
Pteropods	0.008	0.307
Bivalve larvae	-0.224	0.132
Cladocerans	-0.204	0.088
Ostracods	0.128	0.236
Centropages furcatus	0.023	0.173
Eucalanus sp.	0.066	0.061
Undinula vulgaris	-0.082	-0.135
Other Calanoids	-0.009	0.169
Harpacticoids	0.047	0.141
Corycaeus sp.	-0.249	0.172
Oithona sp.	0.286	0.489
Oncaea sp.	-0.162	0.424
Other Cyclopoids	-0.012	0.149
Copepod copepodites	-0.198	0.126
Copepod nauplii	-0.056	0.097
Lucifer faxoni	0.131	0.094
Other shrimp-like forms	0.114	0.129
Crab larvae	0.061	0.017
Other Crustaceans	0.072	0.177
Echinoderm larvae	-0.042	-0.097
Chaetognaths	0.019	0.156
Oikopleuridae	-0.100	0.026
Fritillaridae	0.206	0.155
Other Tunicates	-0.029	0.184
Fish eggs	-0.022	-0.001
Fish larvae	0.137	-0.053
Other zooplankters	0.124	-0.047

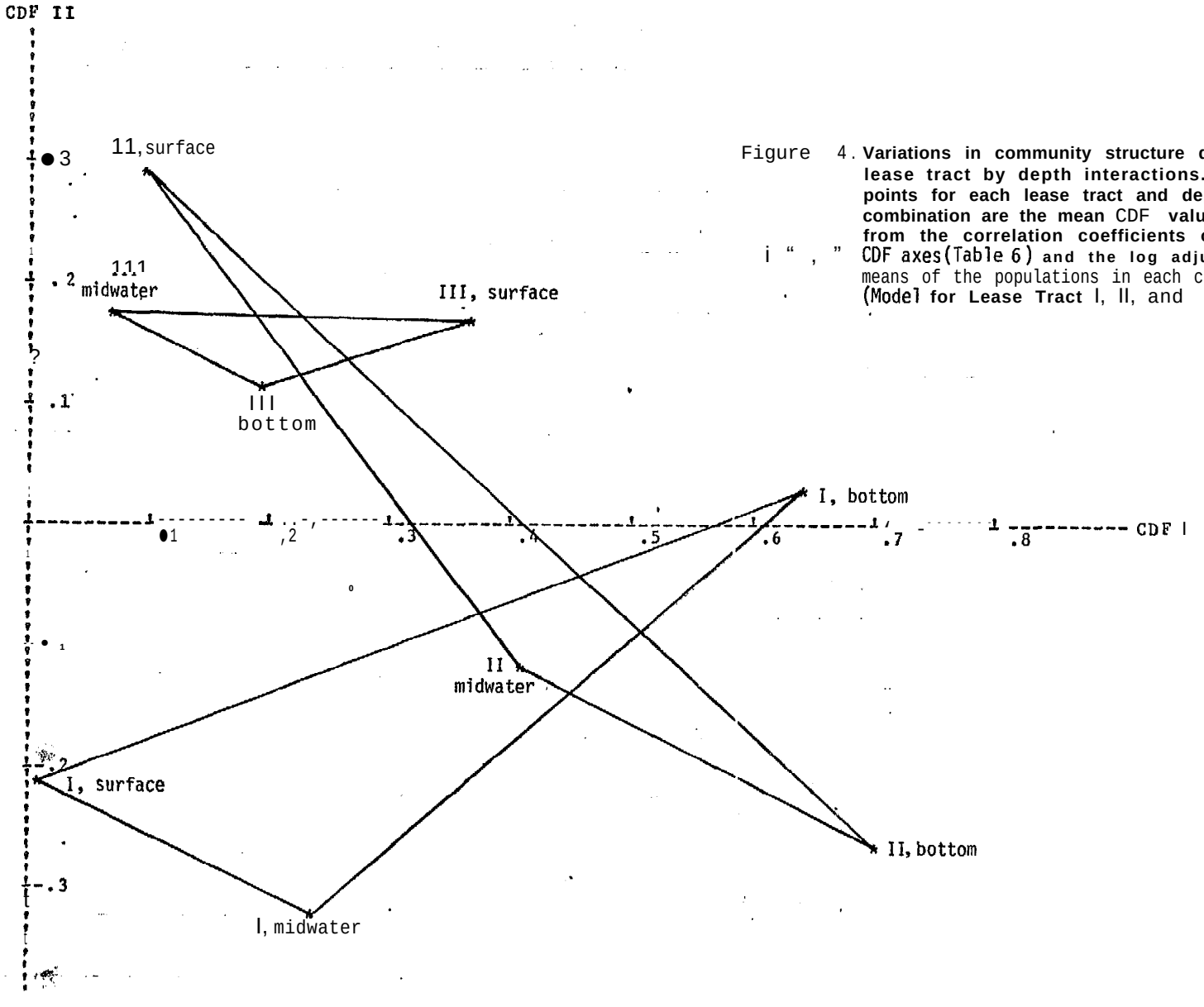


Figure 4. **Variations in community structure due to lease tract by depth interactions. Data points for each lease tract and depth combination are the mean CDF values obtained from the correlation coefficients of the CDF axes (Table 6) and the log adjusted means of the populations in each category. (Model for Lease Tract I, II, and III.**

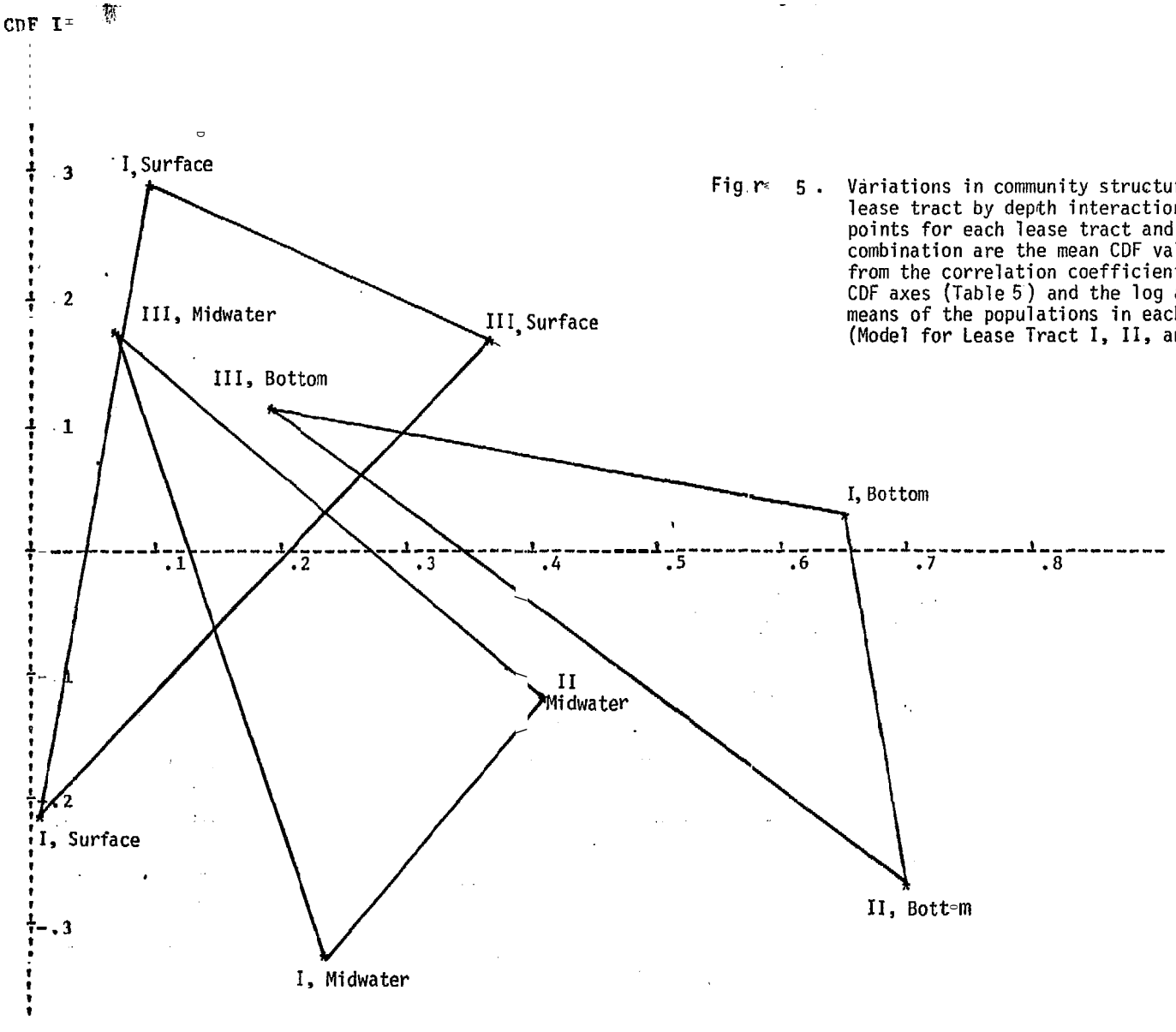


Figure 5. Variations in community structure due to lease tract by depth interactions. Data points for each lease tract and depth combination are the mean CDF values obtained from the correlation coefficients of the CDF axes (Table 5) and the log adjusted means of the populations in each category. (Model for Lease Tract I, II, and III)

different positions on the CDF plane are" separated by differences in community structure which are accounted for by the composition of the CDF axes.

Table 5 shows the basis for construction of the two CDF axes. This table is a list of the correlations of each of the categories with each CDF axis. For instance, changes in CDF I are positively correlated with population levels of categories which have positive values in Table 5.

Examination of the lease tract and depth interaction can be approached from two directions. In both Figures 4 and 5, the same points are graphed on the same CDF variation space. Figure 4 consists of triangles made by connecting all points from the same lease tract. Thus, three triangles are produced -- one triangle made up of surface, midwater, and bottom points for each of the three lease tracts. The information examined in Figure 4 concerns the relative variation of the zooplankton community with depth of the separate lease tracts. Figure 5 consists of triangles made by connecting points from the same depth classification. Using this figure the variation of zooplankton community at the surface, midwater, or bottom can be explained over the three lease tracts.

Figure 4 presents the variation at all three depths for each of the three lease tracts separately. Lease Tract 111 again exhibits smaller variation than the other two lease tracts in this model. Lease Tract I is separated distinctly from Lease Tract III by CDF II. Table 5 reveals that this axis is positively correlated with Corycaeus sp. and siphonophores and negatively correlated with ostracods and Eucalanus sp. The relative positions of the lease tracts on the CDF plane indicate that

Table 5 Correlation coefficients between canonical variables and the dependent variables for lease tract by depth (Model for lease tracts I, II, and III

Categories	CDF I	CDF II
Globigerina	0.099	0.058
Other Protozoans	-0.255	-0.001
Siphonophores	-0.050	0.021
Medusae	-0.023	0.107
Polychaetes larvae	-0.092	-0.065
Gastropod veligers	-0.097	0.108
Pteropods	0.090	0.035
Bivalve larvae	0.268	0.087
Cladocerans	0.241	0.079
Ostracods	0.256	-0.258
Centropages furcatus	0.490	-0.188
Eucalanus sp.	0.188	-0.222
Undinula vulgaris	0.014	0.016
Other Calanoids	0.095	-0.051
Harpacticoids	-0.045	0.062
Corycaeus sp.	0.399	0.347
Oithona sp.	0.216	-0.018
Oncaea sp.	0.297	-0.075
Other Cyclopoids	0.121	0.077
Copepod copepodites	0.149	-0.057
Copepod nauplii	0.022	-0.061
Lucifer faxoni	0.162	-0.013
Other shrimp-like forms	0.159	-0.095
Crab larvae	0.042	0.080
Other Crustaceans	0.21-	-0.039
Echinoderm larvae	0.031	0.143
Chaetognaths	0.234	0.011
Oikopleuridae	0.056	0.140
Fritillaridae	0.131	0.117
Other Tunicates	0.108	0.175
Fish eggs	0.097	-0.038
Fish larvae	-0.144	0.049
Other zooplankters	0.063	0.002

Lease Tract III has more Corycaeus sp. and siphonophores and fewer ostracods than Lease Tract I.

Data shown in Figure 4 indicate that the variational space of Lease Tract II overlaps partially with that of the other lease tracts. Again, this may be interpreted that Lease Tract II occupies an intermediate position in terms of zooplankton community structure. Note also that in each lease tract the point representing the bottom samples is to the right of the point representing midwater samples. From this observation we may conclude that midwater and bottom tows are separated by changes in the populations of zooplankters who are associated with CDF I. Table 5 represents the correlation of the various categories with CDF I. Increased population of Centropages sp., Corycaeus sp. and Oncaea Sp. are correlated with movement from midwater to bottom in all lease tracts of this model .

In Figure 5, all points representing similar depth classification are connected. From this presentation it is apparent that the variation is about the same for each type of depth tow at all three lease tract areas.

Depth by Sunlight Interaction

The depth-sunlight term of the model was found to be significant. This term accounts for migrations associated with sunlight conditions.

As in the previous discussions of lease tract by depth interactions, this interaction term will be approached from two graphical viewpoints. Figure 6 presents the variation over day', dusk, or night conditions for each depth. The correlations of the CDF I and CDF II axis with the population levels for the various categories is presented in Table 6. For this term CDF I accounts for 41.09% of the variation attributable

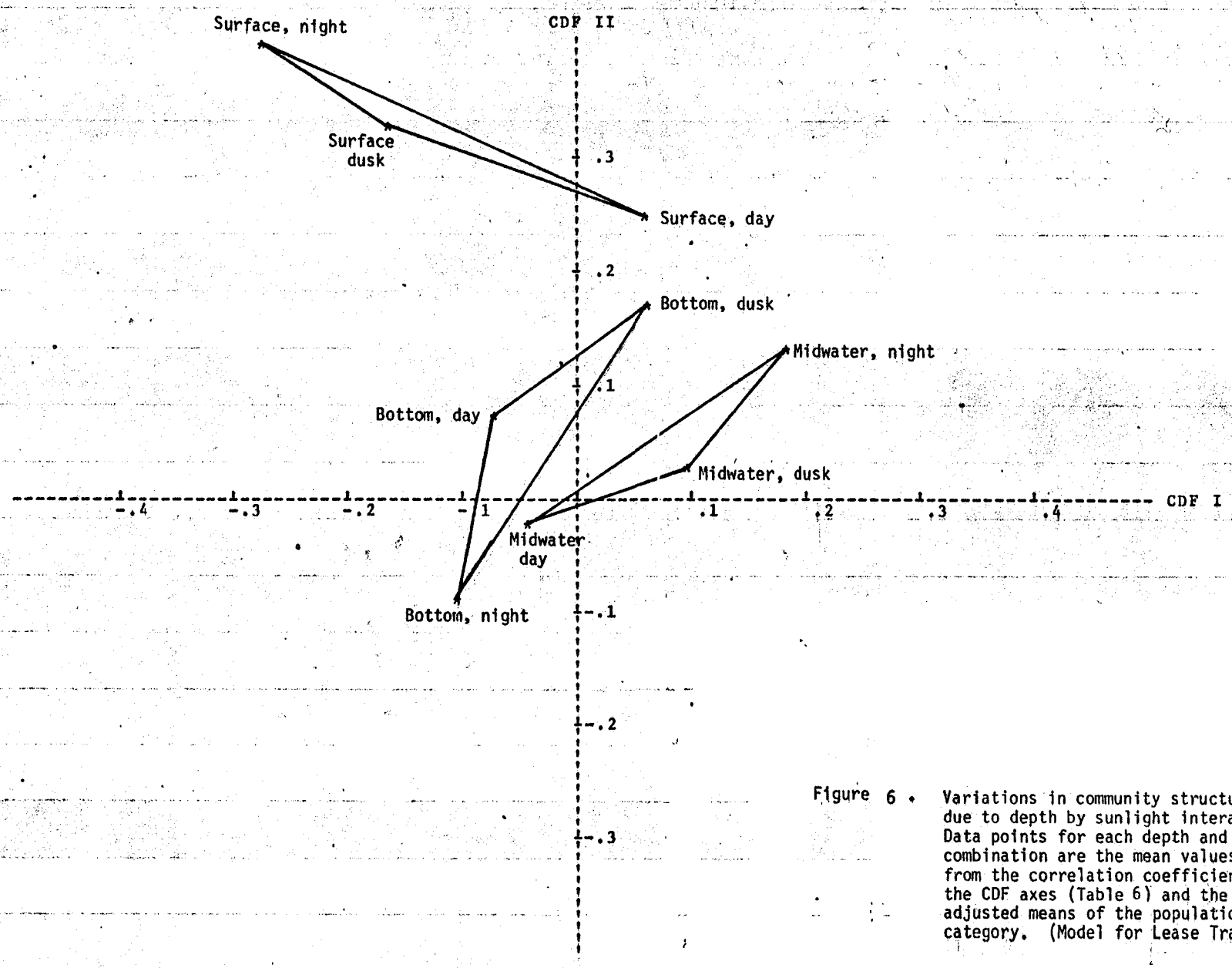


Figure 6. Variations in community structure due to depth by sunlight interactions. Data points for each depth and sunlight combination are the mean values obtained from the correlation coefficients of the CDF axes (Table 6) and the log adjusted means of the populations in each category. (Model for Lease Tract I, II, I I

Table 6 Correlation coefficients between canonical variables and the dependent variables for depth by sunlight (Model for lease tracts 1, 11, and III)

Categories	CDF I	CDF II
Globigerina	-0.050	-0.069
Other Protozoans	0.182	0.015
Siphonophores	-0.046	0.101
Medusae	0.157	0.021
Polychaetes larvae	-0.048	-0.148
Gastropod veligers	0.195	-0.028
Pteropods	0.201	0.154
Bivalve larvae	-0.090	-0.157
Cladocerans	-0.17s	0.034
Ostracods	-0.203	0.055
Centropages furcatus	-0.139	0.153
Eucalanus sp.	-0.061	-0.118
Undinula vulgaris	-0.031	0.081
Other Calanoids	0.103	0.189
Harpacticoids	0.042	0.022
Corycaeus sp.	0.058	0.207
Oithona sp.	0.107	-0.212
Oncaea sp.	0.071	0.150
Other Cyclopoids	0.073	0.108
Copepod copepodites	0.099	0.152
Copepod nauplii	0.052	-0.049
Lucifer faxoni	0.166	-0.118
Other shrimp-like forms	0.138	-0.051
Crab larvae	0.237	0.209
Other Crustaceans	0.028	0.243
Echinoderm larvae	-0.249	0.025
Chaetognaths	-0.094	0.093
Oikopleuridae	-0.101	0.157
Fritillaridae	-0.003	0.032
Other Tunicates	-0.044	0.207
Fish eggs	0.001	-0.261
Fish larvae	0.203	0.004
Other zooplankters	-0.017	0.129

to depth by sunlight interactions, and CDF 11 for 31.86%.

The triangle representing the variation in surface populations at the different sunlight conditions (Figure 6) reveals that night and day tows are separated along the CDF I axis with dusk tows occupying an intermediate position. This, coupled with correlations presented in Table 6, indicates there are more fish larvae, crab larvae, pteropods, gastropod veligers, and Lucifer at the surface during the day than at night. Fewer ostracods and echinoderm larvae were found on the surface during the day than at night. The variations along CDF II for the surface assemblage of animals shows that day samples contained fewer Corycaeus and other crustacea; and more fish eggs, bivalve larvae, and Oithona than night samples. In general, the entire surface assemblage is separated from the midwater and bottom communities by CDF II. The variation with sunlight conditions at the surface takes place largely along CDF I. It is quite probable that the CDF II axis is associated with the categories of zoop?ankton which remain at a similar depth throughout the day and that CDF I is associated with the categories which migrate as dictated by sunlight conditions. Oithona in particular is correlated with CDF II. Th· s organism also seemed to be exhibiting a tendency to remain at a constant depth as was pointed out in the depth section of this discuss" on. Bottom and midwater assemblages seem to have much in common with each other and are separated only by a small distance on the CDF I axis. Positive correlations of crab larvae with both axes might account for the difference between midwater and depth at night, when midwater tows contained more crab larvae than bottom tows. Fish larvae, also seem to be associated with the difference between midwater and bottom tows at night. There are fewer fish larvae

at midwater than at the bottom at night. This part of the model suggests that midwater night tows have fewer fish larvae and more crab larvae than bottom night tows.

Consideration of Figure 7 brings out variation among the three classes of depth considered at each sunlight condition. In general, the variation at night is the greatest. This could mean that the zooplankton community is in its most strongly stratified condition during the night.

Hour

Hour was not found to be significant.

Sunlight .

Sunlight conditions were not found to be significant. The depth-sunlight interaction was found to be significant, indicating that generally the same categories are present at the same population numbers throughout the depth of the water column at all sunlight conditions, but the population is strongly stratified as dictated by light conditions.

CH_4 (methane)

At the levels found by Dr. Sackett, CH_4 was not found to be significant correlated with zooplankton community structure.

C_2H_4 (ethene)

Levels of C_2H_4 were found to be significantly correlated with zooplankton community structure. The correlations of the various categories with levels of C_2H_4 are presented in Table 7. Most of the strong correlations were positive ones. It is essential to emphasize that such, positive correlations do not imply a cause and effect relationship. A possibility is that levels of C_2H_4 are correlated with

Table 7 Correlation coefficients between canonical variables and the dependent variables for levels of C_2H_4 (Model for lease tracts I, II, and III

Categories	CDF I
Globigerina	-0.025
Other Protozoans	0.191
Siphonophores	0.144
Medusae	0.099
Polychaetes larvae	-0.122
Gastropod veligers	0.080
Pteropods	0.278
Bivalve larvae	0.142
Cladocerans	0.147
Ostracods	-0.035
Centropages furcatus	0.055
Eucalanus sp.	-0.072
Undinula vulgaris	-0.062
Other Calanoids	0.181
Harpacticoids	0.164
Corycaeus sp.	0.115
Oithona sp.	0.015
Oncaea sp.	0.312
Other Cyclopoids	0.226
Copepod copepodites	0.137
Copepod nauplii	-0.033
Lucifer faxoni	-0.054
Other shrimp-like forms	-0.092
Crab larvae	0.035
Other Crustaceans	0.070
Echinoderm larvae	-0.097
Chaetognaths	0.182
Oikopleuridae	0.099
Fritillaridae	0.101
Other Tunicates	0.287
Fish eggs	-0.106
Fish larvae	-0.038
Other zooplankters	0.018

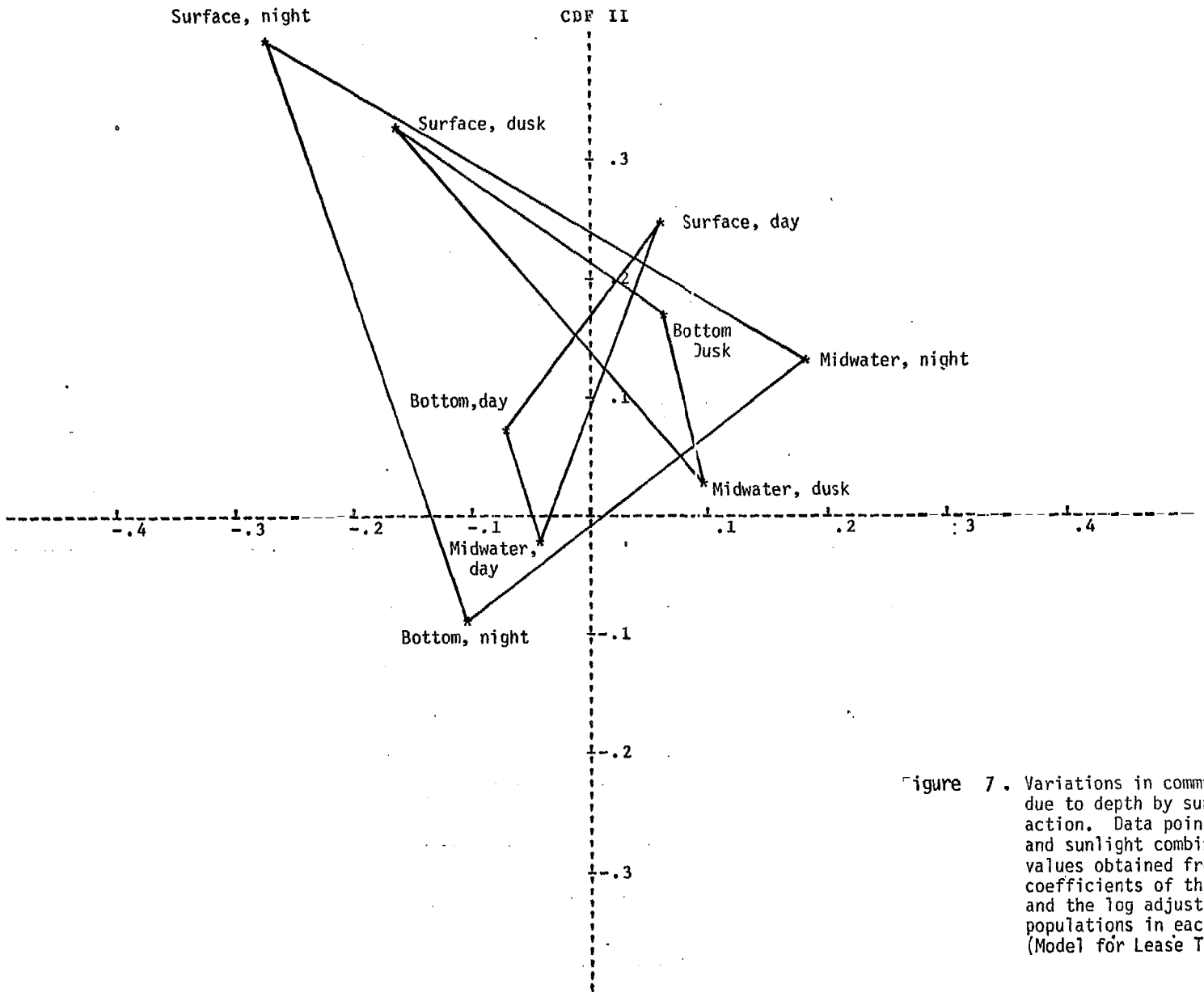


Figure 7. Variations in community structure due to depth by sunlight interaction. Data points for each depth and sunlight combination are the mean values obtained from the correlation coefficients of the CDF axes (Table 6) and the log adjusted means of the populations in each category. (Model for Lease Tracts I, II, and III

some other factor which was not included in the analysis. A more complete model which would include more physical and chemical information might resolve this question. For instance, levels of C_2H_4 might be related to water masses which have certain zooplankton communities determined by other environment parameters.

C_3H_8 (propane)

Level of C_3H_8 were found to be significantly correlated with zooplankton community structure as revealed by the categories included in the model. The correlations of these categories with levels of C_3H_8 are presented in Table 8. Notice that the strongest correlations are negative ones--with fish eggs, fish larvae, *Lucifer*, gastropod veligers, and shrimp larvae. There are also positive correlations with other calanoids, *Oikopleura*, and *Corycaeus*. This picture is somewhat confusing and indicates the strong need for a more extensive modeling effort. However, strong negative correlations with fish eggs and fish larvae may be indicative of some future problems which require more investigation.

Category Assemblage

An effort was made to identify any favored "important" category assemblage which might be found in Lease Areas I, II, and III. This was done by examining the correlations for categories which were often found together throughout all the samples. .

Some of the smaller sized categories seemed to be correlated, perhaps indicating clogging took place on shine tows when more smaller (less than 202 microns) creatures were collected. *Globigerina*, other protozoans, *Corycaeus* and copepod nauplii fall into this group.

A major grouping did present itself. Among its most highly

Table 8 Correlation coefficients between canonical variables and the dependent variables for levels of C_3H_8 (Model for lease tracts I, II, and III

Categories	CDF I
Globigerina	-0.114
Other Protozoans	-0.109
Siphonophores	0.045
Medusae	-0.105
Polychaetes larvae	-0.052
Gastropod veligers	-0.185
Pteropods	-0.041
Bivalve larvae	0.045
Cladocerans	0.168
Ostracods	-0.125
Centropages furcatus	-0.017
Eucalanus sp.	-0.152
Udinula vulgaris	0.078
Other Calanoids	0.218
Harpacticoids	-0.163
Corycaeus sp.	0.169
Oithona sp.	.0.127
Oncaea sp.	0.148
Other Cyclopoids	0.154
Copepod copepodites	0.048
Copepod nauplii	0.031
Lucifer faxoni	-0.277
Other shrimp-like forms	-0.181
Crab larvae	0.060
Other Crustaceans	0.037
Echinoderm larvae	-0.053
Chaetognaths	-0.110
Oikopleuridae	0.195
Fritillaridae	-0.050
Other Tunicates	0.090
Fish eggs	-0.352
Fish larvae	-0.249
Other zooplankters	0.020

correlated members were chaetognaths, Oncaea sp., Oithona sp., other calanoids, shrimp larvae, Oikopleura, copepod copepodites, pteropods, Centropages furcatus, crab larvae, other crustaceans, ostracods, other tunicates, and fish eggs. In general, this is a fairly typical Gulf of Mexico offshore assemblage heavily weighted with meroplankters due to the time of year of the collection (Summary of Knowledge of the Eastern Gulf of Mexico, 1973).

b

B

B

ZOOPLANKTON ANALYSIS FOR LEASE TRACTS IV AND V

Woodmansee, Bennett, Novinska, Loman, Cliburn

The results of the zooplankton analysis are summarized and submitted as Monthly Report VIII; Supplements; Supplement IV which gives the means and ranges in numbers per cubic meter for the three "standard depths" at each master station as well as for Lease Areas V and IV. Each master station mean for each of the three "standard depths" is derived from six replicate sample concentrations. Each area mean for each of the three "standard depths" is derived from the three master station means and therefore is derived from 18 sample concentrations. Single samples were taken at each depth at control stations 4 and 3. A total of 59 different planktonic categories have been tabulated. This total includes 18 copepod genera, a tabulation of total copepods and one for dry weight of the total net plankton.

The composition and certain comparative characteristics of the dominant elements of the copepod communities in Areas V and IV are presented in Table 9 Each concentration is an area total which is a simple summation of the means of the three "standard depths*' and therefore is derived from 54 sample concentrations. All concentrations are given in numbers per cubic meter and do not take into account the existing variations in station depths.

The dominant copepod genus in both areas is Paracalanus, which is 1.9 times more numerous or concentrated in Area V. The second ranked copepod genus in both areas is Acartia, which is 3.7 times as numerous in Area V as in Area IV. It is highly probable that the majority of these forms are Acartia tonsa, which is an important component of the nearshore planktonic copepod community. The higher relative concentration of Acartia in Area V is considered to be a reflection of the lower salinities encountered in this somewhat more inshore area. The ratios of Acartia to the dominant Paracalanus in the two areas illustrate the same pattern.

COPEPOD	V #/M^3	IV #/M^3	V/IV	V x/P	IV x/P
Acartia	2170(2)	585(2)	3.7	0.71	0.37
Centropages	1320(4)	483(4)	2.7	0.43	0.30
Corycaeus	1699 (3)	353	4.8	0.56	0.22
Eucalanus	701	388	1.8		
Oithona	388	395	1.0		
Oncaea	367	496 (3)	.7	0.12	0.31
Paracalanus	3036(1.)	1586(1)	1.9	1.00	1.00

Table 9. Composition and comparative characteristics of the copepod communities in Areas V and IV. The X/P ratios are comparisons of the concentrations of the various copepods to the concentration of Paracalanus.

The third ranked genus in Area V is Corycaeus, which is 4.8: times as concentrated in Area V as in Area IV. The very high relative concentration of Corycaeus in Area V is one of the most, if not the most distinguishing characteristic, of the Area and results from the abundance of Corycaeus amazonicus and Corycaeus americanus, which are also characteristic members of the more coastal plankton community.

The third ranked copepod in Area IV, by contrast, is Oncaea, which is only 0.7 times as numerous in Area V as in Area IV. This feature is also considered to be principally a salinity effect since all local species in the genus Oncaea are offshore forms.

The fourth ranked form in both areas is Centropages, which is 2.7 times as 'numerous in Area V.

In summary, then, the copepod communities of the two areas are somewhat similar in composition in that they have the same genera in ranks one, two and four. The principal difference is to be found in the third ranking form, which is Corycaeus in'Area V and Oncaea in Area IV. Another significant difference is the relatively higher concentration of Acartia in Area V.

MODEL FOR LEASE TRACTS IV AND V

Mature, Hearne, Ingram, Caldwell, Antonielli

The significance of each term of the Lease Tract IV and V model and the percent variation accounted for by the CDF axes for each term are presented in Table 10. Also included are the mean values of the cononical variables which are important for the analyses of each of the terms of the model. Figures 8 thru 10 are based upon these mean values.

Difference Between Lease Tracts

'Significant differences were found to exist between Lease Tracts IV and V. Table 11 shows the correlation coefficients with the CDF of each of the categories. Here only a single axis exists (see Statistical Methods). The means for Lease Tracts IV and V for that CDF axis are 0.114 and 0.546, respectively. Lease Tract V is much higher than Lease Tract IV. This means that Lease Tract V has more *Euterpina*, *Centrogages*, *Corycaeus*, *Oncaea*, bivalve larvae, and *Acartia* than Lease Tract IV. Fewer salps and other cyclopoids were associated with Area v. In general, *Euterpina* and *Acartia* are associated with the more inshore type of environment found in Lease Area V. The fresh water influence of the Mississippi River accounts for an increase in these organisms at Lease Area V.

Station Variations Within Lease Tracts

Figure 8 is a presentation of the various stations and the positions they occuply in the CDF space as defined by CDF I and CDF II axis. CDF I accounts for 55.50% of the variation between stations within lease tracts

Table 10. MANOVA Summary Table for Lease Tracts IV, V

Source	Wilks' λ	Significance	# of CDF Axis Possible	% Variation explained by each CDF axis	CDF Mean Scores: Source	CDF Axis I	CDF Axis II
Lease Tract Site	.2845290	.0001	1	100.	Lease Tract IV	.115	--
					Lease Tract V	.546	--
Station within lease tract site	.0099368	.0001	3	55.5	Tract IV Sta. 2	.173	.231
				31.07	Tract IV Sta. 2	.373	-.425
				9.92	Tract IV Sta. 3	.207	.013
				3.51	Tract V Sta. 2	.644	-.090
					Tract v Sta. 2	.471	-.049
					Tract V Sta. 3	-.149	-.268
Depth	.0318767	.0001	2	76.03	Surface	-.513	.139
				23.97	Midwater	.118	.384
					Depth	.252	.014
Lease Tract by depth	.071835	.0001	2	57.98	Tract IV surface	,220	.288
				42.02	Tract IV midwater	-.367	-.050
					Tract IV depth	-.521	-.238
					Tract V surface	-.380	.446
					Tract V midwater	-.202	.436
					Tract V depth	-.359	-.015
HOUR	.0015136	.0898	NOT SIGNIFICANT				
CH_4	.3984761	.0081	NOT SIGNIFICANT				
C_2H_4	.4017182	,0090	NOT SIGNIFICANT				
C_4H_8	.5145931	.1697	NOT SIGNIFICANT				

Table 11 Correlation coefficients between canonical variables and the dependent variables between lease-tracts (Model for lease tracts IV and V

Categories	CDF I
Pyrocystis	-0.065
Ceratium	-0.051
Foraminiferans	-0.043
Siphonophores	-0.123
Hydromedusae	-0.057
Polychaetes	0.104
Gastropod larvae	(I.110
Bivalve larvae	0.161
Cladocerans	0.044
Acartia sp.	0.120
Calanus sp.	-0.084
Centropages sp.	0.367
Eucalanus sp.	0.034
Euchaeta sp.	0.062
Paracalanus sp.	0.087
Temora sp.	-0.048
Undinula sp.	-0.027
Other Calanoids	-0.086
Euterpina sp.	0.393
Other Harpacticoids	0.065
Corycaeus sp.	0.237
Oithona sp.	0.14'7
Oncaea sp.	0.165
Other Cyclopoids	-0.128
Copepod nauplii	0.005
Decapod larvae	0.091
Other crustaceans	-0.026
Chaetognaths	0.143
Larvaceans	0.067
Sal ps	-0.254
Fish eggs	-0.040
Fish larvae	-0.007

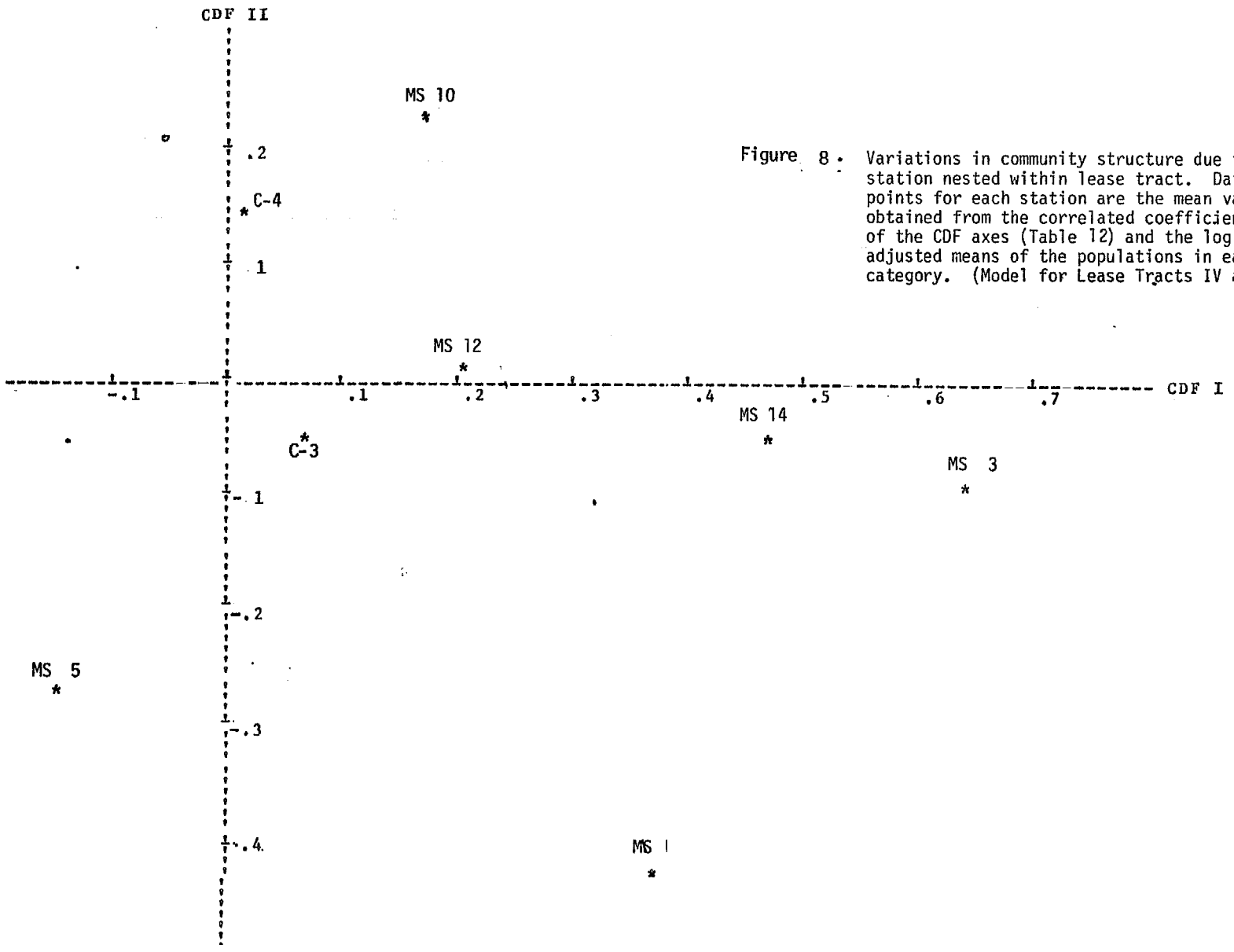


Figure 8. Variations in community structure due to station nested within lease tract. Data points for each station are the mean values obtained from the correlated coefficients of the CDF axes (Table 12) and the log adjusted means of the populations in each category. (Model for Lease Tracts IV and V)

and CDF 11 for 31.07%. The triangles represent the variation of the three stations within each lease tract. Lease Tract IV stations seem to vary most in a vertical direction along the CDF II axis. This axis, as presented in Table 12 is correlated strongly in a negative way with Acartia, Centropages, decapod larvae, and Eucalanus sp. Apparently, MS-11 is separated from MS-10 and MS-12 by having more Acartia. Possibly some peculiarity of water masses resulted in a fresh water intrusion from the Mississippi River into the area of MS-11. More hydrographic data could contribute significantly to the model.

Lease Tract V exhibits the most variation along CDF I. Particularly, MS-15 seems to be separated from the other five master stations in these two lease areas by the CDF I axis. MS-15 is the westernmost station and is also closer inshore. The strong positive correlation with Euterpina of CDF I indicates that MS-15 has fewer of these organisms than MS-14 and MS-13.

It seems that an interesting interaction between freshwater influences of the Mississippi River and Mobile Bay and the saltwater of the open Gulf of Mexico is occurring. More detailed models including hydrographic data for this area will help resolve this problem.

Variations Due to Depth

The depth of collection showed significance in terms of the zooplankton community structure. Figure 9 indicates the variation between surface, mid-water, and depth collections. Table 13 lists the correlation of the various categories with the two CDF axes used.

Surface samples are separated from" midwater and bottom samples along the CDF I axis. This axis has a large negative correlation with Acartia, Eucalanus sp., and Centropages, and its largest positive correlation with Oithona. The surface-samples from these lease areas are strongly correlated with increased populations of Acartia (and to a lesser extent - Euterpina).

Table 12 Correlation coefficients between canonical variables and the dependent variables for station nested within lease tract (Model for lease tracts IV and V)

Categories	CDF I	CDF II
Pyrocystis	-0.161	0.097
Ceratium	-0.043	0.113
Foraminiferans	-0.056	0.134
Siphonophores	0.088	-0.094
Hydromedusae	-0.081	-0.118
Polychaetes	0.117	0.005
Gastropod larvae	-0.096	-0.031
Bivalve larvae	-0.191	0.044
Cladocerans	-0.375	-0.036
Acartia sp.	0.139	-0.329
Calanus sp.	0.099	0.019
Centropages sp.	0.150	-0.324
Eucalanus sp.	0.280	-0.250
Euchaeta sp.	0.216	0.275
Paracalanus sp.	0.081	-0.018
Temora sp.	0.015	0.122
Undinula sp.	-0.027	0.042
Other Calanoids	0.113	0.079
Euterpina sp.	0.323	-0.157
Other Harpacticoids	0.035	0.084
Corycaeus sp.	-0.017	-0.204
Oithona sp.	0.076	0.137
Oncaea sp.	0.270	0.165
Other Cyclopoids	0.084	0.097
Copepod nauplii	-0.038	0.012
Decapod larvae	0.073	-0.280
Other crustaceans	0.346	-0.067
Chaetognaths	0.024	-0.130
Larvaceans	0.184	-0.047
Salps	0.102	-0.184
Fish eggs	0.127	-0.103
Fish larvae	0.102	-0.004

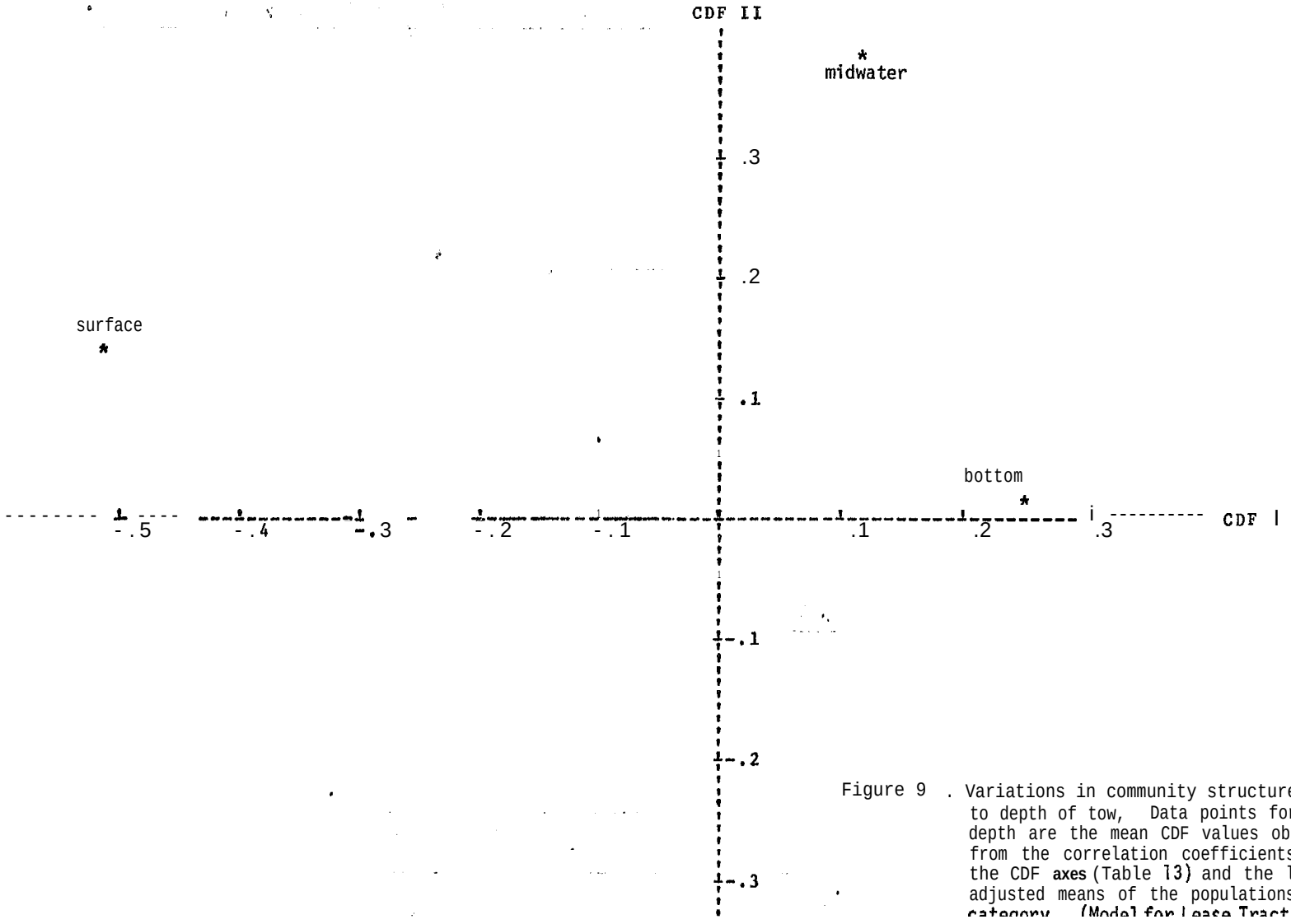


Figure 9 . Variations in community structure due to depth of tow, Data points for each depth are the mean CDF values obtained from the correlation coefficients of the CDF axes (Table 13) and the log adjusted means of the populations in each category. (Model for Lease Tracts IV and V)

Table 13 correlation coefficients between canonical variables and the dependent variables for depths (Model for lease tracts IV and V)

Categories	CDF I	CDF II
Pyrocystis	0.067	0.284
Ceratium	0.126	0.340
Foraminiferans	0.051	0.091
Siphonophores	0.054	0.220
Hydromedusae	-0.102	0.082
Polychaetes	-0.034	0.058
Gastropod larvae	-0.191	-0.004
Bivalve larvae	-0.138	-0.036
Cladocerans	-0.098	0.205
Acartia sp.	-0.344	0.118
Calanus sp.	-0.047	0.151
Centropages sp	-0.281	0.285
Eucalanus sp.	-0.306	.0.262
Euchaeta sp.	0.015	0.179
Paracalanus sp.	-0.096	0.122
Temora sp.	-0.003	-0.045
Undinula sp.	-0.002	0.065
Other Calanoids	0.079	0.064
Euterpina sp.	-0.146	0.246
Other Harpacticoids	0.023	0.142
Corycaeus sp.	-0.205	0.132
Oithona sp.	0.269	0.234
Oncaea sp.	0.195	0.294
Other Cyclopoid	-0.127	0.079
Copepod nauplii	-0.044	0.038
Decapod larvae	-0.036	0.290
Other crustaceans	-0.194	0.276
Chaetognaths	-0.177	0.148
Larvaceans	-0.137	0.088
Salps	-0.049	0.288
Fish eggs	-0.056	0.256
Fish larvae	0.026	0.145

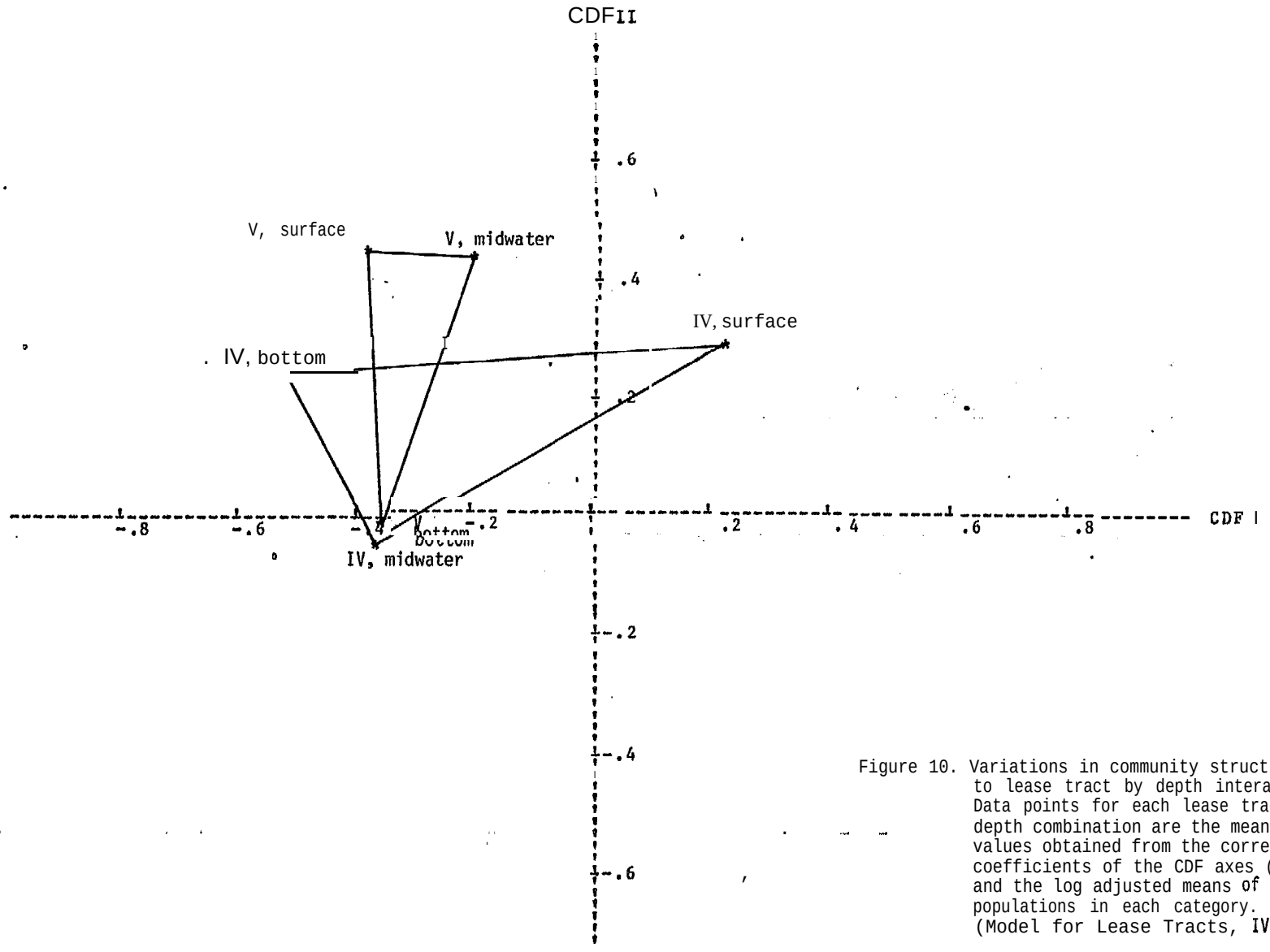


Figure 10. Variations in community structure due to lease tract by depth interaction. Data points for each lease tract and depth combination are the mean CDF values obtained from the correlation coefficients of the CDF axes (Table 14) and the log adjusted means of the populations in each category. (Model for Lease Tracts, IV and V)

This might be the result of reduced salinity in the upper layers of the water column. Again, this could be resolved with more hydrographic and physical data.

Midwater and bottom samples are separated by CDF II. This axis is correlated positively with Ceratium, Oncaea sp., decapod larvae, salps, Centropages sp., and Pyrocystis. It appears that CDF I is correlated strongly with an assemblage of categories which serves to separate surface samples from midwater and bottom. CDF II is correlated more closely with organisms who make up the difference between midwater and bottom.

Only a small number of collections were taken at night in Lease Areas IV and V so that little information is available concerning diurnal migration. Therefore, the information concerning stratification of the zooplankton community is limited to daylight conditions.

Generally, the model for Lease Tract IV and V indicated that surface tows were associated with animals which can be regarded as euryhaline - Acartia and Euterpina. Midwater and bottom tows were separated by the fact that midwater tows have more Ceratium, decapod larvae, Oncaea sp., and Centropages.

Lease Tract Depth Interaction

This term was found to be significant for Lease Tract IV and V. The variations over all depths for each lease tract are presented in Figure 10. It is evident that the variation exhibited by Lease Tract IV is somewhat larger than that of Lease Tract V. The correlations of the various categories with the CDF axes are given in Table 14. The surface station of Lease Tract IV seems to be separated from the other points by CDF I, indicating that levels of gastropod veligers, Temora sp., salps, decapod larvae, and copepod nauplii are generally higher at that station. The bottom station of Lease Tract V appears to be quite different from the surface and midwater

Table 14 Correlation coefficients between canonical variables and the dependent variables for lease tract by depth interaction. (Model for lease areas IV and V)

Categories	CDF I	CDF II
Pyrocystis	0.020	-0.184
Ceratium	-0.026	-0.203
Foraminiferans	0.021	-0.161
Siphonophores	0.132	0.125
Hydromedusae	-0.043	0.018
Polychaetes	-0.047	0.110
Gastropod larvae	0.155	-0.057
Bivalve larvae	0.042	-0.036
Cladocerans	0.037	0.190
Acartia sp.	-0.110	0.104
Calanus sp.	0.059	0.077
Centropages sp.	0.010	0.205
Eucalanus sp.	-0.049	0.501
Euchaeta sp.	-0.129	0.013
Paracalanus sp.	-0.078	0.180
Temora sp.	0.155	0.019
Undinula sp.	-0.039	-0.003
Other Calanoids	0.041	0.016
Euterpina sp.	0.080	0.141
Other Harpacticoids	0.085	0.047
Corycaeus sp.	0.044	0.186
Oithona sp.	-0.072	-0.062
Oncaea sp.	-0.133	0.011
Other Cyclopoids	0.050	0.020
Copepod nauplii	0.144	0.141
Decapod larvae	0.027	0.149
Other crustaceans	-0.109	0.074
Chaetognaths	-0.022	0.067
Larvaceans	0.149	-.166
Salps	0.069	0.241
Fish eggs	-0.086	-0.064
Fish larvae		

station of that area. Its separation is largely due to CDF II, an axis which indicates that the surface station has fewer *Eucalanus*, fish eggs, and *Centropages* and more *Ceratium* and *Pyrocystis*.

Hour

The hour term was not found to be significant in Lease Tracts IV and V.

CH_4, C_2H_4, C_3H_8

None of these low molecular weight hydrocarbons were found to have a significant effect upon zooplankton community structure at the levels measured in Lease Tracts IV and V.

Diversity, Total Density, and Biomass Models

As explained in Statistical Methods, two more compact statistical models were run. The model for Lease Tracts I, 11 and III used Shannon-weaver diversity, total density, and biomass as dependent variables. The effects investigated in this model were lease tract, station nested within lease tract, depth, lease tract by depth interaction, depth by sunlight interactions, hour, sunlight, and concentration of CH_4, C_2H_4, C_3H_8. Of these only lease tract, station nested within lease tract, and lease tract by depth interaction turned out to be significantly correlated with diversity, total density and biomass for this particular model. The appropriate means of the cononical variables for these terms are given in Table 14.

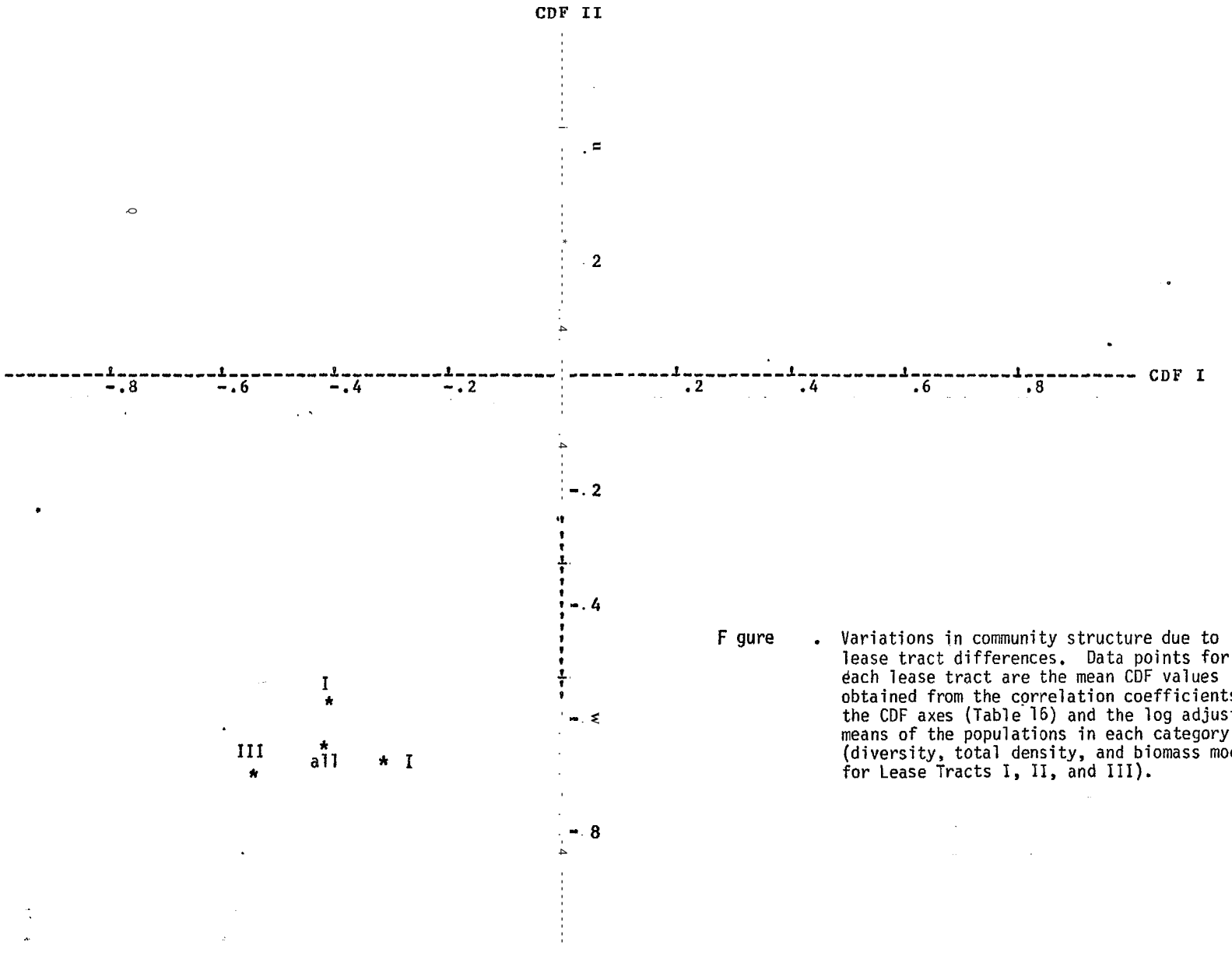
Figure 11 exhibits the position of the three lease tracts with respect to the CDF axes defined in Table 15. The means of the Shannon-Weaver Diversity, total density and biomass for Lease Tracts I, II, and III are listed in Table 16. Examination of this table and Figure 11 reveals that Lease Tract III is different from I and II by having lower density and biomass, but higher diversity. While Lease Tracts I and II have very

Table 15. Summary of MANOVA Results for the Shannon Weaver Diversity, Total Density and Biomass Model for Lease Tracts I, II, and III

Source	Wilks'	Significance	# of CDF Axes Possible	% Factor Variat on Explained by Each CDF Axis	Group	CDF Mean Values CDF I	CDF II
Lease Tract	.7023894	.0001	2	96.37 3.63	Tract 1	-.4079	-.5725
					Tract 11	-.3118	-.6788
					Tract 111	-.5414	-.7004
Station Nested Within Lease Site	.4785992	.0001	3	74.32 17.67 8.01	MS - 1	.6151	.6345
					MS - 2	.6388	.5770
					MS - 3	.6082	.5489
					MS - 4	.8429	.5767
					MS - 5	.8668	.4922
					MS - 6	.5617	.5563
					MS - 7	.7332	.7088
					MS - 8	.7219	.6868
					MS - 9	.7384	.6793
Depth	.8334930	.0072	NOT SIGNIFICANT				
Lease Tract by Depth	.5955646	.0001	3	49.44 46.16 4.40	I Surface	.2096	.6618
					I Midwater	.2295	.9341
					I Bottom	.3577	.8398
					II Surface	.2667	.7722
					II Midwater	.3733	.7971
					II Bottom	.5981	.8872
					III Surface	.2909	.9055
					III Midwater	.2901	.9507
					III Bottom	.3070	1.0390

Table 15. Continued.

Depth by Sunlight	.9074633	.6596	NOT SIGNIFICANT
Hour	.7719565	.9938	NOT SIGNIFICANT
Sunlight	.9541136	.6017	NOT SIGNIFICANT
CH_4	.9475966	.1567	NOT SIGNIFICANT
C_2H_4	.9810933	.6098	NOT SIGNIFICANT
C_3H_8	.8542668	.0020	NOT SIGNIFICANT



F gure . Variations in community structure due to lease tract differences. Data points for each lease tract are the mean CDF values obtained from the correlation coefficients of the CDF axes (Table 16) and the log adjusted means of the populations in each category (diversity, total density, and biomass model for Lease Tracts I, II, and III).

B

Table 16. Correlation coefficients between canonical variables and the dependent variables for between lease tracts (Diversity, total density, biomass model for lease tracts I, II, and III

Categories	CDF I	CDF II
Shannon-Weaver Diversity	-0.596	-0.338
Total density	0.829	-0.445
Biomass	0.659	0.010

D'

Table 17. Means of Shannon-Weaver Diversity, 'Total density, and Biomass for lease tracts I, II, and III

Lease Tract	Shannon-Weaver Diversity	Total Density	Biomass
I	2.11	1594.60	21.52
II "	2.12	3870.88	36.63
III	2.52	10Q4.OO	12.87

Table 18. Correlation coefficients between canonical variables and the dependent variables for station nested within lease tracts (Diversity Total density biomass model for lease tract I, II, and III

Categories	CDF I	CDF II
Shannon-Weaver Diversity	0.417	0.887
Total Density	0.645	-0.249
Biomass	0.293	0.241

similar diversity, Lease Tract II has much higher total density and biomass.

The effects of station nested within lease tract are presented in Figure 12 and the correlation coefficients of the CDF axes are listed in Table 17. Note that the variation among the stations of Lease Tract I and III are much smaller than the variations between the station of Lease Tract II. In particular, MS-6 seems to be separated by lower diversity and total density from the other two stations of Lease Tract II.

The effects of lease tract depth interaction are presented in Figures 13 and 14. The correlation coefficients for diversity, density and biomass-are listed in Table 18. Two figures are presented in keeping with the technique used thus far in the report for interactive terms. In Figure 13, where points from all three depths for each lease tract are connected, several trends are apparent. The largest variation of Lease Tract III is along the CDF II axis, indicating increasing diversity with increasing depth. Lease Tract II varies mostly along CDF I indicating that as depth increases, total density and biomass are also increasing. Typically the variation of Lease Tract 111 is the smallest of the three. The southernmost lease tract, I, exhibits the most variation with respect to-depth when diversity, total density and biomass are considered. Bottom samples of Lease Tract I indicate a larger total density and biomass than surface or midwater tows at the same lease tract. Surface samples at Lease Tract I exhibit the lowest value of density.

When each type of depth tow is considered separately, Figure 14 can be used to indicate the variation over the lease tracts. Surface tows seem to vary the least, but mostly along CDF II. The trend is toward higher diversity with northward movement. Among midwater tows, Lease Tract II exhibits lower diversity but somewhat higher total density and biomass. The bottom collections have the largest variations over the lease tracts

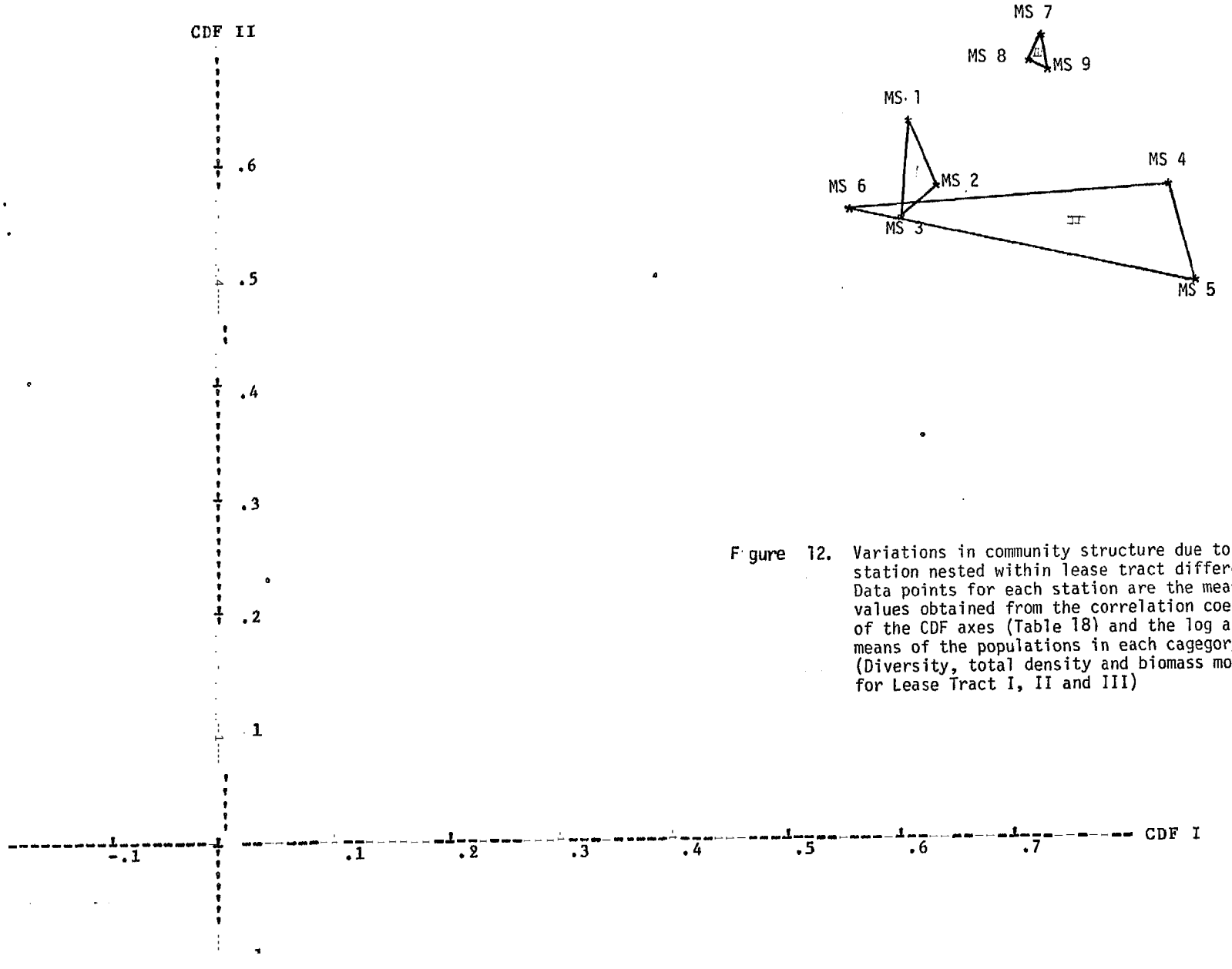


Figure 12. Variations in community structure due to station nested within lease tract differences. Data points for each station are the mean CDF values obtained from the correlation coeffieients of the CDF axes (Table 18) and the log adjusted means of the populations in each cagegory. (Diversity, total density and biomass model for Lease Tract I, II and III)

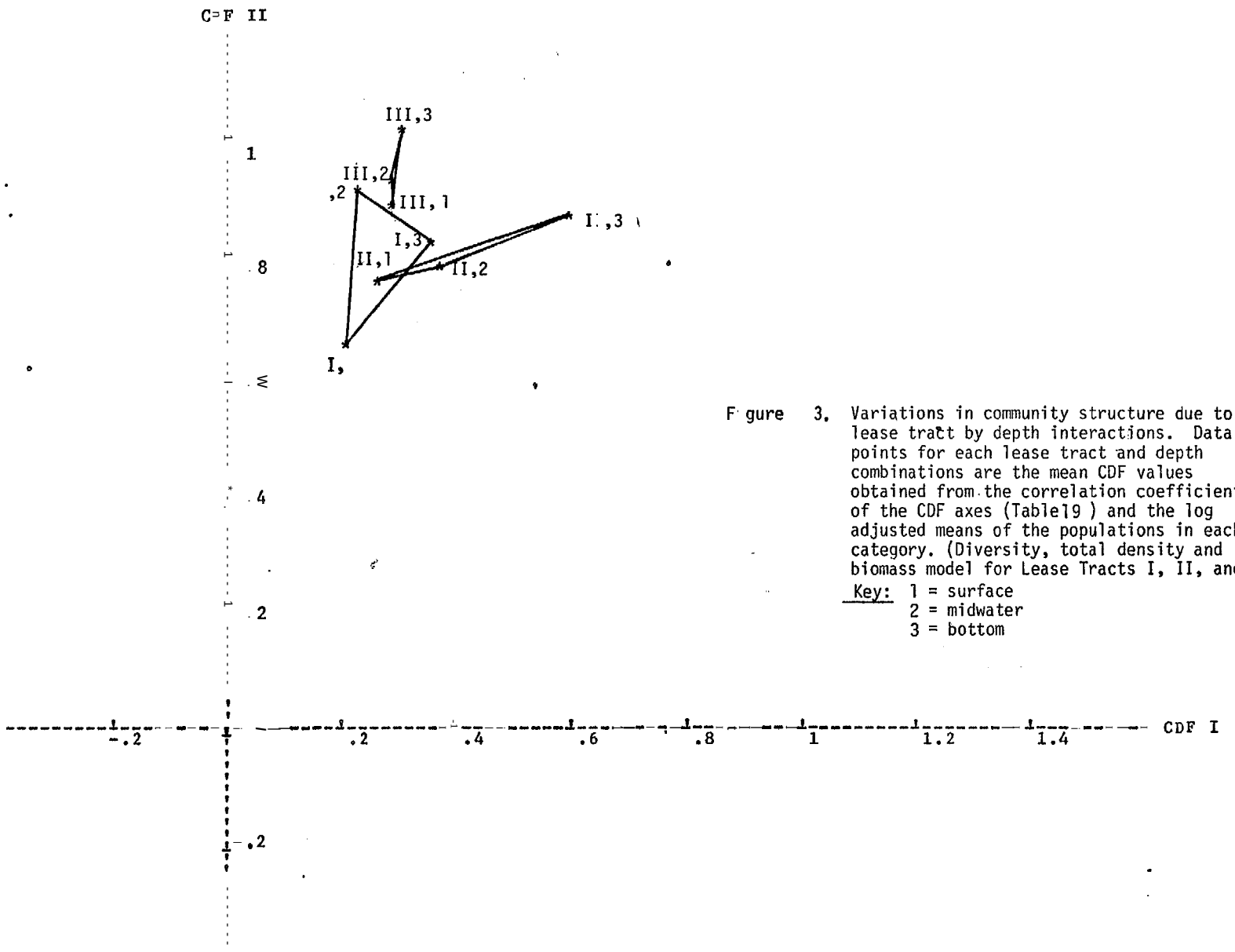


Figure 3. Variations in community structure due to lease tract by depth interactions. Data points for each lease tract and depth combinations are the mean CDF values obtained from the correlation coefficient of the CDF axes (Table19) and the log adjusted means of the populations in each category. (Diversity, total density and biomass model for Lease Tracts I, II, and II

Key: 1 = surface
2 = midwater
3 = bottom

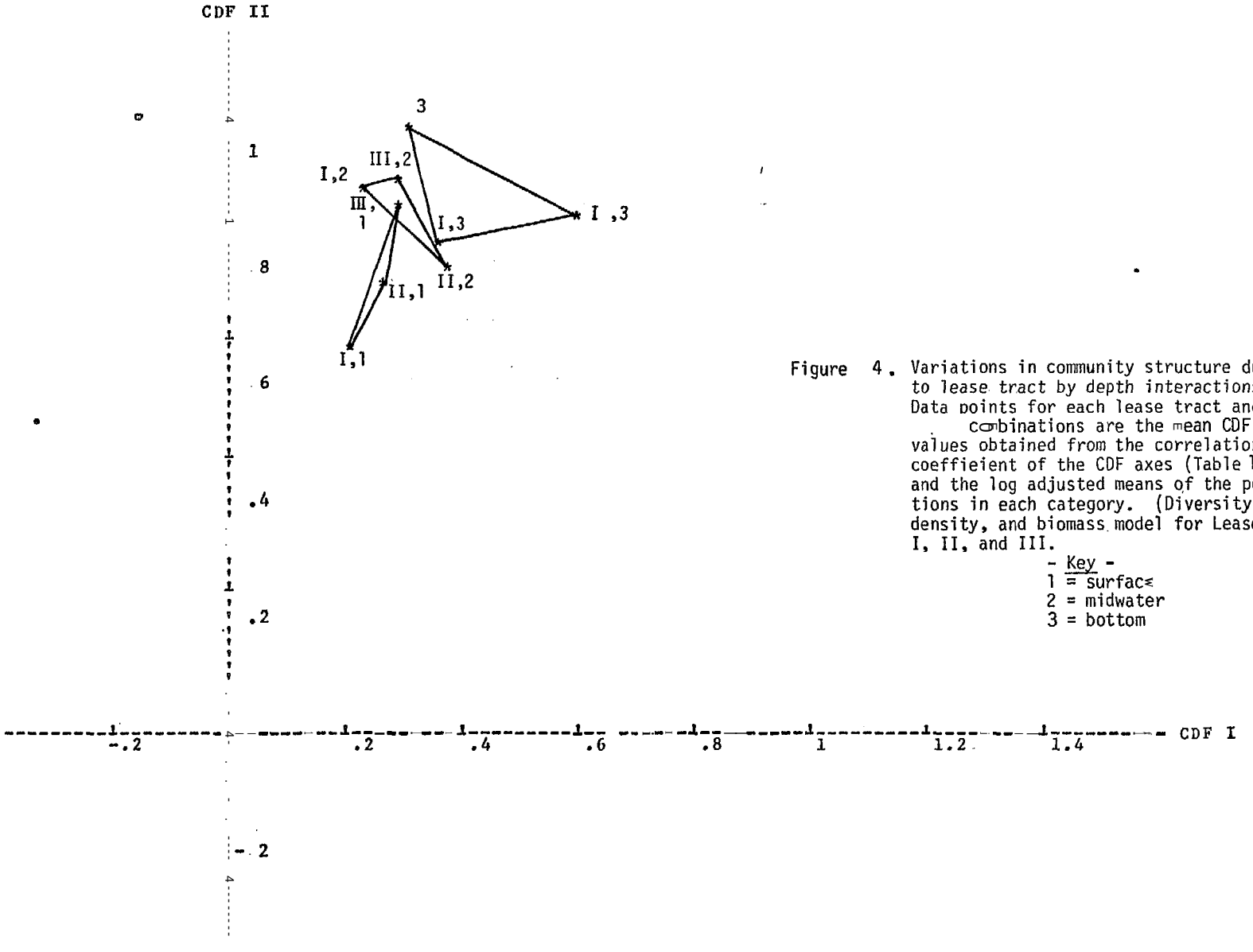


Figure 4. Variations in community structure due to lease tract by depth interactions. Data points for each lease tract and combinations are the mean CDF values obtained from the correlation coeffieient of the CDF axes (Table 19) and the log adjusted means of the populations in each category. (Diversity, total density, and biomass model for Lease Tracts I, II, and III.

- Key -
1 = surface
2 = midwater
3 = bottom

with the bottom tows of Lease Tract II exhibiting larger total density and biomass and bottom tows of Lease Tract III exhibiting the 'largest. diversity.

A similar model to that for Lease Tract I, II, and III was run for Lease Tract IV and V. Total density and diversity were modeled as dependent variables determined by lease tract, station within lease tract, depth, lease tract by depth interaction, hour, and concentration of CH_4, C_2H_4, and C_3H_8. Only depth effects were significant (Milks' λ = .7188 significance = .0001). Figure 15 presents the graph of the depth of tow effects on the CDF space defined in Table 19. The means of the cononical variables for each depth of tow are given in Table 20. Note that CDF 11 which serves to separate midwater tows from surface and bottom tows is characterized by large positive correlations with diversity and large negative correlations with total density. Bottom samples are separated by CDF I indicating , in general, lower density and biomass than in surface and midwater samples (Table 21).

Considerations of Control Stations

The problems involved with including the control stations in the analyses are discussed in the section on statistical methods. A method wasused which allowed comparison of the control stations with the Master Stations within the framework of the CDF space presented in Figure 16 (see Statistical Methods). For this one season sample and for the categories used in the model, C-2 appears to be a fairly good representative of the group of stations made up of MS-4, MS-5, MS-7, MS-8, and MS-9. In other words, C-2 appears, in this model, to be fairly comparable station to the Lease Tract 111 stations. While MS4 and MS-5 of Lease Tract II compare favorably with C-2; MS-6, which is closer geographically, is quite different in terms of this analysis. Control Station 1 appears to be intermediate to

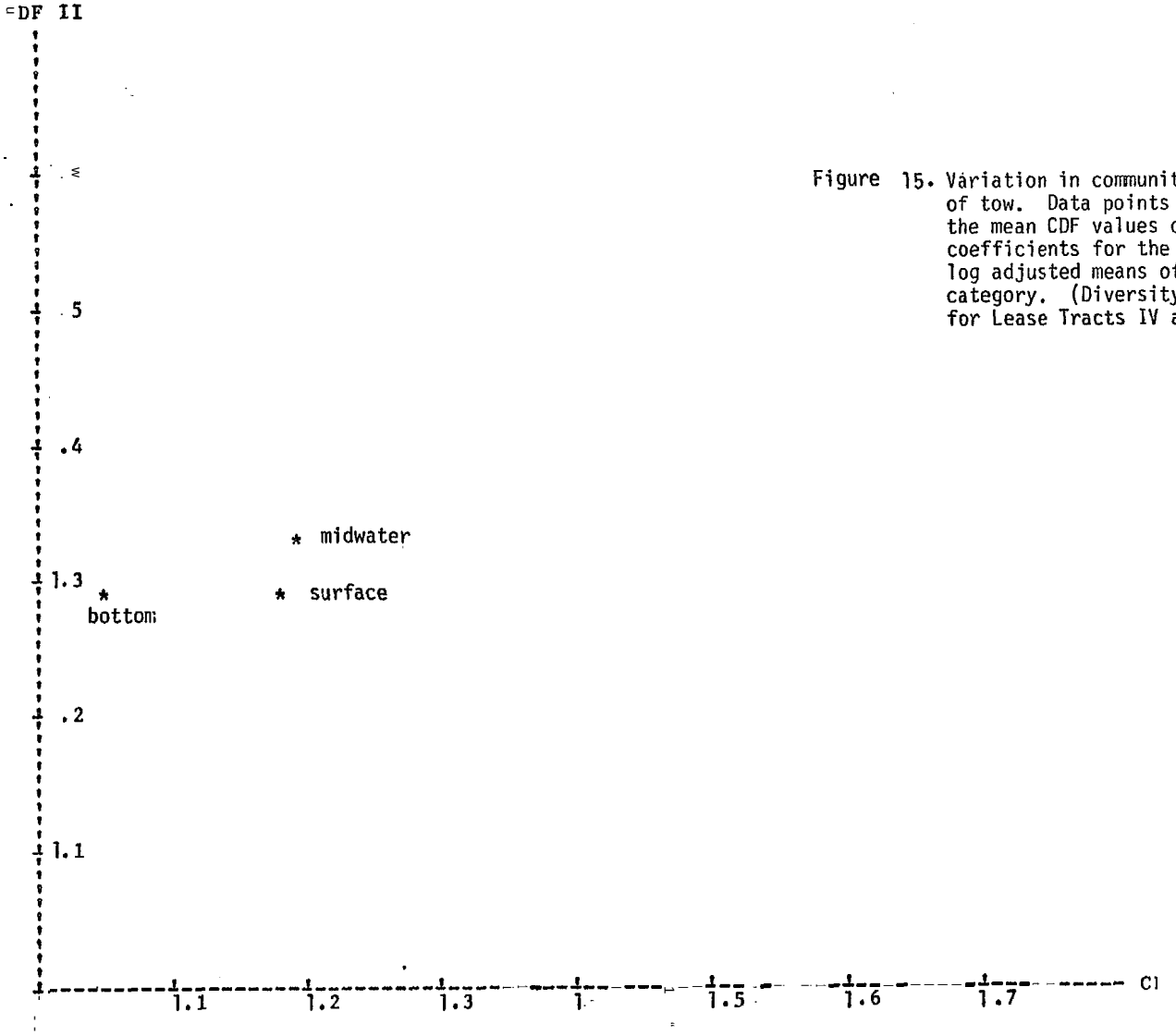


Figure 15. Variation in community structure due to depth of tow. Data points for each depth of tow are the mean CDF values obtained from the correlation coefficients for the CDF axes (Table 21) and the log adjusted means of the populations in each category. (Diversity and total density model for Lease Tracts IV and V)

Table 19. Correlation Coefficients Between Canonical Variables and the Dependent Variable for Lease Tract by Depth Interactions (Diversity, Total Density, Biomass Models for Lease Tract I, II, and III)

Categories	CDF I	CDF II
Shannon-Weaver Diversity	-0.013	0.997
Total Density	0.878	-0.048
Biomass	0.452	0.226

Table 20. Means of the Canonical Variables for Surface, Midwater and Bottom Tows (Diversity and Total Density Model for Lease Tracts IV and V)

Depth	CDF I	CDF II
Surface.	1.1810	1.2882
Midwater	1.1926	1.3280
Depth	1.0487	1.2876

Table 21. Correlation Coefficients Between Canonical Variables and the Dependent Variables for Differences Between Depths (Diversity and Total Density Model for Lease Tracts IV and V)

	Total Density	Shannon-Weaver Diversity
CDF I	0.7977	0.5814
CDF II	-0.6030	0.8136

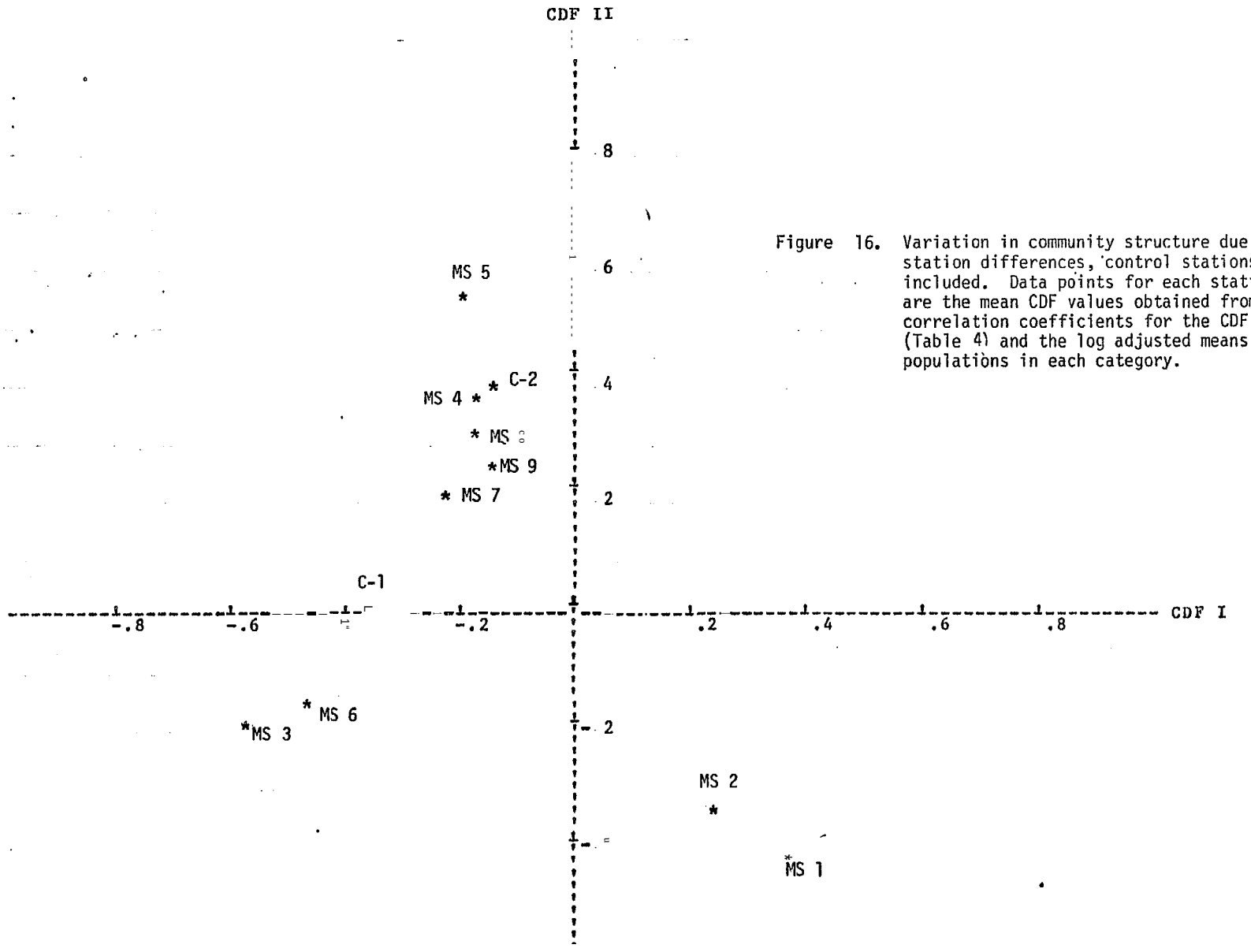


Figure 16. Variation in community structure due to station differences, control stations included. Data points for each station are the mean CDF values obtained from the correlation coefficients for the CDF axes (Table 4) and the log adjusted means of the populations in each category.

the stations of Lease Tract II and III.

Control Station 3 and 4 are compared to the Master Stations of Lease Tract IV and V in Figure 8. C-3 and C-4 have the best association with MS-10 and 12 in the CDF space but little relationship with MS-13, MS-14, and MS-15.

This comparison of control stations to the master stations indicates the need for control areas in the next study and for a better understanding of water masses in the area.

As has been suggested for the monitoring studies, an attempt to sample water masses would be wiser than the simple geographic location of master and control stations.

III. CONCLUSIONS

1. In Lease Tracts I, 11, and 111, an assemblage of organisms composed of chaetognaths, Oncaea sp., Oithona sp., other calanoids, shrimp larvae, Oikopleura, copepod copepodites, pteropods, Centropages furcatus, crab larvae, other crustaceans, ostracods, other tunicates, and fish eggs was typical. This is a group consisting of offshore holoplankters associated with a group of meroplankters rather typical of the spring season.
2. Some samples from Lease Tract IV and V exhibited an inshore, freshwater-influenced community with interesting variations possibly attributable to hydrographic fluctuations.
3. Evidence for stratification of communities due to salinity related water density differences is present for Lease Tract IV and V. However, these conclusions are based largely on knowledge of the tolerance levels of certain organisms rather than upon salinity data. This stratification, if it does indeed exist, would be an important influence in the fate of hydrocarbons introduced into the environment.

4, The master and control stations indicated, through the variations of the zooplankton model, that geographic placement of stations is inferior to sampling of specific water masses in the Eastern Gulf of Mexico.

5. The importance of meroplankters in the community emphasizes the importance of seasonal sampling to further detail the variation of the zooplankton community.

6. Lease Tract I and II have similar diversities, but Lease Tract II in general was an area of higher total density and biomass in keeping with the rich community structure typical of the Florida middle grounds.

7. Lease Tract 111 is an area of low density and biomass but higher diversity when compared to Lease Tracts I and II.

8. In general, when stations within lease tracts and depth of tow are considered, Lease Tract III exhibited smaller variation than Lease Tracts I and II.

9. It appears that much of the variation unaccounted for in this model may be explained by more detailed physical and chemical information.

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ZOOPLANKTON APPENDIX I

DISCUSSION OF TECHNIQUE OF SAMPLE SIZE DE.TERMINATION

Plankton densities were log-adjusted according to the transform $X\text{-}LOG_{10}(X+1)$. This transformed the population of plankter densities to one more closely approximating the multivariate normal distribution. Calculations of sample size were based on the ability to detect changes in these log-adjusted densities equal to or exceeding .25 of the mean value for each category with 99% confidence.

Since the variance-covariance matrix for a multivariate model is crucial to the determination of future sample size, a discriminant analysis procedure was performed on 108 samples using the N.E.R.D.C. IBM 370/165 computer and the Statistical Analysis System (SAS) DISCRIM (Service, 1970) procedure. This allowed us to determine that the variance-covariance matrices of the three lease tract sites were not similar enough to permit the use of a pooled variance-covariance matrix in setting the future sample size. It was decided to approach the sample size problem on an individual lease tract basis (See Morrison, p. 121, 1967).

To determine sample size, it was necessary to solve the following equation for N:

1. $N(D'S^{-1}D) = (N-1)\ (1/N-P)(P)(F_{1\ 2})$,

where N is sample size,

P is the number of dependent variables in the model,

F is the appropriate F-value for the desire level of confidence, , with the applicable degrees of freedom, $_1$= p and $_2$ N-p,

S^{-1} is the inverse of the applicable variance-covariance matrix, "

D is the vector of desired detectable differences, and,

D' is the transpose of D "(Morrison, 1967).

The matrix multiplication, $D'S^{-1}D$, will result in a scalar, here called Y, which allows us to write the equation (with further manipulation) as:

2. $N = (N-1)(1/N-P)(P)(F)(1/Y)$

Further solution is complicated by the fact that the value of F is a function not only of the desired confidence level, but also of the associated degrees of freedom, which requires a knowledge of the sample size, N, the quantity we are seeking to determine.

The calculation of N then reduces to a repetitive technique of making an educated guess at the likely sample size, using that value of N on the right-hand side of equation 2 to solve for N, comparing the result obtained with the original estimate, and adjusting as necessary until general agreement is achieved. The N thus obtained is rounded upward to the next multiple of 9 to conform with the sampling scheme in use.

Water column environmental data are currently being processed by other investigators and are unavailable. When these data are received, a more accurate assessment of sample size will "be possible.

Without these data, only very limited models, expressing the levels of plankton populations as a function of station and sampling depth, have been attempted. These models have focused on either those populous categories which comprise the bulk of the plankters found, or on categories of plankters not so abundant in the smaples but of commercial significance to the MAFLA area, such as fish and shellfish.

In the final model, the loss of degrees of freedom for 2 due to increasing the number of dependent variables, which would necessitate an increase in the number of samples required, should be adequately compensated for by the reduction in variability explained by the inclusion of environmental parameters.

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ZOOPLANKTON APPENDIx II

Review and Bibliography of Effects of Petro-hydrocarbons in the Sea.

J. Mangiordi (Maturo Group)

Introduction

The fate and effects of petroleum - derived hydrocarbons in the open ocean are relatively new and poorly documented problems. The complexity of the problems are very great not only because of the large number of biological and physical factors with which these products can interact, but the petroleum products themselves are geographically unique combinations of 150 or more types. This report examines the effects of these petro-hydrocarbons on marine zooplankton and provides a general bibliography of the chemistry and fate relations of these substances when introduced into the sea.

The effects of petro-hydrocarbons introduced into the marine environment depend on several parameters: 1) type of oil introduced, 2) oceanographic conditions including season, the weather, and water temperature, 3) amount of oil introduced, 4) amount of previous exposure the affected area has had to petroleum pollution, 5) existing biota levels, 6) other pollutants compounding the problem, 7) method in which affected area is treated, and 8) time lag in implementation of treatment (from Evans and Rice, 1974).

Effects on Zooplankton

Prior to a discussion of the effects on zooplankton, it is important to recognize the inherent problems in current investigations of the biological aspects of oil pollution. The absence of comprehensive systematic zooplankton studies coupled with a lack of standardization of results and innacurate measurements of the concentration and composition

of crude petroleum both in solution and in the bodies of plants and animals, have made comparison of different studies difficult. In addition, the complex composition of hydrocarbons substances and the physical and biological processes which alter this composition make it difficult to analyze accurately and to attribute observed biological effects specifically to even a limited fraction of the crude petroleum (Moore, et al., 1974).

Since there are large numbers of conflicting factors in experimentation concerning the influence of petroleum on zooplankton, the results should not be regarded as rigorous standards. Instead, they should serve as guidelines for general interpretation.

The-effects of hydrocarbons on the zooplankton community can be grouped as follows:

1. direct lethal toxicity
2. sublethal disruption of physiological and behavioral activities
3. effects of direct coating by oil
4. incorporation of hydrocarbons in organisms which may cause tainting and/or accumulation in food chains
5. long-range changes of community due to an altered environment.

Direct Lethal Toxicity

Insofar as zooplankton is concerned, the effects may be critical or lethal to one organism, and show little or no effect on another. For example, *Tigriopus californicus* (a tidal pool copepod) died in three days when subjected to a crude oil concentration of 25 ml/liter, whereas, pelagic copepods *Acartia clausi* and *Oithona nana* survived not more than one day when subjected to only 0.1 ml/liter crude oil concentration (Kontogiannis and Barnett, 1973). Nelson-Smith (1972) noted one author's

findings that after testing twenty different crude oils, their toxicity varied from 1-89%. The water soluble, more volatile, and lower. boiling portions are the most toxic, especially if they are high in aromatics (Blumer, 1969 b).

The oil spill in West Falmouth, Massachusetts, in 1969, was extremely dangerous because it contained nearly 45% aromatics. On the west coast of the United States, similar products pumped from a stranded barge completely destroyed a commercial shell fishery (Nelson-Smith, 1972). Wells (1972) noted that emulsions of crude oil are lethal to larvae of the American lobster, Homarus americanus, at concentrations of 100 ppm, and appeared to show sublethal effects in concentrations down to 1 ppm.

Renzoni (1973) noticed that while oil was not as toxic to larvae as might be expected, its influence on the fertilizing capacity of the sperm is "remarkably" high. The toxic effects of oil on larval stages of many marine organisms appear to be 10-100 times more severe than on adults (Moore et al., 1974). Evans and Rice (1973) cited evidence which demonstrates that 0.001 ml of crude oil/liter was toxic to the-eggs of anchovy, scorpion-fish, and sea parrots from the Black Sea. Also tested was crude oil effects on several copepods and a cladoceran. These experiments showed that 0.001 ml/liter accelerated death in all forms and concentrations of 0.1 m./liter caused death in less than one day. Larvae of crabs and shrimp died at 1 ppm concentrations. Some further evidence of petroleum toxicity on zooplankters can be found in Moore et al., 1974.

Blumer (1969b) categorized the toxicity of hydrocarbons by saying that the low boiling saturated hydrocarbons produce at low concentration anesthesia and narcosis and at greater concentrations cell damage and

death in a wide variety of lower animals and that they may be especially damaging to larval and juvenile forms of marine life. Higher boiling saturated hydrocarbons naturally occur in many marine organisms and are probably not directly toxic, but may inhibit chemotaxis and nutrition. Aromatic hydrocarbons are abundant in petroleum and are the most dangerous portion. Lower boiling aromatics (benzene, tolulene, xylenes, etc.) are acute poisons for man as well as other organisms. The high boiling aromatic hydrocarbons are believed to be long-term poisons. These are polycyclic compounds (similar to carcinogenic compounds of cigarette smoke). Nonhydrocarbon portions of crude oil (sulfur, nitrogen, oxygen, and metal compounds) closely resemble the ecological properties of the corresponding aromatic compounds.

Sublethal Disruption of Physiological and Behavioral Activities

Marine organisms employ a diverse set of complex systems for chemotaxic purposes. For most species in the marine environment, the chemical *sense* may be the most dominant. Chemical cues (pheromones) are necessary for: food location, detection and selection of niches, providing the signal for gamete fusion in many species, homing and aggregation behavior, location and selection of sexual partners, and organizing social structure (as in some species of fish). Among crustacea, the female may release a sex pheromone to signal her presence and physiological state (Takahashi and Kittredge, 1973). The studies of Takahashi and Kittredge on chemoreception interference by petrohydrocarbons in crabs suggest that sublethal effects of the water-soluble components of petroleum products may alter species survival. "The basic elements of species survival are reproduction and growth. It has been shown that both of these elements, because of the major role of chemoreception in the marine environment, are sensitive to minute con-

centrations of aromatic hydrocarbon pollution."

Blumer (1969b) believes that artificially introduced hydrocarbons may gravely interfere with chemotaxis by blocking or masking response [larvae of plaice, cod, and herring were noted by one scientist to be unable to avoid well-defined milky clouds of toxic oil dispersants (Evans and Rice, 1974)] or by mimicking natural stimuli which can result in -false responses. Evans and Rice (1974) cited an event which involved the attraction of lobsters to kerosene and to purified hydrocarbon fractions derived from kerosene and observed many lobsters washed ashore following the West Falmouth spill. Walsh and Mitchell (1973) state that "A wide variety of chemical and physical water pollutants is shown to inhibit microbial chemotaxis. Such chemotaxis inhibition may have direct effects on microbial predator-prey relationships or on the rate of degradation of organic substrates in the sea."

The paucity of experimentation within this area involving zooplankton is evident. However, the fact that petro-hydrocarbons interfere with chemotaxis in organisms as diverse as bacteria and fish leaves a range of possibilities for interference with chemoreception in zooplankton creatures, whose members include many phyla.

A thick oil slick on the ocean can reduce light penetration by 90%, prohibiting normal photosynthetic activities of phytoplankton, and possibly interfering with the daily vertical" migration of zooplankton. Some larger members of the zooplankton community feed after visual inspection rather than filtering indescriminantly. Covering portions of the sea by oil, thus preventing light penetration, may affect nutrition and behavior of zooplankters (Nelson-Smith, 1973). Furthermore,

Kittredge (1974) adds: "We have found that minute traces of the sea-water soluble components of crude petroleum (Boylan and Trip, 1971) completely inhibit the feeding response of crabs that is normally induced by dilute solut ons of amino acids. Even lower dilutions of this petroleum fraction will inhibit the response to the sex pheromone. This, combined with the observations of Cook and Elvidge (1951), Muller (1968), and Atema et al. (1971) recording both false responses of algal gametes to lower-hydrocarbons and a 'confusion' response of lobsters to kerosene fractions, strongly suggests that in the marine environment the most detrimental effect of petroleum pollution is an interference with the organisms' facilities for chemoreception. This effect may well result in inhibition of their ability to detect food or sexual partners at concentrations 10^{-9} below those nominally determined as lethal .

Effects of Direct Coating by Oil

Coating affects zooplankton which are unable to leave a contaminated area. If compounded by inclement weather, which increases vertical mixing, it is possible that a large amount of coating can develop. The results of such coating could be asphyxiation and restriction of appendages used for feeding and locomotion (Moore et al., 1974). Conover (1971) noted that after oil was re-introduced by coastal processes as particulate matter mixed with sand (approximately the size range as those food items consumed by zooplankton), small oil particles were shown caught in the interstices of the feeding appendages of Temora longicornis. There has been little documentation concern" ng this petroleum pollution consequence.

Incorporation of Hydrocarbons Causing Tainting and/or Accumulation of Hydrocarbons in Food Chains

Morris (1974)," after collecting samples of near surface crustaceans, fish, and mixed plankton, found large amounts of petroleum hydrocarbons in their lipids (17-33% of the wet weight of those organisms). Since some plankton organisms are capable of ingesting petroleum hydrocarbons, these pollutants are likely to travel along the food chain. Blumer (1969b) has determined that hydrocarbons, once they enter the food chain, are very stable and undergo little or no alterations. He believes that once in the food web, hydrocarbons may not only be retained but also accumulated similar to chlorinated pesticides. "Once assimilated, this oil passes through the marine food chain and eventually reaches organisms that are harvested for human consumption."

Another consequence of hydrocarbon incorporation and accumulation is "tainting" which produces undesirable flavor. Another is the accumulation of carcinogenic compounds (Finnerty et al., 1973). Moore et al. (1974) state "essentially any aquatic organism can be expected to equilibrate chemically with its surrounding media. If the media contain even low (ppb) concentrations of hydrocarbons, these substances may be ingested and accumulate in various tissues." Moore cites additional information which indicates that oil entering an estuary can be found in virtually all organisms. Blumer et al. (1969b) found that several species of copepods displayed different and distinct concentrations of hydrocarbons in lipid composition which can lead to "practical implications for studies of the marine food web and of marine pollution with persistant chemicals,"

Conover (1971) studied large "oil" particles in the foregut of

of Calanus which seemed unable to pass into the posterior region of the gut. He referred to studies which showed that certain Metridia longa, who seemed to be feeding well, became moribund and defunct even though their foreguts were full of food. Upon examination, there proved to be some foreign matter obstructing food passage. He therefore concluded the possibility that a large oil particle might cause such blockage producing fatal consequences. However, Conover declared, "If zooplankton ingested suspended oil in proportion to its concentration, in a suitable size range in the water, and if this oil passed through the animals largely unaltered to be defacated along with other undigested food remains in the form of fecal pellets which would be considerably denser than sea water, zooplankton grazing might constitute an important natural clean up process for disposing of oil on and in the sea." He calculated, after studying Bunker C oil in zooplankton feces following the wreck of the tanker Arrow in Nova Scotia, that 0.21 metric tons of oil could be deposited daily on the bottom of the Chedabucto Bay in zooplankton feces. However, he maintained an assumption that zooplankton were predominantly unaffected during ingestion of hydrocarbons.

It is important to realize that hydrocarbons are synthesized by most and probably all living creatures (Blumer et al., 1970a). Organisms possess specific biosynthetic pathways which favor the production of hydrocarbons in certain size ranges. It. has been shown earlier in this report that copepods may ingest polluting hydrocarbons along with hydrocarbons from natural sources. Thus, it is through such pathways that polluting hydrocarbons become part of the marine food web. Hydrocarbons have a strong resistance towards metabolism which may have the consequences of persisting after the initial source has been lost (Blumer et al., 1970a).

Long-range Changes of Community to an Altering Environment

The community structure of planktonic organisms may be jeopardized, especially in chronically affected areas, by reduction in diversity which may be represented by an increase in more resistant species. This situation is only feasible where the community is broad enough to include such resistant organisms. It is generally held that reduction in diversity is unfavorable since that community may be influenced by further adverse conditions; now reduced in stability the ecosystem may not be stable enough to face further pollution (Nelson-Smith, 1973).

As Moore describes, "An additional 'effect' of oil which can be hypothesized, but is virtually unstudied,-is adaptive changes that may occur in the short-term (less than one generation)--acclimation--or in the long-term (many generations)-- genetic changes. If acclimation occurs, the sensitivity of an individual to effects of oil may decrease noticeably under prolonged or repeated exposure, thereby increasing the individual's resistance to oil. However, the poorly understood complex process of acclimation may involve accompanying changes which further affect the individual's overall probability of survival in an unknown manner.

Genetic adaptation is actually a population level phenomena. Natural selection operates to select those individual progeny in each generation which are best able to cope with the environment as it exists. Persistant petroleum-derived hydrocarbons in the environment may result over many generations in a population of more tolerant individuals. Again, however, this poorly understood process may reaccompanied by

other changes, unrelated to hydrocarbon tolerance--changes which may be desirable or undesirable.

Both of these adaptive effects are essential characteristics of living organisms. However, the process are poorly understood in general, and virtually unknown relative to their implication for responses to and effects of oil. However, it is clear they play important roles in determining the u" timate effect of oil in marine environments.

Discussion

Zooplankton, consisting of a large array of organisms balanced in a web of interrelations, can be affected by hydrocarbons in basically two methods: a single large spill or dumping of petro-hydrocarbons, or chronic sources. Since the most dramatic in immediate consequence is a spillage (which constitutes approximately 10% of hydrocarbon pollution; see Table I) many observations have been made in the aftermath of such events. On the other hand, areas affected by a chronic source of hydrocarbon pollution are altered in a more severe manner (Nelson-Smith, 1973; Evans and Rice, 1974). A large spill may affect an area but afterwards depart, leaving those affected to recover as they caii from the spill. A chronic source would not permit a period of recuperation', therefore accumulating 'more dangerous consequences, especially by constantly exposing zooplankton populations who are subject to currents and tides and cannot easily avoid a chronically contaminated region. It has been shown by Blumer (1969b) that species of zooplankton are capable of incorporating polluting hydrocarbons along with normally synthesized hydrocarbons as both sources assimilate into the lipid pool which may have cumulative effects

TABLE I

ESTIMATED DIRECT PETROLEUM HYDROCARBON LOSSES

TO THE MARINE ENVIRONMENT (NOT INCLUDING: AIRBORNE

'HYDROCARBONS DEPOSITED ON SEA SURFACE).

Millions of Tons

Source	1969	1975 Min.	1975 Max.	1980 Min.	1980 Max.
tankers	.530	.056	.805	.075	1.062
other ships	.500	.705	.705	.940'	.940
offshore production*	.100	.160	.320	.230	.460
refinery operations	.300	.200	.450 "	.440	.650
oil wastes	.550	.825	.825	1.200	1.200
accidental spills	.200	.300	.300	.440	.440
TOTAL	2.180	2.246	3 . 4 0 5	3.325	4.752

* Makes up 19% of present oil product on (from Revelle, et al., 1971).

on higher members of the trophic levels, even man, as in the case of chlorinated pesticides. Oil may block feeding passages by either -clogging the digestive tract or inhibiting the use of feeding appendages as shown by Conover (1971) in the case of some copepods. Planktonic copepods may also have a capacity to aid in dispersing oil, but in this process various effects may accumulate and take their toll "on the population involved. The oil may directly cause the zooplankters to perish by toxic effects or by interfering with chemotaxis.

In long-range projections, theory (Moore et al., 1974) includes the possibility of individual acclimations and population level genetic alterations of zooplankton to a petro-hydrocarbon polluted environment. Since ecological systems must adjust to constantly changing environments, the change of higher petro-hydrocarbon levels on and in the sea may necessitate zooplankters to utilize genetic capacities in order to survive.

Moore et al. (1974) bring up an interesting question, asking what constitutes a significant loss in the pelagic biomass. "If ten square miles of oceanic life is impacted and all organisms in the area are killed by a spill, is that significant? What about an entire generation of copepods over Georges Bank$is that a significant loss?"

He further states, "Rather than define significance in terms of areal, interspecies (predator-prey) or commercial effects, we simply consider impact to be significant if it would detectably (i.e. a difference could be measured with existing techniques) alter for more than a year the size or age distribution of an impacted 'breeding population.'"

In conclusion, it is evident that petro-hydrocarbons pose intricately

complex problems on zooplankton community structures. Not only are the ramifications difficult to assess, so too is the significance in relation to the marine ecosystem. Zooplankters are one of the primary recipients of floating oil consequences in the manner described, but since they constitute a primary link in the chain of trophic positions in marine ecology, the effects shown on their community structure will likewise be revealed in those organisms which depend upon zooplankton for ultimate survival.

Summary

1) Oil pollution consists of hundreds of different hydrocarbon compounds with many complex physical, chemical, and biologically associated properties.

2) Sources of petroleum hydrocarbons in the sea arrive through various means, of which spills account for approximately 10%. The amount of hydrocarbon pollution in the sea has exceeded natural production of hydrocarbons (Blumer, 1969).

3) Hydrocarbons affect zooplankton by direct lethal toxicity coating and subsequent asphyxiation, interference with chemotaxis, ingestion of oil particles by zooplankters causing possible blockage of internal digestive pathways, confining the use of feeding appendages, and incorporation and accumulation of petro-hydrocarbons into the lipid pools of the organisms. In long-range speculation, a change in genetic makeup may be necessary for survival in hydrocarbon polluted seas.

4) Chronic sources of hydrocarbon pollution are more severe than from single dosage sources (i.e. spill's, etc.).

5) Total assessment of damages is difficult owing to limited study and lack of standardized analysis techniques: Many more studies are needed in order to refine the impact of petroleum hydrocarbons on marine zooplankton as well as other marine organisms.

D "

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